بسم الله الرحمن الرحيم

Sudan University Of Science And Technology

College Of Graduate Studies

Changes of Some Haematological Parameters in Chronic Renal Failure patients after Haemodialysis

التغيرات في بعض قياسات الدم لدى مرضى الفشل الكلوي المزمن بعد الغسيل الدموي

A Dissertation Submitted in partial fulfillment of the requirement of M.Sc. degree in Hematology and Immunohematology

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بسم الله الرحمن الرحيم

الآية

قال تعالى :-

(وَمَن يَتَّقِ اللَّهَ يَجْعَل لَّهُ مَخْرَجًا وَيَرْزُقْهُ مِنْ حَيْثُ لَا يَحْتَسِبُ وَمَن يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ حَسْبُهُ إِنَّ اللَّهَ بَالِغُ أَمْرِهِ قَدْ جَعَلَ اللَّهُ لِكُلِّ شَيْءٍ قَدْرًا)

صدق الله العظيم

سورة الطلاق الاية (2-3)

DEDICATION

To the candle who burns to light my lives, who continuously encourage me to success, made me a woman and show me the meaning of love

My Mother

To the one who lives to make his dreams true

My Father

TO those who continuously encourage me to success and always around me, help me to obtain knowledgement and to reach this level

My Teachers

To those who make happiness and fun in my life

My Friends

Acknowledgement

First, I would like to express my deepest thanks and praise to ***Allah*** *for enabling me to accomplish this work.*

Thanks are extended to my supervisor ***Dr.Babiker Ahmed*** *for helping, supporting, assistance and advice.*

Also my thanks to all staff of Haematology Department.

Special thanks to ***Dr.Abdelazim Mohammed Abdelrahim*** *and all the staff of the Armed Forces Medical Services Hospital who provided me with all the required facilities, There are no words to describe their excellent work.*

Finally, I sincerely thank everyone who helped me to accomplish this research.

Abstract

This study was conducted at Haemodialysis Unit in Armed Forces Medical Services Hospital, Omdurman, during the period from February 2015 to May 2015 to evaluate Haematologial Changes in Chronic Renal Failure patients undergo Haemodialysis.

Blood samples were collected-in EDTA- from 100 chronic renal failure patients undergoing hemodialysis before and after dialysis sessions. A questionnaire was used to collect some information about the patients and The samples were tested using automated machine (Sysmex KX-21N).

Males comprises 62% of patients who were aged 20 to 80 years.

The results from the patients undergo Hemodialysis showed that the mean of White Cells count pre Hemodialysis were (5.0×10^3 /µI ±1.5), while the mean of White Cells count post Hemodialysis were (5.6×10^3 /µI ±2.1) and this indicates significant increase after Hemodialysis (p.value 0.000).

The mean of Red Cells count pre Hemodialysis were (3.2×10^6 /µI ±0.6), while the mean of Red Cells count post Hemodialysis were (3.4×10^6 /µI ±0.7) and this indicates significant increase after Hemodialysis (p.value 0.000).

The mean of Hemoglobin pre Hemodialysis were (9.5g/dl ±2.2), while the mean of Hemoglobin post Hemodialysis were (10.2 g/dl ±2.0) and this indicates significant increase after Hemodialysis (p.value 0.000).

The mean of Packed Cell Volume pre Hemodialysis were (30.7% ±6.3), while the mean of Packed Cell Volume post Hemodialysis were (32.5% ±6.6) and this indicates significant increase after Hemodialysis (p.value 0.000).

The mean of Mean Cell Volume pre Hemodialysis were (92.5 fl ±9.9), while the mean of Mean Cell Volume post Hemodialysis were (93.1 fl ±10.1) and this indicates insignificant increase after Hemodialysis (p.value 0.59).

The mean of Mean Cell Hemoglobin pre Hemodialysis were (29.7 pg ±2.0), while the mean of Mean Cell Hemoglobin post Hemodialysis were (29.3 pg±3.3) and this indicates insignificant decrease after Hemodialysis (p.value 0.15).

The mean of Mean Cell Hemoglobin Concentration pre Hemodialysis were (31.7% ±1.1), while the mean of Mean Cell Hemoglobin Concentration post Hemodialysis were (31.5% ±1.2) and this indicates significant decrease after Hemodialysis (p.value 0.03).

The mean of Platelets count pre Hemodialysis were (184×10^3/µI ±68.1), while the mean of Platelets count post Hemodialysis were (188×10^3/µI ±74.6) and this indicates insignificant increase after Hemodialysis (p.value 0.09).

The mean of Lymphocytes count pre Hemodialysis were (1.18% ±0.7), while the mean of Lymphocytes count post Hemodialysis were (1.19% ±0.8) and this indicates insignificant increase after Hemodialysis (p.value 0.97).

The mean of Mixed cells count pre Hemodialysis were (0.57%), while the mean of Mixed cells count post Hemodialysis were (0.61%) and this indicates insignificant increase after Hemodialysis (p.value 0.264).

The mean of Neutrophil count pre Hemodialysis were (3.3%), while the mean of Neutrophil count post Hemodialysis were (3.9%) and this indicates significant increase after Hemodialysis (p.value 0.000).

ملخص الدراسة

أجريت هذه الدراسة بمركز الغسيل الدموي بمستشفى السلاح الطبي بامدرمان ، في خلال الفترة الزمنيه من فبراير 2015 الى مايو 2015 وذلك لدراسة التغيرات التي تطرأ على كريات الدم الحمراء في مرضى الفشل الكلوي المزمن بعد الغسيل الدموي.

تم جمع عينات دم _في حاوية EDTA _ من 100 مريض بالفشل الكلوي المزمن قبل وبعد الغسيل الدموي.تم استخدام الاستبيان لجمع معلومات عن المرضى و تم تشخيص العينات باستخدام جهاز تحليل الدم الالي.

شكل الذكور 62 % من المرضى الذين تتراوح اعمارهم بين 20 و 80 سنة.

اشارت النتائج المتحصل عيها من المرضى الخاضعين لغسيل الدموي الى ان متوسط عدد الخلايا البيضاء قبل الغسيل الدموي ($10^3\times5.0$ /مايكروليتر ±1.5) بينما متوسط عدد الخلايا البيضاء بعد الغسيل الدموي ($10^3\times5.6$ /مايكروليتر ±2.1) هذا يدل على زيادة ذات دلاله معنوية بعد الغسيل (مستوى معنوية 0.00).

متوسط عدد الكريات الحمراء قبل الغسيل الدموي ($10^6\times3.2$ /مايكروليتر ±0.6) بينما متوسط عدد الكريات الحمراء بعد الغسيل الدموي ($10^6\times3.4$ /مايكروليتر ±0.7) وهذا يدل على زيادة ذات دلاله معنوية بعد الغسيل (مستوى معنوية 0.00).

متوسط خضاب الدم قبل الغسيل الدموي (9.5 جم/ديسلتر ±2.2) بينما متوسط خضاب الدم بعد الغسيل الدموي (10.2 جم/ديسلتر ±2.0) هذا يدل على زيادة ذات دلاله معنوية بعد الغسيل (مستوى معنوية 0.00).

متوسط كريات الدم الحمراء المضغوطة قبل الغسيل الدموي (30.7 % ±6.3) بينما متوسط كريات الدم الحمراء المضغوطة بعد الغسيل الدموي (32.5 % ±6.6) وهذا يدل على زيادة ذات دلاله معنوية بعد الغسيل (مستوى معنوية 0.00).

الوسط الحسابي لمتوسط حجم الخليه قبل الغسيل الدموي (92.5 فيمتوليتر ±9.9) بينما الوسط الحسابي لمتوسط حجم الخليه بعد الغسيل الدموي (93.1 فيمتوليتر ±10.1) وهذا يدل على زيادة ذات دلاله غير معنوية بعد الغسيل (مستوى معنوية 0.59).

الوسط الحسابي لمتوسط هيموجلوبين الخليه قبل الغسيل الدموي (29.7 بيكوجرام ±2.0) بينما الوسط الحسابي لمتوسط هيموجلوبين الخليه (29.3 بيكوجرام ±3.3) وهذا يدل على انخفاض ذو دلاله غير معنوية بعد الغسيل (مستوى معنوية 0.15).

الوسط الحسابي لمتوسط تركيز هيموجلوبين الخليه قبل الغسيل الدموي (31.7 % ±1.1) بينما الوسط الحسابي لمتوسط تركيز هيموجلوبين الخليه بعد الغسيل الدموي (31.5 %±1.2) وهذا يدل على انخفاض ذو دلاله معنوية بعد الغسيل (مستوى معنوية 0.03).

متوسط عدد الصفائح الدمويه قبل الغسيل الدموي ($10^3\times184$/ مايكروليتر ±68.1) بينما متوسط عدد الصفائح الدمويه بعد الغسيل الدموي ($10^3\times188$/مايكروليتر ±74.6) وهذا يدل على زيادة ذات دلاله غير معنوية بعد الغسيل (مستوى معنوية 0.09).

متوسط عدد الخلايا الليمفاويه قبل الغسيل الدموي (1.18 % ±0.7) بينما متوسط عدد الخلايا الليمفاويه بعد الغسيل الدموي (1.19 % ±0.8) وهذا يدل على زيادة ذات دلاله غير معنوية بعد الغسيل (مستوى معنوية 0.97).

