

بسم الله الرحمن الرحيم



Sudan University of Science and Technology
College of Graduate Studies

**Evaluation of Complete Blood Count in Sudanese
Patients with Systemic Lupus Erythromatosus (SLE)
in Khartoum State**

**تقويم تعداد الدم الكامل لدي المرضى السودانيين المصابين
بالذئبة الحمراء بولاية الخرطوم**

Athesis is submitted for partial fulfillment for M.Sc.degree
in Hematology and Immunhematology

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صدق الله العظيم

سورة البقرة الآية ١

Dedication

To those who gave me every part of
their lives

(My father and mother)

To the hands that helped and
supported me

(My brothers and sisters)

To my teachers and friends

To all SLE Patients in the world who
are suffering every single day

To the coming generation that I
hope will benefit from this work

I dedicate this work

Acknowledgment

My gratefull thanks firstly to **Alla** for guidance through life, blessings upon me, and for making this work possible.

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Abstract

This is a case control study aimed to evaluate complete blood count in systemic lupus erythomatosus(SLE) , conducted during the period of April to November 2014 at Ommdurman Military Hospital included 50 female patients already diagnosed with SLE with age ranged between 18-62 years, and 50 apparently healthy females as control with matched age range were selected for this study .A questionnaire was designed to obtain basic and clinical data of patients after explaining of objectives for them, 2.5 ml of venous blood was collected from each participant ,for each sample of blood CBC parameters were measured using Sysmex (XT 2000 i) automated analyzer, data were analyzed using the SPSS computer programme.

A significant decrease was observed in SLE patient's Hb($11.2 \text{ g/dl} \pm 1.9$) VS control ($12.7 \text{ g/dl} \pm 1.1$), RBCs count ($4.2 \times 10^6/\mu\text{l} \pm 0.6$) VS ($4.6 \times 10^6/\mu\text{l} \pm 0.5$) , PCV ($34.2\% \pm 9.1$) VS ($38.5\% \pm 3.8$) , MCH ($26.8/\text{fl} \pm 2.9$) VS ($27.9/\text{fl} \pm 2.2$) , Neutrophils ($4.1 \times 10^3/\mu\text{l} \pm 2.1$) VS ($3.0 \times 10^3/\mu\text{l} \pm 1.1$) , Eosinophils ($0.1 \times 10^3/\mu\text{l} \pm 0.1$) VS ($0.2 \times 10^3/\mu\text{l} \pm 0.1$) and Basophils ($0.0 \times 10^3/\mu\text{l} \pm 0.0$) VS ($0.0 \times 10^3/\mu\text{l} \pm 0.0$) with P.value less than 0.05 .No

difference in MCV, MCHC ,Platelets count, WBC,s count,lymphocytes and monocytes differential count in SLE patients compared to control group with P.value > 0.05.

There was no relationship was observed between CBC parameters with duration of disease and age ,P.value>0.3.

There was relationship observed between the duration of disease with history of blood transfusion and complication P.value0.023.

No correlation between WBC,s count,Platelets count,Hemoglobin concentration and duration of disease among SLE(P = 0.66 , .11, .35, R = .062, -.226, -.134 respectively).

This study concluded that there was significant decrease in Hb, RBCs count, PCV, MCH , Neutrophils , Eosinophils and Basophils differential count between Systemic Lupus Erythromatosus patients and control group.

مستخلص البحث

هذه دراسة تحليلية أجريت في الفترة من ابريل إلى نوفمبر 2014 في مستشفى السلاح الطبي أمدرمان, تهدف الي تقييم تعداد الدم الكامل لدى مرضي الذئبة الحمراء شملت الدراسة 50 امرأة مريضة مشخصة مسبقا بمرض الذئبة الحمراء تتراوح أعمارهن ما بين 18- 62 سنة و 50 امرأة صحيحة ظاهريا كعينات ضابطة في نفس المدى العمري, إستخدمت إستبانة لجمع البيانات بعد اطلاع المشاركين علي أهداف الدراسة, تم جمع 2.5 مل من الدم الوريدي من كل مشارك في حاوية مفرغة من الهواء تحتوي علي مانع تجلط .

لكل عينة دم تم إجراء تحليل قياس الدم الكامل بإستخدام جهاز sysmex الذي يعمل أتوماتيكيا لتعداد الخلايا الدموية ومعاملاتها. تم تحليل البيانات بواسطة برنامج الحزم الإحصائية للعلوم الإجتماعية.

أظهرت الدراسة ان هنالك إنخفاض ذو دلالة إحصائية في معدل الهيموكلوبين(خضاب الدم) $(11.2 \pm 1.9 \text{ g/dl})$, تعداد كريات الدم الحمراء $(0.6 \pm 0.6 \times 10^6/\mu\text{l})$ متوسط حجم الخلايا الحمراء $(34.2\% \pm)$

9.1), متوسط هيموكلوبين الخلايا الحمراء ($f\pm 2.9/26.8$) لدى مرضى الذئبة الحمراء عند مقارنتهم مع العينات الضابطة (0 ± 12.7 , 1.1 ± 1.1 g/dl $\pm 0.0, 0.2 \pm 0.1$, , $3.0 \times 10^6/\mu\pm 1.1$, $27.9/f\pm 2.2$ 38.5 ± 3.8 , 4.6 ± 0.5 , ولا يوجد فرق ذو دلالة إحصائية في ,تعداد الصفائح الدموية وتعداد خلايا الدم البيضاء ما بين المجموعتين.

خلصت الدراسة الي ان هنالك إنخفاض في معدل الهيموكلوبين ,تعداد كريات الدم الحمراء متوسط حجم الخلايا الحمراء,متوسط هيموكلوبين الخلية الحمراء لدى مرضى الذئبة الحمراء عند مقارنتهم مع العينات الضابطة ولا يوجد فرق في ,تعداد الصفائح الدموية وتعداد خلايا الدم البيضاء ما بين المجموعتين,وانه لا يوجد تأثير في نتائج تعداد الدم الكامل وفقا للمدة الزمنية لاستمرار المرض او مضاعفاتة, ولا يوجد علاقة مصاحبة ما بين تعداد كريات الدم البيضاء ,الصفائح الدموية وتركيز الهيموكلوبين وبين المدة الزمنية لاستمرارية المرض.

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Abbreviations

ACD	Anemia of chronic disease
AIHA	Autoimmune hemolytic anemia
ALA	AminoLaevulinic Acid
ANA	Anti nuclear antibodies
APA	Antiphospholipid antibodies
APLS	<u>Antiphospholipid syndrome</u>
BFU-E	Burst-forming unit-erythroid
CBC	Complete blood count
CD34	Cluster of Diffrentiation
CFU-E	Colony forming unit erythroid
CRP	c-Reactive Protein
ds-DNA	douple strand deoxyribonucleic acid
ESR	Erythrocyte Sedimentation Rate
G-CSF	Granulocyte colony -stimulating factor
G-CSF	Granulocyte colony-stimulatingfactor (G-CSF),

GM-CSF	Granulocyte macrophage colony –stimulating factor .
GM-CSF	Granulocyte/macrophage colony-stimulating factor
Hb	Hemoglobin
HbA	Hemoglobin A (Adult Hemoglobin)
HbF	Hemoglobin F(Fetal Hemoglobin)
Hct	Hematocrit
HIV	Human Immune deficiency Virus
IDA	Iron Deficiency anemia
IL	Interleukin
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
M-CSF	Monocytic colony –stimulating factor
MCV	Mean cell volume
MDS	Myeloproliferative disease
PCV	Packed cell volume
PLT	Platelets
RBCs	Red blood cells
RNA	Ribonucleic acid
SLE	Systemic lupus erythematosus
TNF	Tumour necrosis factor
WBC,s	White Blood Cells

Chapter One

Introduction and Literature Review

1.1 Introduction

Hematopoiesis is the process of the development of blood cell lineages throughout life. Hematopoiesis is necessary to replenish dying cells with new blood cells. The key role of hematopoietic cells in maintaining hematopoietic homeostasis, host immunity and tissue oxygenation requires that they are highly regulated. The hematopoietic system includes the elements of the blood, marrow, lymph nodes, endothelial cells, thymus gland and spleen that are involved in the production of all blood lineages. This system further includes cytokine - producing cells and stromal elements of the bone marrow and spleen. Blood cells include red blood cells (erythrocytes, RBCs), white blood cells (leukocytes) and platelets (Schmaier and Lazarus., 2012).

