Sudan University of Science and Technology

Faculty of Medical Laboratory Sciences

College of Graduate Studies and Scientific Research

Measurements of Platelets Activity in End Stage Renal Disease Patients in Al-Ribat Hospital – Sudan

From March to May 2014

قياس نشاط الصفائح الدموية لمرضى الفشل الكلوي المزمن في مستشفى الرباط بالسودان في الفترة من مارس إلى مايو

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بسم الله الرحمن الرحيم

الآية القرآنية

Dedication

I dedicate this research :

To my parents who guided me through my early days of life and inspired me to take the road of ever and continuous scientific learning.

To my sister, my brother, and all family members whom help me and being strongly supportive, without their encouragement, this work could not have been completed.

To my friends for being a killer of strength for me when i was weak.

To everyone mentioned directly and indirectly were necessary threads woven in to the fabric of my life.

Namariq

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Abstract

End stage renal disease (ESRD) is a major health problem in Sudan which is stage five of chronic renal failure leading to many complications. Patients need to dialysis as a treatment to survive.

Haemodialysis leading to platelets abnormalities (chronic platelets activation) while the platelets play a major role in haemostasis and they are natural source of growth factors which are play a significant role in repair and regeneration of connective tissues in healthy individuals.

This analytical descriptive cross sectional case-control study was conducted in Al-Ribat Hospital during the period from March to May 2014.The aim of the study was to determine the changes in platelets activity by measuring platelets count and platelets indices (MPV,PDW, and P-LCR) among Sudanese End Stage Renal Disease Patients attending Al-Ribat Hospital in the renal dialysis unit.

Eighty four blood samples were collected from the patients with end stage renal disease (ESRD) and fifty blood samples were collected from apparently healthy (controls) individuals. 2.5 ml of blood was collected from each patient and control , automated haematological analyzer (Sysmex KX-21N) was used to measure platelets count, MPV, PDW, P-LCR. Informative data of age and gender , were collected using a questionnaire during the period of March to May 2014.

Firstly, the Age in this study was divided to three groups (20-40), (40-60), (60-80) years.

The study results revealed that platelets count was significant decreased and MPV was significant elevated on the end stage renal disease patients. The mean value of patients with end stage renal disease of platelets count (185.25± SD) and mean value of MPV (13.02± SD) respectively .The mean value of Platelets count (274.86± SD) and mean value of MPV (9.36± SD) in control group respectively.

The study concluded that there was a significant effectiveness of platelets count and MPV on ESRD patients , the other parameters (PDW and P-LCR) have no significant effectiveness on ESRD patients.

Also, no significant effectiveness of gender on platelets count, MPV, PDW, and P-LCR of end stage renal disease patients, and there was no significant effectiveness of age on PDW and P-LCR of end stage renal disease patients but there was an age effectiveness on platelets count (inverse relation) and also there was an age effectiveness on MPV (direct relation) of end stage renal disease patients.

مستخلص البحث

أجريت هذه الدراسة التحليلية الوصفية (حالة وحالة ضابطة) بمستشفى الرباط الجامعي خلال الفترة من مايو إلى مارس 2014، حيث هدفت الدراسة إلى تحديد التغيرات في نشاط الصفائح الدموية بين مرضى الحالة الأخيرة من الفشل الكلوي المزمن بالسودان الذين يترددون على مستشفى الرباط الجامعي في وحدة غسيل الكلى، وذلك عن طريق قياس الصفائح الدموية ومؤشراتها (متوسط حجم الصفيحات ، عرض توزيع الصفائح الدموية ، نسبة الصفائح الدموية كبيرةالخلية) نظرا لأن المرض الكلوي يشكل مشكلة صحية رئيسة في السودان، ومرحلة خامسة في حالة الفشل الكلوي المزمن الذي بدوره قد يؤدي إلى مضاعفات عديدة.

المرضى بحوجة إلى الغسيل الكلوي كعلاج للبقاء على قيد الحياة ، الغسيل الدموي يؤدي إلى تشوهات الصفائح الدموية (تنشيط الصفائح الدموية المزمنة) في حين أن للصفائح الدموية دوراً" رئيسياً" في عمليات تخثر الدم ومصدر طبيعي لعوامل النمو التي تلعب دوراً" هاماً في اصلاح وتجديد الأنسجة الضامة في الأشخاص الأصحاء.

تم أخذ (84)عينة دم من المرضى الذين يعانون من الحالة الأخيرة من الفشل الكلوي المزمن و (50)عينة دم تم أخذها من أشخاص أصحاء كمجموعة ضابطة وجمع (2.5) مل من دم كل من المرضى والأصحاء واستعمل جهازالمحلل الأوتوماتيكي (Sysmex KX-21N) وذلك لقياس عدد الصفائح الدموية وقياس مؤشراتها (متوسط حجم الصفيحات ، عرض توزيع الصفائح الدموية ، نسبة الصفائح الدموية كبيرةالخلية). وقد تم جمعها من خلال استبانة المعلومات حول العمر والجنس وفترة المرض في الفترة الزمنية المحددة.

قسمت الدراسة مرحليا" وفق الأعمار إلى ثلاث مجموعات أولية (40-20)، (60-40)، (80-60) سنة .

أوضحت نتيجة الدراسة أن عدد الصفائح الدموية قد سجل انخفاضا" كبيرا"، وأن متوسط حجم الصفيحات سجل قيما" ذات أهمية مرتفعة لمرضى الحالة الأخيرة من الفشل الكلوي المزمن.

أشارت نتيجة الدراسة إلى أن متوسط قيمة عدد الصفائح الدموية كان (SD± 185.25) لمرضى الحالة الأخيرة من الفشل الكلوي المزمن ومتوسط قيمة المتوسط لحجم الصفيحات كان (SD±13.02) على التوالي وأن متوسط قيمة عدد الصفائح الدموية كان (SD±274.86) ومتوسط قيمة المتوسط لحجم الصفيحات كان (±SD 9.36) للأصحاء كمجموعة ضابطة على التوالي. وأن متوسط عرض توزيع الصفائح الدموية كان (SD±12.09) لمرضى الحالة الأخيرة من الفشل الكلوي المزمن ومتوسط نسبة الصفائح الدموية كبيرة الخلية كان (SD±22.39) على التوالي، وأن متوسط عرض توزيع الصفائح الدموية كان (SD±11.66) وأن متوسط نسبة الصفائح الدموية كبيرة الخلية كان (SD±20.94) للأصحاء كمجموعة ضابطة على التوالي..

وقد تلاحظ من خلال الدراسة أنه يوجد اختلاف مؤثر لقيم عدد الصفائح الدموية ومتوسط حجم الصفيحات لمرضى الحالة الأخيرة من الفشل الكلوي المزمن، وأن قيم المؤشرات الأخرى لم تسجل اختلافا مؤثرا على هؤلاء المرضى .

وقد استنتجت الدراسة أيضا أنه لايوجد اختلاف مؤثر في كل القيم المأخوذة للقياس تبعاً" للجنس لمرضى الحالة الأخيرة من الفشل الكلوي المزمن في وحدة غسيل الكلى. وقد وجد أنه لاتوجد علاقة بين عمر المريض واختلاف قيم عرض توزيع الصفائح الدموية ،و نسبة الصفائح الدموية كبيرة الخلية، إلا أنه وجد تأثير للعمر على عدد الصفائح الدموية (علاقة عكسية) ووجد ايضا تأثير للعمر على قيم متوسط حجم الصفيحات (علاقة طردية) لمرضى الحالة الأخيرة من الفشل الكلوي المزمن في وحدة غسيل الكلى.

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List of contents

List of abbreviations

ARF: Acute Renal Failure

CKD: Chronic Kidney Disease

CSF: Colony Stimulating Factor

EDTA: Ethylene Diamine Tetra Acetic Acid

ESRD: End Stage Renal Disease

fl: femtolitre

G-CSF: Granulocyte Colony Stimulating Factor

GFR: Glomerular Filtration Rate

GM-CSF: Granulocyte -Macrophage Colony Stimulating Factor

HD: Haemodialysis

HIV: Human Immunodeficiency Virus

HSCs: Haematopoietic Stem Cells

LD: Lower Discriminator

M-CSF: Macrophage- Colony Stimulating Factor

MPV: Mean Platelet Volume

Pct: Platelet crit

PDGF: Platelets Derived Growth Factor

PDW: Platelet Distribution Width

P-LCR: Platelet Large Cell Ratio

RBCs: Red Blood Cells

SCCS: Surface Connected Canalicular System

SCF: Stem Cell Factor

TAR: Thrombocytopenia with Absent Radii

TGF beta: Transforming Growth Factor-beta

THPO: Thrombopoietin

TTP: Thrombotic Thrombocytopenic Purpura

UD: Upper Discriminator

□**l:** Microlitre

□**m:** Micrometre

List of Tables

List of figures

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1. Introduction and literature review

1.1. Introduction:

1.1.1. Blood components and function:

Blood is specialized bodily fluid circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and the venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled. The blood also regulates the body temperature and the body PH with other coagulation, messenger, hydraulic and immunological functions (Dicken and Scott, 2004).

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma is constitutes $55^{\%}$ of blood fluid, is mostly water (91% by volume) , and contains dissolved proteins , glucose , mineral ions , hormones, carbon dioxide (plasma being the main medium for excretory product transportation),platelets and blood cells themselves .The blood cells present in blood are mainly red blood cells and white blood cells including leukocytes , and platelets (Dicken and Scott, 2004).

1.2. Literature Review:

1.2.1. Haematopoiesis:

Haematopoiesis is the formation of blood cellular components. All cellular components are derived from haemopoietic stem cell in healthy adult person, approximately 10^{11}-10^{12} new blood cells are

produced daily in order to maintain steady state levels in the peripheral circulation .

This process mainly include erythropoiesis, leukopoiesis and megakaryopoieis (Alenzi *et al*.,2009).