متوسط عدد الخلايا المختلطة قبل الغسيل الدموي (0.5 % ±0.3) متوسط عدد الخلايا المختلطة (0.6 % ±0.2) وهذا يدل على انخفاض ذو دلاله غير معنوية بعد الغسيل (مستوى معنوية 0.26).

متوسط عدد النيوتروفيل قبل الغسيل الدموي (3.3 % ±1.2) بينما متوسط عدد النيوتروفيل (3.9 %±1.7) وهذا يدل على زيادة ذات دلاله معنوية بعد الغسيل (مستوى معنوية 0.00).

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Abbreviation

ADP	Adenosine DiPhosphate
AKI	Acute Kidney Injury
AoCRF	Acute-On-Chronic Renal Failure
ARF	Acute Renal Failure
ASCP	American Society for Clinical Pathology
ATN	Acute Tubular Necrosis
ATP	Adenosine TriPhosphate
Baso	Basophils
BFU	Burst Forming Unit
BFU-E	Burst Forming Unit- Erythroid
CAP	College of American Pathologists
CBC	Complete Blood Count
CD	Cluster of Differentiation
CFU	Colony Forming Unit
CFU-E	Colony Forming Unit -Erythroid
CFU-GEMM	Colony Forming Unit -Granulocytes-Erythrocytes-Monocytes/Macrophages-Megakaryocytes
CFU-GM	Colony Forming Unit- Granulocyte-Macrophage
CFU-L	Colony Forming Unit- Lymphoid
CFU-MK	Colony Forming Unit-Megakaryocyte
CKD	Chronic Kidney Disease
CLL	Chronic Lymphocytic Leukaemia
DNA	DeoxyriboNucleic Acid
EDTA	Ethylene Diamine Tetra Acetic acid
Eos	Eosinophils
eosinophil-CSF	eosinophil- colony-stimulating factor
EPO	Erythropoietin

ESRD	End-Stage Renal Disease
FBC	Full Blood Count
FBE	Full Blood Exam
Flt-L	FIt ligand
G-CSF	Granulocyte Colony-Stimulating Factor
GFR	Glomerular Filtration Rate
GM-CSF	granulocyte-macrophage colony-stimulating factor
Hct	Hematocrit
HD	HemoDialysis
HGB/Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HSC	Hematopoietic Stem Cell
IgE	immunoglobulin class E
IL	Interleukin
ITTP	Idiopathic Thrombotic Thrombocytopenic Purpura
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
M-CSF	macrophage colony-stimulating factor
MCV	Mean Corpuscular Volume
Mixed cells	Eosinophils, Basophils and Monocytes
Mk-CSF	megakaryocyte colony-stimulating factor
MPV	Mean Platelet Volume
NK	Natural killer cells .
PCV	Packed Cell Volume
PD	Peritoneal Dialysis
PDW	Platelet Distribution Width
PMNs	Polymorph nuclear neutrophil leukocytes
RBCs	Red Blood Cells

RDW	Red blood cell Distribution Width
RNA	RiboNucleic Acid
RRT	Renal Replacement Therapy
SCF	stem cell factor
SLIME	Salicylic acid, Lithium, Isopropanol, Magnesium-containing laxatives, and Ethylene glycol
TNF	Tumour Necrosis Factor
TPO	Thrombopoietin
WBCs	White Blood Cells

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Chapter One

Introduction and literature review

Chapter One
Introduction and literature review

1.1 Introduction

Blood is a specialized bodily fluid that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. In vertebrates, it is composed of blood cells suspended in a pale yellow liquid called blood plasma (Blood, 2015)

Complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel or Hemogram , is a test panel requested by a doctor or other medical professional that gives information about the kinds and number of cells in a patient's blood. The cells that circulate in the blood stream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease (Complete Blood count, 2015).

Kidneys are paired bean shaped organs located in the back of the abdomen. The most prominent function are the removal of un wanted substances from plasma, filter the blood, control the body's fluid balance, regulate the balance of electrolytes and secretion of hormones like Erythropoietin (which controls erythrocyte production) and Thrombopoietin (which controls Thrombocyte production) so, in the clinical laboratory kidney function tests are used in the assessment of renal diseases. **(**Bishop, Fody & Schoeff 2005)

Dialysis is a process for removing waste and excess water from the blood, Dialysis involves the removal of urea and other toxic substances from the plasma as well as the correction of electrolyte imbalance. Dialysis is regarded as a "holding measure" until a renal transplant can be performed, or sometimes as the only supportive measure in those for whom a transplant would be inappropriate. Two methods of dialysis, haemodialysis (HD) is the most commonly used method in which, blood is passed through an extra corporeal circuit and pumped across an artificial semi permeable membrane to bring the blood into contact with the dialysate (Bishop, Fody & Schoeff 2005)
The second method is the intermittent and continuous ambulatory peritoneal dialysis (PD). This method utilizes the peritoneal membrane, as the semi

permeable membrane, with capillaries on one side and high osmotic fluid infused into the peritoneal cavity on the other side. The peritoneal cavity is drained and the cycle is repeated after a suitable time to allow the equilibration of diffusible substances . Both types of dialysis are known to have side effects on the variable blood component. Dialysis is an imperfect treatment to replace kidney function because it does not correct the endocrine functions of the kidney. (Dialysis 2015)

1.2 Literature review

1.2.1 Blood

Blood accounts about 7% of the human body weight, with an average density very close to pure water's density (Shmukler, 2004).The average adult has a blood volume of roughly 5 litters (1.3 gal), composed of plasma and several kinds of cells (occasionally called corpuscles); these formed elements of the blood are erythrocytes (red blood cells, RBCs), leukocytes (white blood cells, WBCs), and Thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%. (Pendse, Singh & Zawada 2008)

1.2.1.1 Constituents of human blood

1.2.1.1.1 Fluid portion (Plasma):

About 55% of blood is plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in colour. The blood plasma volume totals of 2.7–3.0 litters in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Also contain glucose, mineral ions, hormones, blood cells and removes waste products, such as carbon dioxide, urea, and lactic acid. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood (Blood, 2015)

Other important components include:

- Serum albumin.
- Blood-clotting factors (to facilitate coagulation).
- Immunoglobulins (antibodies).
- lipoprotein particles.
- Various other proteins.
- Various electrolytes (mainly sodium and chloride).

Serum: The term refers to plasma from which the clotting proteins have been removed.

1.2.1.1.2 Solid portion (Cells):

One microlitter of blood contains:

- 4.7 to 6.1 million (male), 4.2 to 5.4 million (female) Erythrocytes: Red blood cells contain the blood's haemoglobin and distribute oxygen, They carry oxygen from the lungs to the tissues and return carbon dioxide (CO_2) from the tissues to the lungs to exhaled. The primary function of erythrocytes is gas exchange. Mature red blood cells lack a nucleus and organelles; a large proportion of their cytoplasm consists of the iron-containing oxygen transport molecule haemoglobin. biconcave disk shape gives red blood cells (RBCs)

the flexibility to squeeze their way through capillaries and other small blood vessels. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. RBCs have life span 120 days. (Martini, Timmons &Tallitsch 2006)

- 4,000–11,000 Leukocytes: White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukaemia. Leukocytes are usually divided into granulocytes, which have specific granules, and A granulocytes, which lack specific granules .Granulocytes are divided into neutrophils (with faintly staining granules), eosinophils(with large reddish or eosinophilic granules), and basophils (with large dark blue or basophilic granules). A granulocytes are divided into lymphocytes and monocytes. (Barrett &Ganong 2010)
- 150,000–450,000 Thrombocytes: Also called platelets, anucleate discs with a diameter of ~1 to 4 µm, They have pale blue cytoplasm with reddish-purple granules. Thrombocytes are responsible for blood clotting (coagulation). They change fibrinogen into fibrin. This fibrin creates a mesh onto which red blood cells collect and clot, which then stops more blood from leaving the body and also helps to prevent bacteria from entering the body. Platelets have a life span of approximately 10 days. Senescent platelets are removed by the spleen. Platelets have alpha granules and dense bodies. Platelet granules contain clotting factors, adenosine diphosphate (ADP) and adenosine triphosphate (ATP), calcium, serotonin, and catecholamines; many of these stimulate platelet aggregation or are important in the coagulation cascade. (Barrett &Ganong 2010)

1.2.1.2 Blood functions

blood performs many important functions within the body including: (Blood, 2015)

- Supply of oxygen to tissues (bound to haemoglobin).
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids)).
- Removal of waste such as carbon dioxide, urea, and lactic acid.
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding).

- Messenger functions, including the transport of hormones and the signalling of tissue damage.
- Regulation of body pH.
- Regulation of body temperature: Increasing blood flow to the surface, causes warmer skin, resulting in faster heat loss. In contrast, when the external temperature is low, blood flow to the extremities and surface of the skin is reduced and to prevent heat loss and is circulated to the important organs of the body.
- Hydraulic functions: the restriction of blood flow can also be used in specialized tissues to cause engorgement, resulting in an erection of that tissue; examples are the erectile tissue in the penis and clitoris.