Systemic Lupus Erythematosus is one of the autoimmune diseases in which autoantibodies are against nuclear antigens, including DNA, RNA and nucleic acid binding proteins. The resulting immune complex deposition is widespread throughout the vascular system, giving rise to a 'non-organ-specific' pattern of disease (Playfair and Chain ., 2001).

Hematological abnormalities are the commonest among most manifestations in SLE such as Anemia, leucopenias and thrombocytopenia. They commonly result from an immune mediated bone marrow failure, excessive peripheral cells destruction or certain drugs and infections (Bashal.,2013).

The frequency of Systemic Lupus Erythromatosus disease is rising in recent years with many complications including Hematolodical disease, prevalence was 50-100 cases per 100,000 (D'Cruzeta/ .,2007).

The outcome of this study may help to know the degree of haematological disorders associated with this disease.

1.2 Literature Review

1.2.1 Hematopoiesis:

Hematopoiesis is the process of making blood cells. For the average adult, the bone marrow produces $\sim 5 \times 10^{11}$ cells per day. Production of blood cells is highly regulated and balanced. Hematopoiesis begins in the yolk sac during the first month of embryogenesis but gradually shifts to the liver and, to a lesser extent, the spleen. The liver is the primary site of hematopoiesis during the second trimester, however the bone marrow becomes the primary site of hematopoiesis after the seventh month. After birth, the bone marrow is normally the sole site of hematopoiesis (intramedullary hematopoiesis). Hematopoiesis may resume in the liver and spleen after birth in conditions associated with fibrosis of

the bone marrow (extramedullary hematopoiesis). During infancy and childhood, there is active hematopoiesis in the medullary cavity of virtually every bone. With age, the hematopoietically active marrow (red marrow) is gradually replaced by inactive marrow (yellow marrow), which consists predominantly of adipose tissue. In adults hematopoiesis is restricted to the proximal long bones and the axial skeleton (skull, vertebral bodies, ribs, sternum, and pelvis). The yellow marrow can resume active hematopoiesis under conditions of chronic hematologic stress (chronic bleeding or hemolytic anemia). The medullary cavities contain vascular spaces (sinuses), hematopoietic cells, and specialized stromal cells of various types. All the cells form a complex microenvironment, with numerous intricate and interdependent relationships between stromal cells and hematopoietic cells. The hematopoietic cords (parenchyma) are the extravascular portions of the bone marrow and the site of blood cell production. The sinuses (vascular spaces) of the marrow are lined with specialized endothelial cells, which prevent the premature escape of immature cells into the peripheral blood. The basal lamina is incomplete, allowing mature cells to pass through the wall of the sinuses. The stromal cells compose the supportive tissues of the bone marrow, some of these cells produce hematopoietic growth factors (Kern, 2002).

1.2.1.1 Stem cell model of hematopoiesis

All blood cells are derived from pluripotent hematopoietic stem cells. The progeny of these cells are capable of giving rise to all

the different lines of mature blood cells: erythrocytes, granulocytes, monocytes, and megakaryocytes (platelets). The pluripotent stem cells are capable of self-renewal. They are rare in the bone marrow (~1 per 1,000 to 2,000 marrow cells) and cannot be recognized morphologically. Expression of CD34, a marker of immature cells, is used as a marker for hematopoietic stem cells. However, CD34 is not specific for stem cells, and only a minority of CD34+ cells (1%) are actually pluripotent stem cells. Low numbers of hematopoietic stem cells can be found circulating in the peripheral blood (peripheral blood progenitor cells) (Kern, 2002).

The haemopoietic growth factors and cytokines are the soluble regulators of blood cell production and are produced by several cell types in different sites in the body. They are glycoproteins with little primary amino acid homology (Hoffbrand *et al.*, 2005).

Differentiation of hematopoietic cells in the bone marrow is regulated by the extracellular matrix and microenvironment provided by stromal cells.

These cells, including macrophages, fibroblasts in various stages of differentiation,

endothelial cells, fat cells, and reticulum cells, nurture hematopoietic stem cells and progenitor cells by producing growth factors like granulocyte/macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, or stem cell factor. Other cytokines secreted by stromal cells regulate the adhesion molecules present on

hematopoietic cells, allowing them to remain in the bone marrow or migrate to an area where their respective cell type is needed (Munker *et al.* , 2007)

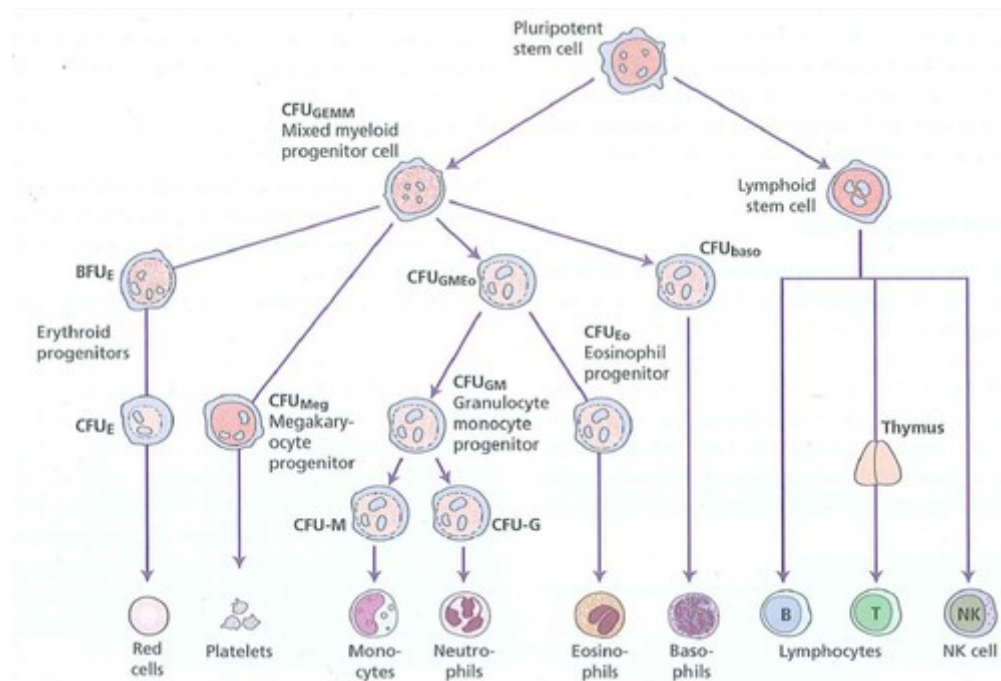


Figure (1.1) Haemopoiesis development pathways
(Hoffbrand *et al.* , 2008)

1.2.2 Erythrocyte Production (Erythropoiesis)

The erythron is the sum of all erythroid cells, including circulating red blood cells (RBCs) and marrow erythroid precursors. The earliest progenitor committed exclusively to erythroid lineage is the burst-forming unit-erythroid (BFU-E), this is followed by the colony forming unit erythroid (CFU-E). The earliest recognizable RBC precursor is the proerythroblast, which is characterized by fine nuclear chromatin and intensely blue cytoplasm. The last

nucleated RBC precursor is the orthochromatophilic erythroblast, which is characterized by well hemoglobinized cytoplasm; the nucleus is then lost, producing the reticulocyte. Reticulocytes are identified using supravital stains such as new methylene blue. Reticulocytes contain ribonucleic acid (RNA) for 4 days normally, the first 3 days are spent in the marrow and the fourth in the blood. However, under intense stimulation by erythropoietin, reticulocytes may be released into the blood early where they may contain RNA for 2.0 to 2.5 days (shift reticulocytes) (Kern, 2002).