1.2.1.1. Haemopoietic stem cells (HSCs):

Haemopoietic stem cells reside in the medulla of the bone marrow and have the unique ability to give rise to all of the different mature blood cells types.

HSCs are self renewing: when they proliferate, at least some of their daughter cells remain as HSCs, so the pool of stem cells does not become depleted. The other daughters of HSCs (myeloid and lymphoid progenitor cells), however can each commit to any of the alternative differentiation pathways that lead to the production of one or more specific types of blood cells, but cannot self- renew. This is one of vital process in the body (Alenzi *et al*., 2009).

1.2.1.2. Location of haematopoiesis:

In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands. As developing processes, blood formation occur in the spleen ,liver and lymph node. When bone marrow develops, it eventually assumes the task of forming of the blood cells for entire organism.

However, maturation, activation and some proliferation of lymphoid cells occurs in secondary lymphoid organs (spleen, thymus and lymph nodes). In children, haematopoiesis occurs in the marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae and sternum. In some vertebrates ,

haematopoiesis can occur wherever there is a loose stroma of connective tissue and slow blood supply, such as gut , spleen, kidneys or ovaries. In some cases the liver, thymus and spleen resume their haematopietic function, if necessary. This is called extra-medullary haematopoiesis. It may cause these organs to increase in size substantially. During fetal development, since bones and thus the bone marrow, develop later, the liver functions as the main haemopoietic organ. Therefore, the liver is enlarged during development (Alenzi *et al*., 2009).

1.2.1.3. Maturation of haematopoiesis:

As stem cell matures it undergoes changes in gene expression that limit the cell types that it can become and moves it closer to a specific cell type. These changes can often be tracked by monitoring the presence of proteins on the surface of the cell. Each successive change moves the cell closer to the final cell type and further limits its potential to become a different cell type (Alenzi *et al*., 2009).

1.2.1.4. Determination of Haematopoiesis:

Cell determination appears to be dictated by the location of differentiation. For instance, the thymus provides an ideal environment for thymocytes to differentiate into a variety of different functional T cells. For the stem cells and other un differentiated blood cells in the bone marrow, the determination is generally explained by the determinism theory of haematopoiesis, saying that colony stimulating factors and other factors of the haematopoietic microenvironment determine the cells to follow a certain path of cell differentiation. This is the classical way of scribing haematopoiesis. In fact, however, it is not really true. The ability of the bone marrow

to regulate the quantity of different cell types to be produced is more accurately explained by a stochastic theory. Un differentiated blood cells are determined to specific cell types by randomness. The haematopoietic microenvironment prevails upon some of the cells to survive and some, on the other hand, to perform apoptosis and die. By regulating this balance between different cells types, the bone marrow can alter the quantity of different cells to ultimately be produced (Alenzi *et al*., 2009).

1.2.1.5. Haematopoietic growth factors:

Red and white blood cells production is regulated with great precision in healthy humans and the production of granulocytes is rapidly increased during infection. The proliferation and self-renewal of these cells depend on stem cell factor (SCF). Glycoprotein growth factors regulate the proliferation and maturation of the cells that enter the blood from the marrow, and cause cells in one or more committed cell lines to proliferate and mature. Three more factors that stimulate the production of committed stem cells are called are colony-stimulating factors (CSFs) and include granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF) and macrophage-CSF (M-CSF). These stimulate much granulocyte formation and are active on either progenitor cells or end product cells. Erythropoietin is required a myeloid progenitor cell to become an erythrocyte.

On the other hand, thrombopoietin makes myeloid progenitor cells differentiate to megakaryocytes (thrombocyte-forming cells)(Alenzi *et al*., 2009).

1.2.1.6. Transcription factors:

Growth factors initiate signal transduction pathways, altering transcription factors, that in turn activate genes that determine the differentiation of blood cells (Alenzi *et al*., 2009).

1.2.1.7. Haematopoiesis classification:

1.2.1.7.1. Erythropoiesis:

Erythropoiesis is the process by which red blood cells (erythrocyte) are produced. It is stimulated by decreased O^2 delivery to the kidneys, which then secrete the erythropoietin hormone. This process occurs either in haemopoietic tissues or extramedullary. In the process of RBCs maturation, a cell undergoes a series of differentiation (Alenzi *et al*., 2009).

1.2.1.7.2. Leukopoiesis:

Leukopoiesis is a form of haematopoiesis in which white blood cells (WBCs or leukocytes) are formed in bone marrow located in bones in adults and haematopoietic organs in the fetus. White blood cells , indeed all blood cells, are formed from differentiation of pluripotent haematopoietic stem cells which give rise to several cell lines with more limited differentiation potential .

These immediate cell lines, or colonies, are proginitors of red blood cells (erythrocytes), platelets (megakaryocytes), and the two main groups of WBCs, myelocytes and lymphocytes (Pike ., *et al* 2014).

1.2.1.7.2.1. Lymphopoiesis:

Lymphopoiesis refers to the generation of lymphocytes, one of the five different types of WBCs, and is also more formally called lymphoid haematopoiesis(Pike ., *et al* 2014).

1.2.1.7.2.2. Myelopoiesis:

Myelopoiesis is the regulated formation of myeloid cells, including eosinophilic granulocytes, basophilic granulocytes, neutrophilic granulocytes and monocytes. In haematology,myelopoiesis is the production of blood cells in the bone marrow. The myeloid progenitor can differentiate in the bone marrow into granulocytes, macrophages (mature monocytes), mast cells (whose blood-bone progenitor is not well defined), and dendritic cells of the innate immune system. The granulocytes also called polymorphonuclear leukocytes because of their oddly shaped nuclei, give rise to three short lived cell types including eosinophils, basophils and neutrophils. Agranulocyte differentiates into a distinct cell type by a process called granulopoiesis. In this process it first transforms from a common myeloblast (myeloid progenitor) to a common promyelocyte. This promyelocyte gives rise to a unique myelocyte that for the first time can be classified as a eosinophil, basophil, or neutrophil progenitor based on the histological staining affinity (eosinophilic, basophilic,or neutral granules). The unique myelocyte next differentiates into a metamyelocyte and then a band cell,with a "c" shaped nucleus, before becoming a mature eosinophil, basophil,or neutrophil . Macrophagescome from monoblast progenitors that differentiate into promonocytes, which mature into monocyte. Monocytes eventually enter the tissues and become macrophages (Pike ., *et al* 2014).

1.2.1.7.3. Megakaryopoiesis:

The process of megakaryopoiesis is complex, with the potential for regulation at multiple stages. Megakaryocytes are derived from the haemopoietic stem cell through successive lineage commitment steps,

and they undergo a unique maturation process that includes polyploidization, development of an extensive internal demarcation membrane system and finally formation of pro-platelet processes . Platelets are shed from these processes into vascular sinusoids within the bone marrow. Megakaryocytic differentiation is regulated both positively and negatively by transcription factors and cytokine signalling . Thrombopoietin is the most important haemopoietic cytokine for platelet production. Clinically, acquired and inherited mutations affecting megakaryocytic transcription factors and thrombopoietin signalling have been identified in disorders ofthrombocytopenia and thrombocytosis (Schulman *etal*., 1960).

1.2.2. Platelets:

1.2.2.1. Platelets definition and their role:

Platelets or thrombocytes are small. Regularly-shaped clear cell fragments (i.e. cells that do not have a nucleus containing DNA), 2-3 μ_m in diameter, which are derived from fragmentation of precursor megakaryocytes cytoplasm (50 μ_m).

The megakaryocyte matures by endomytotic synchronous nuclear replication, at variable stages in development most commonly at eight nucleus stage, the cytoplasm become granular and platelets are liberated. Each megakaryocyte is responsible for the production of about 4000 platelets. The average life span of a platelet is normally just 5 to9days. Platelets play a fundamental role in haemostasis and are a natural source of growth factors such as platelets derived growth factors (PDGF) and TGF beta which are play a significant role in the repair and regeneration of connective tissues with others healing-associated growth factors (Laki ,1972).

They circulate in the blood of mammals and involved in haemostasis, leading to formation of the blood clots. The major regulator of platelets production is thrombopoietin hormone (THPO) which is produced by liver and kidney. Normal platelets count is about $275*10^9$/l (Lewis *et al.*,2001).

If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) orthrombotic thrombocytopenicpurpura (TTP) that typically causesthrombocytosis, or clots, instead of bleeding(Laki ,1972).

1.2.2.2. Platelets structural and functional anatomy:

Under light microscopy, platelet appear as small, a nucleate (that is lacking of nucleus) fragments with occasional reddish granules, measuring approximately 2µm in diameter with a volume of approximately 8fl. By scanning electron microscopy, circulating platelets appear as flat discs with smooth counters, rare spiny filopodia, and random openings of channel system, the surface-connected canalicular system (SCCS),which investigates throughout the platelet and is conduit by which granular contents exocyte after stimulation. They are roughly discoid in shape and contain

cytoplasmic organelles, sytoskeletal elements, invaginating open-canalicular membrane systems, and platelet specific granules called alpha and dense granules. Platelets have numerous intrinsic glycoproteins attached to the outersurface of their plasma membrane that are receptors for such ligands as fibrinogen, collagen, thrombin and thrombospondin to vonwillebrand and fibronectin (White 1971).

In describing detailed platelets anatomy, most information is derived from transmission electron microscopy, and platelets structure is classified in to four general areas (fig.1.1).

- The platelet surface.
- The membranous structure.
- The cytoskeleton (sol-gel-zone).
- The granules (Greer *et al.*,2004).