1.2.1.3 Formation of blood cells (Haematopoiesis)

Haematopoiesis is defined as the production, development, differentiation, and maturation of all blood cells (Kienle et al. 1996) . The term comes from the Greek haima (blood) and poiein (to make). For the average adult, the bone marrow produces ~5 x 10^{11} cells per day. The Haemopoietic system includes the bone marrow, liver, lymph nodes and thymus. Haematopoiesis 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 haematopoiesis during the second trimester; how-ever, the bone marrow becomes the primary site of haematopoiesis after the seventh month. After birth, the bone marrow is normally the sole site of haematopoiesis (intramedullary haematopoiesis). Haematopoiesis may resume in the liver and spleen after birth in conditions associated with fibrosis of the bone marrow (extramedullary haematopoiesis) (Ciesla,2007) .

Table 1.1 Sites of haemopoiesis (Beck, 2009)

Fetus	0-2 months (yolk sac)
	2-7 months (liver, spleen)
	5-9 months (bone marrow)
Infants	Bone marrow (practically all bones)
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

Table1-2 Function of the spleen (Kienle et al. 1996)

Hematopoietic function	Can produce white cell, red cells, and platelets if necessary
Reservoir function	One third of platelets and granulocytes are stored in the spleen
Filtration function	Aging red cells are destroyed, spleen removes inclusion from red cells, if red cell membrane is less deformable or antibody-coated spleen presents a hostile environment leading to production of spherocytes
Immunologic function	Opsonizing antibodies produced, trapping and processing antigens from encapsulated organs

1.2.1.3.1 Postnatal Haematopoiesis

During infancy and childhood, there is active haematopoiesis in the medullar cavity of virtually 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, haematopoiesis is restricted to the proximal long bones and the axial skeleton (skull, vertebral bodies, ribs, sternum, and pelvis). The yellow marrow can resume active haematopoiesis under conditions of chronic hematologic stress (chronic bleeding or haemolytic anaemia) (Ciesla,2007)

All blood cells derive from pluripotent hematopoietic stem cells (HSC). These stem cells are supported by stromal cells which has two properties the first is self renewal, and the second is its proliferation and differentiation in to progenitor cells, committed to one specific cell line (Hoffbrand, Moss& Pettit 2006).

Pluripotent hematopoietic stem cells give rise to committed progenitor cells. This is a multistep process in which the cells become more committed to a specific lineage. These committed progenitor cells are given various names, such as colony-forming unit (CFU) or burst forming unit (BFU). The initial differentiation step is into either CFU-GEMM (GEMM granulocytes-erythrocytes-monocytes/macrophages-megakaryocytes) or CFU-L (L = lymphoid). The CFU-GEMM gives rise to the CFU-GM (granulocyte-macrophage), BFU-E (erythroid), and CFU-Mk (megakaryocyte). Each committed progenitor cell gives rise to a thousand or more mature blood cells (Ciesla,2007). (Figure1-1).

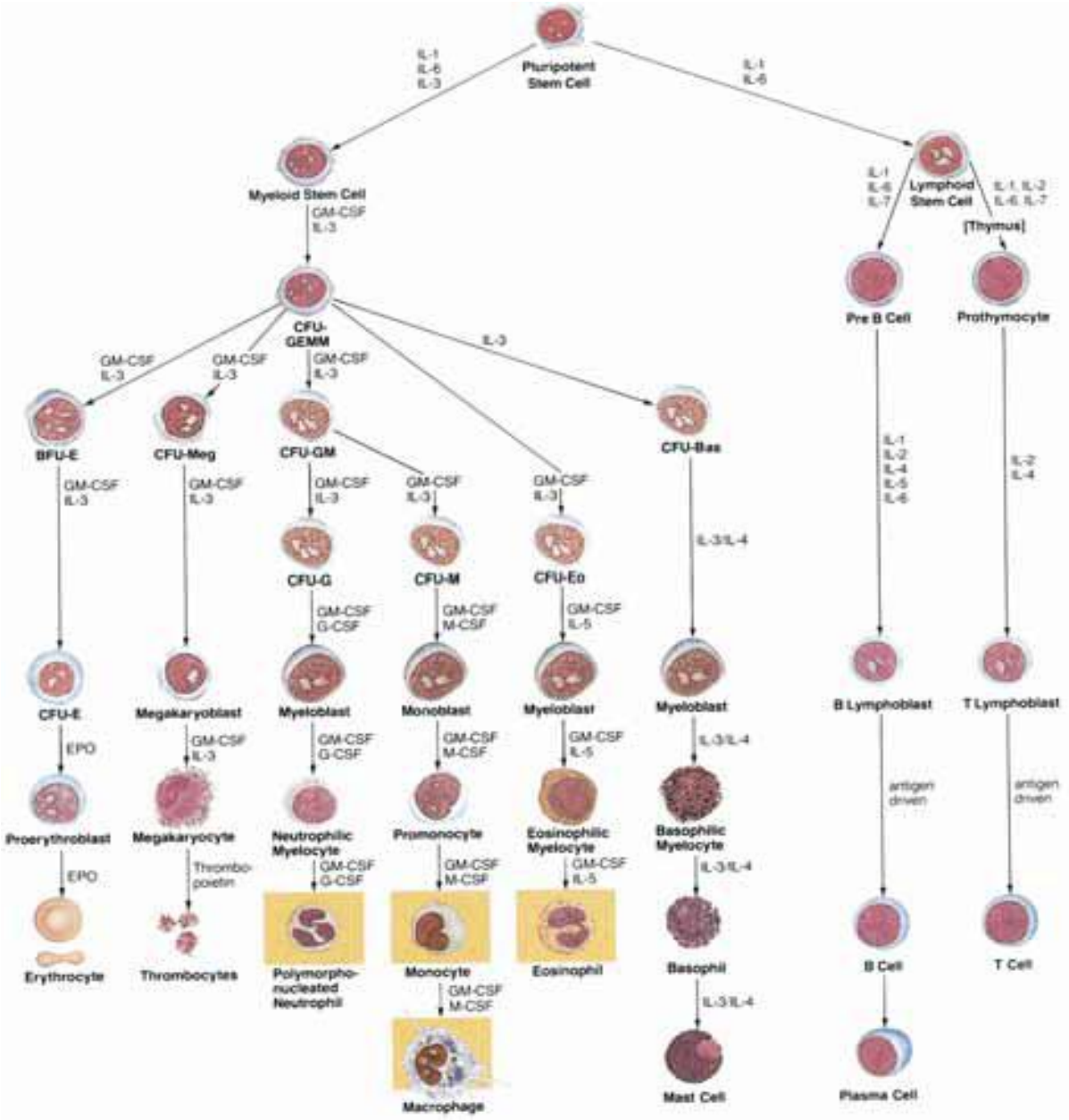

Figure 1-1 Blood cell formation from stem cells to mature cells (Kienle et al. 1996)

1.2.1.3.2 Hematopoietic growth factors

Hematopoietic growth factors are proteins or glycoproteins that regulate the production and differentiation of hematopoietic precursors. They act on specific cell surface receptors on hematopoietic precursor cells and may either stimulate or inhibit cell proliferation and differentiation. The majority are produced within the marrow and act locally. Erythropoietin and thrombopoietin are produced outside the marrow and reach the marrow through the blood (Ciesla,2007).

Examples of important growth factors:

• Stem Cell Factor(Steel factor, c-kit ligand): Stem cell factor is critical at the level of pluripotent stem cells.

• Granulocyte Colony-Stimulating Factor(G-CSF): Stimulates granulocyte differentiation and maturation and stimulates the action of mature neutrophils.

• Granulocyte-Macrophage Colony-Stimulating Factor(GM-CSF, Leukine): GM-CSF stimulates differentiation and maturation of granulocytes and monocytes, and also the function of mature cells.

• Erythropoietin(EPO; Epogen): EPO is hormone which produced predominantly in the kidney(90%) and a small amount is produced in the liver(10%) in response to renal hypoxia. the gene for EPO s on chromosome 7 It is required for erythrocyte production and appears relatively specific for erythroid cells.

• Thrombopoietin(TPO, Mk-CSF): TPO is a glycoprotein hormone critical in megakaryocyte growth and differentiation and is produced predominantly in the liver**.** TPO gene is located on the long arm of chromosome3.Growth factor act at different stages of haemopoiesis (Table 1-3) . Stromal cells are the major source of growth factors except for erythropoietin, 90% of which is synthesized in the kidney and thrombopoietin, made largely in the liver.

