Sudan University of Science and Technology

Collage of Graduate Studies

D-dimer Assay and Complete Blood Count among Women with Systemic Lupus Erythematosus

قياس دي دايمر و التعداد الكامل لخلايا الدم في النساء المصابات بمرض الذئبة الجهازية الإحمرارية

Dissertation submitted for partial fulfillment for the resonant degree of M.Sc. in medical laboratory sciences (haematology and Immunohaematology).

By

Marwa FatehAlrahman Omer MohamedAhmad, Bsc SUST 2006, HD. SUST 2011

Supervisor

Dr. Hiba BadrEldin Khalil, Ph.D

Associate Professor, Department of Haematology

Alneelain University

2018

Sudan University of Science and Technology

Collage of Graduate Studies

D-dimer Assay and Complete Blood Count among Women with Systemic Lupus Erythematosus

قياس دي دايمر و التعداد الكامل لخلايا الدم في النساء المصابات بمرض الذئبة الجهازية الإحمرارية

Dissertation submitted for partial fulfillment for the resonant degree of M.Sc. in medical laboratory sciences (haematology and Immunohaematology).

By

Marwa FatehAlrahman Omer MohamedAhmad, Bsc SUST 2006, HD. SUST 2011

Supervisor

Dr. Hiba BadrEldin Khalil

2018

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قال تعالى : (وَمَا خَلَقْتُ الْجِنَّ وَالإِنسَ إِلاَّ لِيَعْبُدُونِ (٥٦))

صدق الله العظيم

سوره الذاريات الآيه (٥٦)

DEDICATION

TO MY BELOVED AND PLEASED PARENTS WHOM EVERY THINGS FOR ME.

TO MY WONDERFUL SUPERVISOR;

DR. HIBA BADRELDIN KHALIL.

TO MY SPECIAL FRIENDS AND COLLEAGUES.

I DEDICATE THIS WORK

Acknowledgement

The greatest thank to Allah for helping me to finish this research. I would like to express my deep graduate and thanks to everyone who helped me throughout this work at any step of it.

Firstly, I am most grateful to my supervisor **Dr. Hiba BadrEldin Khalil** for this expertise support and guidance

My great thanks also extended to Dr**. Alnoor Alaqeb** (Rheumatology consultant), head of Rheumatology department of military hospital for his valuable assistance and encouragement throughout the research.

Finally, thanks extend to all women with systemic lupus erythematosus that participated in this research.

Abstract

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organ systems with immune complexes and a large array of autoantibodies. Haematological abnormalities are common findings in patients with SLE with increased risk for thrombosis. The study aimed to evaluate the risk of thrombosis and haematological changes on women with Systemic lupus erythematosus.

Materials and Methods: This is an analytical case control study carried out in Khartoum state in military hospital, in the period from August to December 2017. Thirty women diagnosed with SLE and thirty matched control group were recruited in this study. EDTA and citrated blood samples were collected from each patient and control for Complete blood count and D-dimer. Data was analyzed by SPSS version 20.

Results: The mean of D-dimer in SLE patients was (379ng/ml) and in control was (89.58ng/ml) that showed significant increased (P value = 0.00) in SLE patient than control group. Moreover, the highest level of D-dimer (936.5 ng/ml) was related to the most clinical severity patients with significant association between severity of disease and D-dimer level (P value= 0.00). The means of Haemoglobin, Packed cell volume, Total white blood cells, Thrombocyte, Neutrophil/Lymphocyte ratio for SLE patients were 11.6 g/dl, 36.3%, 5.78 x 10^9/L, 262 x 10^9/L, (3.33) respectively, while the means of the same parameters in the same order for the control were 11.96 g/dl, 35.75%, 5.42x10^9/L, 288x10^9/L, (1.61) respectively. No significant differences between patients and controls in CBC, all P values was (P value > 0.05) except in N/L ratio which was significantly increased in patients (P value= 0.026). On the other hand, there

were no significant differences in D-dimer level and CBC in between group of medication and duration of disease, also there were no significant differences in severity of disease for CBC, all P values (P value > 0.05). Adding to that, there were no significant differences in D-dimer on the different haematological situation (P value > 0.05).

Conclusion: The study concluded that, there was a significant increase in the D-dimer level among women with SLE and it is associated to the clinical severity of the disease, also there were no significant differences between patients and control group in CBC.

ملخص الدراسه

مقدمه : مرض الذئبه الجهازيه الأحمراريه هو مرض مناعي يهاجم العديد من الأعضاء في جسم الأنسان بواسطه مركبات مناعيه وأنواع مختلفه من الأجسام المضاده الذاتيه, التغيرات في تعداد الكريات الدمويه يوجد بكثره لدى المرضى بالذئبه الجهازيه الأحمراريه وكذلك لديهم زياده في خطوره احتماليه ظهور الجلطات الدمويه . هذه الدراسه الهدف منها تقييم خطوره أحتماليه الأصابه بالجلطات والتغيرات في تعداد الكريات الدمويه لدى النساء اللاتي يعانين من مرض الذئبه الجهازيه الأحمراريه.

طرق البحث: هذه دراسة تحليليه لحالة مقارنه بحالة ضابطة في ولاية الخرطوم في المستشفى العسكري في الفترة من أغسطس ٢٠١٧ الى ديسمبر٢٠١٧. تم أختيار ثلاثين امرأه تعانين من الذئبه الجهازيه الأحمراريه كحاله دراسه و ثلاثين امرأه من الأصحاء كمجموعه ضبط ، تم أخذ الدم الوريدي في مانع تجلط مناسب لكل نوع فحص من كل مريضه و حاله ضابطه لقياس دي دايمر وتعداد كريات الدم وتم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية اصداره ٢٠ .

النتائج: وجد ان متوسط قيمه الدي دايمر لدى المرضى بالذئبه الجهازيه الأحمراريه كان يساوي (٣٧٩ نانوغرام / مليليتر)، و متوسط قيمه الدي دايمر لدى المجموعه الضابطه كان يساوي (٨٩،٥٨ نانوغرام / مليليتر)، وقد كانت قيمته لدي المرضى مرتفعه عن المجموعه الضابطه مع فرق بدلاله أحصائيه واضحه و بالأضافه الى ذلك وجد أن هذه القيمه تزيد بزياده حده المرض وتبلغ ذروتها لدى المرضى اللاتي يعانين من الحده الأعلى حيث كانت قيمه الدي دايمر لديهن تساوي (٩٣٦،٥ نانوغرام/مليليتر)، تم حساب متوسط النتائج لتعداد الكريات الدمويه كالتالي : متوسط نتائج المرضى وجد ان الهيموغلوبين، و حجم الخلايا المعبأة، و مجموع خلايا الدم البيضاء، و الصفائح الدمويه،و نسبة العدلات / اللمفاويات كانت على التوالي: (٦,١١ غرام/ ديسيليتر)، (٣,٣٦٪)، (٧٨,٥ × $١٠^{٩}$ / ليتر)، (٢٦٢ ×$١٠^{٩}$/ ليتر)، (٣٣,٣). أما بالنسبة للمجموعة الضابطة فقد كانت النتائج لنفس المتغيرات كالتالي : (٩٦,١١ غرام / ديسيليتر)، (٧٥,٣٥٪)، (٤٢,٥× $١٠^{٩}$ / ليتر)، (٢٨٨× $١٠^{٩}$ / ليتر)، (٦١,١).هذه النتائج عند مقارنتها بالمجموعه الضابطه نجد انه لايوجد فروقات ذات دلاله احصائيه ما عدا في نسبه العدلات/اللمفاويات نجد ان هناك ارتفاع ذا دلاله احصائيه لدى المرضى عند مقارنتهم بالمجموعه الضابطه . بالمقابل لم يوجد أي فروقات ذات دلاله أحصائيه في قيمه كل من الدي دايمر و تعداد كريات الدم بالنسبه لمجموعات العلاج المختلفه وأيضا مجموعتي مدة المرض، وكذلك لايوجد فروقات ذات دلاله أحصائيه في قيمة تعداد كريات

الدم بين المجموعات المختلفه لحده المرض، بالأضافه الى ذلك لم يكن هناك فروقات ذات دلاله أحصائيه في قيمة الدي دايمر بين حالات تغير تعداد كريات الدم.

الخلاصة: اشارت المحصلة على انه يوجد زياده ذات دلاله أحصائيه واضحه في مستوى الدي دايمر لدى النساء المرضى بالذئبه الجهازيه الأحمراريه وهذه الزياده مرتبطه بزياده حده المرض ، وكذلك لا يوجد فروقات ذات دلاله أحصائيه واضحه بين المرضى والمجموعه الضابطه في تعداد كريات الدم.

Contents

List of Tables

List of Figures

List of Abbreviations

Term	Abbreviation	Term	Abbreviation
ACA	Anticardiolipin Antibodies	**CVAs**	Cerebrovascular Accidents
ACE	Administer angiotensin-Converting Enzyme	**DVT**	Deep Venous Thrombosis.
ACR	American College of Rheumatology	**DIC**	Disseminated Intravascular Coagulation
ADP	Adenosine Diphosphate	**DNA**	Deoxyribonucleic Acid
AIHA	Autoimmune Haemolytic Anemia	**(ds) DNA**	Double stranded DNA
ANA	Antinuclear Antibodies	**DIL**	Drug-Induced Lupus
APC	Activated Protein C	**EBV**	Epstein– Barr Virus
APLAs	Antiphospholipid Antibodies	**EDTA**	Ethylene Diamine Tetra Acetic acid
ARBs	Angiotensin Receptor Blockers	**ENAs**	Extractable Nuclear Antigens
AT-III	AntiThrombin III	**FDPs**	Fibrin Degradation Products
BCR	B Cell antigen Receptor	**Fc**	Fragment Cluster
BSA	Bovine Serum Albumin	**Fc γ R**	Fc Gamma Receptor
CBC	Complete blood count	**GI**	Gastrointestinal
CTLA-4	Cytotoxic T-Lymphocyte Associated protein 4	**GPI**	Glycoprotein I

HCQ	Hydroxychloroquine	**PZ**	Protein Z
HMW	High Molecular Weight	**RBC**	Red Blood Cell
HGB, Hb	Haemoglobin	**Ro (SS-A)**	anti-Sjogren's Syndrome related Antigen A
IC	Immune Complex	**SLE**	Systemic Lupus Erythematosus
IFNα	Interferon Alfa	**SLICC**	Systemic Lupus International Collaborating Clinics
Ig	Immunoglobulin	**SPSS**	Statistical Package for Social Science
IL	Interleukin	**ssDNA**	Single Stranded DNA
LAC	Lupus Anticoagulant	**TAFI**	Thrombin Activatable Fibrinolysis Inhibitor
LDL-C	Low- Density Lipoprotein Cholesterol	**TF**	Tissue Factor
MRI	Magnetic Resonance Imaging	**TLR**	Tool Like Receptor
NLR	Neutrophil Lymphocyte Ratio	**TNF**	Tumor Necrosis Factor
PBS	Phosphate Buffered Saline	**TxA2**	Thromboxane A2
PE	Pulmonary Embolism	**UV**	Ultraviolet
PLT	Platelet	**VTE**	Venous Thromboembolism
PPP	Platelet Poor Plasma	**WBC**	white Blood Cell

Chapter One

1. Introduction and Literature Review

1.1 Systemic lupus erythematosus

Simply stated, autoimmune disease is caused by failure of the tolerance processes to protect the host from the action of self-reactive lymphocytes. These diseases result from the destruction of self-proteins, cells, and organs by auto-antibodies or self-reactive T cells. Autoimmune disease is estimated to affect between 3% and 8% of individuals in the industrialized world, making this a rising problem in terms of morbidity and mortality around the globe (Owen, *et al*. 2013).

1.1.1 Historical of SLE

The term 'lupus' (Latin for 'wolf') was first used during the middle ages to describe erosive skin lesions evocative of a 'wolf's bite'. In 1846 the Viennese physician Ferdinand von Hebra (1816–1880) introduced the butterfly metaphor to describe the malar rash. He also used the term 'lupus erythematosus' and published the first illustrations in his Atlas of Skin Diseases in 1856. Lupus was first recognized as a systemic disease with visceral manifestations by Moriz Kaposi (1837–1902) (Cervera. 2006).

1.1.2 Epidemiology of SLE

Prevalence rates in lupus are estimated to be as high as 51 per 100 000 people in the USA. The incidence of lupus has nearly tripled in the last 40 years, mainly due to improved diagnosis of mild disease. Estimated incidence rates in North America, South America, and Europe range from 2 to 8 per 100 000 per year (Cervera. and Khamashta. 2006). Women are affected nine times more frequently than men (Cervera, *et al*. 2009). Sixty-five percent of patients

with SLE have disease onset between the ages of 16 and 55 years, 20% present before age 16, and 15% after the age of 55 (Pons-Estel, *et al*. 2010).

1.1.3 Etiology and pathogenesis

The etiology of SLE includes both genetic and environmental components with female sex strongly influencing pathogenesis. These factors lead to an irreversible break in immunological tolerance manifested by immune responses against endogenous nuclear antigens (Urowitz, *et al*. 2012).

1.1.3.1 Genetic factors

Siblings of SLE patients are approximately 30 times more likely to develop SLE compared with individuals without an affected sibling. The rate of gene discovery in SLE has increased during the past few years (Guerra, *et al*. 2012).

1.1.3.2 Epigenetic effects

The risk for SLE may be influenced by epigenetic effects. (Patel DR and Richardson BC, 2013) .such as DNA methylation and post-translational modifications of histones, which can be either inherited or environmentally modified (Javierre, *et al*. 2010).

1.1.3.3 Environmental factors

Candidate environmental triggers of SLE include ultraviolet light, demethylating drugs, and infectious or endogenous viruses or viral-like elements. Sunlight is the most obvious environmental factor that may exacerbate SLE. (Cooper, *et al*. 2010). Epstein– Barr virus (EBV) has been identified as a possible factor in the development of lupus. (Zandman-

Goddard, *et al*. 2012) It is well established that certain drugs induce autoantibodies in a significant number of patients, most of whom do not develop signs of an autoantibody associated disease. Over 100 drugs have been reported to cause drug-induced lupus (DIL) (Bukhari. 2012).

1.1.3.4 Hormonal Factors

In murine models, addition of oestrogen or prolactin can lead to an autoimmune phenotype with an increase in mature high-affinity autoreactive B cells. Oral contraceptive use in the Nurses' Health Study was associated with a slightly increased risk of developing SLE (relative risk 1.9 compared to never users) (Sánchez-Guerrero, *et al*. 2005). Pregnancy may cause in some cases a lupus flare, but this is not due to an increase in estradiol or progesterone in fact, the levels of these hormones are lower in the second and third trimester for SLE patients in comparison with healthy pregnant women (Markle, *et al*. 2013).