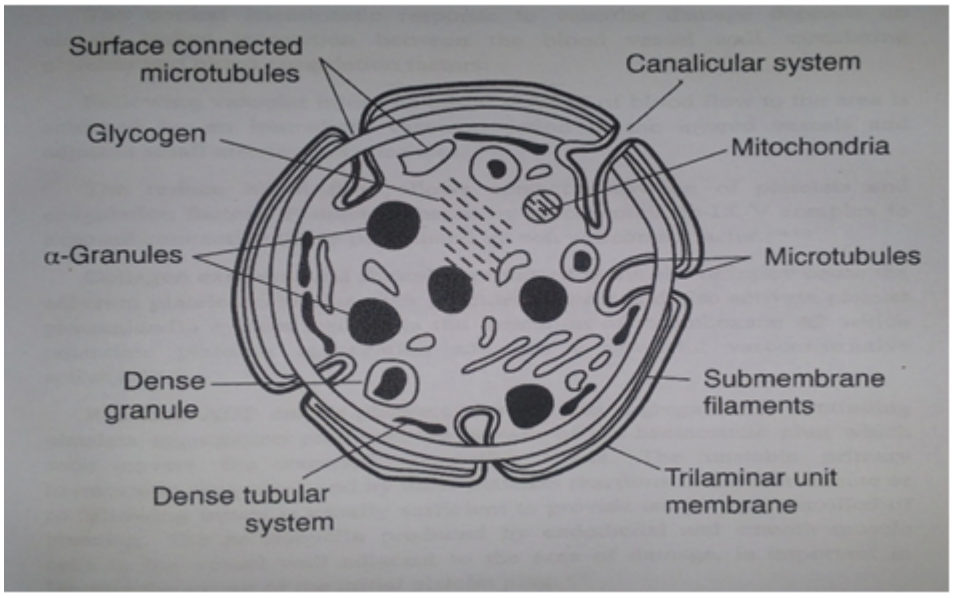

Figure (1.1): platelet structure, **(** Safi 2001).

1.2.2.3. Platelets count:

Platelets count is a test to measure how many platelets you have in your blood, either by manual and automated methods. The number of platelets in your blood can be affected by many diseases. Platelets may be counted to monitor or diagnose diseases, or identify the cause of excess bleeding. Normal results 150,000 to 400,000 platelets per microliter (David and Dugdale, 2012).

The manual method using counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) to calculate the number of cells per liter of blood. The advantages of this method is that the automated analyzers are not reliable at counting abnormal cells .That is, cells that are not present in normal patients and are only seen in the peripheral blood with certain haematological conditions. Manual counting is subject to sampling errors because so few cells are counted compared with automated analysis. The automated method give faster, reliable results but some normal patient's platelets will clump in EDTA anticoagulated blood, which give false low platelets count. The technician view the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets (David and Dugdale, 2012).

1. 2.3. Automated technique:

1.1.3.1. Principle of automated technique:

The blood cells are counted in system based on either Aperture impedance (voltage-pulse) or light-scattering technology (electro-optical counting). It begin as simple counting instrument and has developed in to very sophisticated analyzers capable of producing

simultaneous count of all three blood cells type, haemoglobin values and red cell indices. After that the instrument has been developed that they can produce data related to variation in cell size, as well as being able to produce data on differential white cell count .The most commonly used type in haematology laboratories is the impedance cell counters, firstly describe by Wallace coulter in 1956 (coulter W.H) (Suttor ,1995).

1.2.3.1.1. Aperture impedance technology:

The impedance principle depend on the fact that red cells are poor conductors of electricity while certain diluents are good conductors, this difference form the basis of the counting system used in Beckman-coulter, Sysmex , Abbot, Roche, and a number of other instruments. For cell counting, blood is highly diluted in a buffered electrolyte solution .The rate of this diluted sample is controlled by a mercury siphon (as in original coulter system) or by displacement of tightly fitting piston. This measures the volume of sample passing through an aperture tube of specified dimension, by constant source electricity direct current is maintained between two electrodes, one in the sample beaker or the chamber surrounding the aperture tube, and another inside the aperture tube. As a blood cell is carried through the aperture, it displaces some of the conducting fluid and increased the electrical resistance; this produce change in potential between the electrodes, the height of the pulses produced indicates the volume of the cells passing through. The pulses can be displayed on an oscillograph screen. The pulses are led to a threshold circuit provided the minimal pulse height which will count (Suttor, 1995).

The impedance principle is still the most commonly used method even through, it is unable to provide consistently accurate platelets

counting in thrombocytopenia, this is due to progressive loss of linearity, an increase influence of background noise and sample debris. The main problem is that platelet can't be discriminated from other cellular particles and precipitate that cause similar signals, the main non platelets particles include fragments of RBCs, WBCs, especially myeloblast (M2, M5) and lipid or protein aggregates. In addition, platelet may not be counted if they are too big or too small to fit into the window reserved for normal platelets size (Dickerhoff *et al.*,1995,Kmoiyama *et al.*,1997,Throm ,1990).

1.2.3.1.2. Light scattering method (electro-optical counting):

The other method for counting depend on light scattering, these work on a similar principle reverse dark field microscope, the blood cells counted by means of electro-optical detector (Koht *et al.*, 1996).

The diluted cells flow through the aperture so that the cells pass, in single file. In front of a light source, light is scattered by the cell passing through light beam, scatter light is detected by photomultipliers which converts it in to electrical impulses which accumulated and counted (Nissshiyama *et al*., 2005).

Electro-optical detectors are employed for red cell sizing and counting in Bayer-technician system, and for white cell differential counting in a number of other instruments. The optical method using two dimensions of light scatter are less prone to these problems but there are still some circumstances in which optical analyses may be erroneous. About $70^{\%}$ of sample with an abnormal platelet distribution width showed discrepant value between automated and manual visual counting (Nissshiyama *et al*., 2005).

1.2.3.2. Disadvantages of automation technique:

Because the instruments that depend on impedance principle are set to count only particles with proper size range for exclusion limit. Any cell or material larger or smaller than size exclusion limit will not be counted. Any object in the proper size range is counted, however, even if it is not a platelet. Electronic counting instruments some time produce artificially wrong platelets count. It may arise from an increase or decrease in counting time or volume, if the platelet and other blood cells pass through the counter at the same time, the instrument will not count the large cell because of the size exclusion limit, which will cause the instrument to accidentally miss the platelets. Clumps of the platelets will not be counted because clumps exceed the upper size exclusion limit for platelets. Re-circulation of cells and recounting, presence of non platelet particle called pseudo platelet such as bubble and another extraneous particle. If the sample has high white blood cell count, electronic counting may yield an unusually low platelet count because white blood cells may filter out some of platelets before the sample counted. On the other hand, if the red cells in the sample have burst, their fragments will be falsely counted as platelets (Baker *etal*., 1985).

To some extent most of these errors had been limited in automated counters by adjusting volume and time of counting using higher dilutions, filter and sweep flow of counted cells eliminated much of these errors so that automated counters can give more reliable results than manual techniques (Suttor 1995, Frank *et al* .,1989, Chanarian *et al* .,1989,Brown,1993).

1.2.4. Platelets parameters:

1.2.4.1. Platelets count:

1.2.4.1.1. Causes of increase platelet counts:

- Inflammatory disorders.
- Myeloproliferative states.
- Acute blood loss.
- Haemolytic anaemias.
- Carcinomatosis.
- Status post-splenoctomy.
- Exercise.

1.2.4.1.2. Causes of decreased platelets count:

- decreased platelet production: production defect such as:

- Wiskott-Aldrich syndrome.

- May-Hegglin anomaly.

- Bernard-Soulier syndrome.

- Chediak-Higashi anomaly.

- Fanconi's anaemia.

- A plastic anaemia.

- Bone marrow replacement.

- Megaloblastic and severe iron deficiency anaemias.

- Uraemia.

- Increased platelet destruction or consumption:

- Consumption defects with autoimmune thrombocytopenias.

- Disseminated Intravascular Coagulation.

- Thrombotic Thrombocytopenic purpura.

- Increased splenic sequestration (capturing of circulating platelets in the spleen):

-Hypersplenism (Siamak *et al.*,2014).

1.2.4.2. Mean Platelet Volume (MPV):

1.2.4.2.1. Value of MPV:

The mean platelet volume is an indication of platelet size, and the most extensively studies platelet activation markers. It is one of platelets indices measured by haematology analyzers (Gurney *et al.*, 2002,Coban *et al.*,2005).Normal MPV ranges are approximately 7 to 11 fl (Corooran *et al.*,2002).

The mean platelet volume can be an indication of platelet turnover because younger platelets tend to be larger (Taril *et al.*, 2007).

A spectrum of platelet size is seen in patient with rapid turnover (Corooran *etal*., 2002).

MPV is recently taken as a determinant for platelet function, as it is positively associated with platelets reactivity function (Bath *et al*., 2004).

Elevated MPV levels have been identified as independent risk factor for myocardial infarction in patient with coronary heart disease and for death or recurrent vascular event after myocardial infarction.

Moreover, increased platelets size has been reported in patient with vascular risk factor such as diabetes, and smoking. Previous studies have demonstrated higher levels of MPV in patient with acute ischemic stroke than in control subject. In contrast, data regarding the association between MPV and stroke severity or stroke outcome have been controversial (Greisem egger *et al* .,2004).

1.2.4.2.2. Causes of raised MPV:

- Idiopathic thrombocytopenic purpura.
- Bernard-Soulier disease.
- May -Hegglin anomaly (Ishii *et al* .,1990).

1.2.4.2.3. Causes of low MPV:

- A plastic anaemia.

- Wiskott-Aldrich syndrome.

-Thrombocytopenia with absent radii (TAR).

-Storage pool disease (Tschope *et al*.,1991).

- Pernicious anaemia(Lewis *et al*., 2001).

* Calculation: MPV (fl) = $\frac{pct(\%)}{PLT(*10^3/\mu L)}$

1.2.4.3. Platelet distribution width (PDW):

The measure of platelet anisocytosis and the platelet crit which is the product of MPV and platelet count , by analog with the haematocrit may be seen as indicative of the volume of circulating platelets in a unit volume of blood (Lewis *et al*., 2001).

MPV and PDW are investigated as prospective platelet activation markers However, not all haematology analyzers examine these indices. Normal PDW is approximately 9-13 fl (Boss *et a.,* 2007, Van Cott *et al.,*2005).