Table1-3 Haemopoietic growth factors **(**Beck,2009)

Act on stromal cells
IL-l
TNF
Act on pluripotential stem cells
SCF
Flt-L
Act on multipotential progenitor cells
IL-3
GM-CSF
G-CSF
Thrombopoietin
Act on committed progenitor cells
G-CSF*
M-CSF
IL-5 (eosinophil-CSF)
Erythropoietin
Thrombopoietin

1.2.1.3.3 Erythrocyte Production (Erythropoiesis)

The erythron is the sum of all erythroid cells, including circulating red blood cells (RBCs) and marrow erythroid precursors. Erythroid precursors are derived from the CFU-GEMM. 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 (Table1-4). The last nucleated RBC precursor is the orthochromatophilic erythroblast, which is characterized by well-hemoglobinized cytoplasm; the nucleus is then lost, producing the reticulocyte. Reticulocyte are identified using supravital stains such as new methylene blue; they cannot be definitively identified with routine Wright-Giemsa stains. Reticulocyte contain ribonucleic acid (RNA) for 4 days; normally, the first 3 days are spent in the marrow and fourth in the blood. However, under intense stimulation by erythropoietin, reticulocyte may be released into the blood early where they may contain RNA for 2.0 to 2.5 days (shift reticulocyte) (Ciesla,2007)

Table 1-4 Erythropoiesis (Ciesla,2007)

Cell	Appearance
Proerythroblast	14–19 µm diameter; small amount of deeply basophilic cytoplasm; large round nucleus with fine chromatin, nucleolus
Basophilic erythroblast	12–17 µm diameter; deeply basophilic cytoplasm; nuclear chromatin begins to condense
Polychromatophilic erythroblast	12–15 µm diameter; greyish cytoplasm; nucleus is smaller with increased chromatin condensation
Orthochromatophilic erythroblast	8–12 µm diameter; cytoplasm red to pale gray; small totally opaque nucleus
Reticulocyte	7–10 µm diameter; nucleus extruded; ribonucleic acid visible on reticulocyte stain
Erythrocyte	7–8 µm diameter; reddish cytoplasm; anucleate

- Red cell production is effective when the bone marrow responds to anaemic stress by producing an increased number of reticulocyte and nucleated red cells.
- Ineffective red cell production is described as death of red cell precursors in the bone marrow before they can be delivered to the peripheral circulation.

Table1-5 College of American pathologists(CAP) vs. American society for clinical pathology(ASCP) terminology for Red Cells (Kienle et al. 1996)

CAP	ASCP
Pronormoblast	Rubriblast
Basophilic normoblast	Prorubricyte
Polychromatophilic normoblast	Rubricyte
Orthochromic normoblast	Metarubricyte
Reticulocyte	Reticulocyte
Erythrocyte	Erythrocyte

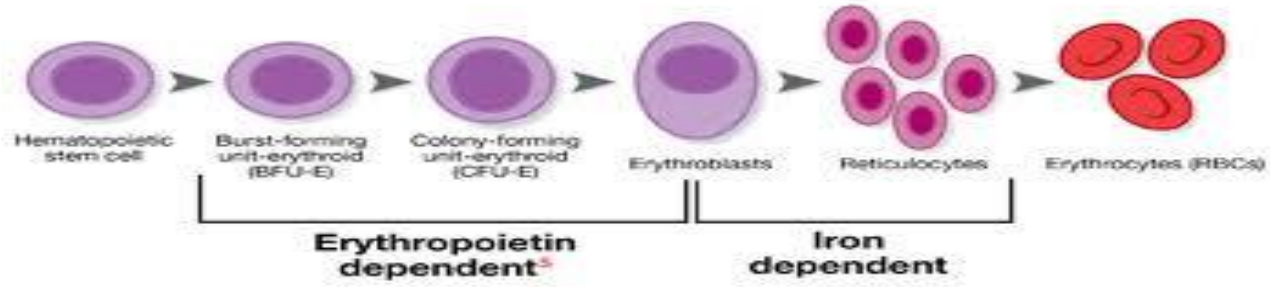

Figure 1-2 Erythropoiesis (Kienle et al. 1996)

1.2.1.3.4 Granulocyte Production (Granulocytopoiesis)

Neutrophils, eosinophils, and basophiles 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 (Table 1-6). Neutrophil, eosinophil, and basophil maturation follows parallel pathways; they can be differentiated at the myelocyte stage, when secondary (specific) granules appear. The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2 : 1 to 12 : 1 (Beck,2009)

Table 1-6 Granulocytopoiesis (Beck,2009)

Cell	Appearance
Myeloblast	10–20 µm diameter; large round nucleus with fine chromatin, nucleolus; high nucleus to cytoplasm ratio; small amounts of light gray to pale blue cytoplasm without granules
Promyelocyte	Distinguished by presence of large, reddish-purple primary granules; immature nucleus with nucleolus; grayish to dark blue cytoplasm

Myelocyte	Distinguished by presence of secondary granules; cytoplasm begins to turn light yellow-orange; round nucleus with early chromatin condensation; ± nucleolus
Metamyelocyte	Resembles myelocyte, but with indented (kidney bean–shaped) nucleus with increased chromatin condensation
Band neutrophil	Deeply indented (horse shoe-shaped) but not segmented nucleus; mature cytoplasm
Segmented neutrophil	Nucleus segmented into distinct lobes

Neutrophils

Neutrophils are the most common type of WBCs in adults. Two types are described: segmented neutrophils and band neutrophils:

• Segmented neutrophils (also called polymorph nuclear neutrophil leukocytes [PMNs]) have a nucleus divided into multiple distinct lobes connected by thin strands of chromatin. The cytoplasm has fine granules that stain lightly with the usual blood stains. normally comprise ~50 to 70% of total WBCs .

• Band neutrophils(“bands,” or “stabs”) have a horse-shoe-shaped nucleus, without the distinct lobes of PMNs. They are an earlier stage than segmented neutrophils but are fully functional. Bands normally represent ~2 to 6% of all WBCs.

The primary function of neutrophils is phagocytosis, predominantly of Bacteria: killed by antimicrobial agents contained or generated within neutrophil granules. Neutrophils circulate in the blood for ~10 hours and may live 1 to 4 days in the extravascular space. The number of Neutrophils increases with acute stress or infection (Ciesla,2007)

Eosinophils (“Eos”)

Eosinophils contain large granules that stain reddish-orange (eosinophilic) with usual blood smear stains. The nucleus is segmented (often bilobed). Functions of eosinophils include phagocytosis of antigen-antibody complexes and defence against parasitic infection. The normal eosinophil count is ~2 to 4% of total WBC. The number of eosinophils increases with allergic reactions and parasitic infections (Ciesla,2007).

Basophils (“Baso”)

Basophils contain large dark blue or purple (basophilic) granules, which often obscure the nucleus. The nucleus is segmented. Basophils are the least common type of leukocytes, normally ≤1% of total WBCs. The basophil granules contain heparin (an anticoagulant), histamine (a fast vasodilator), the slow-reacting substance of anaphylaxis (a slow vasodilator), and other

compounds. Basophils appear to be involved in immediate hypersensitivity reactions related to immunoglobulin class E (IgE) (Beck,2009).

1.2.1.3.5 Monocyte Maturation

Monocytes normally comprise ~3 to 8% of leukocytes, derived from the CFU-GEMM. Monoblast developed into promonocyte which develop into mature monocyte. Monocytes are large cells, with abundant light gray to light blue finely granular cytoplasm. The nucleus has very finely granular chromatin and is often folded, bean shaped, or irregular . Monocytes circulate through the blood and then after 8 to 14 hours enter the tissues to become either phagocytes (macrophages also called histiocytes) or professional antigen presenting cells (Langerhans' cells and dendritic reticulum cells) (Ciesla,2007).

1.2.1.3.6 Lymphocyte Maturation

lymphoblast developed into prolymphocyte which develop into mature lymphocyte. Lymphocyte maturation begins 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 await 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 (Ciesla,2007) .

Lymphocytes("Lymphs") are the second most common type of leukocytes in adults (~20–40% of WBC).

• Resting lymphocytes: are usually small (7–10 μm), with a dark round to oval nucleus and scant amounts of pale blue cytoplasm . The nucleus is small, resting lymphocyte is about the same diameter as a normal erythrocyte.

• Reactive ("atypical") lymphocytes: are larger, with more abundant pale blue cytoplasm and larger nuclei with less condensed chromatin, and perhaps a nucleolus. These are designated reactive or atypical lymphocytes.

• Large granular lymphocytes: A small number of lymphocytes in normal blood are slightly larger than resting lymphocytes, with reddish-purple (azurophilic) granules. This appearance generally corresponds to natural killer(NK) cells (Ciesla,2007)

Functionally, there are two main types of lymphocytes: B lymphocytes and T lymphocytes .

B lymphocytes:

The primary effectors of the humoral (antibody-mediated) immune system, They develop in the bone marrow and are found in lymph nodes, the spleen and other organs, as well as the blood. After antigen stimulation, B lymphocytes may develop into plasma cells, which are the primary antibody-producing cells (Beck,2009).

T lymphocytes:

The main effectors of cell-mediated immunity. T lymphocytes are the control cells of the entire immune system: they stimulate or inhibit the function of other cells of the immune system, including B lymphocytes, monocytes and macrophages, and other T cells. T cell precursors originate in the bone marrow but develop and mature in the thymus (T = thymic dependent).T cells are divided into two main subtypes: (Ciesla,2007)

• T helper lymphocytes, which are the major regulatory cells of the immune system, usually express a surface antigen designated CD4.

• T suppressor/cytotoxic lymphocytes are involved in the destruction of virally infected cells and rejection of transplanted organs. They usually express the CD8 surface antigen.

Unlike other leukocytes, which make a one-way trip between blood and tissues, lymphocytes can recirculate between blood, tissue, and lymph fluid.