The normal adult reticulocyte count is $25-125 \times 10^9/L$. Erythropoiesis is regulated by erythropoietin hormone which is a heavily glycosylated polypeptide of 165 amino acids. Normally 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver. Erythropoietin production increases in anemia. The normal adult red blood cell count is $4.5-6.5 \times 10^{12}/L$ (Male) and $3.9-5.6 \times 10^{12}/L$ (Female) (Hoffbrand *et al.*, 2006).

1.2.2.1. Red cell indices

The three basic red blood cell parameters which can be measured are: The concentration of hemoglobin per unit volume of blood after lysis of the red cells (hemoglobin concentration) determined spectrophotometrically after conversion to cyanmethemoglobin. The number of red blood cells per unit volume of blood (red cell count). The red cell count is determined using electrical impedance or light-scattering techniques. The hematocrit: Prior to automation, blood was centrifuged in tubes of

standard specification under a fixed centrifugal force for a fixed time to determine the packed cell volume (PCV). The hematocrit and PCV are not directly comparable as the value obtained for the PCV includes the volume of some plasma trapped between the red cells. From the values obtained for the hemoglobin concentration, red cell count and hematocrit, it is possible to calculate the mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Some automated blood-counting machines determine the MCV using electrical impedance or light scattering techniques and calculate the hematocrit from the measured MCV and red cell count. Others determine the hematocrit directly by summing all the pulses in the red cell channel. Normal range of PCV in adult male 0.40–0.51 and adult female 0.36–0.4, for MCV for both adult male and female 82–99(fl), MCH for both adult male and female 27–32.8(pg), and for MCHC for adult male and female 32–34(g/dl) (Porwit *et al.*, 2011).

1.2.2.2 Haemoglobin synthesis:

The main function of red cells is to carry oxygen (O₂) to the tissue and return carbon dioxide (CO₂) from the tissue to the lung, in order to achieve this gaseous exchange they contain the specialized protein haemoglobin. Each red cell contains approximately 640 million haemoglobin molecules. Each molecule of normal adult haemoglobin HbA the dominant haemoglobin in blood after the age of 3-6 months which consists of four polypeptide chains ($\alpha_2\beta_2$) each with its own haem group. Normal

adult blood also contain small quantities of two other haemoglobin: HbF and HbA₂. These also contain α chains , but with γ and δ chains respectively instead of β . The major switch from fetal haemoglobin occurs 3-6 months after birth. Haem synthesis occurs largely in the mitochondria by a series of glycine and succinyl coenzyme A under the action of the key rate limiting enzyme δ -aminolaevulinic acid (ALA) synthase. Pyridoxal phosphate (vitamin B₆) is a coenzyme for this reaction which is stimulated by erythropoietin . ultimately protoporphyrin combines with iron in the ferrous Fe²⁺ state to form haem , each molecule of which combines with a globin chain made on the polyribosomes .A tetramer of four globin chains each with its own haem group in a pocket is then formed to make up a haemoglobin molecule(Hoffbrad *et al* .,2006).

Polycythaemia: An elevation in packed cell volume (PCV), rather than a raised haemoglobin concentration, defines polycythaemia. A raised packed cell volume (0.51 in males, 0.48 in females) (Provan,2003).

The World Health Organization (WHO) has defined anemia in adults as a hemoglobin of <13 g/dL males (a hematocrit [Hct] of about 39) and <12 g/dL in females (Hct about 36) (Tkachuk and Hirschmam,2007).

1.2.3 Leucopoiesis

The white blood cells (leucocytes) are divided into phagocytes immunocytes, and granulocytes which include three types of

cels| neutrophils(polymorphs), eosinophils and basophils-together with monocytes comprise the phagocytes, the immunocytes include lymphocytes(Hoffbrand *et al.*,2006).

Neutrophils, eosinophils, and basophils go through similar and parallel maturation processes. The earliest two stages of the three pathways are not distinctive (myeloblast and promyelocyte); the appearance of specific (secondary) granules at the myelocyte stage differentiates the three cell types(Kern,2002).

The blood granulocytes and monocytes are formed in the bone marrow from common precursor cell in the granulopoietic series progenitor cells ,myeloblasts, promyelocytes, and myelocytes from mitotic pool while the metamyelocyte, band and segmented granulocytes make up a post-mitotic maturation compartment, following their release from bone marrow, granulocytes spend only 6-10 hours in the circulation before moving to tissues where they perform phagocytic function. In the blood-stream there are two pools(circulating pools and marginating pools) usually of about equal size. Monocytes circulate for 20-40 hours then transform in the tissues to macrophages with lifespan several months or even years. Many growth factors are involved in this maturation process including interleukin (IL1,3,5,6,11),granulocyte macrophage colony -stimulating factor (GM-CSF), granulocyte CSF(G-CSF) and monocyte CSF (M-CSF) and tumor necrosis factor (TNF), the maturation of granulocytes was completed by the appearance of secondary granules(specific) and lobulation of nucleus .Lymphocytes are the immunological competent cells that

assist the phagocytes in the defense of the body against infection and other body invasion they include T and B lymphocytes and Natural Killer cells (Hoffbrand *et al.*, 2006).

Lymphocytes maturation begin in the bone marrow, B cells complete initial development in the marrow and then circulate to peripheral lymphoid tissues (lymph node, spleen, and mucosal surfaces) to wait antigen exposure and final maturation into plasma cells. T-cell maturation also begins in the bone marrow, T-cell precursors then travel to the thymus (initially the cortex of the thymus, progressing down into the medulla of the thymus), where they complete maturation before being released into the blood to travel to tissues. Differentiation into T helper and T suppressor subsets occurs in the thymus (Kern, 2002).

1.2.3.1 White blood cell abnormalities:

Neutrophilia is defined as an absolute neutrophil count $>7.5 \times 10^9/L$ occur due to Infection (bacterial, viral, fungal, spirochaetal, rickettsial), Inflammation (trauma, infarction, vasculitis, rheumatoid disease, burns). Chemicals e.g. drugs. Hormones., Physical agents e.g. cold, heat, burns, labour surgery, anaesthesia. Haematological e.g. myeloproliferative disease, CML, chronic neutrophilic leukaemia (Provan *et al.*, 2003).

Neutrophilia defined as absolute peripheral blood neutrophil count of $<2.0 \times 10^9/L$. It can be congenital or acquired due to viral infection, e.g. Influenza, HIV, hepatitis, overwhelming bacterial sepsis, drugs anticonvulsants (e.g. Phenytoin), Immune mediated

Autoimmune (antineutrophil antibodies) and SLE(Provan *etal.*,2003).

Lymphocytosis is said to occur when the absolute count of lymphocytes in the blood exceeds the upper limit of normal of $4 \times 10^9/\text{fl}$ (Firkin *etal.*,1998).

Lymphopenia (peripheral blood lymphocytes $<1.5 \times 10^9/\text{L}$) can be due to malignant disease e.g. Hodgkin's disease, non-haematopoietic cancers, angioimmunoblastic lymphadenopathy., collagen vascular disease e.g. rheumatoid, SLE, Infections e.g. HIV, chemotherapy and surgery (Provan *et al.*.,2004).

Eosinophilia when the eosinophil count in the peripheral blood of normal subjects ranges from 0.04 to $0.4 \times 10^9/\text{fl}$ (Firkin *etal.*,1998).

Commonly due to Drugs (huge list e.g. gold, sulphonamides, penicillin), erythema multiforme(Stevens-Johnson syndrome), and Parasitic infections: hookworm, Ascaris, tapeworms, filariasis, amoebiasis, schistosomiasis and Allergic syndromes—asthma, eczema, urticaria(Provan *et al.* ;2003).

Monocytosis other than in neoplastic involvement of the myeloid series is usually associated with only moderate elevation of the absolute count of monocytes in the blood beyond the upper limit of normal(Firkin *etal.*,1998).

Monocytosis occur due to Malaria, trypanosomiasis, typhoid (commonest world-wide causes), Post-chemotherapy or stem cell transplant, Tuberculosis and Myelodysplasia (MDS) (Provan, *etal.*.,2003).