1.1.4 Pathogenesis and pathophysiology of SLE

Immune responses against endogenous nuclear antigens are characteristic of SLE. Autoantigens released by apoptotic cells are presented by dendritic cells to T cells leading to their activation. Activated T cells in turn help B cells to produce antibodies to these self-constituents by secreting cytokines such as interleukin 10 (IL10) and IL23 and by cell surface molecules such as CD40L and CTLA-4 (Bertsias, *et al*. 2009). In addition to this antigen-driven T cell-dependent production of autoantibodies, recent data support T cell-independent mechanisms of B cell stimulation via combined B cell antigen receptor (BCR) and TLR signaling. (Bertsias, *et al*. 2015). The pathogenesis of SLE involves a multitude of cells and molecules that participate in

apoptosis, innate and adaptive immune responses (Dias, *et al.* 2009). Increased amounts of apoptosis-related endogenous nucleic acids stimulate the production of IFNα and promote autoimmunity by breaking self-tolerance through activation of antigen-presenting cells (figure: 1.1). Once initiated, immune reactants such as immune complexes amplify and sustain the inflammatory response (Tsokos. 2011).

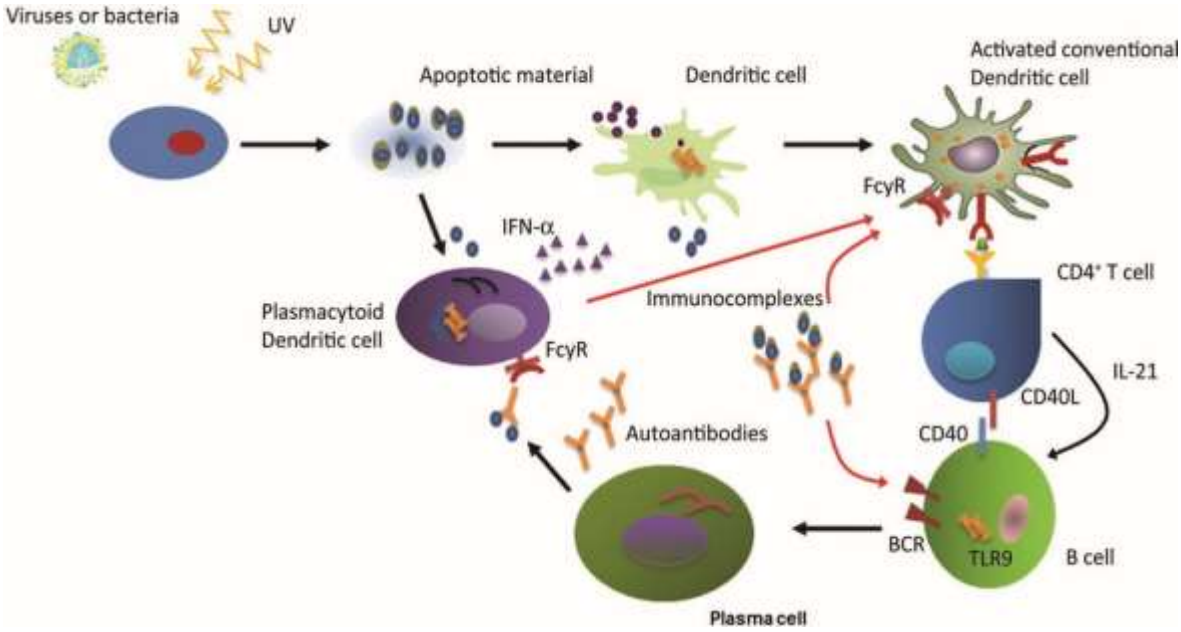


Figure 1.1: SLE Pathogenesis: The key events adopted by (Bertsias, *etal.* 2010)

1.1.5 Disease mechanisms and tissue damage

Immune complexes and complement activation pathways mediate effector function and tissue injury In healthy individuals, immune complexes are cleared by Fc and complement receptors; failure to clear immune complexes results in tissue deposition and tissue injury at sites. Tissue damage is mediated by recruitment of inflammatory cells, reactive oxygen intermediates, production of inflammatory cytokines, and modulation of the coagulation cascade (Fausta, *et al.* 2010). Locally produced cytokines, such as IFNα and tumor necrosis factor (TNF), contribute to affected tissue injury and

inflammation. These mediators, together with the cells producing them (macrophages, leucocytes, dendritic cells and lymphocytes), are the subject of investigation as potential therapeutic targets in lupus. Recent studies have also highlighted the role of locally expressed factors for the protection of tissues under immune attack (Navarra and Leynes. 2010). It is possible that TNF-α is a proinflammatory cytokine with the strongest prothrombotic action. TNF-α stimulates monocyte and neutrophil adhesion to endothelium, inhibits protein C system, impairs fibrinolysis and increases TF expression on the cell surface. (Swadzba, *et al*. 2011). Vascular damage in SLE has received increased attention in view of its relationship with accelerated atherosclerosis (McMahon, *et al*. 2009).

1.1.6 Clinical features of SLE

SLE can have a lot of clinical features in fact with many complications including Mucocutaneous features (Figure 1.2), musculoskeletal features, renal features, nervous system features, cardiovascular features, pleura and lungs features, lymphadenopathy and splenomegaly, haematologic features, liver and GI tract features and ophthalmic features (Bertsias, *et al*. 2015).

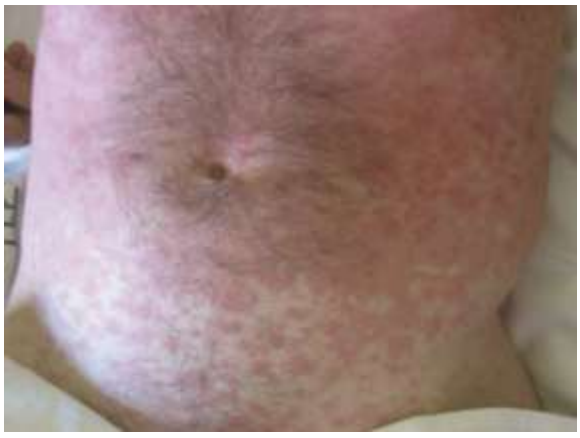
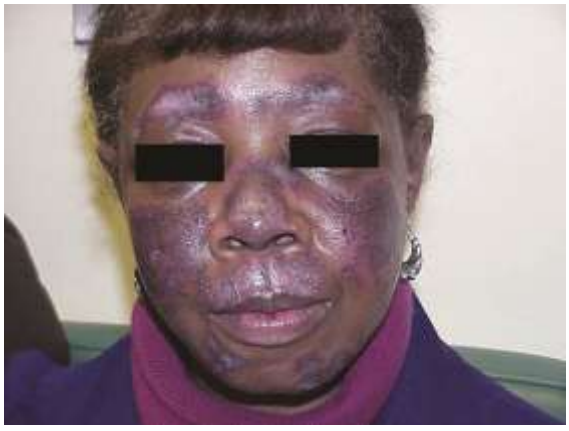
A  **B** 

Figure 1.2: **A**. **Acute cutaneous lupus erythematosus. B. Facial discoid lupus rash with a malar distribution**

1.1.7 Classification criteria of SLE

Criteria for SLE classification were developed in 1971, revised in 1982, and revised again in 1997 (table: 1.1). These criteria distinguish patients with the disease in question from those without the disease. The American College of Rheumatology (ACR) classification criteria were developed for clinical studies of lupus to ensure that cases reported in the literature do in fact have the disease (Pons-Estel, *et al*. 2014). In addition to the wide variety of manifestations, SLE runs an unpredictable course. The dynamic nature of the disease often makes its diagnosis challenging (Petri, *et al*. 2012). While the SLICC (Systemic Lupus International Collaborating Clinics) 2012 criteria are currently used to diagnose SLE (Table 1.2) (Stephenson and Shipman. 2014).

Table 1.1: The American College of Rheumatology revised classification criteria for systemic lupus erythematosus. Adapted from Hochberg 1997

Criteria	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring occurs in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
Arthritis	Non-erosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling or effusion
Serositis	a. Pleuritis: convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion or b. Pericarditis: documented by ECG or rub or evidence of pericardial effusion
Renal disorder	a. Persistent proteinuria >0.5 g per day or >3+ if quantitation is not performed or b. Cellular casts: may be red cell, haemoglobein, granular tubular, or mixed

Neurological disorder	a. Seizures: in the absence of off ending drugs or known metabolic derangements (eg, uraemia, acidosis, or electrolyte imbalance) or b. Psychosis: in the absence of off ending drugs or known metabolic derangements (eg, uraemia, acidosis, or electrolyte imbalance)
Haematologic disorder	a. Haemolytic anaemia with reticulocytosis, or b. Leucopenia: <4000/mm3, or c. Lymphopenia: <1500/mm3, or d. Thrombocytopenia: <100 000/mm3 in the absence of off ending drugs
Immunologic disorder	a. Anti-DNA: antibody to native DNA in abnormal titer, or b. Anti-Sm: presence of antibody to Sm nuclear antigen, or c. Positive finding of antiphospholipid antibodies based on: (1) an abnormal serum concentration of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) a false positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilisation or fluorescent treponemal antibody absorption test
Antinuclear antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with 'drug-induced lupus' syndrome

Table 1.2: SLE diagnostic criteria SLICC (Bazzan, *et al*. 2015)

Systemic lupus erythematosus: diagnostic criteria (2012 – SLICC)	
Criteria	**Items**
Cutaneous manifestation	Acute Cutaneous Lupus Erythematosus/Subacute Cutaneous Lupus Erythematosus Chronic Cutaneous Lupus Erythematosus Oral ulcers Non-scarring alopecia
Joints	Synovitis > 2 peripheral joints (pain, tenderness, swelling or morning stiffness > 30 min)
Serositis	Pleuritis, typical pleurisy ≥ 1 day, history, rub, evidence of pleural effusion, pericarditis, typical pericardial pain ≥ 1 day, EKG evidence of pericardial fusion

Renal disorder	Urine protein/creatinine ratio or urinary protein concentration of 0.5 g of protein/24 h, Red blood cell casts
Haematological disorder	Haemolytic anaemia Leukopenia (<4000/mm3) or lymphopenia (<1000/mm3) separately at least once Thrombocytopenia (<100,000/mm3) at least once
Immunologic abnormal	Positive ANA Positive anti-dsDNA (except ELISA) on ≥ 2 occasions Anti-Sm Antiphospholipid antibody (including lupus anticoagulant, false-positive RPR, anti-cardiolipin, anti-beta2glycoprotein1) Low complement (C3, C4 or CH50) Direct Coombs test in the absence of haemolytic anaemia
Diagnosis	Fulfil 4 items (at least one clinical and one immunologic item)

1.1.8 Laboratory Diagnosis of SLE

1.1.8.1 Antinuclear Antibodies (ANA)

The ANA assay is an ideal screening test because of its sensitivity (95% when using human cultured cells as the substrate) and simplicity. The entity of 'ANA-negative lupus' described in previous years is usually associated with the presence of other cytoplasmic autoantibodies such as anti-Ro (SS-A) and anti-ribosomal P protein. The specificity of ANAs for SLE is low, since they are found in many other conditions (Meroni and Schur. 2010). In contrast to the low positive predictive value of ANA testing, a patient with a negative test has less than a 3% chance of having SLE; thus, a negative ANA test is useful for excluding the diagnosis of SLE. However, in the presence of typical features of lupus, a negative ANA test does not exclude the diagnosis (Bertsias, *et al*. 2015).

1.1.8.2 Antibodies to extractable nuclear antigens (ENAs)

The nucleosome (a complex of DNA and histones) was the first identified lupus autoantigen (Nossent, *et al*. 2010). Autoantibodies to single stranded DNA (ssDNA) and individual histones are common in SLE as well as in drug-induced lupus. Antibodies to double stranded (ds) DNA are found in up to 70% of SLE patients at some point during the course of their disease, and are 95% specific for SLE, making them a valuable disease marker (Bertsias, *et al*. 2013).

1.1.9 SLE treatment

The current treatment approach includes antimalarials, steroidal and non-steroidal anti-inflammatory agents and immunosuppressive drugs, including cyclophosphamide, azathioprine, mycophenolic acid and methotrexate. Although there is a dramatic improvement in the prognosis for SLE patients, treatment of those with active disease refractory to traditional therapies continues to be a real challenge. On the horizon are new targeted therapies specifically designed to block pathways involved in disease pathogenesis. As to understand the initiation and progression of the disease better, considering therapeutic options that focus on blocking defined phases of disease pathogenesis is recommended (Yildirim-Toruner and Diamond, 2011).

1.1.9.1 Adjunctive therapy

Hydroxychloroquine should be used as adjunctive therapy in lupus nephritis because of the potential for reduction in rates of disease flare; damage accrual, including renal damage; and risk of thrombotic events (Doria and Briani 2008). Administer angiotensin-converting enzyme (ACE) inhibitors or

angiotensin receptor blockers (ARBs) to all patients with lupus nephritis, except pregnant women, who have proteinuria of 0.5 g or more per 24 hours (Contreras, *et al*. 2004). Statin therapy is recommended in patients with low-density lipoprotein cholesterol (LDL-C) levels greater than 100 mg/dl because both renal dysfunction alone and SLE alone are independent risk factors for accelerated atherosclerosis (Ferreira, *et al*. 2007). Vitamin D insufficiency and deficiency are more common in patients with SLE than in the general population. Vitamin D supplementation may decrease disease activity and improve fatigue. In addition, supplementation may improve endothelial function, which may reduce cardiovascular diseases (Christie and Muller. 2017). Bone mineral density measurement should be considered in SLE patients at high risk of osteoporosis, particularly those starting corticosteroids and in postmenopausal women. Calcium and vitamin D supplementation provide general prophylaxis and are a suitable first-line option (Sen and Keen. 2001).

1.1.10 Hematological changes in SLE

Haematological abnormalities are common findings in patients with SLE. Sometimes, haematological abnormalities can be caused by the pathophysiology of SLE itself, but at other times they can be found in patients with SLE but not be a manifestation of SLE. Thus, it is important to distinguish haematological abnormalities as either manifestations of SLE, consequence of SLE treatment or as part of another blood cell dyscrasia (Fayyaz, *et al*. 2015).

1.1.10.1 Leucopenia

According to the ACR and SLICC criteria for classification of SLE, leucopenia is defined as <4000/mm^3 on two or more occasions (Petri, *et al*. 2012). Along with the pathogenic mechanism of disease itself, several other factors such as immunosuppressive drugs may contribute towards low white cell count in these patients. Leucopenia, that is, low total white blood cell (WBC) count, constitutes a paucity of granulocytes as well as lymphocytes, yet a greater absolute deficiency of granulocytes than lymphocytes is usually found (Martinez-Banos, *et al*. 2006). Mild neutropenia is a common finding in SLE that requires no specific therapy. Whether or not this leads to immune suppression is not known. However, a small percentage of patients with SLE develop severe, even life-threatening, neutropenia, which may be caused by a variety of mechanisms drugs may contribute towards low white cell count in these patients (Sugimoto, *et al*. 2006).