1.2.1.3.7 Platelet (Thrombocyte) Production(Thrombopoiesis)

Platelets are derived from bone marrow megakaryocytes, which are large cells with multilobated nuclei and abundant finely granular light gray-blue cytoplasm. Megakaryocytes have cytoplasmic projections extending through the walls of sinuses into the lumen; fragments of cytoplasm break off into the sinus as platelets. [(12)] Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets.

Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys (Ciesla,2007).

1.2.2 Complete blood count

A test produced by automated haematology analyzers, requested to gives information about the cells in a patient's blood. It can be used to Screen for a wide range of conditions and diseases , Help diagnose various conditions, such as anaemia, infection, inflammation, bleeding disorder or leukaemia,

Monitor the condition and/or effectiveness of treatment after a diagnosis is established and Monitor treatment that is known to affect blood cells, such as chemotherapy or radiation therapy (Complete Blood count, 2015).

1.2.2.1 Principle of automated blood count

The blood is well mixed (not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Sensors count the number of cells passing through the tubing, and can identify the type of cell; this is flowcytometry. The two main sensors used are light detectors and electrical impedance. One way the instrument can tell what type of blood cell is present is by size. Other instruments measure different characteristics of the cells to categorize them. Certain abnormal cells in the blood may not be identified correctly, requiring manual review of the instrument's results. In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated haematology analyzers also measure the amount of haemoglobin in the blood and within each red blood cell (Complete Blood count, 2015)

1.2.2.2 Red blood cells

- Total red blood cells count: given as an absolute number per litre.
- Haemoglobin: expressed in grams per decilitre.
- reticulocyte count: which is a measurement of the absolute count or percentage of young red blood cells in blood.
- Hematocrit (Hct) or packed cell volume (PCV): This is the fraction of whole blood volume that consists of red blood cells.
- Red blood cell indices:

_ Mean corpuscular volume (MCV): the average volume of the red cells, measured in femtolitres.

_ Mean corpuscular haemoglobin (MCH): the average amount of haemoglobin per red blood cell, in picograms.

_ Mean corpuscular haemoglobin concentration (MCHC): the average concentration of haemoglobin in the cells.

- Red blood cell distribution width (RDW): the variation in cellular volume of the RBC population (Complete Blood count, 2015)

1.2.2.3 White blood cells

All the white cell types are given as a percentage and as an absolute number per litre.

A complete blood count with differential will also include:

-Neutrophil granulocytes: May indicate bacterial infection. May also be raised in acute viral infections.

- Lymphocytes: Higher with some viral infection . Also raised in chronic lymphocytic leukaemia (CLL). Can be decreased by HIV infection.

-Monocytes: May be raised in tuberculosis, malaria, monocytic leukaemia.

-Eosinophil granulocytes: Increased in parasitic infections, asthma, or allergic reaction.
-Basophil granulocytes: May be increased in bone marrow related conditions such as leukaemia or lymphoma**.** (Complete Blood count, 2015)

1.2.2.4 Platelets

Platelet numbers are given, as well as information about their size and the range of sizes in the blood.
- Mean platelet volume (MPV): It is a measurement of the Platelate average size.
- Platelet distribution width (PDW): It is a measurement of the variation of platelet size. (Complete Blood count, 2015)

1.2.3 Kidney

Each kidney contains around a million units called nephrons, each of which is a microscopic filter for blood. It's possible to lose as much as 90% of kidney function without experiencing any symptoms or problems.
Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time. The GFR is typically recorded in units of volume per time that is ml/min (Fadem et al. 1986)

1.2.3.1 Renal failure

Renal failure can be divided into two categories: acute kidney injury and chronic kidney disease. Renal failure is mainly determined by a decrease in glomerular filtration rate This is detected by a decrease in or absence of urine production or determination of waste products (creatinine or urea) in the blood. Other factors that may help differentiate acute kidney injury from chronic kidney disease include anemia and the kidney size on ultrasound. Chronic kidney disease generally leads to anemia and small kidney size (Wikipedia, 2015)**.**

1.2.3.2 Acute kidney injury (AKI)

previously called acute renal failure (ARF), Acute (sudden) kidney failure is the sudden loss of the ability of the kidneys to remove waste and concentrate urine without losing electrolytes**,** generally characterized by oliguria (decreased urine production, quantified as less than 400 mL per day in adults, less than 0.5 mL/kg/h in children or less than 1 mL/kg/h in infants); and fluid and electrolyte imbalance. AKI can result from a variety of causes, generally classified as prerenal, intrinsic, and postrenal. An underlying cause must be identified and treated to arrest the progress, and dialysis may be necessary to bridge the time gap required for treating these fundamental cause (Klahr& Steven 1998).

Causes, and Risk Factors

There are many possible causes of kidney damage. They include:
- Acute tubular necrosis (ATN)
- Autoimmune kidney disease
- Acute nephritic syndrome ,Interstitial nephritis
- Decreased blood flow due to very low blood pressure
- Disorders that cause clotting within the kidney's blood vessels:
Haemolytic-uremic syndrome
Idiopathic thrombocytopenic thrombotic purpura (ITTP)
Malignant hypertension
Transfusion reaction and Scleroderma
-Infections that directly injury the kidney such as:
Acute pyelonephritis and Septicemia
-Pregnancy complications, including:
Placenta abruption and Placenta prevail

1.2.3.3 Chronic kidney disease (CKD)

Also called chronic renal failure, is slow loss of kidney function over time. It may be so slow that symptoms do not occur until kidney function is less than one-tenth of normal. The final stage of chronic kidney disease is called end-stage renal disease (ESRD). The kidneys no longer function and the patient needs dialysis or a kidney transplant (Scott, 2013) .

1.2.3.3.1 Causes, & Risk Factors

The most common causes of CKD are diabetes mellitus and long-term, uncontrolled hypertension. Polycystic kidney disease is another well-known cause of CKD, Other genetic illnesses affect kidney function.

Overuse of common drugs such as aspirin, ibuprofen, and acetaminophen (paracetamol) can also cause chronic kidney damage.

Some infectious diseases, such as hantavirus, can attack the kidneys,causing kidney failure.

Chronic kidney disease leads to a build of fluid and waste products in the body

This condition affects most body systems and functions, including red cell production, blood pressure control, and vitamin D and bone health (Perneger et al.1994)

1.2.3.3.2 Stages:-

All individuals with a glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for 3 months are classified as having chronic kidney disease.

Stage 1

Slightly diminished function; kidney damage with normal or relatively high GFR (≥90 mL/min/1.73 m^2). Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies.

Stage 2

Mild reduction in GFR (60–89 mL/min/1.73 m^2) with kidney damage. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies.

Stage 3

Moderate reduction in GFR (30–59 mL/min/1.73 m^2).[17] British guidelines distinguish between stage 3A (GFR 45–59) and stage 3B (GFR 30–44) for purposes of screening and referral.

Stage 4

Severe reduction in GFR (15–29 mL/min/1.73 m^2) Preparation for renal replacement therapy.

Stage 5

Established kidney failure (GFR <15 mL/min/1.73 m^2, permanent renal replacement therapy (RRT) or end stage renal disease (ESRD). When one reaches stage 5 CKD, renal replacement therapy is usually required, in the form of either dialysis or a transplant (National Kidney Foundation, 2002)

Acute kidney injuries can be present on top of chronic kidney disease, a condition called acute-on-chronic renal failure (AoCRF). The acute part of AoCRF may be reversible, and the goal of treatment, as with AKI, is to return the patient to baseline renal function, typically measured by serum creatinine. Like AKI, AoCRF can be difficult to distinguish from chronic kidney disease if the patient has not been monitored by a physician and no baseline (Perneger et al.1994)

1.2.4 Renal dialysis Principle

Dialysis works on the principles of the diffusion of solutes and ultrafiltration of fluid across a semi-permeable membrane. Blood flows by one side of a semi-permeable membrane, and a dialysate, or special dialysis fluid, flows by the opposite side. A semi permeable membrane is a thin layer of material that contains holes of various sizes, or pores. Smaller solutes and fluid pass through the membrane, but the membrane blocks the passage of larger substances (for example, red blood cells, large proteins). This replicates the filtering process that takes place in the kidneys, when the blood enters the kidneys and the larger substances are separated from the smaller ones in the glomerulus. The two main types of dialysis, haemodialysis and Peritoneal dialysis, remove wastes and excess water from the blood in different ways. Haemodialysis removes wastes and water by circulating blood outside the body through an external filter, called

a dialyzer, that contains a semipermeable membrane. The blood flows in one direction and the dialysate flows in the opposite. The counter current flow of the blood and dialysate maximizes the concentration gradient of solutes between the blood and dialysate, which helps to remove more urea and creatinine from the blood. The concentrations of solutes (for example potassium, phosphorus, and urea) are undesirably high in the blood, but low or absent in the dialysis solution, and constant replacement of the dialysate ensures that the concentration of undesired solutes is kept low on this side of the membrane. The dialysis solution has levels of minerals like potassium and calcium that are similar to their natural concentration in healthy blood. For another solute, bicarbonate, dialysis solution level is set at a slightly higher level than in normal blood, to encourage diffusion of bicarbonate into the blood, to act as a pH buffer to neutralize the metabolic acidosis that is often present in these patients. In peritoneal dialysis, wastes and water are removed from the blood inside the body using the peritoneal membrane of the peritoneum as a natural semipermeable membrane. Wastes and excess water move from the blood,

across the peritoneal membrane, and into a special dialysis solution, called dialysate, in the abdominal cavity which has a composition similar to the fluid portion of blood. (Pendse, Singh & Zawada 2008)

1.2.4.1 Types

There are three primary and two secondary types of dialysis: haemodialysis (primary), peritoneal dialysis (primary), hemofiltration (primary), hemodiafiltration (secondary), and intestinal dialysis (secondary).