Monocytopenia (peripheral blood monocytes $<0.2 \times 10^9/L$) caused by autoimmune disorders e.g. SLE., Hairy cell leukaemia, and drugs e.g. glucocorticoids, chemotherapy (Provan *et al.*, 2003).

Table(1.1):Reference range for the leukocytes in peripheral venous blood of healthy adults(Porwit *et al.*, 2011).

Type of WBC,s	Count $\times 10^9/l$
Leukocytes	3.6–10.2
Neutrophils	1.3–7.4
Lymphocytes	1.45–3.75
Monocytes	0.21–1.05
Eosinophils	0.3–0.72
Basophils	0–0.16

1.2.3 Platelet Production(megakaryopoiesis)

The mature megakaryocyte progenitor proceeds down a regimented pathway, forming promegakaryoblasts, which generate megakaryoblasts, and in turn produce megakaryocytes. Megakaryocytes resulting in production of progressively larger cells with complex nuclei platelets are generated by fragmentation of the mature megakaryocytes cytoplasmic pseudopodial projections, called proplatelets. The sliding of microtubules over

one another drives the elongation of proplatelet processes and organelle transportation (into the proplatelets) in a process that consumes the megakaryocyte and results in production of 2000--3000 platelets from each mature megakaryocyte (Saba and Mufti., 2011).

1.2.3.1 platelete Disorder

Thrombocytosis: occur when platelet count exceeds the upper limit of normal range it can be primary thrombocytosis or secondary. Congenital abnormalities of platelets can be divided into disorders according to platelets production and those of platelet function. Normal platelet count for all age groups is $150-450 \times 10^9/l$ (Provan, 2003).

1.2.4 Systemic Lupus Erythematosus(SLE)

Systemic lupus erythematosus (SLE) is a generalized autoimmune disorder associated with multiple cellular and humoral immune abnormalities and protean clinical manifestations. It is most common in females of child-bearing age (Virella., 2001).

In systemic lupus erythematosus the autoantibodies are against nuclear antigens, including DNA, RNA and nucleic acid binding proteins. The resulting immune complex deposition is widespread throughout the vascular system, giving rise to a 'non-organ-specific' pattern of disease. Like the 'organ specific' diseases, non-organ specific diseases tend to occur together (Playfair and Chain., 2001).

1.2.4.1 Clinical manifestations

The clinical expression of SLE varies among different patients. The kind of organ (vital and Nonvital) that becomes involved determines the seriousness and the overall prognosis of the disease (Virella .,2001) .

Table(1.2) Main clinical manifestation of Systemic Lupus Erythematosus (SLE)(Virella ,2001).

Manifestation	% of patients
Musculoarticular	95
Renal disease	60
Pulmonary disease (pleurisy, pneumonitis)	60
Cutaneous disease (photosensitivity, alopecia, etc.)	80
Cardiac disease (pericarditis, endocarditis)	20
Fever of unknown origin	80
Gastrointestinal disease (hepatomegaly, ascites, etc.)	45
Hematological/Reticuloendothelial (anemia, leukopenia, splenomegaly)	85
Neuropsychiatric (organic brain syndrome, seizures, peripheral neuropathy, etc.)	20

Figure (1.2) symptoms and complications of Systemic Lupus Erythromatosus (Ruiz and Ramos.,2010)

1.2.4.2 Pathogenesis of Systemic Lupus Erythromatosus:

Multiple environmental, hormonal, genetic, and immunoregulatory factors are involved in the expression of the disease. In any given patient, different factors contribute variably to the expression of the disease (Virella.,2001) .

1.2.4.2.1 Genetic Factors

Several studies indicated that genetic factors play a role in the pathogenesis of human SLE. Serum DNA and T-cell antibodies as well as cellular abnormalities are present in healthy relatives of lupus patients. There is a moderate degree of clinical disease concordance among monozygotic twins. The fact that the clinical concordance between twins is only moderate strongly indicates that genetic factors alone may not lead to the expression

of the disease and that other factors are needed. The genes that could play a role, probably in synergy with environmental factors, have not been identified. Current evidence indicates that in humans, as in mice, these genes are probably linked to the Molecule Human Complex (MHC). For example, the HLA-DR2 haplotype is overrepresented in patients with SLE. Also an SLE-like disease develops frequently in individuals with C4 and C2 deficiencies (C4 and C2 genes are located in chromosome 6, in close proximity to the MHC genes). Also, individuals lacking C1q are also prone to developing lupus (Virella., 2001).

1.2.4.2.2 Immune response abnormalities

SLE is a disease associated with profound immunoregulatory abnormalities, affecting both humoral and cell-mediated responses. Increased numbers of B cells and plasma cells are detected in the bone marrow and peripherallymphoid tissues secreting immunoglobulins spontaneously. The number of these cells correlates with the disease activity. Only a limited number of light and heavy-chain genes are used by autoantibodies, demonstrating that the autoantibody response involves only a few of all B-cell clones available. Furthermore, the changes appearing in their sequence over time strongly suggest that they undergo affinity maturation, a process that requires T cell help. It also suggests that a few antigens drive the response. Any infection that induces B-cell activation is likely to cause a clinical relapse in patients with inactive SLE (Virella., 2001).

1.2.4.2.3 Hormonal Effects

The extent of the hormonal involvement in human SLE cannot be proven directly, but the large female predominance (9:1 female-to-male ratio) as well as the influence of puberty and pregnancies at the onset of the disease, or the severity of the disease's manifestations, indicates that sex hormones play a role in the modulation of the disease. Investigations suggest that estradiol causes an increased expression of calcineurin, which could lead to increased synthesis of pro-inflammatory cytokines, this could lead to an exaggeration of humoral immunity abnormalities during pregnancy, which in turn could exacerbate the inflammatory processes secondary to immune complex (IC) formation (Virella, 2001).

1.2.4.2.4. Environmental Factors

- Sunlight exposure: was the first environmental factor influencing the clinical evolution of human Systemic lupus erythematosus (SLE) to be identified. This could be related to the fact that the Langerhans cells of the skin and keratinocytes release significant amounts of interleukin-1 upon exposure to UV light and could thus represent the initial stimulus tipping off a precarious balance of the immune system.
- Infections also seem to play a role. The normal immune response to bacterial and viral infections may spin off into a state of B-cell hyperactivity, triggering a relapse.
- Drugs, particularly those with DNA-binding ability, such as hydantoin, isoniazide, and hydralazine, can cause a drug-induced lupus-like syndrome. These drugs are known to cause DNA hypomethylation, because hypomethylated genes are transcribed

at higherrates, it is theoretically possible that they cause SLE by increasing the transcription rate ofgenes involved in the expression of the disease.Anti-nuclear antibodies (ANA) antibodies appear in 15–70% of patientstreated with any of these drugs for several weeks. Patients with drug-induced SLE usually have a milder disease,without significant vital organ involvement (Virella. , 2001)

1.2.4.3 Hematological abnormalities in Patients with Systemic Lupus Erythematosus:

Antibodies mediated distruction of peripheral blood cells may cause neutropenia,lymphopenia,thrombocytopenia or hemolytic anemia.The degree of leucopenia most commonly lymphopenia is often good guide to disease activity(Boonet *al* .,2006).

1.2.4.3.1 Anemia in SLE

It is a common hematological abnormality in SLE that is defined as hemoglobin levels of < 12g/dL for women and <13.5 g/dL for men. It is categorized into the anemia of chronic disease (ACD), which is the most common form (60%-80%), iron deficiency anemia (IDA), autoimmune hemolytic anemia (AIHA), and anemia due to chronic renal insufficiency. In a cohort comprising 132 anemic patients with SLE, ACD was found in 37.1% of the cases, IDA in 35%, AIHA in 14.4% and other causes of anemia in 12.9% of the patients .ACD results from suppressed erythropoiesis secondary to chronic inflammation (normocytic and normochromic, with a relatively low reticulocyte count, low to normal serum iron, adequate bone marrow iron stores and elevated serum ferritin level) . Low levels of erythropoietin due to chronic inflammation or renal insufficiency and presence of anti-erythropoietin autoantibodies which are associated with European Consensus

Lupus Activity Measurement (ECLAM) high score are found in some patients. IDA is defined by serum ferritin below 20 µg/dl, it is common and may be the result of menorrhagia or increased gastrointestinal blood loss because of long term use of corticosteroids. AIHA is characterized by elevated reticulocyte counts, low haptoglobin levels, increased indirect bilirubin concentration and a positive direct Coombs' test. It has been noted in up to 10% of patients with SLE. The presence of hemolytic anemia may associate with manifestations of severe disease such as renal disease, seizures and serositis. The presence of both immunoglobulin and complements on red blood cells is usually associated with some degree of hemolysis, while presence of complements alone (C3 and /or C4) is often not associated with hemolysis (Bashal, 2013).