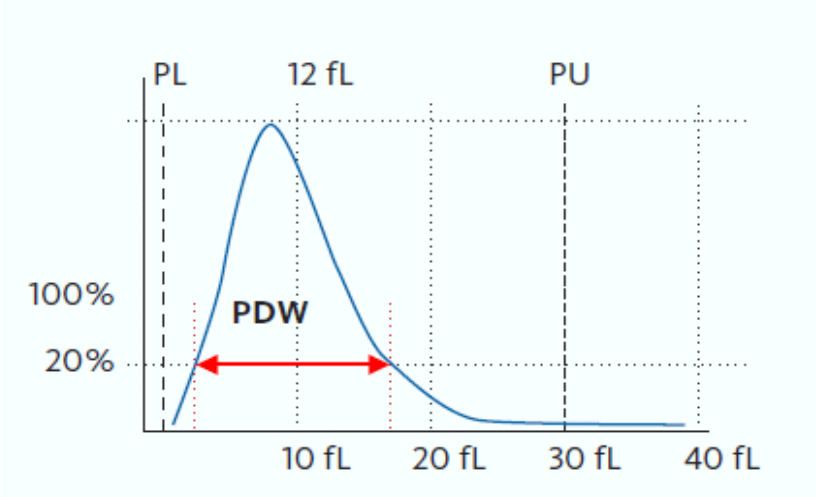

Figure (1.2): indicate display of PDW

1.2.4.3.1. Causes of increased PDW:

- Essential thrombocythaemia.
- Polycythemia rupra vera.
- Chronic granulocytic leukaemia.
- Myelofibrosis.
- Pernicious anaemia (Suttor ,1995).

PDW is normal in normal subject and those with reactive thrombocytosis. (Suttor ,1995).

1.2.4.4. Platelet large cell ratio (P-LCR):

The P-LCR indicates the percentage of large platelets with a volume >12 fl. Aside from the two flexible discriminators which delimit the volume distribution curve, there is additionally a fixed discriminator at 12 fl (Fig. 1.3). The share of platelets >12 fl in the total platelet number is presented in%. The standard range is 15–35%. An increase of the parameter may be an indication for platelet aggregates, microerythrocytes and giant platelets (Sysmex,2011).

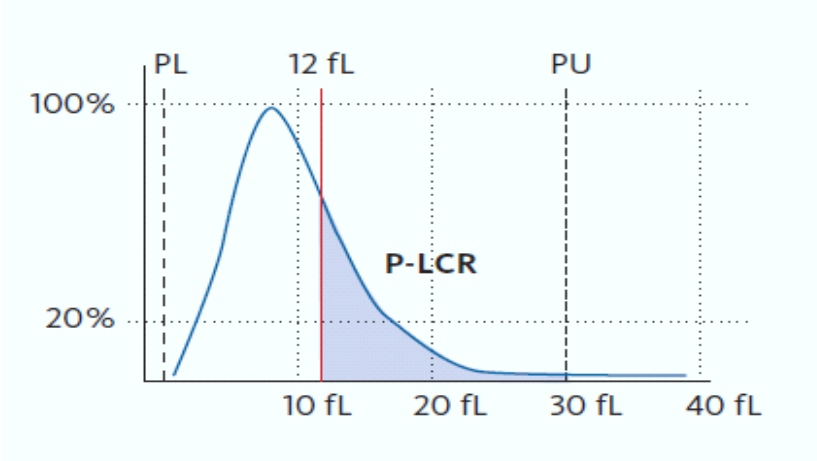

Figure (1.3): Indicate display of P-LCR.

1.2.4.5. Pct (platelet crit):

The platelet crit is equivalent to the sum of platelet impulses which are individually detected by means of the impedance measurement principle and thus it is the equivalent to the haematocrit of the red blood cells (Sysmex,2011).

1.2.4.6. Platelets discriminator:

As the platelet lower discriminator and upper discriminator, the optimum position 2-6 fl and 12-30 fl, respectively are automatically determined by microcomputer. Platelets are calculated from the particles counted between this lower discriminator and upper discriminator (approx 2-30 fl) (Sysmex Corporation,1999).

1.2.4.7. Platelet histogram:

Platelet histogram is analyzed using three discriminators: two discriminators (LD) and(UD) determined automatically between 2-6fl, and between 12-30 fl, respectively and fixed discriminator at12 fl. Regarding platelet histogram, check is made to see that there are no relative frequency errors at discriminators (LD) and (UD), distribution width error, and there is a single peak (Sysmex Corporation, 1999).

1.2.4.8. Platelet Histogram Error Flag:

When the platelet histogram is not normal, histogram error flag is added to parameters of analysis value. Those histogram error flag-used with the parameter analysis unit mounted, are listed in the order of higher priority, when two or more flag are applicable to parameter, the highest priority flag is used (Sysmex Corporation,1999).

As follows:

PL: Relative frequency for LOWER discriminator (LD) exceeds the range, probable is the effect of noise and etc.

PU: Relative frequency for UPPER discriminator (UD) exceeds the range of platelets agglutination, noise interference, and etc.

MP: Two or more peaks in histogram.

DW: Particle distribution width error for 20% frequency with the peak taken as the 100% when the 20% frequency does not cross the histogram two times, this flag is attached (Sysmex Corporation,1999).

1.2.5. Physiology of kidneys:

The kidneys are playing a major role in the control of the constancy of the internal environment. The blood flowing in the kidneys is first filtered (glomerular filtration) so that all the blood constituents except blood cells and plasma proteins go into micro tubular system. In these tubules, modification of the filtrate take place, so that useful substances include most of the filtered water, is quickly reabsorbed (tubular reabsorption) back in to the blood (Sukkar *etal*., 2000).

Un wanted substances that escape filtration are actively secreted into the tubular lumen (tubular secretion). The final concentration of electrolytes and other constituents of the urine are adjusted according to requirement of regulation of extracellular fluid composition. Glomerular filtration, tubular reabsorption and tubular secretion are rightly described as renal mechanisms that allow the kidney to undertake its various homeostatic functions (Sukkar *et al*., 2000).

Several hormones specially antiduretic hormones ADH and aldosterone) act on the kidney to enable it to adjust the final composition of urine in response to changes in the internal environment (Sukkar *et al*., 2000).

1.2.6. Renal disease:

1. 2.6.1. Definition:

Renal disease or kidney disease (formerly called renal insufficiency) describes a medical condition in which the kidneys fail to adequately

filter toxins and waste products from the blood. The two forms are acute (acute kidney injury) and chronic (chronic kidney disease), a number of other diseases or health problems may cause either form of renal disease to occur . Renal disease is described as a decrease in glomerular filtration rate (GFR). Biochemically, renal disease is typically detected by an elevated serum creatinine level. Problems frequently encountered in kidney malfunction include abnormal fluid levels in the body, increased acid levels, abnormal levels of potassium, calcium , phosphate, and (in the longer term) anaemia as well as delayed healing in the broken bones. Depending on the cause, hematuria (blood loss in the urine) and proteinuria (protein loss in the urine) may occur. Long –term kidney problems have significant repercussion on other diseases, such as cardiovascular disease (Liao *et al.*, 2012).

1.2.6.2. Classification of kidney disease:

Renal disease can be divided in to two categories: acute kidney injury or chronic kidney disease. The type of renal disease is determined by the trend in the serum creatinine. Other factors which may help differentiate acute kidney injury from chronic kidney disease include anaemia and the kidney size on ultrasound. Chronic kidney disease generally leads to anaemia and small kidney size.

1.2.6.2.1. Acute kidney injury:

Acute kidney injury (AKI), previously called acute renal failure (ARF)(Moori *et al.*, 2012, Ricci *et al.*,2012), is a rapidly progressive loss of renal function , generally characterized by oliguria [decreased in urine production, quantified as less than400ml per day in adults (Klahr *etal.*,1998),less than 0.5ml/kg/h in children or less than

1ml/kg/h in infants] and fluid and electrolytes imbalance. AKI can result from a variety of causes, generally classified as pre-renal, intrinsic, and post-renal. 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 causes (Klahr *etal.*,1998).

1.2.6.2.2. Chronic kidney disease:

Chronic kidney disease (CKD) can develop slowly and , initially, show few symptoms. CKD can be the long term consequence of irreversible acute disease or part of a disease progression, the most common causes of CKD are diabetes mellitus and uncontrolled hypertension (Kes *etal.*,2011).

1.2.6.3. Causes of renal disease:

The loss of kidney function in the acute renal disease can occur following:

- A traumatic injury with blood loss.
- Damage from certain drugs or toxins.
- Obstruction of urine flow.
- Pregnancy complication.

In the chronic stage, there is a kidney damage and decreased function that last longer than 3 months with no symptoms and the most common causes are immune system conditions such as lupus and chronic viral illness (e.g. HIV), urinary tract infections, inflammation, poly cystic kidney disease, drugs and toxins **(**Kimball Johnson, 2005**)**.

1.2.6.4. Stages of renal disease:

Glomerular filtration rate (GFR) is the amount of blood which the kidneys filter in one minute, which should be at least 90 milliliters per

minute. The National Kidney Foundation established the five stages of chronic renal failure based on GFR. In stage one of CR, diminished renal reserve, the only clinical finding is protein in the urine with a normal GFR and patients have no symptoms. Stage two shows a decreased GFR between 60 to 89 mL/min. According to Scott and White hospital patient information on kidney disease, patients may not have symptoms until the condition is advanced. Early symptoms include a general feeling of illness, fatigue, dry and itchy skin, headaches, a loss of appetite and nausea (Israni *et al.*,2011).Stages three and four are referred to as the renal insufficiency stage where the GFR can be as low as 15 mL/min. Azotemia increases. Symptoms are more severe with difficulty concentrating, weakness, easy bruising , swelling, insomnia, muscle twitching and almost no urine output. End-stage renal disease (ESRD) is stage five of chronic renal failure. Patients need dialysis if they wish to survive. When GFR is less than 10 mL/min, patients may apply to become a candidate for a kidney transplant (Durford *et al.*,2013).