- **Haemodialysis**

In haemodialysis, the patient's blood is pumped through the blood compartment of a dialyzer . exposing it to a partially permeable membrane. The dialyzer is composed of thousands of tiny synthetic hollow fibres. The fibre wall acts as the semipermeable membrane. Blood flows through the fibres, dialysis solution flows around the outside of the fibres, and water and wastes move between these two solutions (Ahmad et al. 2008). The cleansed blood is then returned via the circuit back to the body. Ultrafiltration occurs by increasing the hydrostatic pressure across the dialyzer membrane. This usually is done by applying a negative pressure to the dialysate compartment of the dialyzer. This pressure gradient causes water and dissolved solutes to move from blood to dialysate, and allows the removal of several litres of excess fluid during a typical 3- to 5-hour treatment. In the US, haemodialysis treatments

are typically given in a dialysis centre three times per week .Studies have demonstrated the clinical benefits of dialyzing 5 to 7 times a week, for 6 to 8 hours. This type of haemodialysis is usually called "nocturnal daily haemodialysis", which a study has shown a significant improvement in both small and large molecular weight clearance and decrease the requirement of taking phosphate binders. These frequent long treatments are often done at home while sleeping, but home dialysis is a flexible modality and schedules can be changed day to day, week to week. In general, studies have shown that both increased treatment length and frequency are clinically beneficial. (Ronco & Levin 2007)

1.3 Rationale

In developing countries, where the regular medical check up is rare complications may be observed, this study was conducted in Sudan to study Changes of Some Haematological Parameters in Chronic Renal Failure patients after Haemodialysis in Armed Forces Medical Services Hospital, Omdurman (2015).

Omdurman city where inhabitants come from different parts of Sudan, many tribes were living there. The socio-economical status of most inhabitants was low, these condition made the research in this particular part are very important. The finding of this study may help to solve the health problems associated with Chronic Renal Failure

1.4 Objectives

1.4.1 General objective

1) To evaluate haematological changes in chronic renal failure patients after haemodialysis.

1.4.2 Specific objectives

1) To determine change in WBCs, RBCs, PLTs, Lymphocyte, Mixed cells, Neutrophil, Hb, PCV and red cell indices in chronic renal failure patients on haemodialysis

2) To compare the results of some Haematological parameters in ESRD patients before and after haemodialysis treatment

3) To find out frequency of age, sex and duration of dialysis

Chapter Two

Materisals and Methods

Materials and Methods

2.1 Study design

It is Cross sectional study.

2.2 Study area and duration

This study was carried out during the period from February 2015 to May 2015 in Armed Forces Medical Services Hospital (hemodialysis unit).

2.3 Study population Character

Patients with Chronic Renal Failure on hemodialysis program randomly selected and by means of questionnaire at different ages, from different tribes and sex.

2.4 Study variables

CBC in hemodialysis patients was considered as dependent variable and the following variables as independent variables:

Age: measured by years.

Gender: described by male and female.

Duration of dialysis: described by years.

2.5 Sample size

One hundred samples, including pre and post dialysis sampling

2.6 Data Collection

Data was collected using questionnaire and blood sample (Whole blood were collected (2.5 mls) using evacuated tube and drawn into EDTA anticoagulant).

2.7 Method

Sysmex KX-21N.

2.7.1 Principle

Based on the fact that the blood cells are poor conductors of electricity, where as certain diluents are good conductors. For a cell count blood is highly diluted in a buffered electrolyte solution. The flow rate of this diluted sample is controlled by a mercury siphon or by displacement of a tightly fitting piston. This result in a measured volume of the sample passing through an aperture

tube of specific dimension By means of constant source of electricity, a direct current is maintained between two electrodes, one is the sample beaker or the chamber surrounding the aperture tube and another inside the aperture tube. As blood cell is carried through the aperture, it displaces some of the conducting fluid and increases the electrical resistance. This produce a corresponding changes in potential between the electrolyte which last as the blood cells takes to pass through the aperture, the highest of the produced indicates the volume of the cells passing through.

2.7.2 Operation steps

Turn on the power, self check, background check, select whole blood mode, set sample number to be sample probe, press the start switch, execute analysis, end analysis.

2.7.3 Analysis parameter:

This instrument analysis the following parameter using three detectors block and tow kind of reagent

-WBCs analysis principle: DC detection method

WBCs count in 1ml of Whole blood

-LYM% (W-SRC)(WBC-small cell ratio), ratio % of small lymphocyte to Whole blood.

-MXD% (W-MRC)(WBC-middle cell ratio), ratio% of summation of basophiles, eosinophils and Monocyte (middle cell to Whole WBC).

-LYM# (W-SCC) (WBC-small cell count)

Absolute count of small lymphocyte in one micro liter of whole blood

-MXD%(W-MCC) (WBC-middle cell count) absolute count of the basophiles, eosinophils and Monocyte middle cell in 1ml of whole blood

-NEUT# (W-LCC) (WBC-large cell count)

Absolute count of the neutrophils in one micro liter of whole blood

RBCs (red blood corpuscular) analysis principle DC detection method

RBC count in 1mm of whole blood.

-HBG (hemoglobin) analysis principle non0cyanide hemoglobin analysis method volume (gram)of hemoglobin in 1dl of whole blood.

-HTC (hematocrit volume) analysis principle: RBC plus high detection method. Ratio% of whole RBC volume in whole blood.

-MCV (mean RBC volume) by FL in whole blood.

-MCH (mean hemoglobin concentration) by pg per RBC.

-MCHC (mean RBC hemoglobin concentrating) by g/dl.

-RDW-CV (RBC distribution width- coefficient of variation) .

-RDW-SD (RBC distribution width-standard deviation).

-PLT (platelet count in whole blood).

-PDW (platelet distribution width) .
-P-LCR (platelet large cell ratio).

2.7.4 Start up procedure

Inspection before turning on the power Inspection of reagent, check to see the reagent needed for the number of the sample to be proceeding for the day When reagent run out during analysis, the instrument will automatically come to stop.
Number of sample can be analyzed with one pack of reagent cell
pack =600sample/20ml
Stromatolyser= 470 sample/500ml

2.7.5 Reagents

Instrument reagent system is composed of 5 reagents for testing and cleaning
Pack cell---------- dilute sample
Cell wash ---------- RBC dilution
WDTM lyses ---------- WBC dilution
Stromatolyser CTM ----- hemoglobin dilution
20% clorax ---------- cleaning

2.8 Data analysis

The data off this research were analyzed by SPSS software program to obtain mean, standard deviations and P.value by Paired-samples T test. Data is presented in form of tables and figures.

2.9 Ethical Consideration

Participants were informed simply about the study and its benefits, method of sample collection. The data was kept in highly security mode, the hospital administration was informed.

Chapter Three

Results

Results

The results of the study showed that:

Males comprise 62% of patients while Female comprises 38% (Table 3-1).

Figure 3-1 show distribution of study group according to Age (years), Patients who were aged 20 to 80 years divided into 3 groups; 20 to 40 years comprises 54%. 41 to 60 years comprises 34%. 61 to 80 years comprises 12% (Mean 1.58 ± 0.70).

Figure 3-2 show distribution of study group according to duration (years) duration of disease 1 to 21 years divided into 3 groups; 1 to 7 years comprises 41%, 8 to 14 years comprises 31%, 15 to 21 years comprises 28% (Mean 1.87 ± 0.82).

The Change in Haematological parameter after Hemodialysis. Table 3-2 show that The mean of RBCs, PCV, HB, WBCs and Neutrophil show significant increase after HD (P.value 0.00).

The mean of MCV and Mixed cells show insignificant increase after HD (P.value 0.59, 0.26 respectively).

The mean of MCH show insignificant decrease after HD (P.value 0.15).

The mean of MCHC show significant decrease after HD (P.value 0.03).

The mean of Plts and Lymphocyte show insignificant increase after HD (P.value 0.09, 0.97 respectively) .

Figure 3-3 show distribution of study group according to Family history, 2% of patients have family history while 98% have no family history (Mean 1.98 ± 0.15).