1.2.4.3.2 Leucopenia and Thrombocytopenia in SLE

It is a typical feature of SLE which may occur as a result of lymphopenia, neutropenia or a combination of both. The prevalence of lymphopenia in SLE ranges from 20 to 81% and its degree may correlate with disease activity. Both T and B lymphocytes are reduced, while natural killer cells are typically increased. Reduced surface expression of complement regulatory proteins CD55 and CD59 has been found in leucopenic patients with SLE. Deficiency of these proteins may make these cells susceptible to complement-mediated lysis. There is increasing evidence that endogenous production of type 1 interferons is implicated in the pathogenesis of neutropenia and lymphopenia in SLE. Elevated serum levels of IFN-α in SLE correlate inversely with leucocyte numbers. It has a reported prevalence ranging from 7 to 30% in large series of patients with SLE. Increased peripheral

destruction of platelets and presence of anti-platelet antibodies, is the most likely pathogenic mechanism. Presence of antiphospholipid autoantibodies in some patients has a role. Antibodies against thrombopoietin, the thrombopoietin receptor c-Mpl and CD40L have been found in some thrombocytopenic patients with SLE (Bashal. ,2013).

1.2.4.4 Diagnosis of SLE:

1.2.4.4.1 Clinical diagnosis of Systemic Lupus Erythematosus

The American College of Rheumatology(1999) suggests that a person may be classified as having systemic lupus erythematosus (SLE) if 4 or more of the following 11 criteria are present (which do not have to occur at the same time but can be cumulative over a number of years.

Malar rash, Discoid lupus ,Photosensitivity ,Oral or nasopharyngeal ulcers ,Non-erosive arthritis involving 2 or more peripheral joints ,Pleuritis or pericarditis ,Renal involvement with persistent proteinuria or cellular casts ,Seizures or psychosis ,Hematological disorder:hemolytic anaemia or leukopenia or lymphopenia or thrombocytopenia ,Immunological disorder: anti-DNA antibody or anti-Sm or antiphospholipid antibodies ,A positive antinuclear antibody (American College of Rheumatology, 1999).

1.2.4.3 .2 Laboratory diagnosis :

A. Immunological Investigations:

- ANA: screening test with a sensitivity of 95% but not diagnostic in the absence of clinical features.
- Anti-dsDNA: high specificity but sensitivity only 70%. The level reflects disease activity. The value often varies with disease activity and sometimes guides changes in therapy. A rise in antibody titre may indicate that immunosuppression needs to be increased.
- Anti-Sm is the most specific antibody but sensitivity is only 30-40%.
- Antiphospholipid antibodies: anticardiolipin antibodies and lupus anticoagulant should be checked in lupus patients, as they are associated with [antiphospholipid syndrome](#) (APLS).
- Complement C3 and C4 levels are decreased and C3d (a degradation product) increased with increased disease activity([Bartels et al.,2011](#)).

B. Hematological Investigations

1) Complete Blood Count (CBC): Mild normochromic normocytic anemia is common. Anemia in patients with lupus may be drug-induced or due to chronic disease, but it is sometimes due to haemolytic anemia. In this case, Coombs' antibody, reticulocyte count and [haptoglobins](#) may need to be checked. Leukopenia and thrombocytopenia occur frequently but can also be due to [immunosuppressive therapy](#).

2) Erythrocyte Sedimentation Rate (ESR) is raised but c-Reactive Protein([CRP](#)) may be normal unless there is intercurrent infection or serositis([Bartels et al.,2011](#)).

1.2.4.5 Previous studies:

Study conducted by (Voulgarelis *et al.*,2000) found that hemoglobin levels < 12g/dL for women with SLE.

Study done by (Schur and Berliner, 2012) concludes that Leucopenia is a typical feature of SLE which may occur as a result of lymphopenia, neutropenia or a combination of both. The prevalence of lymphopenia in SLE ranges from 20 to 81% .

Wataru *et al.*,(2004)concluded thatNeutropenia is a common feature of SLE, with a prevalence rate of 47%, it may be mediated by anti-neutrophil antibodies degree that may correlate with disease activity.

Study conducted by (Hepburn *et al* .,2010) concluded that Thrombocytopenia has a high prevalence ranging from 7 to 30% in large series of patients with SLE.

There is no available prevalence in Sudan .

1.3 Rationale:

The frequency of Systemic Lupus Erythromatosus disease is increasingly rising in recent years with many complications including Hematological disease, prevalence was 50-100 cases per 100,000 in UK (D'Cruz *et al* .,2007).

The outcome of this study may help to know the degree of haematological disorders associated with this disease, for example anemia which has clinical significance especially among women .In addition it will be helpful to know other hematological

complications such as leukopenia and thrombocytopenia, and avoid effect of this complication.

1.4 Objectives

1.4.1 General Objective:

To evaluation of Complete blood count of Sudanese patients with Systemic Lupus Erythematosus in Khartoum state.

1.4.2 Specific Objective:

1. To measure Hb,PCV,RBCs count ,MCV,MCH,MCHC, WBC,s countand platelet count among Sudanese patients with SLE in Khartoum state,and compared with a control group .
2. To evaluate the relationship between complete Blood Count parameters with duration of disease and age .
3. To evaluate the relationship between duration of disease with history of blood transfusion and disease complication .
4. To detect correlation between duration of disease with WBC,s count , Platelets count and Hemoglobin concentration among SLE patient.

Chapter Two

Materials and Methods

2.1 Study design:

This is a descriptive case control study conducted during the period from April to November 2014 at Ommdurman Military

hospital .The aim of the study is to evaluate complete blood count of patients with systemic lupus erythomatosus(SLE).

2.2 Study population:

The study included 50 female patients already diagnosed with SLE who attended Ommdurman Military hospital during the period of April to November 2014 with age range 18-62 years, and 50 females apparently healthy with matched age as control.

2.2.1 Inclusion criteria: Patients already diagnosed with SLE by clinical symptoms and ANA profile test attended Ommdurman Military hospital during the period of April to November 2014.

2.2.2 Exclusion criteria:

Patients with any hematological disease that may affect the parameters under study such as thalathemia and Iron Deficiency anemia

2.3 Data Collection:

Adesigned questionnaire was use to collect basic and clinical data such as the age, gender, duration of the disease , complications of disease and history of blood transfusion.

2.4 Sample Collection:

Venous blood (2.5 ml) was collected from each participant into vaccotainer tube contain Ethylene Di-amine Tetra-acetic acid (EDTA) as anticoagulant usinig disposable needle and plastic holder after using of 70% alcohol as disinfectent Each sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation.

2.5 Methods :

2.5.1 Complete Blood Count (CBC) using Sysmex (XT 2000 i) heamatological analyzer :

For each sample of blood the following haematological parameters were measured using Sysmex (XT 2000 i) heamatological analyzer : Red blood cells (RBCs), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), meanAcorpuscular hemoglobin (MCH), platelets count and differential leucocytes count .

2.5.1.1 Principle of Sysmex (XT 2000 i) heamatological analyzer:

Sysmex (XT 2000 i) performs analysis based on the Electric Detecting Method(Hydro Dynamic focusing Method), Flow Cytometry Method using semiconductor laser and SLS-Hemoglobin method (see appendix 1).