1.2.7. End Stage Renal Disease (ESRD):

A diagnosis of end-stage kidney disease means the final stage of kidney disease, and the kidneys are not functioning well enough to meet the needs of daily life. The kidneys are responsible for filtering waste and excess water from the blood in the form of urine. At end-stage kidney disease, also referred to as end-stage renal disease (ESRD), kidneys are functioning below 10 percent of their normal function. This may mean that the kidneys are barely functioning or not functioning at all. Kidney disease is usually progressive. It typically does not reach the end stage until 10 to 20 years after diagnosed with

chronic kidney disease, which may also develop slowly (Christine Dimaria and Marijane Leonard ,2012).

1.2.7.1. Causes of ESRD:

Many types of kidney diseases attack the nephrons which are the tiny units in the kidneys that actually do the filtering. The result is blood not being filtered properly and, eventually, ESRD.

Diabetes and hypertension (high blood pressure) are the two most common causes of ESRD. Diabetics cannot break down glucose (sugar) correctly, and levels remain high in the blood. High levels of glucose in the blood damage the glomeruli in the nephrons. If there is hypertension, the increased pressure that is forced upon the small vessels in the kidneys leads to damage that makes the vessels unable to perform their blood filtering duties (Christine Dimaria and Marijane Leonard ,2012).

1.2.7.2. Main groups at risk for ESRD:

The two main groups at risk for ESRD are diabetics and those with hypertension, also more likely to develop the condition if you have relatives with the disease. The risk of developing ESRD also rises when you have any type of kidney disease or condition, including: polycystic kidney disease, interstitial nephritis, pyelonephritis, certain autoimmune conditions, such as lupus (Christine Dimaria and Marijane Leonard,2012).

1.2.7.3. Symptoms of ESRD:

A wide range of symptoms including: a decrease in urine output, inability to urinate, Fatigue, general ill feeling , headaches, un explained weight loss, loss of appetite and other symptoms. ESRD can be treated by dialysis or kidney transplant (Christine Dimaria and Marijane Leonard, 2012).

1.2.7.4. Complication of ESRD:

There are many complications including skin infections, hyper parathyrodism, hepatitis, dialysis related amyloidosis and other complications.

End-stage kidney disease is not preventable, but should keep the blood glucose levels and the blood pressure of the patient under control (Christine Dimaria and Marijane Leonard ,2012).

1.2.7.5. Treatment of renal disease:

1.2.7.5.1. Dialysis:

One of the methods of treatment of renal failure (renal disease) is dialysis. In medicine, dialysis (from Greek dialysis, meaning dissolution, die, meaning through. And lyses' meaning loosing or splitting) is a process from removing waste and excess water from the blood, and is used primarily to provide an artificial replacement for lost kidney function in people with renal failure (William *et al.*,2014).

Dialysis may be used for those with an acute disturbance in kidney function (acute kidney injury, previously acute renal failure),or progressive but chronically worsening kidney function-a state known as chronic kidney disease stage5 (previously chronic renal failure or end-stage renal disease). The latter from may develop over months or years, but in constant to acute kidney injury is not usually reversible, and 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 in appropriate (Pendse *et al.*,2008).

The kidneys have important roles in maintaining health. When healthy, the kidneys maintain the body's internal equilibrium of water

and minerals (sodium, potassium, chloride, calcium, phosphorus, magnesium, sulphate).The acidic metabolism end-products that the body cannot get rid of via respiration are also excreted through the kidneys. The kidneys also function as a part of the endocrine system, producing erythropoietin and calcitirol. Erythropoietin is involved in the production of red blood cells and calcitirol plays a role in bone formation (Brundage ,1992).

Dialysis is an imperfect treatment to replace kidney function because it does not correct the endocrine functions of the kidney. Dialysis treatments replace some of these functions through diffusion (waste) removal and ultra filtration (fluid removal (Robert ,2011).

1.2.7.5.1.1. Principle:

Dialysis works on the principles of the diffusion of solutes and ultra filtration of fluid across a semi-permeable membrane. Diffusion describes a property of substances in water. Substances in water tend to remove from the area of high concentration to an area of low concentration (Mosby,2006).

Blood flows by one side of semi-permeable membrane, and dialysate, or special dialysis fluid, flow 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 fluids pass through the membrane, but the membrane blocks the passage of larger substances (for example, red blood cells and 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 glomerulas (Mosby,2006).

1.2.7.5.1.2. Haemodialysis (HD):

- Haemodialysis schematic
- Haemodialysis and home 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 fibers. The fiber will act as the semi- permeable membrane. Blood flows through the fibers, dialysis solution flows around the outside the fibers, and water and wastes move between these two solutions. (Ahmed *et al.*,2008**).**

The cleaned blood is then returned via the circuit back to the body. Ultra filtration 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 liters of excess fluid during a typical 3-to 5- hour's treatment. In the US, haemodialysis treatments are typically given in a dialysis center three times per week (due in the US to Medicare reimbursement rules); however, as of 2007 over 2.500 peoples in the US are dialyzing at home more frequently for various treatment lengths (Pfuntner *etal.*, 2011).

Studies have demonstrated the clinical benefits of dialyzing 5 to7 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 (Rocco, 2007).

1.2.7.5.1.2.1. Complication of haemodialysis:

Access create significant problems for renal practitioners the health care system and especially for individuals living with end stage renal disease.

Chronic haemodialysis complications include thrombosis infection, ischemic steal syndrome, aneurysms, venous hypertension hematomas, heart failure and prolonged bleeding and result in frequent interventions and morbidity and mortality. In addition access interventions are often costly challenging and may require special

Surgical expertise (Oliver, 2008).Approximately, 100 white individuals per million populations (pmp) require renal replacement therapy in the United Kingdom each year. The corresponding figure among people of African and Asian origin in the United Kingdom is 300-400 pmp (Kumarp *et al.*,2002). With 70-80 new patients, pmp accepted for long term dialysis annually (Edwardi *et al.*, 1992).

In Sudan according to ministry of health records, the prevalence is increasing through the few past years, approximately 70-140 new patient pmp undergo dialysis each year. This frequency is thought to be due to epidemic malarial infection which as well known to cause glomerulonephritis (Abboud *etal.*,1989).

1.2.7.5.2. Kidney transplant:

Kidney transplant surgery involves removing the diseased kidneys and replacing them with a donated organ. One healthy kidney is need. This means that donors are often living because they can donate one and continue to function normally. Kidney transplants are among the most-common transplant surgeries in the U.S. (Christine Dimaria and Marijane Leonard, 2012).

1.2.8. Platelets and end stage renal disease:

Patients with end-stage renal disease(ESRD) develop haemostatic disorders mainly in the form of bleeding diatheses. Haemorrhage can occur at cutaneous, mucosal, or serosal sites. Retroperitoneal or intracranial haemorrhages also occur. Platelet dysfunction is the main factor responsible for hemorrhagic tendencies in advanced kidney disease. Anaemia, dialysis, the accumulation of medications due to poor clearance, and anticoagulation used during dialysis have some role in causing impaired haemostasis in ESRD patients. Platelet dysfunction occurs both as a result of intrinsic platelet abnormalities and impaired platelet–vessel wall interaction. The normal platelet response to vessel wall injury with platelet activation, recruitment, adhesion, and aggregation is defective in advanced renal failure. Dialysis may partially correct these defects, but cannot totally eliminate them. The haemodialysis process itself may in fact contribute to bleeding. Haemodialysis is also associated with thrombosis as a result of chronic platelet activation due to contact with artificial surfaces during dialysis. Desmopressin acetate and conjugated estrogen are treatment modalities that can be used for uremic bleeding. Achieving a hematocrit of 30% improves bleeding time in ESRD patients (Dinkar Kaw and Deepak Malhotra ,2006).

1.2.9. Previous Studies:

A study using Coulter Model S-Plus Counter provides a measure of the platelet count and the mean platelet volume (MPV). An analysis of 5000 unselected blood specimens showed an inverse relationship between the number of circulating platelets and their MPV. In nearly $95^{\%}$ of normal adults the platelet count varied from 150 to $450*10^{9}$/L and the MPV from7.0 to10.5fl. Thrombocytosis was found in iron deficiency anaemia, after trauma and acute blood loss and in rhematoid arthritis. Although there is a normal platelet distribution in pregnancy, patients with pre-eclampsia and uncomplicated hypertension in late pregnancy tended to have lower platelet counts and larger platelets than controls. A variable platelet pattern was found in infection, renal failure and treated malignant disease (Giles ,1981).

A second retrospective study of 78 patients with chronic renal failure undergoing either haemodialysis (n=57) or intraperiotoneal dialysis (n=21) was aimed to investigate effect of erythropoietin treatment over six months on the patients compared with other control groups. The result showed there was a significant increase in MPV ($p<0.01$) over six months period, but no change in either total platelets count or PDW in the patients receiving erythropoietin and the control groups showed no significant change in MPV. The study concluded that erythropoietin affect thrombopoiesis and may be a part of humoral factors contributing to megakaryocyte development and maturation. Larger platelets are more reactive and may contribute to the increased risk of thrombosis associated with erythropoietin (Sharpe *et al* .,1994).

A third study was aimed to investigate whether an increase in platelet size was present before the manifestation of the disease and therefore could be used to predict the progression of the disease before it is clinically apparent. It is concluded that increasing platelet size can predict which patients are likely to progress to severe disease before it becomes clinically obvious (Walker*et al.*,1989).

A fourth study done by Vamseedhar Annam with others at 2011 to evaluate the platelet indices and platelet counts and their significance in pre-eclampsia and eclampsia .The result showed that, the platelet counts were lower while the mean platelet volume, platelet distribution width and platelet large cell ratio were increased in pre-eclampsia and eclampsia as compared to control group and concluded a relationship between platelet indices and severity of pre-eclampsia. The estimation of platelet count and platelets indices can be considered as an early, simple and rapid procedure in the assessment of severity of pre-eclampsia (Vamseedhar Anna*met al*., 2011).