Table 3-1 Distribution of study group according to sex

Gender	Frequency	Means	S.D	Total
M	62	1.38	0.49	100
F	38			

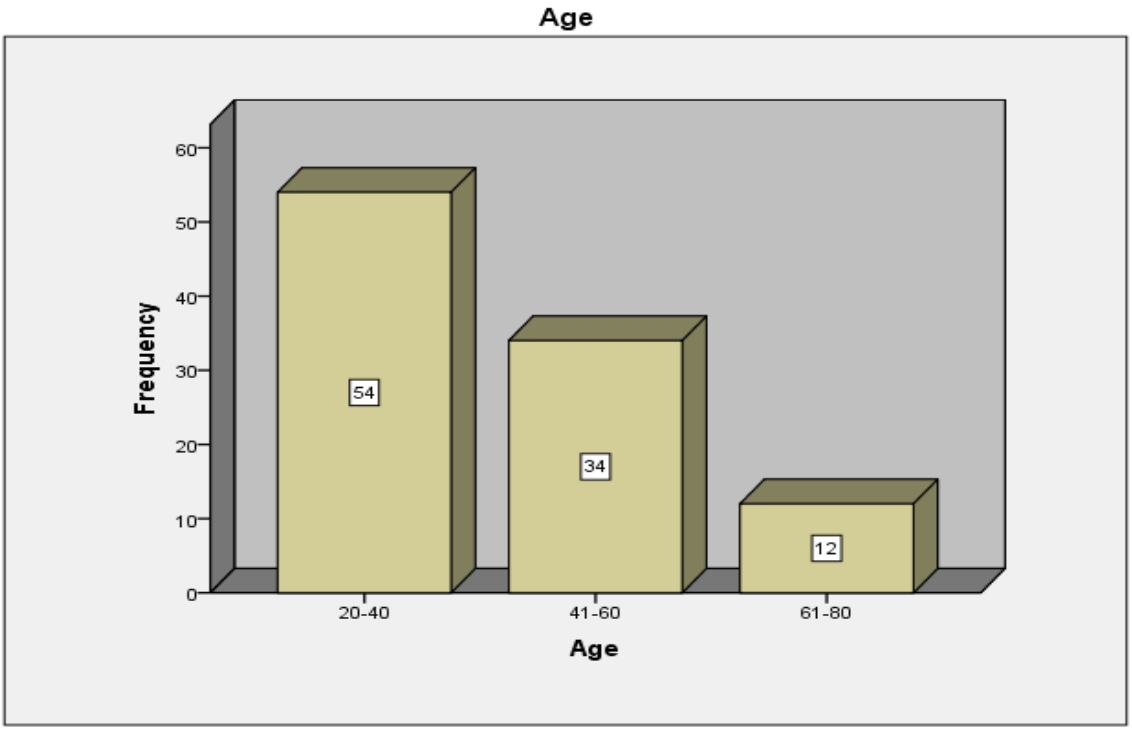

Figure 3-1 Distribution of study group according to Age (years)

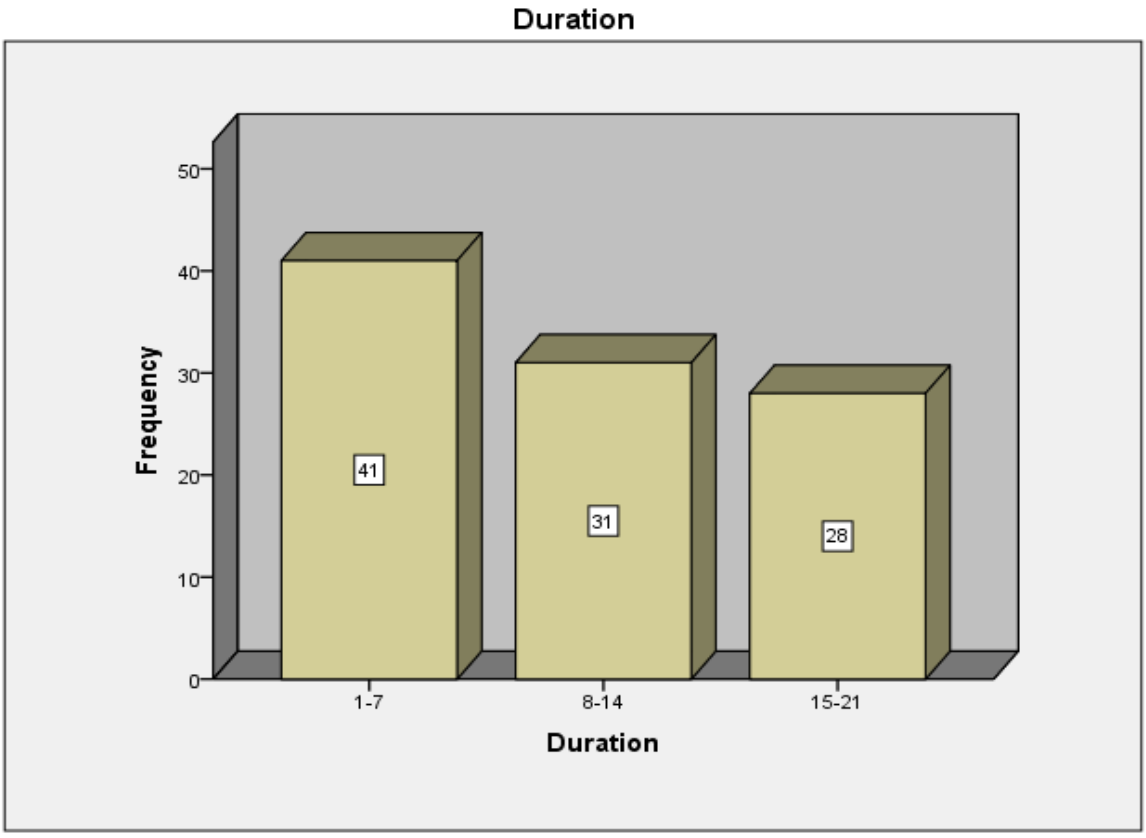

Figure 3-2 Distribution of study group according to duration (years)

Table 3-2 The Change in Haematological parameter after Hemodialysis

Parameters	Mean ± SD N=100	P.value
TWBCs.b	5.0 ±1.5	0.00
TWBCs.a	5.6 ±2.1	
TRBCs.b	3.2 ±0.6	0.00
TRBCs.a	3.4 ±0.7	
HB.b	9.5 ±2.2	0.00
HB.a	10.2 ±2.0	
HCT.b	30.7 ±6.3	0.00
HCT.a	32.5 ±6.6	
MCV.b	92.5 ±9.9	0.59
MCV.a	93.1 ±10.1	
MCH.b	29.7 ±2.0	0.15
MCH.a	29.3 ±3.3	
MCHC.b	31.7 ±1.1	0.03
MCHC.a	31.5 ±1.2	
PLT.b	184 ±68.1	0.09
PLT.a	188 ±74.6	
Lympocyte.b	1.18 ±0.7	0.97
Lympocyte.a	1.19 ±0.8	
Mixed cells.b	0.5 ±0.3	0.26
Mixed cells.a	0.6 ±0.2	
Neutrophil.b	3.3 ±1.2	0.00
Neutrophil.a	3.9 ±1.7	

N= number of subjects

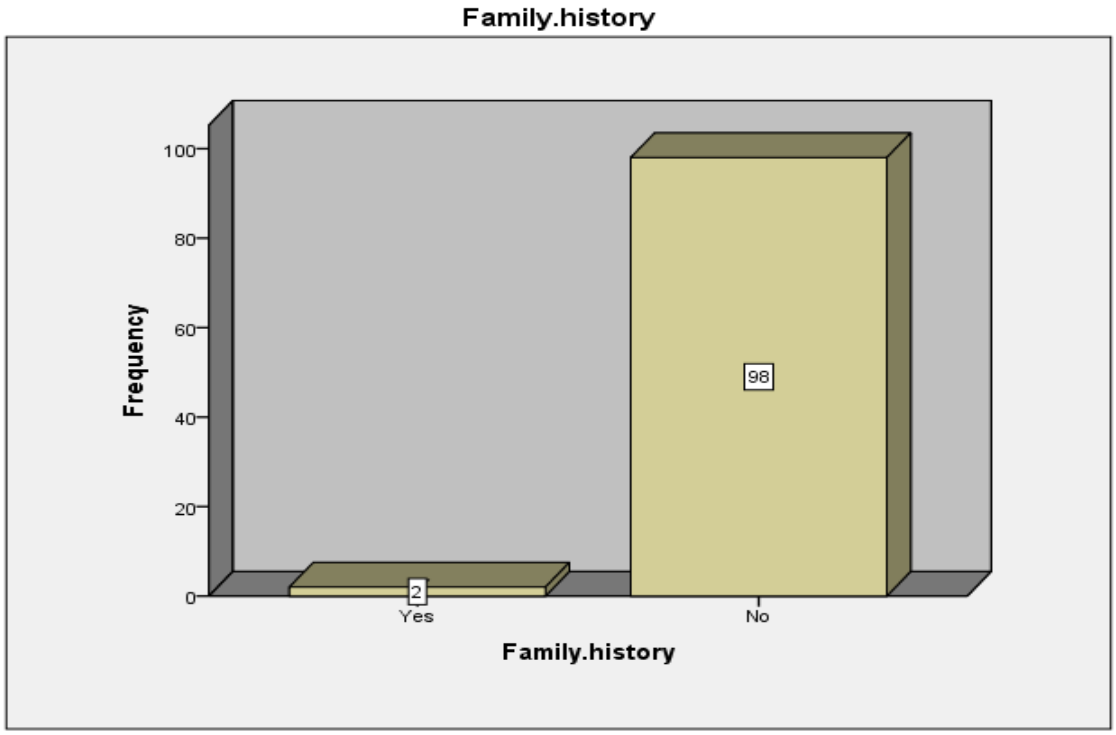

Figure 3-3 Distribution of study group according to Family history

Chapter Four

Discussion, Conclusion and Recommendation

Chapter Four
Discussion, Conclusion, Recommendation

4.1 Discussion

Chronic Renal failure is a major health problem and affects the economic and social status of patients. In Sudan, according to ministry of health records, the prevalence of renal failure is increasing approximately 70 to 140 new patients undergo dialysis each year. This high frequency is thought to be due to epidemic malarial infection, which is well known to cause glomerulonephritis (Pendse, Singh & Zawada 2008).