2.5.1.2 Reagents:

- **Cell back (diluent).**

Active ingredients: sodium chloride- 0.64, Boric acid – 0.10%, Sodium Tetraborate-0.02% and EDTA-2k-0.02%.(Sysmex,2002).

- **Stromatolyser-4DL(diluent).**

Active ingredients: Non- ionic Surfactant-0.18%, and organic quaternary ammonium salt -0.08%.(Sysmex,2002).

Stromatolyser-FB(diluent).

Active ingredients: Non- ionic Surfactant-0.40%, and organic quaternary ammonium salt -0.01%.(Sysmex,2002).

Sulfolyser

Active ingredients: Sodium lauryl sulphate-0.17%.(Sysmex,2002).

Stromatolyser-4DS

Active ingredients: Polymethine dye-0.002%, Methanol- 3% and ethylene glycol-96.90%. (Sysmex, 2002).

Cellclean

Active ingredients: Sodium Hypochloride-5%. (Sysmex, 2002).

2.5.1.3 procedure:

2.5.1.3.1 RBC/PLT analysis procedure

Well mixed EDTA blood was aspirated from the sample probe into the sample rotar valve, then 4 µl of blood measured by the sample rotar valve, was diluted to a ratio of 1:500 with 1.996 ml of Cell pack, and then was sent to the sample chamber as the diluted sample, then sheath injector piston sends 11.7 µl of diluted sample slowly to the RBC/PLT detector, the RBC detector counts the RBC and PLT via the Hydro Dynamic focusing Method (DC Detection). At the same time the hematocrit (HCT) was calculated via the RBC pulse height detection method. (Sysmex, 2002).

2.5.1.3.2 Total WBC count:

Well mixed EDTA blood was aspirated from the sample probe into the sample rotar valve, 20 µl of blood measured by sample rotar valve, was diluted with 0.980 ml of Stromatolyser -FB to a ratio of 1:50 and then sent to the reaction chamber as diluted sample. After reacting for about 14 seconds in this condition, the red cells were hemolyzed. The sheath injector piston sends 40 µl of diluted sample to the optical detector block where the sample is analyzed via flow cytometry method utilizing a semiconductor laser. (Sysmex, 2002).

2.7.1.3.3 Hemoglobin analysis procedure

Well mixed EDTA blood was aspirated from the sample probe into the sample rotar value, 4.0 µl of blood measured by sample rotar value, was diluted to a ratio of 1:250 with 0.996 ml of cell pack and then sent to the flow cell. At the same time 0.5 ml of Sulfolyser was added to hemolyze the red blood cells to make 1:375 diluted sample, and the hemoglobin was converted into Sodium Lauryl Sulfate (SLS) hemoglobin. Light (of wavelength 555 nm) emitted from the light emitting diode passes through the lens and into the sample in hemoglobin cell. The concentration of Sodium Lauryl Sulfate (SLS) hemoglobin was measured as light absorbance, and is calculated by comparison with the absorbance of the diluent measured before the sample was added. (Sysmex, 2002).

2.5.1.3 .4 Differential analysis procedure

Differential analysis was used to identify and analyze: Lymphocytes, neutrophils, monocyte, eosinophils, and basophils. (Sysmex, 2002).

Well mixed EDTA blood was aspirated from the sample probe into the sample rotar value, 20 µl of blood measured by sample rotar value, was diluted with 0.980 ml of Stomatolyser -4DL and then sent to the reaction chamber as diluted sample. At the same time 40 µl of Stomatolyser+-4DS is added to dilute the sample to a ratio of 1:52. After reacting for about 22 seconds in this condition

,the red blood cells were hemolyzed and the white blood cells were stained . The sheath injector piston sends 40 µl of diluted sample to the optical detector block where the sample was analyzed via flow cytometry method utilizing a semiconductor laser. (Sysmex,2002).

2.6 Ethical consideration:

The research proposal was approved at Hematology Department Research Committee of Post Graduate Studies of Sudan University of Science and Technology.

Participants were informed about the research and its benefit and verbal informed consent was obtained before blood was collected

2.7 Data analysis:

The data were analyzed by using independent T test to calculate mean of both case and control, Correlation test and Chi square test using the SPSS computer programmed version 11.5, the significant level set at (≤ 0.05).

Chapter Three

Results

The results of present study showed that 46% of patients were less than 40 years table(3.1) . According to disease duration 68% of patients had disease duration less than 5 years table (3.2) . 66% of patients did not suffer from complications table (3.3) .34% of patients did not receive blood table (3.4) .

There was significant decrease in Hb ($11.2\text{g/dl} \pm 1.9$) Vs($12.7\text{g/dl} \pm 1.1$), RBCs count ($4.2 \times 10^6/\mu\text{l} \pm 0.6$)Vs($4.6 \times 10^6/\mu\text{l} \pm 0.5$) , PCV ($34.2\% \pm 9.1$)Vs($38.5\% \pm 3.8$) ,MCH ($26.8/\text{fl} \pm 2.9$)Vs($27.9/\text{fl} \pm 2.2$) , Neutrophils($4.1 \times 10^3/\mu\text{l} \pm 2.1$)Vs($3.0 \times 10^3/\mu\text{l} \pm 1.1$) , Eosinophils ($0.1 \times 10^3/\mu\text{l} \pm 0.1$)Vs($0.2 \times 10^3/\mu\text{l} \pm 0.1$) and Basophils($0.0 \times 10^3/\mu\text{l} \pm 0.0$) Vs(0.0 ± 0.0) with P.value less than 0.05. No difference in MCV ($80.8\text{fl} \pm 7.4$)Vs ($82.9\text{fl} \pm 7.2$) MCHC ($32.9\text{g/dl} \pm 1.9$)Vs ($33.3\text{g/dl} \pm 1.7$), Platelets count ($293.5 \times 10^3/\mu\text{l} \pm 123.5$)Vs($262.4 \times 10^3/\mu\text{l} \pm 72.7$) , WBC,s count($6.2 \times 10^3/\mu\text{l} \pm 2.4$)Vs ($4.6 \times 10^3/\mu\text{l} \pm 2.2$) ,lymphocytes ($2.0 \times 10^3/\mu\text{l} \pm$

1.3)Vs($2.4 \times 10^3/\mu\text{l} \pm 0.8$), and monocytes($0.4 \times 10^3/\mu\text{l} \pm 0.2$)Vs($0.4 \times 10^3/\mu\text{l} \pm 0.1$)(P.value more than 0.05, table(3.5).

Table (3.6) shows that there was no significant variation in CBC parameters between the subject and control group with regard to the disease duration according P.value>0.05.

As can be seen in table (3.7)no significant difference in CBC parameters between the subject and control group with regard to the age,P.value>0.05.

According to table (3.8) there was no association between the duration of disease and complication ,P.value 0.39.

Table (3.9) shows that there was association between the duration of disease and history of blood transfusion,P.value 0.023

As can be seen in figure (3.1),(3.2),(3.3) respectively no correlation between duration of disease with WBC,s, Platelets count and Hemoglobin concentration (P = 0.66 ,R = .062), (P =.11, R = -226), (P =0.35, R = -.134) .

Table (3.1) Distribution of population according to age

Age group		Frequency of case	Percent% of case	Frequency of control	Percent% of control
Less than 40		32	64	30	60
More than 40		18	36	20	40
Total		50	100	50	100

Table(3.2)Distributionof SLE patients according to disease duration

Duration of Disease	Frequency	Percent%
Less than 5years	34	68
more than 5years	16	32
Total	50	100

Table(3.3)Distributionof patient according to Complication of SLE.

Disease Complications	Frequency	Percent%
NO Complications	33	66
Lupus Nephritis	10	20
Other	7	14
Total	50	100

Others: Cerebral lupus , Antiphospholipid syndrome.

Table(3.4)Distribution of SLE patient according to History of Blood Transfusion.

History of Blood Transfusion	Frequency	Percent%
Yes	17	34
	51	

No	33	66
Total	50	100

Table (3.5) Complete blood count in cases and controls.