1.3. Rationale:

End stage renal disease is a major health problem and greatly affects the economic and social status of affected patients worldwide. In Sudan, according to ministry of health records, the prevalence of ESRD is increasing through the few past years and might cause many complications of high mortality and morbidity.

Few data is available about the effect of ESRD on the platelets count and platelets indices , this study was designed to determine to which extend changes occur in platelets activity by measuring platelets count and platelets indices (MPV, PDW, and P-LCR) in Sudanese individuals with ESRD attending Al-Ribat Hospital in haemodialysis unit.

1.4. Objectives:

1.4.1. General objective:

To assess platelets activity change using platelets count and platelets indices in Sudanese patients at end stage renal disease referred to University Al-Ribat Hospital from March up to May 2014.

1.4.2. Specific objectives:

-To measure platelets count and platelets indices (MPV, PDW, and P-LCR) in Sudanese patients at end stage renal disease.

-To demonstrate the variation in these parameters between patients and control group.

-To determine platelets count, MPV, PDW, and P-LCR in Sudanese patients at end stage renal disease according to gender effect.

-To determine platelets count, MPV, PDW, and P-LCR in Sudanese patients at end stage renal disease according to age effect.

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and methods

2.1. Study design:

Non-interventional analytical case control study.

2.2. Study area:

The study was conducted in (Al- Ribat) Hospital at renal dialysis unit.

2.3. Study period:

Three months during the period of March to May (2014).

2.4. Study population:

A total of (84) patients with ESRD who attended (Al- Ribat) Hospital, already diagnosed as cases and (50) healthy individuals as control were included in this study. The selected patients and healthy subject had included both genders with three age groups (20-40)،(40-60)،(60-80) years old. The patients were (56) males and (28) females.

2.5. Inclusion criteria:

All patients with ESRD available at the time of the study with no medical condition or drugs that affect the results were included in the study.

2.6. Exclusion criteria:

Healthy individuals with known haematological parameters and newly diagnosed patients with acute renal disease were excluded from the study.

2.7. Sampling:

2.7.1. Sample collection:

2.5ml of venous blood were collected from each individual using disposable syringe from anticubital vein cleaned by 70% alcohol and transferred to labeled containers containing sequestered EDTA. Gentle mixing of the specimens was achieved to avoid haemolysis and clotting or platelets aggregation. Any haemolyzed, turbid, clotted samples were rejected.

2.7.2. Sampling method:

Probability sampling (simple random sampling).

2.8. Data collection:

The data were collected using a direct interviewing questionnaire. The questionnaire was used to collect data regarding patient number, age and gender (See appendix -1).

- Laboratory investigations to obtain the platelet parameters.

2.9. Methodology:

2.9.1. Materials and equipments:

●Sysmex KX-21(1992)

●Cell packed reagent

●Rotary mixer (Machine)

●Cotton

●70% alcohol (spirit)

●Syringes

●Tourniquet

●K_2 EDTA containers

●Examination gloves

2.9.2. Test applied:

2.9.2.1. Measurement of platelets parameters:

A blood cell counter Sysmex KX-21 was used to measure platelets parameters.

All measurements (Platelets count , Mean Platelet Volume , Platelet Distribution Width and Platelet- large cell ratio) were measured in whole blood anticoagulated with K_2 EDTA.

All measurements of collection were made between half and hour of collection. The whole blood mode (WB) was selected to analyze the whole blood sample without pre dilution. The sample number was entered before each sample, this procedure was followed:

● A well mixed anticoagulated sample was set to the sample probe, and the start switch was pressed till the aspirating process was finished (volume aspirate approximately 50□L).

● The sample was removed straight down and the sample probe was automatically cleaned.

● The aspirated sample was then automatically suspended in to different blocks and different parameters were measured.

● The results of parameters were then viewed on the screen and subsequently printed out.

* **Quality control:** Sysmex(Kx-21) device was calibrated daily using control sera before analysis was made.

2.9.2.2. Data analysis:

The data was collected in master sheet paper, (raw data) subjected to statistical analysis using Statistical Professional for Social Science (SPSS) computed program, version (16).

2.9.2.3. Ethical considerations:

- The hospital permission was taken prior to data and sample collection.
- Verbal consent was obtained from all participants.
- No names were published and data will be kept confidently.

CHAPTER THREE

RESULTS

3. Results

Table (3.1): The frequency of case and control groups in the study population.

Study population.	Cases	Controls
Frequency	84	50
Percentage	62.7	37.3

Table (3.2): Gender distribution among case and control groups.

Study groups						
Case			Control		Total	
Gender	Frequency	(%)	Frequency	(%)	Frequency	(%)
Male	56	69.1	25	30.9	81	100
Female	28	52.8	25	47.2	53	100
Total	84	62.7	50	37.3	134	100

Table (3.3): The Mean, SD, Maximum and Minimum values of platelets count and platelets indices in the study population.

	Control				Case			
	Mean	**SD**	**Maximum**	**Minimum**	**Mean**	**SD**	**Maximum**	**Minimum**
Platelets count $*10^3/\mu l$	**274.86**	**81.93**	**493**	**155**	**185.25**	**62.72**	**359**	**10**
MPV (fl)	**9.36**	**0.94**	**11**	**8**	**13.02**	**12**	**16**	**12**
PDW(fl)	**11.66**	**1.64**	**15**	**9**	**12.09**	**2.17**	**19**	**8**
P-LCR (%)	**20.94**	**6.54**	**33**	**8**	**22.39**	**7.47**	**42**	**8**

Table (3.4): The relationship between platelets count and platelets indices in case and control group in the study population .

Parameters	Samples	Number	Mean	SD	P.value
Platelets count $*10^3/\mu l$	Case	84	185.25	62.72	0.000
	Control	50	274.86	81.93	
MPV (fl)	Case	84	13.02	12	0.000
	Control	50	9.36	0.75	
PDW(fl)	Case	84	12.09	2.17	0.222
	Control	50	11.66	1.64	
P-LCR (%)	Case	84	22.39	7.47	0.25
	Control	50	20.94	6.54	

Number of cases 84,Control 50

P.value significant at $P < 0.01$, Non significant at $P > 0.05$

Table (3.5): The relationship between platelets count and platelets indices in male and female among case group.

Parameters	Gender	Frequency	Mean	SD	P.value
Platelets count $*10^3/\mu l$	**Male**	56	177.02	57.88	0.089
	Female	28	201.71	69.63	
MPV(fl)	**Male**	56	9.5	1.14	0.327
	Female	28	9.79	1.45	
PDW(fl)	**Male**	54	12.04	2.15	0.728
	Female	28	12.21	2.25	
P-LCR (%)	**Male**	56	22.43	7.22	0.951
	Female	28	22.32	8.08	

Number of cases 84 ,Control 50

P.value significant at $P < 0.01$, Non significant at $P > 0.05$

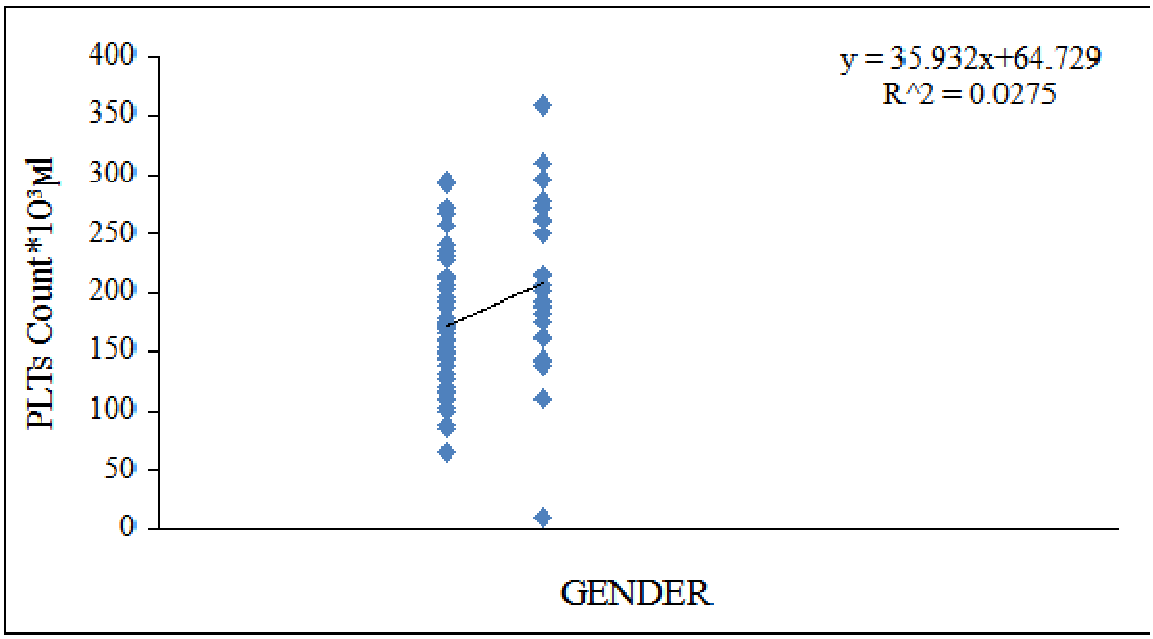

Figure (3.1): Shows correlation between gender and platelets count among case group (P.value =0.09).