1.1.10.2 Lymphopenia

Lymphopenia is defined as <1.5×109 lymphocytes/L on two or more occasions according to the ACR and SLICC criteria (Petri, *et al*. 2012). Low lymphocyte counts commonly occur in SLE with a prevalence ranging from 20% to 93% and are observed frequently in patients with active or severe disease (Odendahl, *et al*. 2000). Lymphopenia may occur by interplay of different mechanisms. (Massardo, *et al*. 2009), therapy for lymphopenia is not indicated in patients with SLE, but lymphopenia, and its degree, may be related to the disease activity (Chen and Lin. 2011).

1.1.10.3 Thrombocytopenia

For the purpose of the ACR classification criteria for SLE and the new SLICC criteria, the definition of thrombocytopenia is a platelet count <100 000/mm^3 (or 100×10^9/L) without any other identifiable cause (Petri, *et al*. 2012). True thrombocytopenia can occur by three mechanisms: impaired production of platelets in the bone marrow, sequestration of platelets in the spleen or accelerated destruction of platelets in the peripheral circulation. The majority of patients with SLE with thrombocytopenia have increased peripheral destruction that is commonly mediated by antiplatelet antibodies, but the other two mechanisms play a role in some patients (Kapouzas. 2013). Many patients with thrombocytopenia as a manifestation of SLE can be watched without specific treatment directed at the low platelet count, and the great majority of those requiring treatment can be successfully managed (Hepburn, *et al*. 2010). Splenectomy results in a 50–66% remission rate, but the only controlled trial in regard to splenectomy as a therapy for thrombocytopenia in SLE indicates a very high rate of subsequent infection, which may be life threatening. Thus, splenectomy should be reserved as a last resort in patients with SLE (Alarcon-Segovia. 2002).

1.1.10.4 Autoimmune haemolytic anemia

The ACR and SLICC criteria recognize AIHA with reticulocytosis as one of the criteria for the classification of SLE, while the SLICC criteria also include a positive Coombs test as a criterion (Petri, *et al*. 2012). Anti-erythrocyte antibodies in SLE are mainly warm-type IgG. APL antibodies associate with Coombs-positive haemolytic anaemia in patients with SLE. ACL antibodies, IgG and IgM, are more common in patients with SLE with AIHA (Costallat

GL, *et al*. 2012). AIHA is one of the common etiologies of severe anaemia in patients with SLE. Reports regarding its diverse clinical presentation and heterogeneous association to other autoimmune manifestations make prompt attention essential (Abdwani and Mani. 2009).

1.1.10.5 Neutrophil to lymphocyte ratio

In medicine neutrophil to lymphocyte ratio (NLR) is used as a marker of subclinical inflammation. It is calculated by dividing the number of neutrophils by number of lymphocytes, usually from peripheral blood sample (Wang, *et al*. 2014).

Lupus nephritis is the most common and severe clinical manifestation of SLE, though overall mortality has decreased remarkably in SLE patients over the last decades. Kidney failure is also the leading cause of death in these patients. Thus the early diagnosis of LN is helpful for patients. Immune complex (IC) formation and deposition are the main cause of SLE kidney damage mechanism. IC can activate complement and inflammatory cell infiltration, thus cause kidney damage. Many studies have shown that NLR is positively associated with inflammatory, different malignancies, ischemic injury, cardiovascular disease and diabetic nephropathy. However, the relationship between NLR and SLE as well as LN has not been well studied so far (Li, *et al*. 2015). Normal NLR values in an adult, non-geriatric, population in good health are between 0.78 and 3.53 (Forget, *et al*. 2017).

1.2 Haemostasis balance

Haemostasis defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop (Thornton and Douglas. 2010). This

thrombohaemorhagic balance is maintained in the body by complicated interactions between coagulation and the fibrinolytic system as well as platelets and vessel wall. Usually, the coagulation process is under the inhibitory control of several inhibitors that limit the clot formation, thus avoiding the thrombus propagation. This delicate balance is interrupted whenever the procoagulant activity of the coagulation factors is increased, or the activity of naturally occurring inhibitors is decreased (Previtali, *et al*. 2011).

1.2.1 Primary haemostasis

Primary haemostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial 'platelet plug'. The formation of the platelet plug involves a series of steps (Palta, *et al*. 2014).

1.2.1.1 Platelet adhesion

After vascular injury vWF acts as a bridge between endothelial collagen and platelet surface receptors GpIb and promotes platelet adhesion. The platelet glycoprotein complex I (GP-Ib) is the principal receptor for vWF (Heemskerk, *et al*. 2002).

1.2.1.2 Platelet secretion

After adhesion, degranulation from both types of granules takes place with the release of various factors. Release of calcium occurs here. Calcium binds to the phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors.

1.2.1.3 Platelet aggregation

Thromboxane A2 produced by activated platelets provide stimulus for further platelet aggregation. TxA2 along with ADP enlarge this platelet aggregate leading to the formation of the platelet plug, which seals off vascular injury temporarily. ADP binding also causes a conformational change in GpIIb/IIIa receptors presents on the platelet surface causing deposition of fibrinogen. Thrombin generation also catalyses the conversion of this fibrinogen to fibrin which adds to the stability of the platelet plug and is now known as secondary haemostasis (Andrews and Berndt. 2004). Prostacyclin inhibits platelet aggregation (platelet anti aggregating effect) and the balance between TxA2 and prostacyclin leads to localized platelet aggregation thus preventing extension of the clot thereby maintaining the vessel lumen patency (Palta, *et al*. 2014).

1.2.2 Secondary hemostasis

Secondary hemostasis consists of the cascade of coagulation serine proteases (Table: 1.3) that culminates in cleavage of soluble fibrinogen by thrombin, Thrombin cleavage generates insoluble fibrin that forms a crosslinked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Furie.2009). It has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation (Figure: 1.3).

Table1.3: Nomenclature of the coagulation proteins/clotting factors (Palta, *et al*. 2010)

Clotting factor number	Clotting factor name	Function	Plasma half-life (h)	Plasma concentration (mg/L)
I	Fibrinogen	Clot formation	90	3000
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65	100
III	TF	Co factor of VIIa	-	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-	-
V	Proacclerin, labile factor	Co-factor of X-prothrombinase complex	15	10
VI	Unassigned			
VII	Stable factor, proconvertin	Activates factors IX, X	5	0.5
VIII	Antihaemophilic factor A	Co-factor of IX-tenase complex	10	0.1
IX	Antihaemophilic factor B or Christmas factor	Activates X: Forms tenase complex with factor VIII	25	5
X	Stuart-Prower factor	Prothrombinase complex with factor V: Activates factor II	40	10
XI	Plasma thromboplastin antecedent	Activates factor IX	45	5
XII	Hageman factor	Activates factor XI, VII and prekallikrein		-
XIII	Fibrin-stabilising factor	Crosslinks fibrin	200	30
XIV	Prekallikerin (F Fletcher)	Serine protease zymogen	35	
XV	HMWK- (F Fitzgerald)	Co factor	150	
XVI	vWf	Binds to VIII, mediates platelet adhesion	12	10 µg/mL
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72	0.15-0.2 mg/mL
XVIII	Heparin cofactor II	Inhibits IIa	60	-
XIX	Protein C	Inactivates Va and VIIIa	0.4	-
XX	Protein S	Cofactor for activated protein C		-

HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

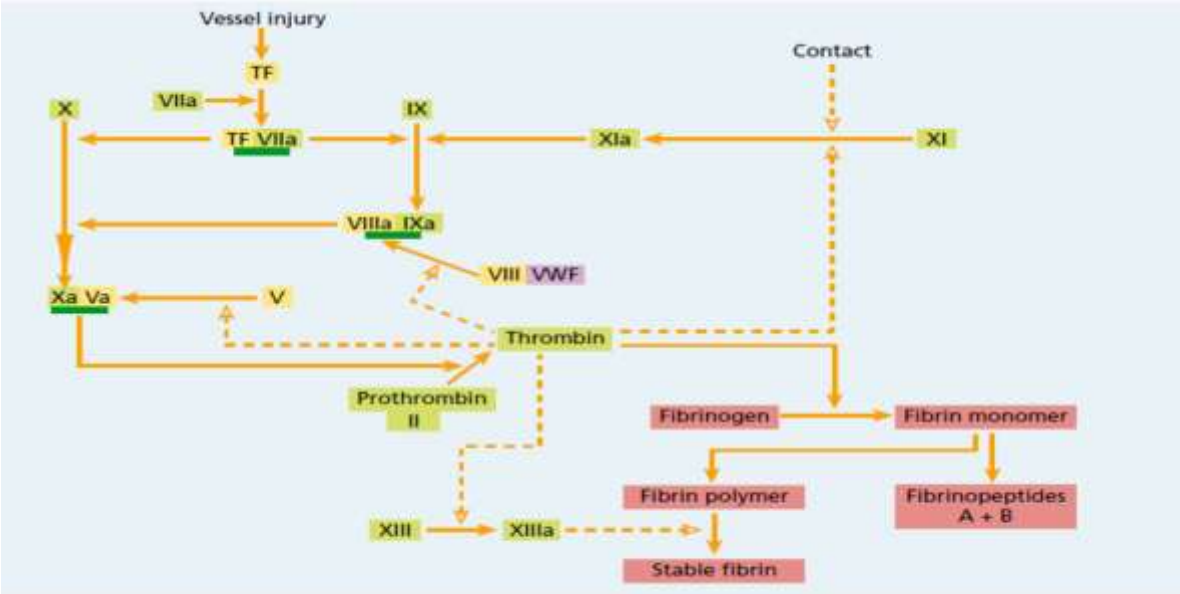


Figure1.3: The pathway of blood coagulation cascade. (Hoff brand and Moss. 2011)

1.2.2.1 Extrinsic pathway

It is considered as the first step in plasma mediated haemostasis. It is activated by TF, which is expressed in the subendothelial tissue (Lasne, *et al*. 2006). Under normal physiological conditions, normal vascular endothelium minimizes contact between TF and plasma procoagulants, but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa (Owens III and Mackman. 2010).

1.2.2.2 Intrinsic Pathway

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallekerin and factor XI (contact family) which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor (factor VIII) to form tenase complex on a phospholipid surface to activate factor X (Hall. 2010).

1.2.2.3 Common pathway

Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilises the clot and forms a definitive secondary haemostatic plug (Kumar V, *et al*. 2010).

1.2.3 Naturally occurring anticoagulants in the body

The anticoagulant system exerts a regulatory role over the procoagulant activity in blood thus localizing the thrombus formation (Colvin. 2004). The main anticoagulant mechanisms naturally present in the body include: Antithrombin (AT) previously known as AT III is the main inhibitor of thrombin, also tissue factor plasminogen inhibitor It acts as a natural inhibitor of the extrinsic pathway by inhibiting TF-VIIa complex, adding to them Protein C pathway in which Protein C a serine protease. It is activated by thrombin to form activated protein C (APC) and acts by inhibiting activated factors V and VIII (with Protein S and phospholipids acting as cofactors) in addition, protein S it also causes direct reversible inhibition of the prothrombinase (FVa–FXa) complex (Rigby and Grant. 2004). In a recently described protein Z dependent protease inhibitor which is component of the anticoagulant system that is produced in the liver and it inhibits Factor Xa in reaction requiring PZ and calcium (Corral, *et al*. 2007).

1.2.4 Fibrinolytic system

Fibrinolytic system is a parallel system which is activated along with activation of coagulation cascade and serves to limit the size of clot. Fibrinolysis is an enzymatic process that dissolves the fibrin clot into fibrin degradation products (FDPs) by plasmin originating from fibrin bound plasminogen in liver. This reaction is catalyzed by tissue Plasminogen activator (tPA) or urokinase plasminogen activator (u-PA) released from vascular endothelium. The release of t-PA is stimulated by tissue occlusion, thrombin, epinephrine, vasopressin and strenuous exercise (Figure 1.4).

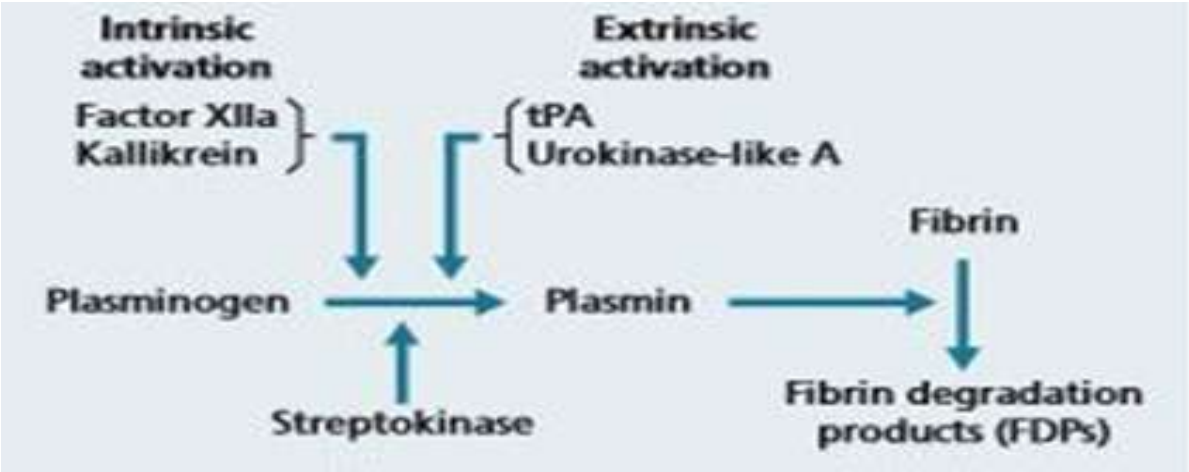


Figure 1.4: the fibrinolytic system (A.V. Hoff brand and P.A.H. Moss. 2011)

Plasmin activity is tightly regulated by its inhibitor (α-2 antiplasmin) thus preventing widespread fibrinolysis (Cesarman-Maus and Hajjar. 2005). In vivo activity of the fibrinolytic system is assessed clinically by measuring the FDP's. D-dimers are produced by digestion of cross linked fibrin and are specific indicators of fibrinolysis used in the assessment and diagnosis of pulmonary embolism, DIC or deep vein thrombosis (Colvin.2004). Since plasmin has the potential to degrade fibrinogen leading to deleterious consequences, the fibrinolytic activity is limited by following factors:

- Plasminogen activator inhibitor - It is the main physiological inhibitor of fibrinolysis and acts by inhibiting t-PA and u-PA irreversibly

- TAFI- thrombin activatable fibrinolysis inhibitor It is a plasma proenzyme synthesized by liver and activated by thrombin. It decreases the affinity of plasminogen to fibrin and augments the action of anti-trypsin in inhibiting plasmin

- Plasmin inhibitors: α2 antiplasmin and α2Macroglobulin are the glycoproteins that exert action by virtue of plasmin inhibition (Ejiofor JA. 2013).