Parameter	Mean \pm SD		P. value
	Case(N=50)	Control (N=50)	
HB g/dl	11.2 \pm 1.9	12.7 \pm 1.1	0.000
WBC $\times 10^3/\mu\text{l}$	6.2 \pm 2.4	6.1 \pm 1.2	0.688
RBC $\times 10^6/\mu\text{l}$	4.2 \pm 0.6	4.6 \pm 0.5	0.000
Platelet $\times 10^3/\mu\text{l}$	293.5 \pm 123.5	262.4 \pm 72.7	0.128
PCV %	34.2 \pm 9.1	38.5 \pm 3.8	0.003
MCV / fl	80.8 \pm 7.4	82.9 \pm 7.2	0.153
MCH /pg	26.8 \pm 2.9	27.9 \pm 2.2	0.032
MCHC g/dl	32.9 \pm 1.9	33.3 \pm 1.7	0.200
Neutrophile $\times 10^3/\mu\text{l}$	4.1 \pm 2.1	3.0 \pm 1.1	0.002
Lymphocyte $\times 10^3/\mu\text{l}$	2.0 \pm 1.3	2.4 \pm 0.8	0.065
Monocyte $\times 10^3/\mu\text{l}$	0.4 \pm 0.2	0.4 \pm 0.1	0.529
Eosinophile $\times 10^3/\mu\text{l}$	0.1 \pm 0.1	0.2 \pm 0.1	0.005
Basophile $\times 10^3/\mu\text{l}$	0.0 \pm 0.0	0.0 \pm 0.0	0.003

*The mean difference is significant at ≤ 0.05 level.

Table (3.6) Complete blood count of SLE patients according to disease duration.

Parameter	Mean \pm SD		P. value
	Less than 5 years	More than 5 years	
HB g/dl	11.3 \pm 1.7	11.0 \pm 2.4	0.592
WBC $\times 10^3/\mu\text{l}$	6.1 \pm 1.9	6.5 \pm 3.1	0.605
RBC $\times 10^6/\mu\text{l}$	4.3 \pm 0.6	4.0 \pm 0.6	0.221
Platelet $\times 10^3/\mu\text{l}$	307.35 \pm 129.9	264.2 \pm 106.6	0.253
PCV %	34.7 \pm 10.2	33.1 \pm 6.4	0.575
MCV / fl	80.4 \pm 7.3	81.7 \pm 7.7	0.556
MCH/pg	26.7 \pm 2.4	27.1 \pm 3.8	0.590
MCHC g/dl	32.8 \pm 1.6	33.1 \pm 2.5	0.585
Neutrophile $\times 10^3/\mu\text{l}$	3.9 \pm 1.6	4.4 \pm 2.9	0.539
Lymphocyte $\times 10^3/\mu\text{l}$	2.1 \pm 1.5	1.7 \pm 1.0	0.321
Monocyte $\times 10^3/\mu\text{l}$	0.4 \pm 0.2	0.5 \pm 0.2	0.228
Eosinophile $\times 10^3/\mu\text{l}$	0.1 \pm 0.1	0.1 \pm 0.1	0.779
Basophile $\times 10^3/\mu\text{l}$	0.0 \pm 0.0	0.0 \pm 0.0	0.600

*The mean difference is significant at ≤ 0.05 level.

Table (3.7) Complete blood count of SLE patients according to age.

Parameter	Mean \pm SD		P. value
	Less than 40 years	More than 40 years	
HB g/dl	11.0 \pm 2.2	11.5 \pm 1.4	0.413
WBC $\times 10^3/\mu\text{l}$	6.3 \pm 2.5	5.9 \pm 2.2	0.577
RBC $\times 10^6/\mu\text{l}$	4.0 \pm 0.7	4.4 \pm 0.4	0.087
Platelets $\times 10^3/\mu\text{l}$	289.6 \pm 142.9	300.6 \pm 81.4	0.767
PCV %	33.7 \pm 10.9	35.1 \pm 4.7	0.591
MCV/fl	81.6 \pm 7.7	79.4 \pm 6.8	0.318
MCH/pg	27.0 \pm 3.2	26.4 \pm 2.1	0.409
MCHC g/dl	32.8 \pm 2.2	32.8 \pm 1.3	0.959
Neutrophile $\times 10^3/\mu\text{l}$	4.3 \pm 2.4	3.7 \pm 1.6	0.404
Lymphocyte	1.9 \pm 1.5	2.2 \pm 1.2	0.615

10 ³ /μl			
Monocyte			0.521
×10 ³ /μl	0.4± 0.2	0.4 ± 0.2	
Eosionphile×10 ³			0.511
/μl	0.1 ± 0.1	0.1 ± 1.0	
Basophile			0.366
×10 ³ /μl	0.0± 0.0	0.0 ± 0.0	

*The mean difference is significant at ≤ 0.05 level.

Table (3.8) Association between disease duration and complications of SLE .

Duration groups	Complications			P. value
	No	Lupus Nephritis	Others	
Less than 5 Years	24	5	5	0.393

More than 5 9 5 2

Years

*The mean difference is significant at ≤ 0.05 level.

Table (3.9) Association between the disease duration and history of blood transfusion

Duration groups	Blood transfusion		P. value
	Yes	No	
Less than 5 Years	8	26	0.023
More than 5 Years	9	7	

*The mean difference is significant at ≤ 0.05 level.

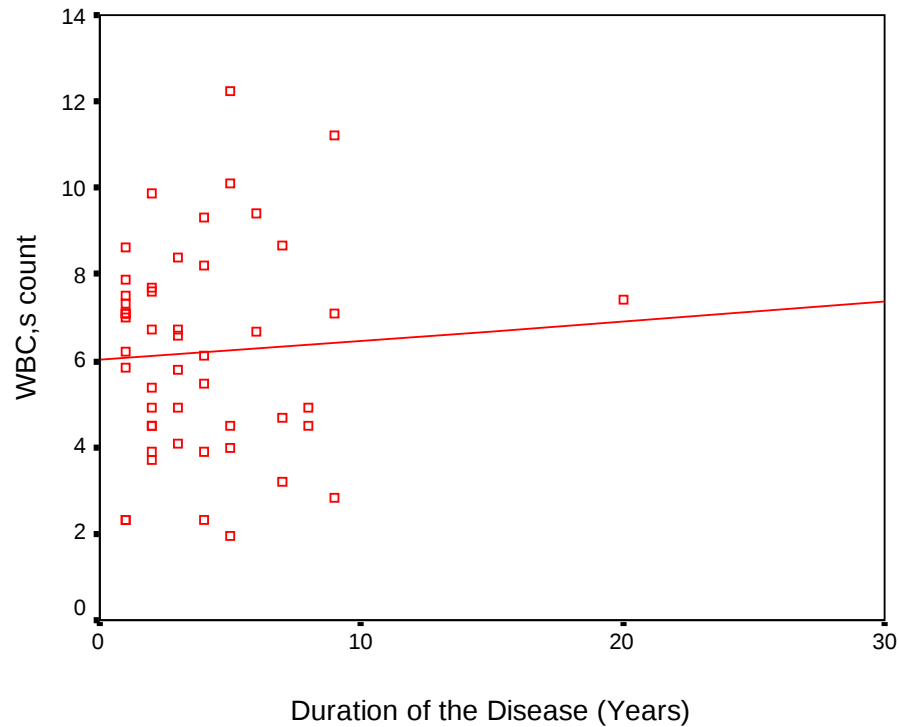


Figure (3.1) Correlation between WBC,s count and duration of disease among SLE patient.

No significant correlation was found between WBC,s count and duration of disease among SLE patient.

(P = 0.66 ,R= .062).