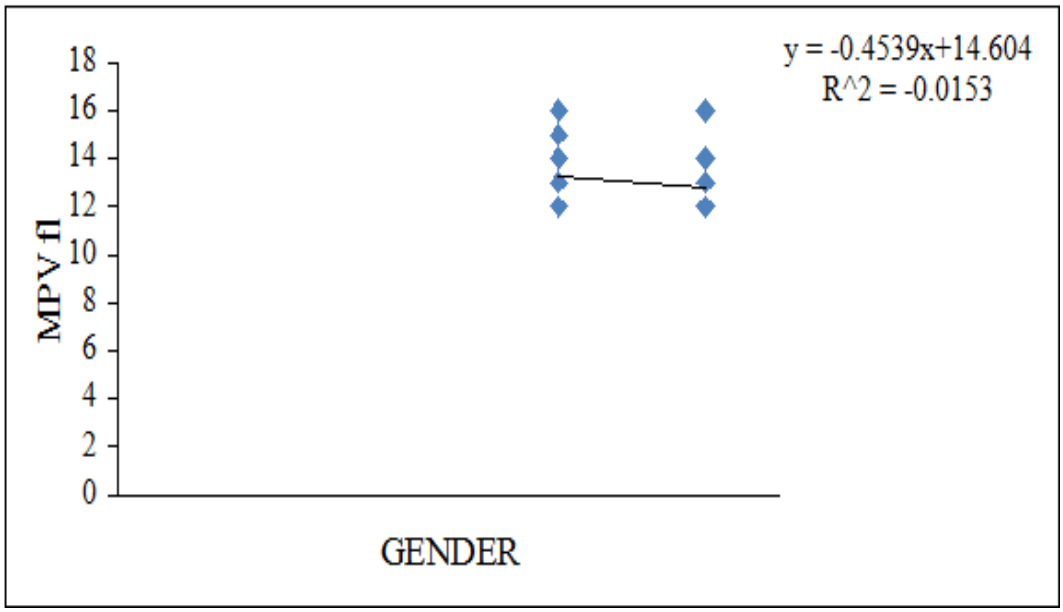

Figure (3.2): Shows correlation between gender and MPV among case group (P.value =0.33).

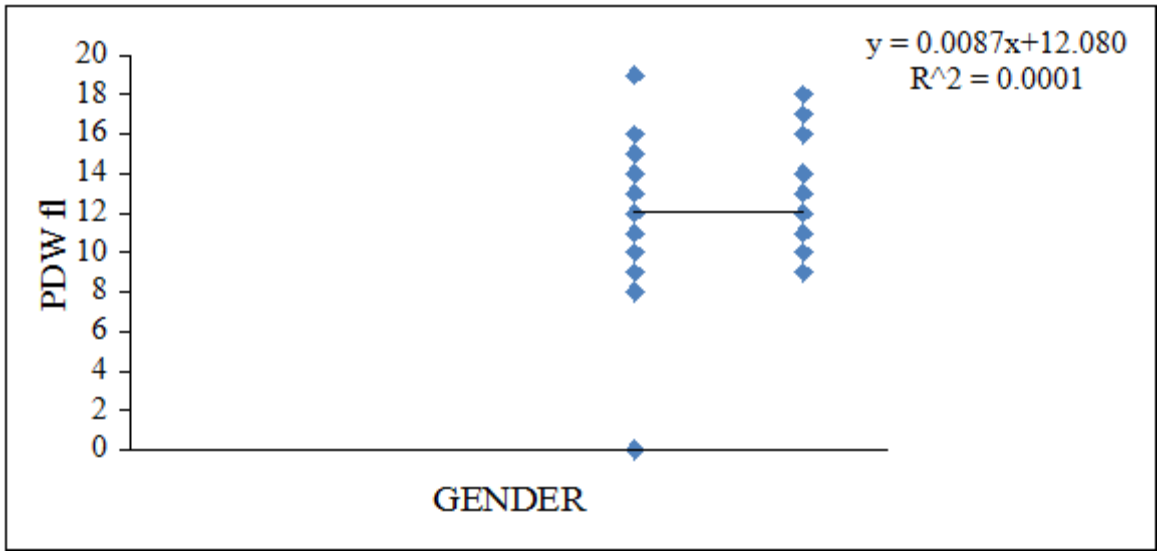

Figure (3.3): Shows correlation between gender and PDW among case group (P.value =0.73).

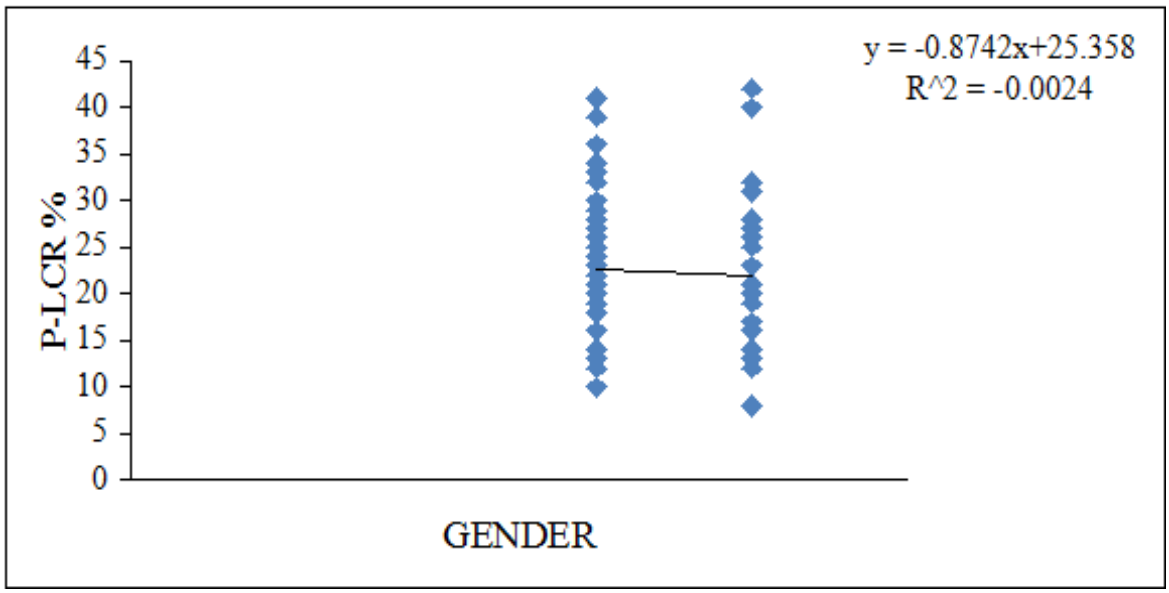

Figure (3.4): Shows correlation between gender and P-LCR among case group (P.value =0.95).

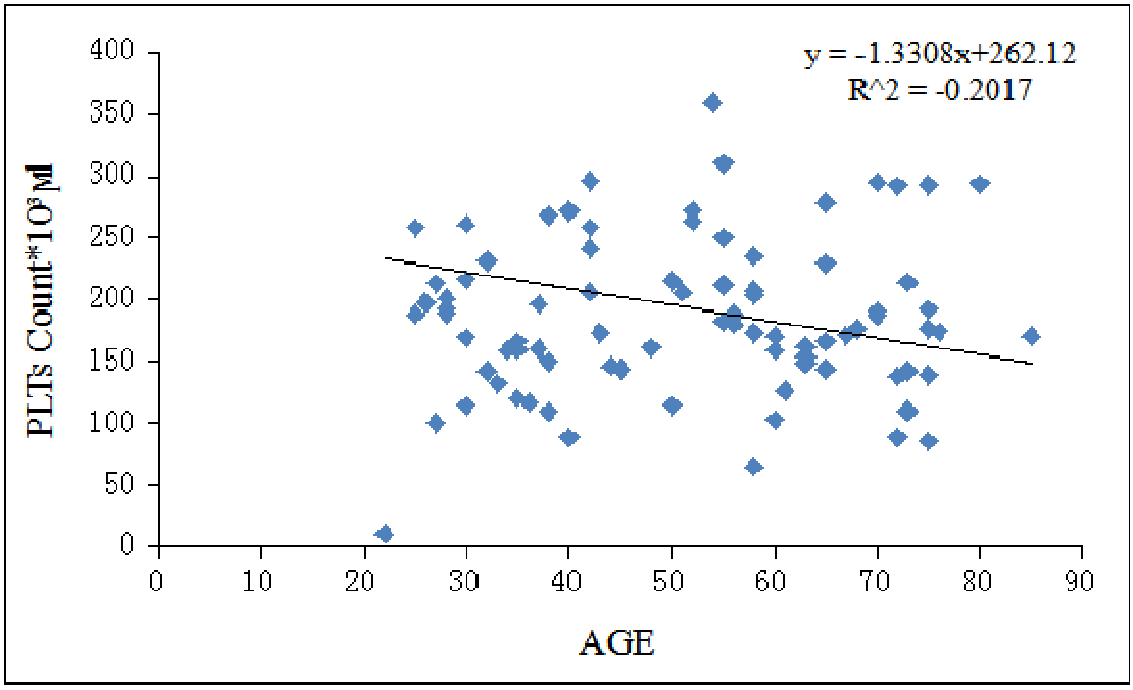

Figure (3.5): Shows correlation between age and platelets count among case group (P.value =0.002).

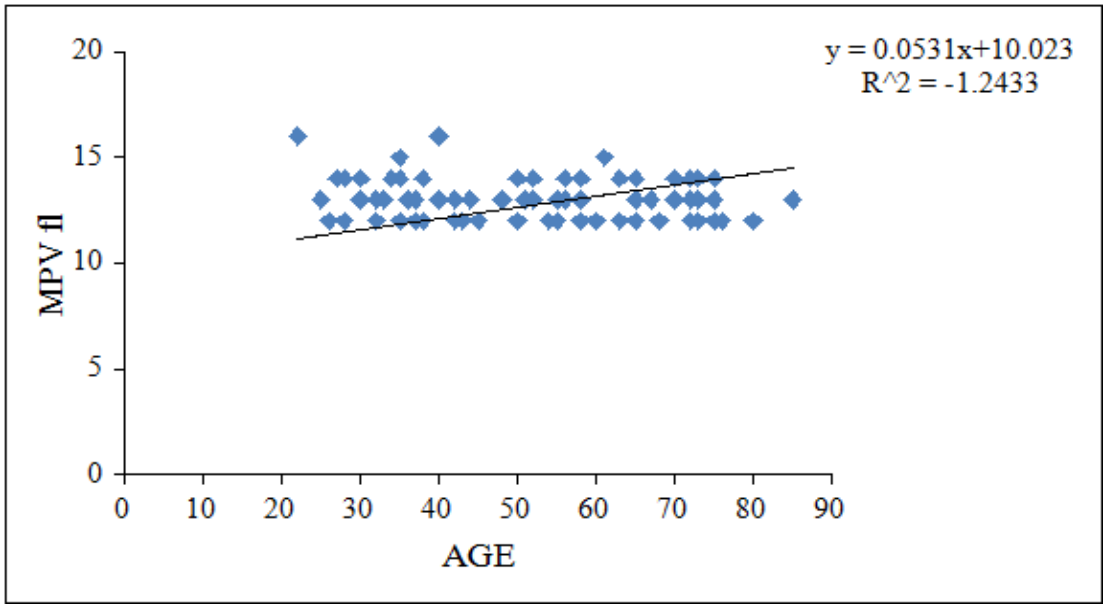

Figure (3.6): Shows correlation between age and MPV among case group (P.value =0.000).