1.3 SLE and risk of thrombosis

There is strong evidence for an association between SLE and an increased risk of VTE (Palatinus and Adams. 2009). The most important risk factor is the presence of antiphospholipid antibodies (APLAs). However, approximately 40% of adults with SLE who are negative for APLA are diagnosed with thrombosis, indicating the importance of other risk factors. Thus, the thrombosis risk factors should be evaluated extensively and regularly and treated aggressively in every patient with systemic lupus erythematosus (Al-Homood. 2012).

However, it not all SLE patients who develop thrombosis have antiphospholipid antibodies. Other mechanisms such as inflammation, acquired protein S deficiency, and microparticles may also contribute to the thrombotic risk among SLE patients (Ardoin, *et al*. 2007). Comparative studies suggest that coagulation and innate immunity have a shared evolutionary origin. It is therefore unsurprising that the immune and coagulation systems are linked, with many molecular components being important for both systems. Systemic inflammation modulates thrombotic responses by suppressing fibrinolysis, upregulating procoagulant, and downregulating anticoagulants, and autoimmune disorders such as systemic lupus erythematosus (SLE) have been linked to an increased risk of VTE (Zöller, *et al*. 2012).

1.3.1 VTE and SLE mechanism

Several hypotheses have been proposed to explain the pathogenic effects of these autoantibodies (Antiphospholipid Antibodies (APLAs) Anticardiolipin antibodies (ACA) and the lupus anticoagulant (LAC) anti-ß2-glycoprotein I (anti-ß2-GPI) and their role in the development of thrombosis. They attach to the negatively charged phospholipid surface that may induce platelet activation, interfere with coagulation inhibitors such as protein-C, inhibit anti thrombin and fibrinolysis, and then initiate the formation of a thrombus. It is well established that APLAs are associated with both arterial and venous thrombosis. In Large cohort studies, the lupus anticoagulant has been shown to be a significant risk factor for myocardial infarction and stroke (Petri. 2004). Antiphospholipid antibodies affect the coagulation cascade and inflammation. In a process mediated by β2 glycoprotein I, antiphospholipid antibodies bind to platelets and endothelial cells, activating endothelial cells and inducing a procoagulant state. Antibody binding also activates complement (Duckitt and Harrington. 2005). Resulting in recruitment of other inflammatory cells activation of tissue factor, endothelial damage, and finally thrombosis (Giannakopoulos, *et al*. 2009). Approximately 40% of adults with SLE who are negative for APLA are diagnosed with thrombosis (Amoroso, *et al*. 2003). Thus, the precise mechanism(s) responsible for thrombosis in these patients remains unclear and indicating the role of other factors. The age at onset of thrombosis in SLE patient is lower than that of general population which is a major concern. The incidence of thrombosis increased in the first year.

Possible reasons for this early higher incidence of thrombosis could be the high levels of disease activity and circulating immune complexes, cytotoxic

antibodies, or a higher inflammatory state (Al-Homood. 2012). According to the Virchow triad (Figure: 1.5), VTE results from altered blood coagulation stasis, or abnormalities in the vessel wall. Hypercoagulable states and endothelial dysfunction may result from multiple interactions between inherited and acquired risk factors. Several studies have identified age, immobilization following cerebrovascular accidents (CVAs), heart failure, lower leg fracture, surgery, diabetes and cancer as acquired risk factors for VTE (Lijfering, *et al*. 2010). However, two recent reports have linked a large number of autoimmune disorders/ immune-mediated disease to an increased risk of PE (Zöller, *et al*. 2012), and VTE (Ramagopalan, *et al*. 2012).

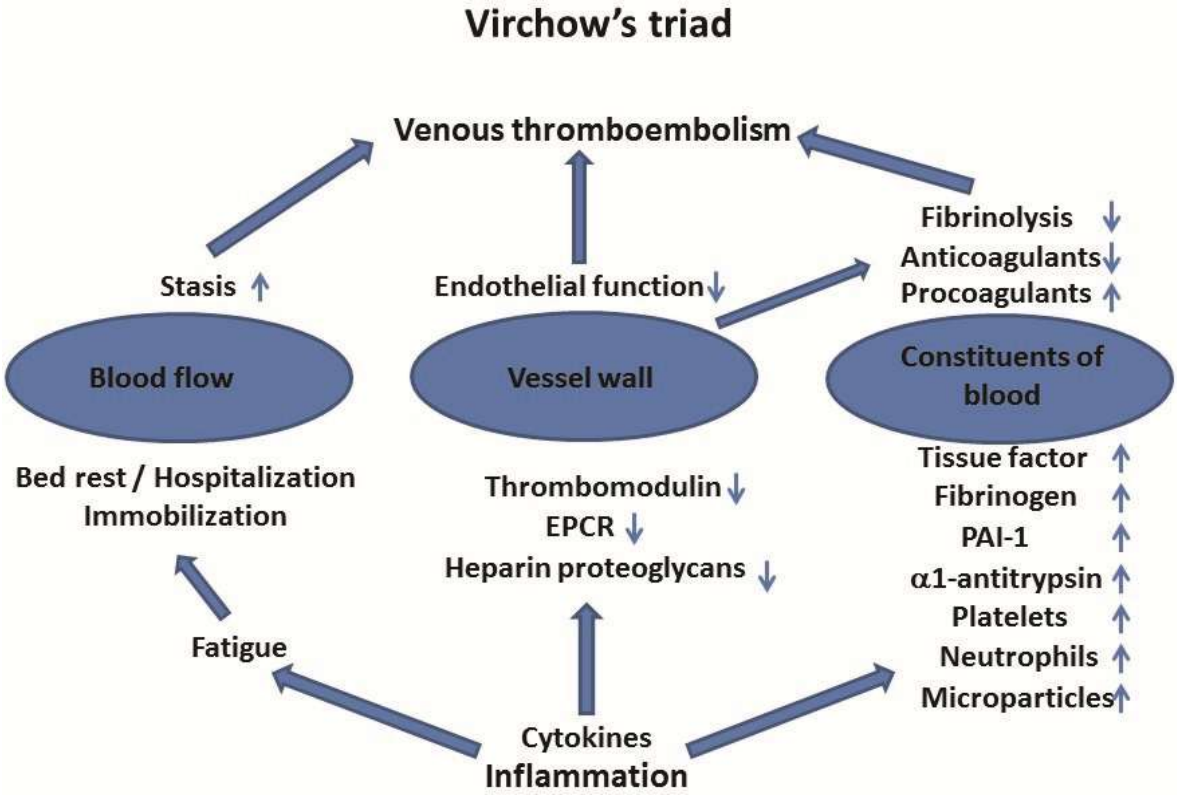


Figure1.5: Virchow's triad and some of the extensive inflammatory changes that may contribute to the development of venous thromboembolism. PAI1=plasminogen activator inhibitor 1, EPCR=endothelial protein C receptor (Bengt Zöller, *et al*. 2012)

1.3.2 Drugs and Thrombosis in SLE

Glucocorticoids are commonly used for treatment of various manifestations of SLE. Glucocorticoids have been associated with thrombosis, probably mediated by endothelial damage and accelerated atherosclerosis (Calvo-Alén, *et al*. 2005). When administered in high doses, glucocorticoids have also been associated with abnormalities in the coagulation cascade. Hydroxychloroquine (HCQ) is commonly prescribed antimalarial agent for SLE. It has a very reasonable safety profile and it decreases the probability of flares, also it has antithrombotic effect. The antithrombotic effect is probably mediated by inhibition of platelet aggregation and adhesion, and arachidonic acid release from stimulated platelets (Yoon. 2002).

1.4 Thrombosis and D-dimer

1.4.1 Deep vein thrombosis (DVT) and pulmonary embolism (PE)

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are manifestations of potentially lethal venous thromboembolism (VTE). Patients affected by DVT most often present with swelling, discoloration and discomfort of the affected leg. Clinicians can diagnose DVT by using non-invasive venous ultrasound, venography, magnetic resonance imaging (MRI), or pathology of the thrombus following its removal during surgery. With concomitant PE, DVT can be a dangerous condition (Tzoran, *et al*. 2012). Patients with PE may present with hypoxemia and/or dyspnea, chest pain, and anxiety or nervousness. The diagnosis of PE can be made on the basis of the results of the D-dimer blood test, lung scan, spiral computed tomography scan, pulmonary angiography, MRI, or pathology of the thrombus following its removal during surgery (Chung, *et al*. 2014).

1.4.2 D-dimer

D-dimer is a specific antigen derived from the degradation of factor XIIIa cross-linked fibrin. Monoclonal antibodies specific for D-dimer antigen were developed to provide clinicians with a laboratory test that could distinguish between products derived from fibrinogen degradation and those derived from fibrin degradation. The D-dimer antigen measured in clinical samples is derived from the degradation of fibrin formed by the combined action of thrombin, factor XIIIa, and plasmin (Adam, *et al*. 2009). There are 4 distinct settings in which D-dimer testing can be considered in hematology/oncology practice: (1) to rule out DVT; (2) to rule out PE; (3) to detect the presence and extent of DIC; and (4) to determine whether a hyperfibrinolytic disorder is causing a thrombohemorrhagic state (Greenberg. 2017).

1.4.3 D-dimer measurement

Each manufacturer of D-dimer tests uses a specific monoclonal antibody and unique detection technology to quantify D-dimer in clinical samples. These tests can be grouped according to their sensitivity at detecting D-dimer–related antigen (Greenberg. 2017).

1.4.4 D-dimer and DVT in SLE

D-dimers are detectable at levels above 500 ng/mL in virtually all patients with VTE. Because of its good sensitivity and poor specificity, the D-dimer test is best used diagnostically to exclude VTE rather than to confirm it (Latella, *et al*. 2010). Periodic D-dimer measurements can be justified in patients with recurrent SLE disease; if D-dimers are elevated (particularly when >1.0 ug/ml), then evaluation for clotting is appropriate; and if no

clotting is found, then close follow-up and the routine use of aspirin, antimalarial, and statins, which are thought to mitigate the clotting process in SLE , may be appropriate. Aspirin alone, however, may not be sufficient (Wu, *et al*. 2008).

1.5 Previous studies

- In a new interested study at January 2018 by **Le Minh, *et al*.** done in Portland USA, The aim of this work was to examine a possible role of clot contraction/retraction in thrombotic complications of systemic lupus erythematosus. In conclusion, autoantibodies in SLE can affect platelet contractility, resulting in reduced ability of clots and thrombi to shrink in volume, which increases vessel obstruction and may aggravate the course and outcomes of thrombotic complications in SLE.
- Moreover, in 2016 **Njoroge, *et al***. at Kenyatta National Hospital, Nairobi, done cross-sectional hospital based descriptive study to determine the prevalence of haematological abnormalities, among SLE patients on follow up and he conclude that haematological abnormalities were the second most common manifestation of the disease after arthritis and arthralgia among SLE patients on follow up. Though majority of these abnormalities were mild to moderate and clinically asymptomatic, the proportions of anaemia, leucopenia and thrombocytopenia were substantially high.
- Also to see the relationship between the inflammation situation and the severity of disease combine with the organ damage, there were two study support the hypothesis that there are strong relationship between inflammation state and hypercoagubility in SLE patients, first one in 2015 by **Li, *et al***. in the second Xiangya Hospital of central south university, Changsha, Hunan, China, retrospectively analyzed the hospital records of all patients diagnosed

as SLE (N=79), 20 of whom have Lupus nephrites, he conclude that NLR is independently associated with SLE. And NLR is independently associated with Lupus nephrites last stage as well give high NLR. It may be a promising marker that reflects renal involvement in patients with SLE. The second study, in 2015 by **Liang, *et al***. in china, he investigate the possible relationships of clinical manifestations and laboratory abnormalities with hypercoagulability in systemic lupus erythematosus especially the inflammatory marker investigation like erythrocyte sedimentation rate (ESR) as well as between C3 and ESR. He concluded that Lupus nephritis, pleuritis, pericarditis, fever (≥38°C), active disease and increased ESR were associated with hypercoagulability in SLE. There was a significant interaction between active disease and increased ESR for hypercoagulability in SLE.

- Also in 2015 **Yusuf, *et al*.** in Atlanta USA concluded that having SLE was associated with an increased likelihood of a VTE event, and more research is necessary to develop better understanding of VTE occurrence among people with autoimmune diseases.
- In support to all these study there were recent prospective observational study of LA-positive individuals in 2015 by **Gebhart, *et al*.** concluded, that occurrence of a thrombotic event is associated with higher mortality in patients with LA. Consequently, the prevention of thromboembolic events in LA positives SLE patients might improve survival.
- In the other hand, in 2014 **Aleem, *et al***. at King Khalid University hospital, Riyadh, Saudi Arabia, done a retrospective study included patients who were diagnosed and treated for SLE and concluded, that haemolytic anaemia are very common at the time of diagnosis and during follow-up in SLE, and some of these abnormalities are associated with organ damage.

- In 2014 **Chung, *et al*.** a nationwide cohort study at China Medical University Hospital, Taichung, Taiwan, concluded that the risks of DVT and PE are significantly higher in SLE patients than in the general population.
- In addition, in 2010 **Lood, *et al***. in Lund University Hospital, Lund, Sweden did the same in a cohort study, but with type 1 TNF in SLE patient that develop VTE to evaluate the risk of vascular disease and thrombosis. Patients with systemic lupus erythematosus (SLE) have a markedly increased risk to develop cardiovascular disease (CVD), and traditional cardiovascular risk factors fail to account for this increased risk. He suggest that interferogenic immune complexes stimulate production of IFN- α that up-regulates the megakaryocytic type I IFN-regulated genes and proteins. This could affect platelet activation and contribute to development of vascular disease in SLE. Platelets with the type I IFN signature might constitute a novel marker of vascular disease in SLE. Further studies are required to clarify the mechanisms involved and prospective studies are needed to investigate its usefulness as a predictive marker of CVD.
    - In 2009 **Tektonidou, *et al*.** in Austria, made longitudinal study included all SLE patients with positive aPL but without previous thrombosis or pregnancy morbidity and they conclude that Independent predictors of thrombosis for aPL-positive patients were male sex, LAC, and constantly positive aCL, and for aPL-negative patients were male sex and hypertension. The duration of low-dose aspirin use played a protective role against thrombosis in aPL-positive patients as did the duration of hydroxychloroquine in both groups.
- In 2008 in the Ohio, United States a SLE Study done by **Wu, *et al.*** hypothesized that elevated levels of D-dimer would predict clinical manifestations of thrombosis in SLE. They concluded that (1) Periodic D-

dimer measurements can be justified in patients with recurrent SLE disease; (2) if D-dimers are elevated (particularly when >1.0 ug/ml), then evaluation for clotting is appropriate; and (3) if no clotting is found, then close follow-up and the routine use of aspirin, antimalarials, and statins, which are thought to mitigate the clotting process in SLE, may be appropriate. Aspirin alone, however, may not be sufficient. In the same line and deep review.