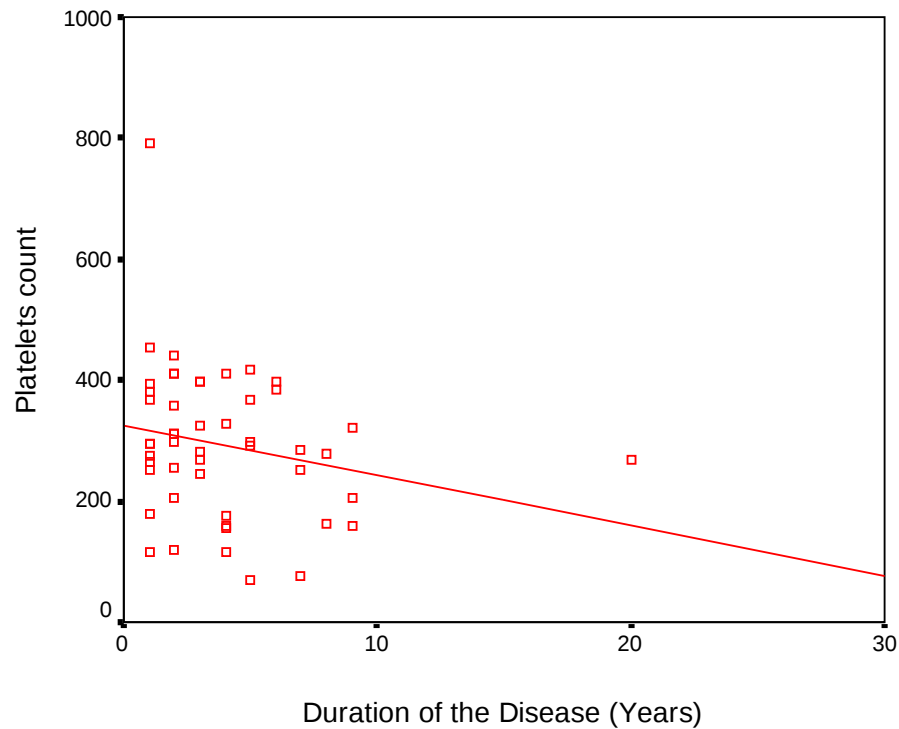


Figure (3.2) Correlation between Platelets count and duration of disease among SLE patient .

No significant was found correlation between Platelets count and duration of disease among SLE patient ($P = .11$, $R = -.226$).

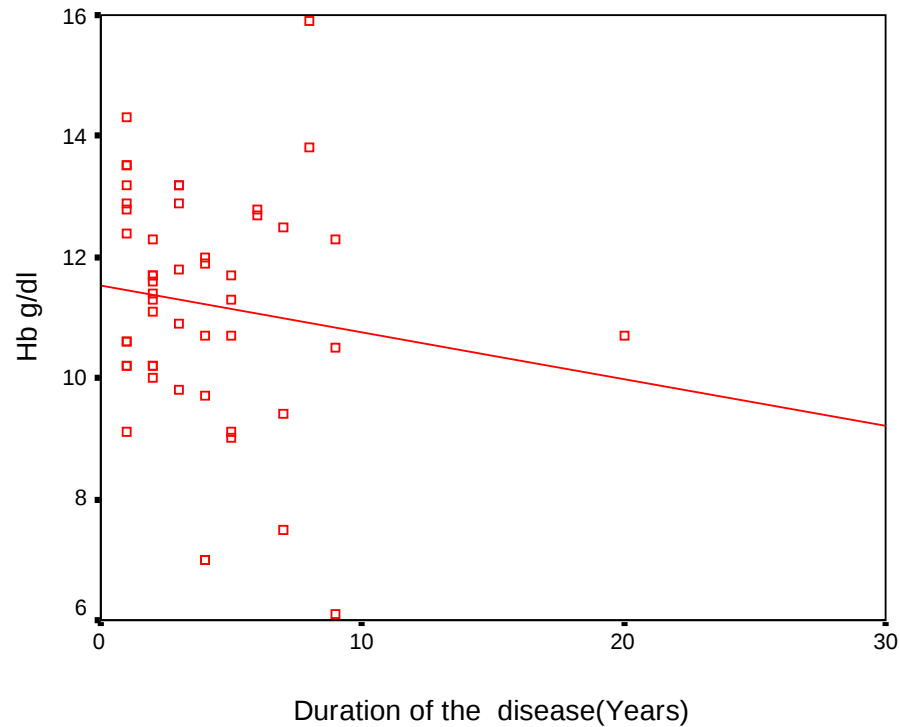


Figure (3.3)Correlation between Hemoglobin concentration and duration of disease among SLE patient.

No significant correlation was found between Hemoglobin concentration and duration of disease among SLE patient ($P = 0.35$, $R = -.134$) .

Chapter Four

Discussion Conclusion and Recommendation

4.1 Discussion

Hematological abnormalities are the commonest among most manifestations in SLE such as Anemia, leucopenias and thrombocytopenia (Bashal, 2013).

In this study Hb, RBCs count, PCV, MCH, were significantly lower in SLE group compared to control group $p\text{-value} > 0.05$. which is online with the study done in Sudan by AL zain .A, (2014) who concluded that there was significant decrease in Hb, Hct, RBCs count, and also agreed with study by Voulgarelis *et al.*, (2000) reported that hemoglobin levels was $< 12\text{g/dL}$ for SLE female patients. In this study showed no significant difference in MCV, MCHC between two groups $p\text{-value} < 0.05$ which consistent with study done in Sudan by AL zain .A, (2014) concluded that there was no difference in MCV and MCHC between two groups.

The results of the present study showed that Neutrophils, Eosinophils and Basophils differential count were significantly lower in SLE group $p\text{-value} > 0.05$ Which consistent with the study of Al Arfaj and Khalil (2009) the study done in Sudia Arabia included 126 patients with SLE, the results showed that 47% had neutropenia Other study concluded that Steroid therapy may cause low eosinophils counts, the basophils counts may also be decreased in SLE, particularly during active disease (Schur and Berliner ;2012).

No significant difference in WBC,s count, Lymphocytes and Monocytes differential count between SLE patient and control, p.value <0.05 which disagreed with results obtained by Schur and Berliner (2012) with prevalence of lymphopenia in SLE ranges from 20 to 81% .

The finding of this study showed that there was no significant difference in plateletes count results p.value <0.05, which disagreed with study conducted by Hepburn *et al.*, (2010) conclude that Thrombocytopenia has a reported prevalence ranging from 7 to 30% in large series of patients with SLE.

Disagreement of this study with other studies may be due to different life style and number of population, technical and laboratories variation also due to treatment and stage of disease

4.2 Conclusion:

This study concluded that:

- There was significant decrease in Hb, RBCs count, PCV, MCH , Neutrophils , Eosinophils and Basophils differential count between Systemic Lupus Erythromatosus patients and apparently healthy individuals .
- No significant difference in MCV, MCHC, Platelets count, WBC, s count , lymphocytes and monocytes differential count between two groups .

No relationship between complete Blood Count parameters with duration of disease and age .

There was relationship between duration of disease with history of blood transfusion.

- No correlation between WBC, s count , Platelets count , Hemoglobin concentration and duration of disease among SLE patient.

4.3 Recommendation

This study recommended:

More studies should be conducted all over the country .

CBC should be done as regular follow up for SLE patients.

- Other studies should be done using more advanced techniques.

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Appendix(1):Questionnaire

Sudan university of Science and Technology

Collage of post graduate studies

Medical Laboratory master program

Department of Hematology

Questionnaire

Name:.....

Sample Serial NO().

Age:.....

Duration of disease :.....

Blood transfusion:.....

Complication:

:.....

Tel. No:.....

Presence of hematological disease/s.....

Type of medication:.....

Investigation results: CBC

.....

Signature:.....

Date:.....

بسم الله الرحمن الرحيم

Appendix(2)

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا- برنامج الماجستير- مختبرات طبية

تخصص علم الدم ومبحث المناعة

إقرار الموافقة

.....:الإسم

سوف يتم أخذ عينة دم من الوريد بواسطة حقنة طعن وذلك بعد مسح مكان أخذ العينة بواسطة مطهر كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية .
وأنا أقر بأن العينات سوف يتم تحليلها فقط لغرض البحث مع مراعاة السرية .
أوافق أنا المذكور أعلاه بأخذ عينة لإجراء الدراسة .

.....:الإسم

.....:الإمضاء

Appendix (3)

Sysmex XT 2000i haematology analyzer