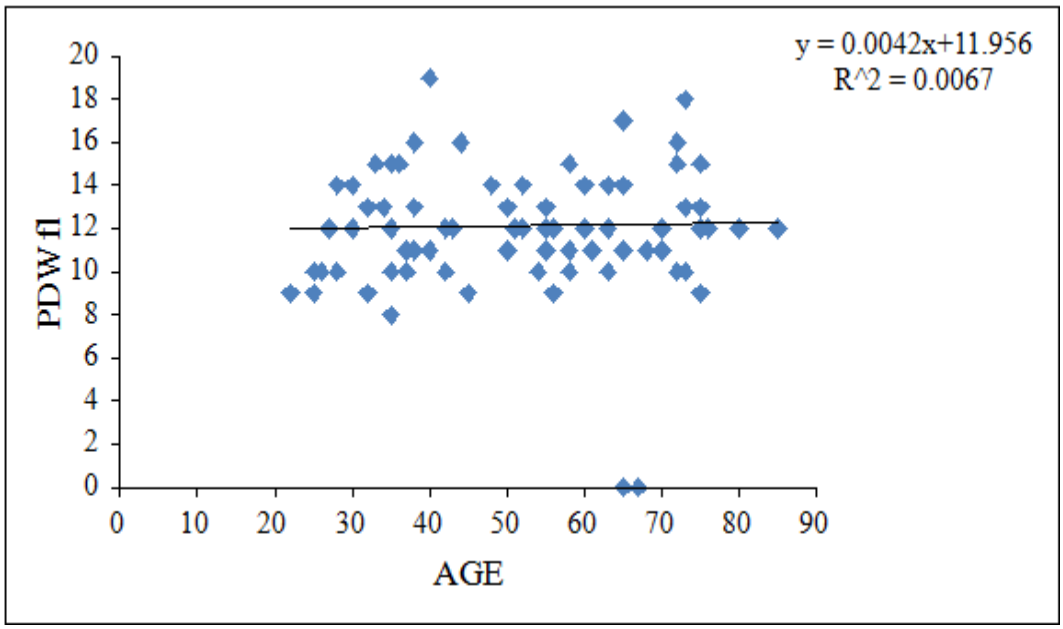

Figure (3.7): Shows correlation between age and PDW among case group (P.value =0.94).

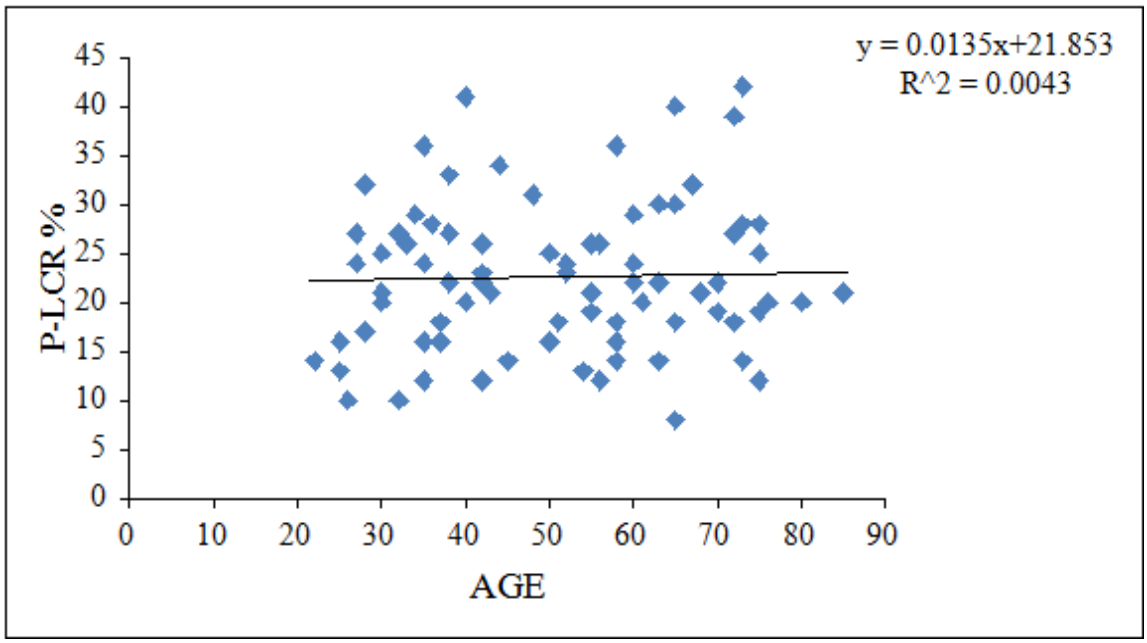

Figure (3.8): Shows correlation between age and P-LCR among case group (P.value =0.99).

CHAPTER FOUR

DISCUSSION

CONCLUSION

RECOMMENDATION

4. Discussion, Conclusion and recommendation

4.1. Discussion:

This study involved a total of eighty four end stage renal disease patients, and fifty healthy persons as control presented in table (3.1). The study group responded to our questionnaire, the frequency of the study participants in case and control groups according to gender, the males were 56 (69.1%), females were 28 (52.8%), table (3.2).

The study reflects that mean Platelets count, MPV, PDW, PLCR of case group were 185.25, 13.02, 12.09, and 22.39 respectively, presented in table (3.3). The results obtained from normal healthy control group revealed that the mean of Platelets count, MPV, PDW, PLCR were 274.86, 9.36, 11.66, and 20.94 respectively , presented in table (3.3).

The study showed that, the mean of platelets count level in Sudanese end stage renal disease patients is significantly lower than the mean of control group (P value < 0.05) showed in table (3.4) and the mean of MPV level is significantly higher than the mean of control group (P.value < 0.05) showed in table (3.4) which was agreed with the first study using Coulter Model S-Plus Counter which obvious that an inverse relationship between the number of circulating platelets and their MPV (Giles,1981).

The study explained that MPV level in Sudanese ESRD patients was higher than values obtained from control group , showed in table (3.4) which similar to the results of the second retrospective study of chronic renal failure undergoing haemodialysis (Sharpe *et al.*,1994).

The study explained affecting of ESRD on platelets count (lower), MPV (higher) as compared to control group which similar to the results of the third study done by Walker with others (Walker *et al*.,1989) , and the results of the fourth study done by Vamseedhar Annam (Vamseedhar Annam*et al*.,2011).

The apparent variation in Platelets count, MPV seen in this study was mostly attributed to the anisocytosis which due to platelets activity abnormalities due to contact with artificial surface during dialysis in the end stage renal disease patients.

The study explained no effectiveness of gender on platelets count and platelets indices (MPV, PDW, P-LCR) on ESRD patients, showed in table (3.5), figures (3.1),(3.2),(3.3), and (3.4) respectively according to result finding.

The study explained no effectiveness of age on PDW and P-LCR of ESRD patients, showed in figures (3.7), and(3.8) respectively, but there was an effectiveness of age on platelets count (inverse relation) of ESRD patients , also there was an age effect on MPV (direct relation) of ESRD patients , showed in figures (3.5) and (3.6) respectively.

4.2. Conclusion:

-There is a significant effect of Platelets count (low), MPV(high) on the end stage renal disease patient and the other parameters (PDW and P-LCR) have no significant effect on ESRD patient.

-No significant effectiveness of gender on Platelets count , MPV,PDW and P-LCR of end stage renal disease patient.

-No significant effectiveness of age on (PDW and P-LCR) of end stage renal disease patient but there is an age effectiveness on Platelets count and MPV of that patients.

4.3. Recommendation:

-More research is needed to elaborate on the important of platelets count and platelets indices in end stage renal disease diagnosis to cover more population with different categories.

-This study can be purposeful in the diagnosis as laboratory finding on end stage renal disease patient (when platelets count was decreased and MPV was increased, the patient request renal function test for check).

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APPENDICES

Appendices

Appendix (1): questionnaire

Sudan University of Science and technology

Collage of graduate studies

Department of hematology and immunohematology

Questionnaire

1-Patient number.....

2-Age.....

3-Tel.No.....

4-Gender:

Male ( ) Female ( )

5-Duration of disease.....

6- Results:

A- Platelets count.....*10^3/□l

B- MPV.....fl

C- PDW.....fl

D- PLCR.....(%)

* Worker sign.....

Appendix (2): Consent form

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

دراسة لنيل درجة الماجستير

الاسم/الرقم.....

سيتم أخذ عينة من الدم بحجم 2.5 مل من الوريد بواسطة حقنة الطعن ، وذلك بعد تعقيم منطقة أخذ العينة بواسطة مطهر.

جميع الأدوات المستخدمةلأخذ العينة معقمة ومتبع فيها كل سبل السلامة المعملية وليس هناك آثار جانبية للعملية، ربما يحدث تورم بسيط في منطقة أخذ العينة وسوف يزول بعد فترة قصيرة.

الغرض من أخذ العينة هو البحث العلمي وسوف يسلم المريض نسخة من النتائج في سرية تامة.

أوافق أنا المذكور أعلاه على أخذ عينة دم لإجراء الدراسة.

الامضاء.....

التأريخ.....