1.5 Rationale

Systemic Lupus Erythematosus (SLE) is an acquired, multiorgan, autoimmune disease. Clinical presentation is extremely variable and heterogeneous. It has been shown that SLE itself is an independent risk factor for developing both arterial and venous thrombotic events. Clotting is a serious complication of systemic lupus erythematosus (SLE) for several reasons. First, clotting can cause persistent disability and death or fetal loss in pregnant women. Second, the clinical manifestations of clotting are often misleading in patients with SLE, leading to inappropriate drug use and delayed administration of anticoagulation. Third, although positive test results for antiphospholipid antibodies and lupus anticoagulant can predict lupus associated disability, and routine tests that reliably predict clotting events in these individuals have not been established. Fewer studies are available for SLE patients and thrombosis. When SLE patients develop a thrombotic event, it is of great clinical relevance since it is potentially life-threatening. D-dimer is a cross-linked peptide derived from fibrin thrombus and are elevated in patients who have formed clots, elevated D-dimers have been used to predict the risk for recurrent DVT & PE, thus Normal D-dimer levels have also been used to exclude clinically significant clot burden to exclude DVT or pulmonary embolism. However haematological abnormalities are present in

the majority of SLE patients at the time of diagnosis and continue to be present in a substantial number of patients during the follow-up even after many years. This present study may help in better management planning of SLE patients to avoid DVT & PE possibility for early detect of thrombosis, and assess any early haematological changes, also for the better prognosis and management of the disease.

1.7 Objectives

1.7.1 General objective

To assess D-dimer level and complete blood count among SLE female patients.

1.7.2 Specific objectives

- To compare the differences in D-dimer, hemoglobin, packed cell volume, Leukocyte count, thrombocyte count and Neutrophil /lymphocyte ratio between SLE patients and healthy individual.
- To estimate the D-dimer levels according to the severity of the disease, treatment type groups, duration of disease and age.
- To correlate between the different groups of treatment, severity, age and duration of the disease on the D-dimer levels.
- To estimate the complete blood count and correlate the result with the treatment and severity of the disease.
- To correlate between D-dimer levels with different blood count statuses on SLE patients.
- To correlate between D-dimer levels with different inflammation degree on SLE patients.

Chapter Two

2. Materials and Methods

2.1 Materials

A comparative case control study conducted from Aug to Nov 2017 in Khartoum state. The study was approved by the faculty of medical laboratory science, Sudan University of Science and Technology, Khartoum, Sudan. The practical work and patients' selection were performed at the Rheumatology Clinics and the Haematology Department of the Military Hospital (Omdurman). EDTA and citrated venous blood samples were collected from 30 women diagnosed clinically and immunologically by SLE after informed of consent and structured non self-questionnaire. Moreover, 30 control samples were collected from healthy individual recruited in this study. All EDTA samples in cases and control were assessed by automated analyzer for CBC parameters and the citrated samples were used for D-dimer measurement.

2.1.1 Inclusion criteria

- Diagnosed systemic lupus erythematosus Sudanese women patients.
- Healthy Sudanese women as control group for comparing.

2.1.2 Exclusion criteria

- Patients on anticoagulant therapy.
- Patient had recent thrombosis.
- Patients had recent infection.

- Patient with the known risk factor of thrombosis (hereditary criteria for thrombosis, heart failure, lower leg fracture, surgery, diabetes, obesity and cancer).
- Pregnant women.

2.2 Methods

2.2.1 Sampling

Venous blood collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol ,the blood was divided into two tubes; first one was 1.8 ml blood added to the anticoagulant at ratio of 9:1 of citrate (3.2%) buffered sodium citrate and gently mixed. The sample was centrifuged at 1300 rpm for 15 min to obtain platelet poor plasma (PPP).The (PPP) laced into plastic tubes and have been analyzed immediately for D-dimer. The second tube had 3 ml of blood added to K3EDTA anticoagulant, ratio between EDTA and whole blood must be between 1 to 2 mg per ml of blood and gently mixed. The sample was immediately analyzed for full blood count.

2.2.2 D-dimer using i-chroma™ Reader

Principle

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocelluose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection

antibody reflects amount of antigen captured and is processed by i-chroma™ Reader to show D-Dimer concentration in the specimen. The working range of i-chroma™ D-Dimer test is 50 – 10,000 ng/ml. * Reference Value: 500 ng/mL (FEU: Fibrinogen equivalent units)

Components and Reagents

I-chroma™ D-dimer consists of Cartridge, an ID Chip, and Detection Buffers. The test cartridge contains a test strip; on the membrane of which, antibodies against D-dimer and streptavidin have been immobilized at the test line and the control line respectively. Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip. The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescent labeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative. The detection buffer is dispensed in each detection buffer tube. 25 detection buffer tubes are packed in a separate pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.

Test procedure for i-chroma™ D-dimer

Ten µL of serum/plasma/control sample was transferred using a transfer pipette to a tube containing the detection buffer. The lid of the detection buffer tube was closed and mixed the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately). Pipetted out 75 µL of a sample mixture and dispensed it into the sample well on the test cartridge. Leaved the sample-loaded test cartridge at room temperature for 12 minutes. For scanning, inserted it into the test cartridge holder of the i-chroma™

Reader. Ensured proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose. Pressed 'Select' button on the i-chroma™ Reader to start the scanning process. i-chroma™ Reader started scanning the sample-loaded test cartridge immediately. The test result was been reading on the display screen of the i-chroma™ Reader.

Interpretation of the results

- i-chroma™ Reader calculates the test result automatically and displays D-Dimer concentration of the test sample as ng/mL.
- Working range of i-chroma™ D-Dimer is 50-10,000 ng/ml.
- Reference value of i-chroma™ D-Dimer is 500 ng/ml. (FEU: Fibrinogen equal units).

2.2.3 Full blood count using BC-3000 Mindary

Principles

The blood counters use the impedance technology to measure the number of cells in a diluted blood sample which pass through an aperture located between two electrodes where a constant electrical current is applied. The dilution is done with an isotonic solution which is a conductor and does not lyse the blood cells. The conductivity of the isotonic diluent allows the passage of the electrical current between the two electrodes. When a particle is aspirated through the micro-orifice, it moves its own volume of electrolyte. This applies a modification of the resistance between both electrodes and generates an electrical pulse. The amplitude is directly proportional to the volume of the particle. Two separate dilutions are prepared for WBC/HGB and for RBC/PLT.

Components and Reagents

- Diluent: is designed for diluting the whole blood prior to counting and sizing of RBC/WBC/PLT. It maintains stability RBC/PLT during counting. Cleaning Reagent is designed to remove protein contaminants from the measurement system analyzer after each blood sample analysis.
- Enzymatic cleaner forte: is designed to remove protein contaminants from the measurement system analyzer after each blood sample analysis. The presence of an enzyme reduces the formation of proteins deposit.
- Lysing reagent CN free: Lysing agent to obtain the measurement of the haemoglobein, counting and differentiation of the white blood cells. Use in combination with the diluent, this reagent lyses the red blood cells and protects the state of the leukocytes to permit the differentiation in three populations (lymphocytes, monocytes, granulocytes). Diluent, lysing reagent, cleaner or enzymatic cleaner are the functional set to perform blood sample analysis on haematology analyzer.

Test procedure

Sample of whole blood collected on EDTA tubes. The ratio between EDTA and whole blood must be between 1 to 2 mg per ml of blood. The samples should be used at room temperature no longer that 4 hours after collection. If the analysis can't be done in the time, the samples should be stored at 4°C. Sample volume whole blood 13 µL.

Performance Parameter	Linearity Range Precision	(CV %)
WBC (10^9/ L)	0.3-99.9	2.5 (7.0-15.0)
HGB (g / L)	10-250	1.5 (110 -180)
MCV (fL)		0.5 (80.0-110.0)
PLT (109 / L)	10-999	(150-500)

Sample mode was selected as whole blood, then pressed main key go to count screen. Pressed ID key to enter patient only. Blood sample mixed 10 times gently. Uncap vile and placed in position No.1 of sample tube holder, sample compartment door closed and pressed the aspirate button. After 55 seconds results have been displayed. Auto print option has been activated and result was printed

Interpretation of the results

According to normal range of hematological parameter globally and the parameter induced in the research:

- Hemoglobin HGB (g / dl) for female (12 - 15 g/dl)
- Total white blood cell WBC (10^9/ L) the rang (3 - 11 x 10^9/l)
- Thrombocytes PLT (10^9 / L) the rang (150 – 450 x 10^9/l)
- Packed cell volume (%) the rang for female (36 – 46 %)
- Mean cell volume MCV(fL) the rang (78 – 99 fl)
- Mean cell hemoglobin MCH (pg) the rang (27 – 32 pg)
- Neutrophil (10^9 / L) the rang (1.6 – 6.8 x 10^9/l)

- Lymphocyte(10^9 / L) the rang (1.2 – 4.9 x 10^9/l)
- Neutrophil/Lymphocyte ratio the rang (0.78 – 3.53)

2.2.4 Data analysis

The collected data proceed for analysis using SPSS version 20 computerized program (Independent t-test, one way ANOVA) and the data presented in form of tables and figures.

Chapter Three

3. Results

3.1 The age groups in SLE patients and D-dimer

The SLE patients were divided into 2 age groups; 56.66% of patients, their age less than 35 years and 43.33% of patients more than 35 years and the means of the D-dimer levels for each group were 330.24 ng/ml and 442.96ng/ml respectively (Figure3.1). By using independent t-test the result shows there were no significant differences in D-dimer level between two groups P. value = 0.251(P. value > 0.05). But the older age shows the higher level (Table 3.1)

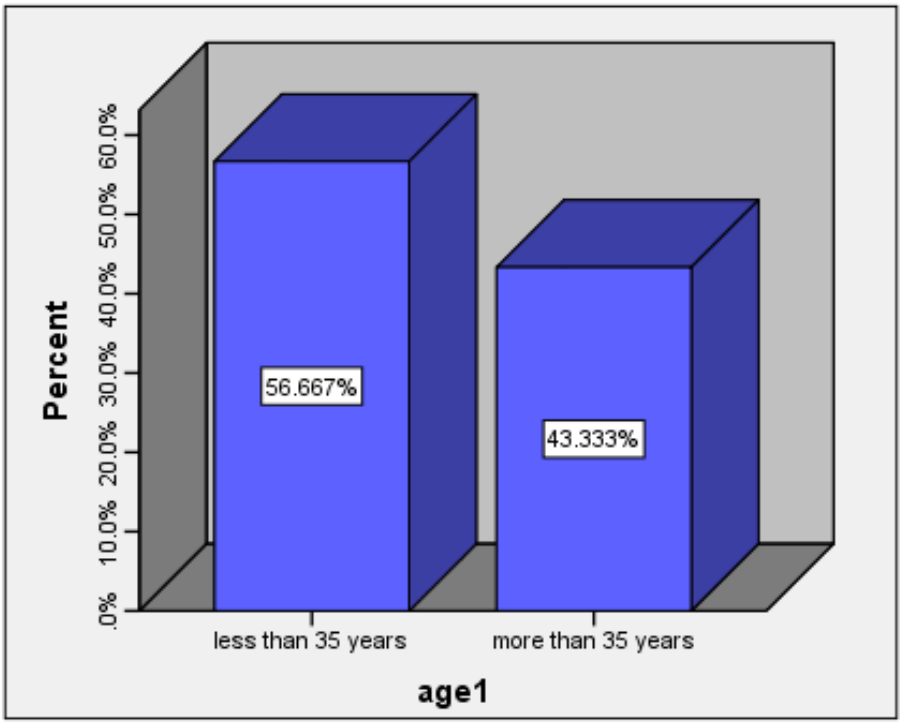


Figure 3.1: age groups of SLE patients

Table 3.1: The significant difference in D-dimer level between age groups

	Age	Number of patient	Mean of D-dimer	P.value
Cases	Less than 35 years	17	330.23	0.251
	More than 35 years	13	442.96	

3.2 The duration of disease in SLE patients and D-dimer

Thirteen patients (43.33%) had SLE less than 2 years of disease duration with mean of D-dimer level 451.47 ng/ml, while the other 17 patients (56.66%), with disease duration more than 2 years with mean 323.73 ng/ml (Figure 3.2). By using independent t-test there were no significant differences in D-dimer level between two groups P. value = 1.92 (P. value > 0.05) (Table 3.2).

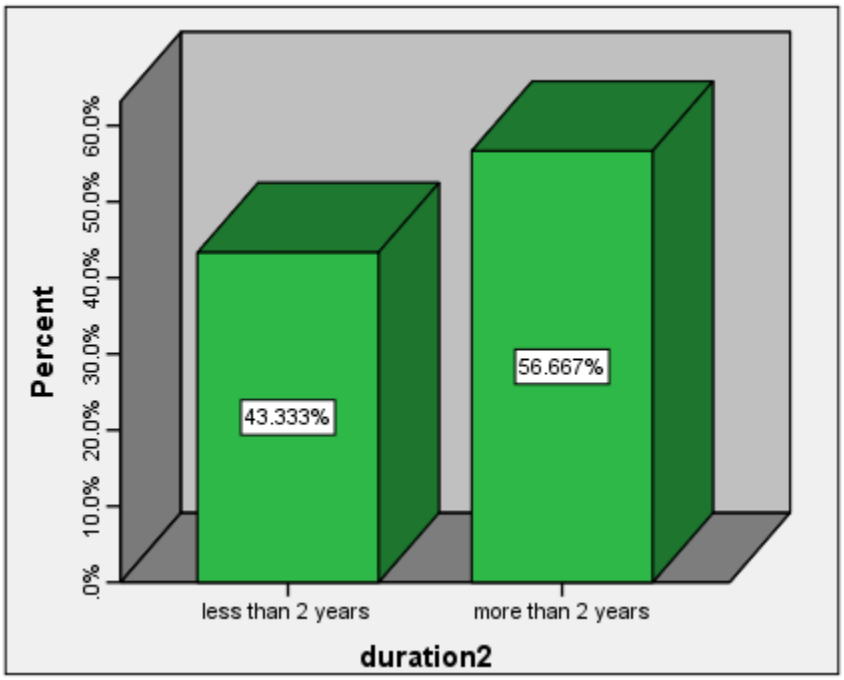


Figure 3.2: the duration of disease groups

Table 3.2 the significant differences in D-dimer level between duration disease groups

	Duration	Number of patient	Mean of D.dimer	P.value
cases	Less than 2 years	13	451.47	0.192
	More than 2 years	17	323.73	

3.3 The frequency of disease severity and medication groups with comparability in D-dimer level between groups

The means of D-dimer level according to the severity of disease as mild (66.67%), moderate (23.33%), and severe (10%) were 257.28 ng/ml, 488.19 ng/ml and 936.57 ng/ml respectively (Figure 3.3). For Medication condition: No medication (10%), HCQ+ anti-inflammatory+ immunosuppressive (26.67%) and HCQ+ anti-inflammatory+ immunosuppressive+ Adjunctive (63.33%) the means of D-dimer for each one were: 273.74 ng/ml, 301.30 ng/ml, 273.74 ng/ml respectively (Figure 3.4).