The result of the study showed that patients with renal diseases on regular HD display various degrees of changes in haematological parameters. The result showed that : -

The mean of WBCs count in patients pre HD were ($5.0 \times 10^3/\mu I \pm 1.5$), and in patients post HD were ($5.6 \times 10^3/\mu I \pm 2.1$), (P.value0.00). this indicates significant increase after HD.

The mean of RBCs count in patients pre HD were ($3.2 \times 10^6/\mu I \pm 0.6$), and in patients post HD were ($3.4 \times 10^6/\mu I \pm 0.7$), (p.value 0.000).this indicates significant increase after HD.

The mean of Hb level in patients pre HD were (9.5 g/dl ±2.2), and in patients post HD were (10.2 g/dl ±2.0), (p.value 0.000). this indicates significant increase after HD.

The mean of PCV level in patients pre HD were (30.7% ±6.3), and in patients post HD were (32.5% ±6.6), (p.value0.000). this indicates significant increase after HD.

This finding is in contrast to the study reported by Mohamed et al. 2008.

The mean of MCV level in patients pre HD were (92.5fl ±9.9), and in patients post HD were (93.1 fl ±10.1), (P.value 0.59). this indicates insignificant increase after HD.

The mean of MCH level in patients pre HD were (29.7pg ±2.0), and in patients post HD were (29.3pg ±3.3), (P.value 0.15). this indicates insignificant decrease after HD.

The mean of MCHC level in patients pre HD were (31.7% ±1.1), and in patients post HD were (31.5% ±1.2), (P.value 0.03). this indicates significant decrease after HD.

The mean of PLTs count in patients pre HD were (184×10^3/µI ±68.1), and in patients post HD were (188×10^3/µI ±74.6), (P.value 0.09). this indicates insignificant increase after HD.

The mean of Lymphocytes count in patients pre HD were (1.18% ±0.7), and in patients post HD were (1.19% ±0.8), (P.value 0.097). this indicates insignificant increase after HD.

The mean of Mixed cells count in patients pre HD were (0.5% ±0.3), and in patients post HD were (0.6% ±0.2), (P.value 0.026). this indicates insignificant increase after HD.

The mean of Neutrophils count in patients pre HD were (3.3% ±1.2), and in patients post HD were (3.9% ±1.7), (P.value 0.00). this indicates significant increase after HD.

In this study TWBCs tend to decrease in HD patients pre dialysis and this is disagree with study of Inagaki *et al*., (2001) Who reported a significant decrease in leukocyte count in patients undergoing HD.

In this study The PLTs tend to increase post HD , and this is disagree with the study of Vickers *et al*., (1998) ,Who assessed there was a small decrease in circulating platelets. also reveal contradictory results researchers have described increase such as study of Mohamed *et al*., (2008) compared the platelets count before and after dialysis sessions. They found that the platelets count increased insignificantly after HD sessions and that the duration of HD did not affect the platelets count the reduction in the various haematological parameters during the HD session may not be attributable entirely to the HD procedure, but rather caused by the supine position and consequent hemodilution caused by redistribution of water from the extra-to intravascular space. [(31)]

The increase of each WBCs. RBCs, PLTs count, Hb, PCV, MCV levels post-HD were explained by the fact that before HD, patients are usually hypervolemic and the values of each RBCs count, Hb, PCV levels are also lower.

The study show that males at greater risk of developing renal failure than females.

4.2 Conclusion

- Hemodialysis cause increase in WBCs, RBCs, Hb, PCV, Neutrophil.
- No significant effectiveness on PLTs, Lymphocytes and Mixed cells
- Red cell indices not affected by HD and remain within normal range
- Male more affected than female as well as elder people.

4.3 Recommendation

1) Patients on haemodialysis should be investigated before and after dialysis to control the risk of anemia.
2) More studies should be done on iron status, platelets function and coagulation factors on haemodialysis patients
3) Genetic studies should be done to detect relationship between renal diseases and family history.
4) Increase rates of awareness among renal failure patients how to behave with their disease and how to protect themselves from complications.
5) Further studies should be done with more number of patients and more haematological parameters in addition to haemostatic parameters.

References and Appendices

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Appendices(1): Questionnaire

بسم الله الرحيم الرحمن

Sudan University Of Science And Technology

Collage Of Medical Laboratories Sciences

Department Of Haematology

Questionnaire about Changes of Some Haematological Parameters in Chronic Renal Failure patients after Haemodialysis

No:.....

Sex:.....

Age:..... years

Duration of hemodialysis:.....years

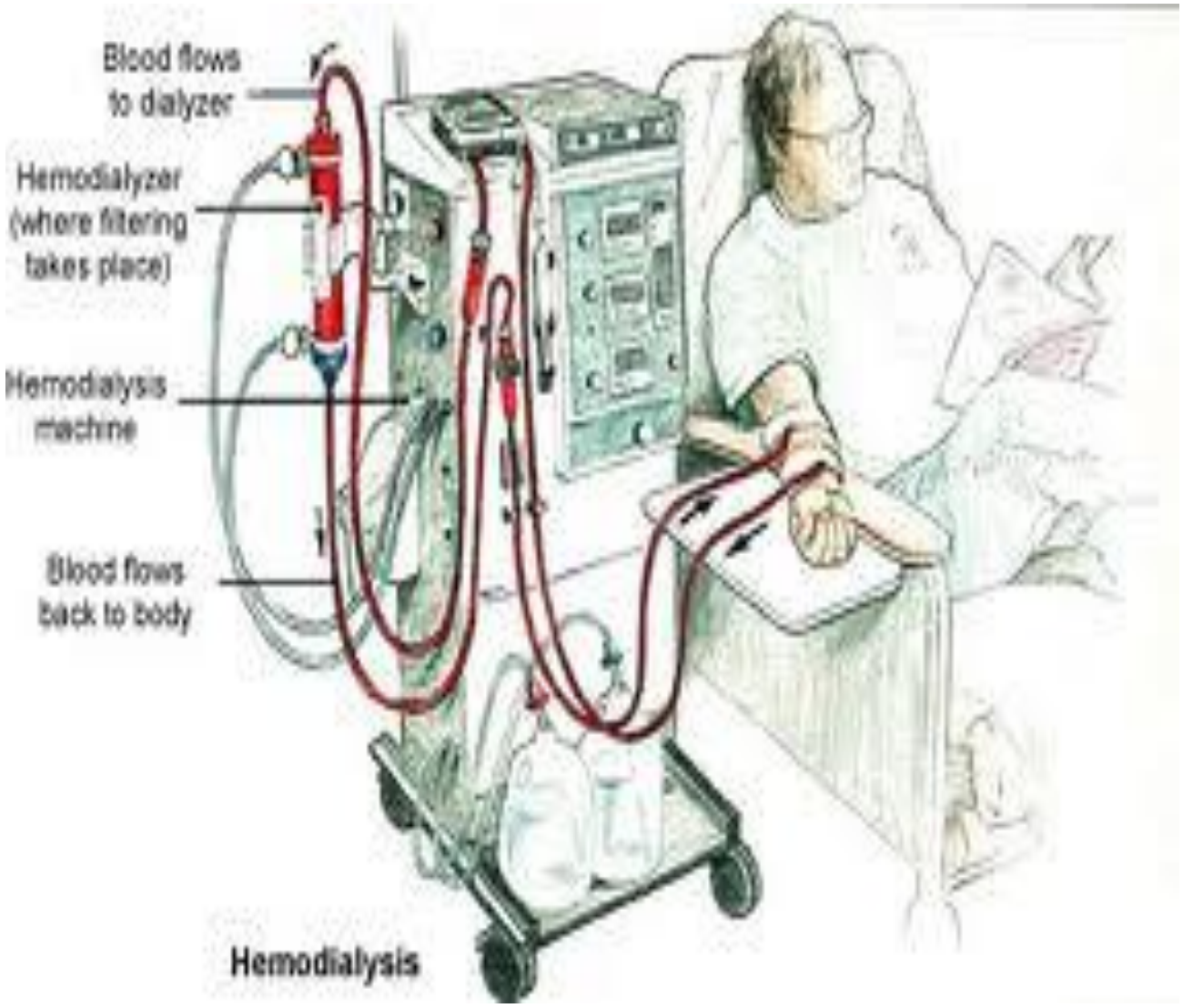
Causes:-
Family history: Yes ( ) No( )

Lab. Results:-

TWBCs..... /µI
TRBCs..... /µI
HB ..... g/dl
HCT..... %
MCV..... fl
MCH..... pg
MCHC..... %
PLT..... µI
Lymphocytes..... %
Eosinophils..... %
Neutrophils..... %

Date:.....
Sig:.....

Appendix(1): Images



Hemodialysis: The patient's blood is pumped through the blood compartment of a dialyzer