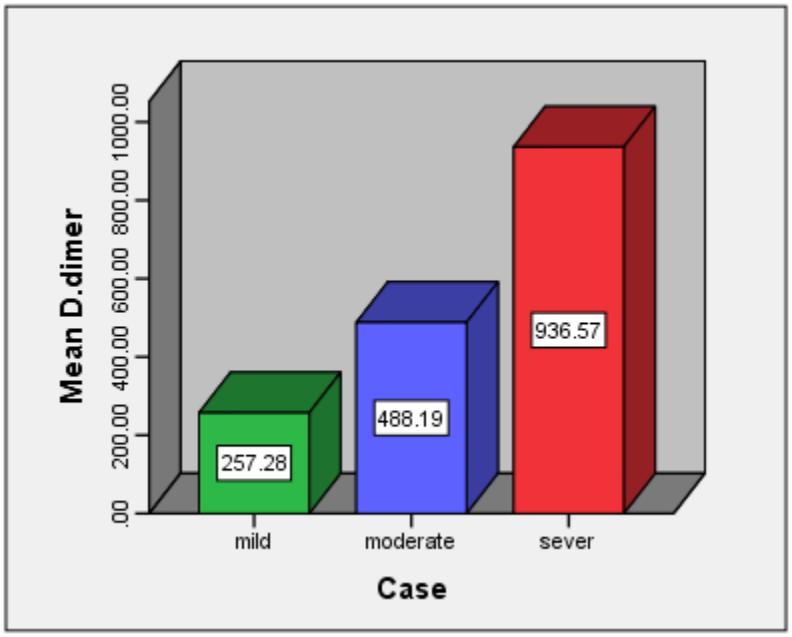


Figure 3.3: the mean of D-dimer in all severity of disease in the SLE patients

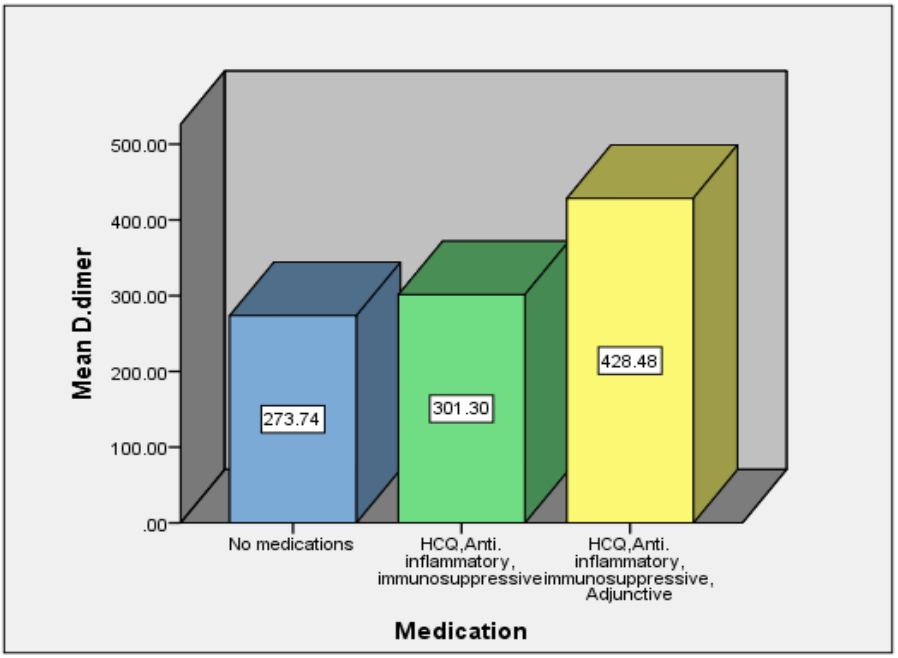


Figure 3.4: medication type and D-dimer level for each group among patients

To compare the differences in D-dimer level with all severity disease groups and medication type groups, One way ANOVA test used to show that there were a significant differences in D-dimer level according to severity of the disease (mild, moderate, severe) based on the result of P.value = 0.00 (P.value less than 0.05) (table 3.3), in the other hand One way ANOVA test shows that there were no significant differences in D-dimer level according to medication type groups the P value= 0.410 (P.value >0.05) (table 3.4).

Table 3.3: the significant differences in D-dimer level between SLE severity groups

SLE patient	Groups	% of cases	Mean of D-dimer	P.value
Severity of disease	mild	66.67	257.28	0.00
	moderate	23.33	488.19	
	sever	10	936.57	

Table 3.4: the significant differences in D-dimer level between medication groups

SLE patient	Groups	% of cases	Mean of D-dimer	P.value
Medication groups	No medication	10	273.74	0.410
	HCQ+ anti-inflammatory+ immunosuppressive	26.67	301.30	
	HCQ+ anti-inflammatory+ immunosuppressive+ Adjunctive	63.33	273.74	

3.4 D-dimer levels with different blood count statuses in SLE patients

There were no significant differences in D-dimer concentration between haematological statuses groups of SLE patients in TWBCs count (leukopenia & Normal TWBCs count), haemoglobein and PCV (anaemic & non anaemic), and Platelet count (Thrombocytopenia & normal PLT count) using independent t- test, the P. value > 0.05 (table 3.5).

Table 3.5: the significant differences in D-dimer level between blood count statuses

Haematological Situation	**groups**	**% of cases**	**Number of patients**	**Mean of D-dimer**	**P.value**
TWBCs count	Leukopenia	16.67	5	477.91	0.366
	Normal TWBCs count	83.33	26	359.32	
Haemoglobin & PCV	Anaemic	46.67	14	432.97	0.302
	Non anaemic	53.33	16	331.97	
Platelet count	Thrombocytopenia	6.67	2	138.24	0.185
	Normal PLTs count	93.33	28	396.29	

3.5 The RBC indices in SLE patients

According to the MCV and MCH results on the SLE patients the RBC picture can be classified to normocytic, microcytic defined by (MCV1) and normochromic, hypochromic defined by (MCH1). The percentage for each category of RBC as: Anaemic SLE patients RBCs (microcytic hypochromic 16.76%, Normocytic hypochromic 6.66% & normocytic normochromic 23.33%). Non anaemic SLE patients RBCs (normocytic hypochromic 13.33%, normocytic normochromic 40%). The results shows that the majority RBCs character in anaemic SLE patients were normocytic normochromic (figure 3.5).

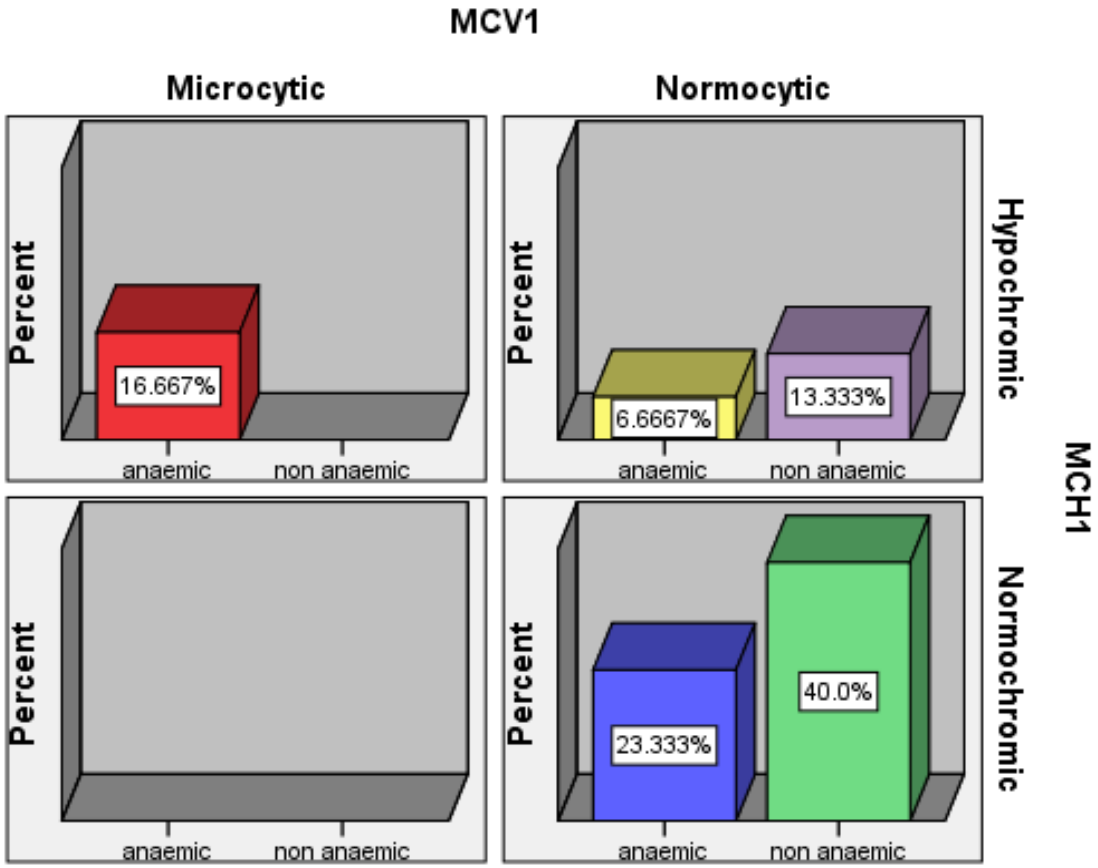


Figure 3.5: the percentage of all RBC categories in anaemic and non anaemic SLE patients

3.6 The mean of D-dimer level and CBC results in SLE patients and control group with the differences between them

The means of the analytical results for SLE patients were: D-dimer (379 ng/ml), haemoglobin (11.6 g/dl), packed cell volume (36.3%), total white blood cells (5.78 x 10^9/L), thrombocyte (262 x 10^9/L), neutrophil/lymphocyte ratio (3.33). For the control group the mean of the analytical results were: D-dimer (89.58ng/ml), haemoglobin (11.96 g/dl), packed cell volume (35.75%), total white blood cells count (5.42x10^9/L), thrombocyte (288x10^9/L), neutrophil/lymphocyte ratio (1.61). There were significant differences between SLE patients and control group in D-dimer level (P.value = 0.00), and N/L ratio (P.value = 0.026) using independent t-test (P. value < 0.05), On the other hand, there were no significant differences in other analytical results between SLE patients and control group (P.value > 0.05) (table 3.6).

Table 3.6: differences in D-dimer and CBC results between SLE patients and control

Haematological parameter	Sample	Number	Mean	P. value
Haemoglobin	Patients	30	11.6	0.358
	Control	30	11.9	
TWBC count	Patients	30	5.78	0.495
	Control	30	5.42	
PCV	Patients	30	36.33	0.532
	Control	30	35.57	
Thrombocytes	Patients	30	262	0.196
	Control	30	288	
N/L ratio	Patients	30	3.32	0.026
	Control	30	1.61	
D-dimer	Patients	30	379.08	0.000
	Control	30	89.58	

3.7 Comparability in CBC results between different disease severity groups also medication type groups

One way ANOVA test shows that there were no significant differences in CBC results with the disease severity groups and type of medications (table 3.7) all P. values were insignificant (P. value > 0.05).

Table 3.7: comparison in CBC results between groups of disease severity also medication type groups.

disease Severity groups				
Complete blood count	**Means of CBC results**			**P value**
	mild	**moderate**	**sever**	
Haemoglobin	11.67	11.72	10.83	0.683
Total WBC count	6.0	4.9	6.2	0.649
PLT count	250.35	289.00	278.33	0.534
PCV	36.26	37.43	34.33	0.585
N/L ratio	3.4	1.7	6.59	0.226
Medication types groups				
Complete blood count	**Means of CBC results**			**P value**
	No medication	**HCQ+ anti-inflammatory+ immunosuppressive**	**HCQ+ anti-inflammatory+ immunosuppressive+ Adjunctive**	
Haemoglobin	12.3	11.7	11.45	0.687
Total WBC count	4.0	6.6	5.7	0.337
PLT count	259.67	258.75	264.0	0.988
PCV	38.3	36.7	35.8	0.626
N/L ratio	1.18	3.08	3.76	0.600

3.8 D-dimer and degree of inflammation according to N/L ratio

One way ANOVA test (table 3.8) shows that there were no significant differences in D-dimer level and the degree of inflammation according to N/L ratio range (0.78 - 3.53) divided to 3 groups:

1- Low inflammation risk the N/L ratio less than 0.77 the mean of D-dimer for this group was 479.20 ng/ml

2- Normal ratio between 0.78 and 3.53 the mean of D-dimer for this group was 341.37 ng/ml

3- High inflammation risk the N/L ratio more than 3.54 the mean of D-dimer for this group was 506.98 ng/ml

The P.value = 0.374 (P. value >0.05)

Table 3.8: the significant differences in D-dimer level between different degrees of inflammation

Degree of inflammation	Number of patients	% of SLE patients	Mean of D-dimer	P.value
Low inflammation risk	1	3.33	479.2	0.374
Normal	23	76.67	341.37	
High inflammation risk	6	20	506.98	

Chapter Four

4. Discussion, Conclusions and Recommendations

4.1 Discussion

The study showed that the mean of D-dimer concentration in SLE patients was significantly increased when compared with the control group (P. value < 0.05). Moreover the concentration of D-dimer showed increased level according to the severity of disease above the cut off of the normal level 500 ng/ml in the severe patients. Both results indicated that D-dimer level in SLE patients was increased and they were at risk of thrombosis especially if the disease getting severe. This observation is agreed with **Wu, *et al*, 2008,** who concluded that all patients with SLE had elevated D-dimer levels long before or shortly before the clinical diagnosis of the clotting event, and that is similar to the gradual increased in D-dimer level on SLE patients going with severity of the disease. Moreover a study done in 2009 by **Tektonidou, *et al*** supported this result, and concluded that, an extensive investigation and a rigorous management of traditional and SLE related risk factors for thrombosis is warranted.. In addition, a nationwide cohort study in 2014 done by **Chung, *et al,*** concluded that, the risks of DVT and PE are significantly higher in SLE patients than in the general population. Also the recent study in 2015 by **Yusuf, *et al***, observed that SLE was associated with an increased likelihood of a VTE event. Moreover a study in 2015 by **Gebhart, *et al,*** confirmed, the occurrence of a thrombotic event in SLE patients is associated with higher mortality. On the other hand there were no significant differences in the means of haemoglobin, TWBC count, PLT count and PCV between SLE

patients and control group (p. value > 0.05). But around 25% of patients were showed normocytic normochromic anemia. These results were in contrast with study done by **Aleem, *et al,*** in 2014 who showed that haemolytic anaemia are very common at the time of diagnosis and during follow-up in SLE, and some of haematological abnormalities are associated with organ damage. In fact, most patients in this current study were founded without appearance of organ damage complication (mild case 66.67%). This finding supported by **Njoroge, *et al*, 2016** , who concluded that, the haematological abnormalities in SLE patient were mild to moderate and clinically asymptomatic.

In the present study there were no correlation between the D-dimer concentration in the age groups as well as medication groups showed no effect on the D-dimer results, and also in the degree of inflammation and duration of the disease for the SLE patients (P. value > 0.05), it was obvious that D-dimer level showed the higher concentration in the older patients (>35 years) and in less than 2 years of disease duration. On the other hand of this study, there was a significant increase in the mean of Neutrophil/Lymphocyte ratio in SLE patients comparing with control group (P. value < 0.05), and the highest level of D-dimer was found in cases with severe inflammation (>3.5) without significant correlation. These findings were agreed with both studies done by **Li, *et al*, 2015** and **Liang**, ***et al*, 2016** showed that, the degree of inflammation increase the D-dimer level. Other study done by **Le Minh, *et al*, 2018** interpreted that, the risk of thrombosis in SLE patients is related to the autoantibody itself rather than the inflammatory event. Also the SLE samples were showed a variation in the degree of anemia, leukopenia and thrombocytopenia without significant correlation with the D-dimer level.

These results indicated that the haematological abnormalities may contribute in the prognosis of SLE.

4.2 Conclusions

- D-dimer concentration was significantly increased in women with SLE.
- The Neutrophil/Lymphocyte ratio showed significant increase in SLE patients compare with normal individual.
- The older patients showed the higher level of D-dimer.
- Patients with disease duration less than 2 years showed the higher level of D-dimer.
- The most severe cases of SLE, showed the highest D-dimer level.
- About 25% of patients showed normocytic normochromic anemia.

4.3 Recommendations

- D-dimer should be considered for patients with SLE for follow up and management to avoid thrombosis.
- Careful monitor for complete blood count to assess any mild change for better prognosis of the disease.

References

- **Abdwani, R. and Mani, R.,** 2009. Anti-CD20 monoclonal antibody in acute life threatening haemolytic anaemia complicating childhood onset SLE. *Lupus*, *18*(5), pp.460-464.
- **Adam, S.S., Key, N.S. and Greenberg, C.S.,** 2009. D-dimer antigen: current concepts and future prospects. *Blood*, *113*(13), pp.2878-2887.
- **Afeltra, A., Vadacca, M., Conti, L., Galluzzo, S., Mitterhofer, A.P., Ferri, G.M., Del Porto, F., Caccavo, D., Gandolfo, G.M. and Amoroso, A.**, 2005. Thrombosis in systemic lupus erythematosus: congenital and acquired risk factors. *Arthritis Care & Research: Official Journal of the American College of Rheumatology*, *53*(3), pp.452-459.
- **Alarcon-Segovia, D.,** 2002. Splenectomy has a limited role in the management of lupus with thrombocytopenia. *The Journal of rheumatology*, *29*(1), pp.1-2.
- **Aleem, A., Al Arfaj, A.S., Khalil, N. and Alarfaj, H.,** 2014. Haematological abnormalities in systemic lupus erythematosus. *Acta Reumatol Port*, *39*(3), pp.236-41.
- **Al-Homood, I.A., 2012.** Thrombosis in systemic lupus erythematosus: a review article. *ISRN rheumatology*, *2012*.
- **Amoroso, A., Mitterhofer, A.P., Del Porto, F., Garzia, P., Ferri, G.M., Galluzzo, S., Vadacca, M., Caccavo, D. and Afeltra, A.,** 2003. Antibodies to anionic phospholipids and anti-β2-GPI: association with thrombosis and thrombocytopenia in systemic lupus erythematosus. *Human immunology*, *64*(2), pp.265-273.
- **Andrews, R.K. and Berndt, M.C., 2004.** Platelet physiology and thrombosis. *Thrombosis research*, *114*(5-6), pp.447-453.
- **Ardoin, S.P., Shanahan, J.C. and Pisetsky, D.S.,** 2007. The role of microparticles in inflammation and thrombosis. *Scandinavian journal of immunology*, *66*(2- 3), pp.159-165.

- **Bartels C.M, Muller D.**, Systemic Lupus Erythematosus (SLE) Treatment & Management , Medscape, nov 14/2017

- **Bazzan, M., Vaccarino, A. and Marletto, F.,** 2015. Systemic lupus erythematosus and thrombosis. *Thrombosis journal*, *13*(1), p.16.

- **Bertsias, G., Cervera, R. and Boumpas, D.T.,** 2012. Systemic lupus erythematosus: pathogenesis and clinical features. *EULAR textbook on rheumatic diseases, Geneva, Switzerland: European League Against Rheumatism*, pp.476-505.

- **Bertsias, G.K., Nakou, M., Choulaki, C., Raptopoulou, A., Papadimitraki, E., Goulielmos, G., Kritikos, H., Sidiropoulos, P., Tzardi, M., Kardassis, D. and Mamalaki, C.,** 2009. Genetic, immunologic, and immunohistochemical analysis of the programmed death 1/programmed death ligand 1 pathway in human systemic lupus erythematosus. *Arthritis & Rheumatism*, *60*(1), pp.207-218.

- **Bertsias, G.K., Salmon, J.E. and Boumpas, D.T.,** 2010. Therapeutic opportunities in systemic lupus erythematosus: state of the art and prospects for the new decade. *Annals of the rheumatic diseases*, *69*(9), pp.1603-1611.

- **Bertsias, G.K., Pamfil, C., Fanouriakis, A. and Boumpas, D.T.,** 2013. Diagnostic criteria for systemic lupus erythematosus: has the time come?. *Nature Reviews Rheumatology*, *9*(11), p.687- 694.

- **Bukhari, M.,** 2012. Drug-induced rheumatic diseases: a review of published case reports from the last two years. *Current opinion in rheumatology*, *24*(2), pp.182-186.

- **Calvo- Alén, J., Toloza, S.M., Fernández, M., Bastian, H.M., Fessler, B.J., Roseman, J.M., McGwin Jr, G., Vilá, L.M., Reveille, J.D., Alarcón, G.S. and LUMINA Study Group,** 2005. Systemic lupus erythematosus in a multiethnic US cohort (LUMINA): XXV. Smoking, older age, disease activity, lupus anticoagulant, and glucocorticoid dose as

risk factors for the occurrence of venous thrombosis in lupus patients. *Arthritis & Rheumatism*, *52*(7), pp.2060-2068.

- **Cervera, R.,** 2006. Systemic lupus erythematosus in Europe at the change of the millennium: lessons from the "Euro-Lupus Project". *Autoimmunity reviews*, *5*(3), pp.180-186.

- **Cervera, R. and Khamashta, M.A.,** 2006. Epidemiology of systemic lupus erythematosus at the change of the millennium: lessons from the Euro-Lupus and the LUMINA projects. *Lupus*, 15:1-2.

- **Cervera, R., Khamashta, M.A. and Hughes, G.R.V.,** 2009. The Euro-lupus project: epidemiology of systemic lupus erythematosus in Europe. *Lupus*, *18*(10), pp.869-874.

- **Cesarman- Maus, G. and Hajjar, K.A.,** 2005. Molecular mechanisms of fibrinolysis. *British journal of haematology*, *129*(3), pp.307-321.

- **Chappell, F.M., Andras, A., Welch, K., Di Nisio, M., Robertson, L., Stewart, M. and Crawford, F.,** 2016. D- Dimer tests for the diagnosis of deep venous thrombosis in symptomatic hospital outpatients with a clinical prediction rule. *The Cochrane Library*. , Issue 9. Art. No.: CD012356. DOI: 10.1002/14651858.CD012356 John Wiley & Sons, Ltd.

- **Chen, W. and Lin, J., 2011.** Lymphopenia relating to T-lymphocyte apoptosis in systemic lupus erythematosus. *Clinical rheumatology*, *30*(11), pp.1515-1516.

- **Chung, W.S., Lin, C.L., Chang, S.N., Lu, C.C. and Kao, C.H., 2014.** Systemic lupus erythematosus increases the risks of deep vein thrombosis and pulmonary embolism: a nationwide cohort study. *Journal of Thrombosis and Haemostasis*, *12*(4), pp.452-458.

- **Colvin, B.T.,** 2004. ES04. 01 Physiology of haemostasis. *Vox sanguinis*, *87*, pp.43-46. Colvin BT.

- **Contreras, G., Pardo, V., Leclercq, B., Lenz, O., Tozman, E., O'nan, P. and Roth, D.,** 2004. Sequential therapies for proliferative lupus nephritis. *New England Journal of Medicine*, *350*(10), pp.971-980.

- **Cooper, G.S., Wither, J., Bernatsky, S., Claudio, J.O., Clarke, A., Rioux, J.D., CaNIOS GenES Investigators and Fortin, P.R.,** 2010. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. *Rheumatology*, *49*(11), pp.2172-2180.

- **Corral, J., González- Conejero, R., Hernández- Espinosa, D. and Vicente, V.,** 2007. Protein Z/Z- dependent protease inhibitor (PZ/ZPI) anticoagulant system and thrombosis. *British journal of haematology*, *137*(2), pp.99-108**.**

- **Costallat, G.L., Appenzeller, S. and Costallat, L.T.L.,** 2012. Evans syndrome and systemic lupus erythematosus: clinical presentation and outcome. *Joint Bone Spine*, *79*(4), pp.362-364.

- **Dias, A.M.B., Do Couto, M.C.M., Duarte, C.C.M., Inês, L.P.B. and Malcata, A.B.,** 2009. White Blood Cell Count Abnormalities and Infections in One- year Follow- up of 124 Patients with SLE. *Annals of the New York Academy of Sciences*, *1173*(1), pp.103-107**.**

- **Doria, A. and Briani, C.,** 2008. Lupus: improving long-term prognosis. *Lupus*, *17*(3), pp.166-170.

- **Duckitt, K. and Harrington, D.,** 2005. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *Bmj*, *330*(7491), p.565.

- **Ezihe-Ejiofor, J.A. and Hutchinson, N.,** 2013. Anticlotting mechanisms 1: physiology and pathology. *Continuing Education in Anaesthesia, Critical Care & Pain*, *13*(3), pp.87-92.

- **Faust, T.W., Chang, E.H., Kowal, C., Berlin, R., Gazaryan, I.G., Bertini, E., Zhang, J., Sanchez-Guerrero, J., Fragoso-Loyo, H.E., Volpe, B.T. and Diamond, B.,** 2010. Neurotoxic lupus autoantibodies alter brain function through two distinct mechanisms. *Proceedings of the National Academy of Sciences*, *107*(43), pp.18569-18574.

- **Fayyaz, A., Igoe, A., Kurien, B.T., Danda, D., James, J.A., Stafford, H.A. and Scofield, R.H.,** 2015. Haematological manifestations of lupus. *Lupus science & medicine*, *2*(1), p.e000078.

- **Ferreira, G.A., Navarro, T.P., Telles, R.W., Andrade, L.E.C. and Sato, E.I.,** 2007. Atorvastatin therapy improves endothelial-dependent vasodilation in patients with systemic lupus erythematosus: an 8 weeks controlled trial. *Rheumatology*, *46*(10), pp.1560-1565.

- **Forget, P., Khalifa, C., Defour, J.P., Latinne, D., Pel, M.C. and Kock, M.,** 2017. What is the normal value of the neutrophil-to-lymphocyte ratio?. *BMC research notes*, *10*(1), p.12.

- **Furie, B.,** 2009. Pathogenesis of thrombosis. *ASH Education Program Book*, *2009*(1), pp.255-258.

- **Gebhart, J., Posch, F., Koder, S., Perkmann, T., Quehenberger, P., Zoghlami, C., Ay, C. and Pabinger, I.,** 2015. Increased mortality in patients with the lupus anticoagulant: the Vienna Lupus Anticoagulant and Thrombosis Study (LATS). *Blood*, pp.blood-2014.

- **Giannakopoulos, B., Passam, F., Ioannou, Y. and Krilis, S.A.,** 2009. How we diagnose the antiphospholipid syndrome. *Blood*, *113*(5), pp.985-994.

- **Greenberg, C.S.,** 2017. The role of D-dimer testing in clinical hematology and oncology. *Clinical advances in hematology & oncology: H&O*, *15*(8), pp.580-583.

- **Guerra, S.G., Vyse, T.J. and Graham, D.S.C.,** 2012. The genetics of lupus: a functional perspective. *Arthritis research & therapy*, *14*(3), p.211.

- **Hall, J.E.,** 2010. Hemostasis and blood coagulation. *Guyton and Hall textbook of medical physiology e-Book*. 11th ed. Elsevier Health Sciences. Philadelphia. p. 457-9

- **Heemskerk, J.W., Bevers, E.M. and Lindhout, T.,** 2002. Platelet activation and blood coagulation. *Thrombosis and haemostasis*, *88*(02), pp.186-193.

- **Hepburn, A.L., Narat, S. and Mason, J.C.,** 2010. The management of peripheral blood cytopenias in systemic lupus erythematosus. *Rheumatology*, *49*(12), pp.2243-2254.

- **Hoff brand, A.V., Moss, P.A.H.** 2011, Essential haematology, 6th ed., John Wiley & Sons Ltd PO19 8SQ, UK, p321- 326

- **Javierre, B.M., Fernandez, A.F., Richter, J., Al-Shahrour, F., Martin-Subero, J.I., Rodriguez-Ubreva, J., Berdasco, M., Fraga, M.F., O'Hanlon, T.P., Rider, L.G. and Jacinto, F.V.**, 2010. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome research*, *20*(2), pp.170-179**.**

- **Karpouzas, G.A.,** 2013. Hematologic and lymphoid abnormalities in SLE. In *Dubois' Lupus Erythematosus and Related Syndromes (Eigth Edition)* Philadelphia: Elsevier, (pp. 426-437).

- **Kumar V, Abbas AK, Fausto N, Aster JC.** 2010. Robbins and Cotran. Pathologic Basis of Disease. 8th ed. Philadelphia, PA: Saunders Elsevier; Hemodynamic disorders, thromboembolic disease and shock; pp. 118–20.

- **Lasne, D., Jude, B. and Susen, S.,** 2006. From normal to pathological hemostasis. *Canadian Journal of Anesthesia*, *53*(2), pp.S2-S11.

- **Latella, J., Desmarais, S., MIRON, M.J., Roussin, A., Joyal, F., Kassis, J., Solymoss, S., Desjardins, L., Ginsberg, J.S. and Kahn, S.R.,**

2010. Relation between D- dimer level, venous valvular reflux and the development of post- thrombotic syndrome after deep vein thrombosis. *Journal of Thrombosis and Haemostasis*, *8*(10), pp.2169-2175.

- **Le Minh, G., Peshkova, A.D., Andrianova, I.A., Sibgatullin, T.B., Maksudova, A.N., Weisel, J.W. and Litvinov, R.,** 2018. Impaired contraction of blood clots as a novel prothrombotic mechanism in systemic lupus erythematosus. *Clinical Science*, p.CS20171510. *132*(2), pp.243-254.

- **Liang, Y., Leng, R.X., Pan, H.F. and Ye, D.Q.,** 2016. Effects of Disease Activity and Inflammatory Response on Hypercoagulability in Patients with Systemic Lupus Erythematosus. *Archives of medical research*, *47*(7), pp.573-579.

- **Lijfering, W.M., Rosendaal, F.R. and Cannegieter, S.C.,** 2010. Risk factors for venous thrombosis–current understanding from an epidemiological point of view. *British journal of haematology*, *149*(6), pp.824-833.

- **Li, L., Xia, Y., Chen, C., Cheng, P. and Peng, C.,** 2015. Neutrophil-lymphocyte ratio in systemic lupus erythematosus disease: a retrospective study. *International journal of clinical and experimental medicine*, *8*(7), p.11026-11031.

- **Lood, C., Amisten, S., Gullstrand, B., Jönsen, A., Allhorn, M., Truedsson, L., Sturfelt, G., Erlinge, D. and Bengtsson, A.A.,** 2010. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. *Blood*, pp.blood-2010. 116(11):1951-1957.

- **Markle, J.G., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K.D., Macpherson, A.J. and Danska, J.S.,** 2013. Sex differences in the gut

microbiome drive hormone-dependent regulation of autoimmunity. *Science*, *339*(6123), pp.1084-1088.

- **Martinez-Banos, D., Crispin, J.C., Lazo-Langner, A. and Sánchez-Guerrero, J.,** 2006. Moderate and severe neutropenia in patients with systemic lupus erythematosus. *Rheumatology*, *45*(8), pp.994-998.

- **Massardo, L., Metz, C., Pardo, E., Mezzano, V., Babul, M., Jarpa, E., Guzmán, A.M., André, S., Kaltner, H., Gabius, H.J. and Jacobelli, S.,** 2009. Autoantibodies against galectin-8: their specificity, association with lymphopenia in systemic lupus erythematosus and detection in rheumatoid arthritis and acute inflammation. *Lupus*, *18*(6), pp.539-546.

- **McMahon, M., Grossman, J., Skaggs, B., FitzGerald, J., Sahakian, L., Ragavendra, N., Charles- Schoeman, C., Watson, K., Wong, W.K., Volkmann, E. and Chen, W.,** 2009. Dysfunctional proinflammatory high- density lipoproteins confer increased risk of atherosclerosis in women with systemic lupus erythematosus. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *60*(8), pp.2428-2437.

- **Meroni, P.L. and Schur, P.H.,** 2010. ANA screening: an old test with new recommendations. *Annals of the rheumatic diseases*, *69*(8), pp.1420-1422.

- **M. Petri,** "The lupus anticoagulant is a risk factor for myocardial infarction (but not atherosclerosis): Hopkins Lupus Cohort," Thrombosis Research, vol. 114, no. 5-6, pp. 593–595, 2004

- **Navarra, S.V. and Leynes, M.S.N.,** 2010. Infections in systemic lupus erythematosus. *Lupus*, *19*(12), pp.1419-1424.

- **Njoroge, J.W., Oyoo, G.O., Kitonyi, G., Barasa, A. and Odhiambo, A.,** 2016. Haematological parameters in systemic lupus erythematosus patients at Kenyatta National Hospital, Nairobi. *African Journal of Rheumatology*, *4*(2), pp.57-62.

- **Nossent, J., Kiss, E., Rozman, B., Pokorny, G., Vlachoyiannopoulos, P., Olesinska, M., Marchesoni, A., Mosca, M., Påi, S., Manger, K. and Schneider, M.,** 2010. Disease activity and damage accrual during the early disease course in a multinational inception cohort of patients with systemic lupus erythematosus. *Lupus*, *19*(8), pp.949-956.

- **Odendahl, M., Jacobi, A., Hansen, A., Feist, E., Hiepe, F., Burmester, G.R., Lipsky, P.E., Radbruch, A. and Dörner, T.,** 2000. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *The Journal of Immunology*, *165*(10), pp.5970-5979.

- **Owens III, A.P. and Mackman, N.,** 2010. Tissue factor and thrombosis: The clot starts here. *Thrombosis and haemostasis*, *104*(3), p.432-439**.**

- **Owen, J.A., Punt, J. and Stranford, S.A.,** 2013. *Kuby immunology*. Chapter 16 tolerance, autoimmunity, and transplantation (p. 692). New York: WH Freeman.

- **Palatinus, A. and Adams, M.,** 2009, October. Thrombosis in systemic lupus erythematosus. In *Seminars in thrombosis and hemostasis* (Vol. 35, No. 07, pp. 621-629). © Thieme Medical Publishers**.**

- **Palta, S., Saroa, R. and Palta, A.,** 2014. Overview of the coagulation system. *Indian journal of anaesthesia*, *58*(5), p.515.

- **Patel, D.R. and Richardson, B.C.,** 2013. Dissecting complex epigenetic alterations in human lupus. *Arthritis research & therapy*, *15*(1), p.201.

- **Petri, M., Orbai, A.M., Alarcón, G.S., Gordon, C., Merrill, J.T., Fortin, P.R., Bruce, I.N., Isenberg, D., Wallace, D.J., Nived, O. and Sturfelt, G.,** 2012. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis & Rheumatism*, *64*(8), pp.2677-2686.

- **Pons-Estel, G.J., Alarcón, G.S., Scofield, L., Reinlib, L. and Cooper, G.S.,** 2010, February. Understanding the epidemiology and progression of

systemic lupus erythematosus. In *Seminars in arthritis and rheumatism* (Vol. 39, No. 4, pp. 257-268). WB Saunders.

- **Pons-Estel, G.J., Wojdyla, D., McGwin Jr, G., Magder, L.S., Petri, M.A., Pons-Estel, B.A., Grupo Latino Americano De Estudio del Lupus (GLADEL) and, Alarcón, G.S. and LUMINA cohort,** 2014. The American College of Rheumatology and the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus in two multiethnic cohorts: a commentary. *Lupus*, *23*(1), pp.3-9.

- **Previtali, E., Bucciarelli, P., Passamonti, S.M. and Martinelli, I.,** 2011. Risk factors for venous and arterial thrombosis. *Blood Transfusion*, *9*(2), p.120-138.

- **Ramagopalan, S.V., Wotton, C.J., Handel, A.E., Yeates, D. and Goldacre, M.J.,** 2011. Risk of venous thromboembolism in people admitted to hospital with selected immune-mediated diseases: record-linkage study. *BMC medicine*, *9*(1), p.1-8.

- **Rigby, A.C. and Grant, M.A.,** 2004. Protein S: a conduit between anticoagulation and inflammation. *Critical care medicine*, *32*(5), pp.S336-S341.

- **Sánchez-Guerrero, J., Uribe, A.G., Jiménez-Santana, L., Mestanza-Peralta, M., Lara-Reyes, P., Seuc, A.H. and Cravioto, M.D.C.,** 2005. A trial of contraceptive methods in women with systemic lupus erythematosus. *New England Journal of Medicine*, *353*(24), pp.2539-2549.

- **Sen, D. and Keen, R.W.,** 2001. Osteoporosis in systemic lupus erythematosus: prevention and treatment. *Lupus*, *10*(3), pp.227-232.

- **Stephenson, J.L. and Shipman, A.R.,** 2014. The Systemic Lupus International Collaborating Clinics criteria have replaced the A merican College of Rheumatology guidelines for the diagnosis of systemic lupus erythematosus. *Clinical and experimental dermatology*, *39*(3), pp.431-432.

- **Sugimoto, T., Soumura, M., Tanaka, Y., Uzu, T., Nishio, Y. and Kashiwagi, A.,** 2006. Early morning neutropenia in a patient with systemic lupus erythematosus. *Modern rheumatology*, *16*(4), pp.267-268.

- **Swadzba, J., Iwaniec, T. and Musial, J.,** 2011. Increased level of tumor necrosis factor-α in patients with antiphospholipid syndrome: marker not only of inflammation but also of the prothrombotic state. *Rheumatology international*, *31*(3), pp.307-313.

- **Tamaki, H. and Khasnis, A.,** 2015. Venous thromboembolism in systemic autoimmune diseases: A narrative review with emphasis on primary systemic vasculitides. *Vascular Medicine*, *20*(4), pp.369-376.

- **Tektonidou, M.G., Laskari, K., Panagiotakos, D.B. and Moutsopoulos, H.M.,** 2009. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. *Arthritis Care & Research*, *61*(1), pp.29-36.

- **Thornton, P. and Douglas, J.,** 2010. Coagulation in pregnancy. *Best practice & research Clinical obstetrics & gynaecology*, *24*(3), pp.339-352.

- **Tsokos GC.** 2011, Systemic lupus erythematosus. N Engl J Med 365(22):2110–2121. doi: 10.1056/NEJMra1100359**.**

- **Tzoran, I., Saharov, G., Brenner, B., Delsart, D., Román, P., Visoná, A., Jiménez, D., Monreal, M. and Riete Investigators,** 2012. Silent pulmonary embolism in patients with proximal deep vein thrombosis in the lower limbs. *Journal of Thrombosis and Haemostasis*, *10*(4), pp.564-571.

- **Urowitz, M.B., Gladman, D.D., Ibanez, D., Fortin, P.R., Bae, S.C., Gordon, C., Clarke, A., Bernatsky, S., Hanly, J.G., Isenberg, D. and Rahman, A.,** 2012. Evolution of disease burden over five years in a multicenter inception systemic lupus erythematosus cohort. *Arthritis care & research*, *64*(1), pp.132-137.

- **Wang, X., Zhang, G., Jiang, X., Zhu, H., Lu, Z. and Xu, L**., 2014. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: a meta-analysis of observational studies. *Atherosclerosis*, *234*(1), pp.206-213.

- **Wu, H., Birmingham, D.J., Rovin, B., Hackshaw, K.V., Haddad, N., Haden, D., Yu, C.Y. and Hebert, L.A.,** 2008. D-dimer level and the risk for thrombosis in systemic lupus erythematosus. *Clinical Journal of the American Society of Nephrology*, *3*(6), pp.1628-1636.

- **Yildirim-Toruner, C. and Diamond, B.,** 2011. Current and novel therapeutics in the treatment of systemic lupus erythematosus. *Journal of Allergy and Clinical Immunology*, *127*(2), pp.303-312**.**

- **Yoon, K.H.,** 2002. Sufficient evidence to consider hydroxychloroquine as an adjunct therapy in antiphospholipid antibody (Hughes') syndome. *The Journal of rheumatology*, *29*(7), pp.1574-1575.

- **Yusuf, H.R., Hooper, W.C., Grosse, S.D., Parker, C.S., Boulet, S.L. and Ortel, T.L.,** 2015. Risk of venous thromboembolism occurrence among adults with selected autoimmune diseases: a study among a US cohort of commercial insurance enrollees. *Thrombosis research*, *135*(1), pp.50-57.

- **Zandman-Goddard, G., Solomon, M., Rosman, Z., Peeva, E. and Shoenfeld, Y.,** 2012. Environment and lupus-related diseases. *Lupus*, *21*(3), pp.241-250.

- **Zöller, B., Li, X., Sundquist, J. and Sundquist, K.,** 2012. Autoimmune diseases and venous thromboembolism: a review of the literature. *American journal of cardiovascular disease*, *2*(3), p.171- 183.

- **Zöller, B., Li, X., Sundquist, J. and Sundquist, K.,** 2012. Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. *The Lancet*, *379*(9812), pp.244-249.

Appendices

Sudan University of sciences and technology

Collage of higher education

Collage of medical laboratory sciences

Haematology department

D-dimer and Haematological Parameters among Women with Systemic Lupus Erythematosus

Questioner 1

Name: ……...

Age……

Gender: ……

Are you diagnosis with SLE? When?

…… ( ) Yes ( ) No. …….

What kind of treatment you use? .....

Can you tell that you're diagnosis Serologic tests was positive? ..... ( ) Yes ( ) No

Have you record History of thrombosis?

…… ( ) Yes ( ) No

Exclusions character Questioner 2

Are you pregnant? …… ( ) Yes ( ) No

Have you suffer from hypertension? ……. ( ) Yes ( ) No

Are you smoking? **.....** …… ( ) Yes ( ) No

Are you diagnosis with diabetes? ……**.** ( ) Yes ( ) No

Are you suffering from chronic kidney disease? ……..… ( ) Yes ( ) No

Have you diagnosed with cancer? …… ( ) Yes ( ) No

Are you diagnosed with asthma? …….. ( ) Yes ( ) No

Have you been under surgery before? …… ( ) Yes ( ) No

Are you suffer from chronic disease other than SLE? …… ( ) yes ( ) No

Are you suffer from other inflammatory disease other than SLE? () Yes ( ) No

Are you under any anticoagulant therapy? …… ( ) Yes ( ) No

After understanding the contents of this questionnaire and the aim of research I agree …… ……. To collect the sample.

The researcher admitted not to use the blood sample in any other porpoises.

Signature: …….**Date**……..

Laboratory investigation

D-Dimer Result: ……

Haematological parameters

test	Result	Ref. value	unit
TWBCs		4.0-11.0	X 10^3/ul
RBCs		F:3.8-4.8	Million/ul
Hb		F:12-15	g/dl
HCT		F:36-46	%
MCV		78-99	fl
MCH		27-32	pg
MCHC		31-37	g/dl
Platelets		150-450	X10^3/ul
Neutrophils		1.6-6.8	X10^3/ul
Lymphocytes		1.2-4.9	X10^3/ul