Sudan University of Science and Technology

Collage of Graduate Studies

Assessment of Complete Blood Count in Renal Failure patients on Hemodialysis attending Omdurman Teaching Hospital- Khartoum State

قياس تعداد الدم الكامل لدى مرضى الفشل الكلوي الذين يتعرضون للغسيل الدموي في مستشفى امدرمان التعليمي بولاية الخرطوم

Dissertation submitted for partial fulfillment for the Requirement of M.Sc. degree in Hematology and Immunohematology

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May 2014

ا لايــه

بسم الله الرحمن الرحيم

(اللهُ نُـوْرُ الـسَّمَـاوَاتِ وَالأرْضِ مَـثَـلُ نُـوْرِهِ كَـمِـشْكَـاةٍ فِـيْـهَـا مِـصْبَـاحٌ الْـمِـصْبَـاحُ فِـيْ زُجَـاجَـةٍ الـزُّجَـاجَـةُ كَـأنَّـهَـا كَـوْكَـبٌ دُرِّيٌّ يُـوْقَـدُ مِـنْ شَجَرَةٍ مُـبَـارَكَـةٍ زَيْـتُـوْنَـةٍ لاَ شَرْقِـيَّـةٍ وَلاَ غَـرْبِـيَّـةٍ يَـكَـادُ زَيْـتُـهَـا يُـضِـيْءُ وَلَـوْ لَـمْ تَـمْسَسْهُ نَـارٌ نُـوْرٌ عَـلَـى نُـوْرٍ يَـهْـدِي اللهُ لِـنُـوْرِهِ مَـنْ يَـشَـاءُ وَيَـضْرِبُ اللهُ الأمْـثَـالَ لِـلـنَّـاسِ وَاللهُ بِـكُـلِّ شَـيْءٍ عَـلِـيْـمٌ).

النور:35

DEDICATION

To the candle which burns to light my life

My Mother

To the one who I live for making his dream truer

My Father

To special person who inspired and give me meaning of being

My

To those who have made it possible

My Teacher

To whom encouraged me

My Brothers and Friends

Acknowledgment

I would to thank Allah who guided our steps in this study.

Thanks to my supervisor Dr.FathElrahman Mahdi Hassan Gameel who paved the way for this study completion and those who supported me and devoted time and effort for my.

The administration of Omdurman TeachingHospital, All the staff of dialysis unit in Omdurman, For allowing the collection of sample and patients examination.

For all above we convey our thanks and appreciation.

Abstract

This is Cross-sectional study was conducted to determined the changes induced by hemodialysis in complete blood count (CBC) and show the peripheral blood picture(PBP) in patients with end stage renal disease (ESRD) who attended hemodialysis sessions in Omdurman Teaching Hospital in Khartoum state. Three mls of venous blood samples were collected using disposable syringes were added slowly to containers K3-EDTA for CBC analysis from50 Renal failure patients(pre and post dialysis) under hemodialysis at different ages, from different tribes and sexes(31from male and 19 from female).The patients wereexposed to different periods of hemodialysis were the recruited for this study, between March to April 2014.

The results of study showed there are increase in the mean of Hb,RBC,PCV,and WBC after hemodialysis(P.value 0.00 for all), Hb=(pre dialysis 8.68g/dL/ post dialysis 9.97g/dL), PCV=(pre dialysis 27.55%/post dialysis 30.56%),RBC=(pre dialysis3.03×10^{6}/ml/post dialysis 3.59×10^{6}/ml)and WBC=(pre dialysis5.12×10^{3}/ml / post dialysis6.11×10^{3}/ml).

There are no any different in the mean of MCV(P.value=0.62).MCH(P.Value=0.96)and MCHC(P.Value=0.34) in pre and post hemodialysis.

Also there isdecrease in the mean of Plt after hemodialysis(pre dialysis218.04×10^{3}/ml/ post dialysis194.86×10^{3}/ml)andP.Value(0.00) .In peripheral blood picture (PBP) there is no significant different in the morphological picture in pre and post hemodialysis the RBC normocytic normochromic,WBC and Plt in normal picture. Also there is decrease in weight of patients after dialysisP.Value0.00.

المستخلص

هذه دراسة تحليلية لتحديد التغيير الناتج عن الغسيل الدمويللكلى في تعداد الدم الكامل و اظهار الصورة الدموية المحيطية في المرضى الذين يعانون من نهاية مرحلة المرض الكلوي (الداء الكلوي بمراحله الأخيرة) الذين حضروا جلسات غسيل الكلى في مستشفى أم درمان التعليمي في الخرطوم. وقد جمع ثلاثة مل من الدم الوريدي من 50 مرضى الفشل الكلوي (قبل وبعد الغسيل الدموي)باستخدام المحاقن وأضيف ببطء إلى حاويات K3- EDTA لتحليل تعداد الدم الكامل منأعماروقبائل مختلفة و الجنسين (31 من الذكورو19 من الاناث) ، ويتعرضون لفترات مختلفة من غسيل الكلى ، بين مارس.-أبريل. عام 2014.

اظهرت الدراسة هناك فروقات ذات دلاله إحصائية في متوسط خضاب الدم ، خلايا الدم الحمراء، حجم الخلايا الحمراء المكدسة، وخلايا الدم البيضاء بعد الغسيل الكلوي (مستوى المعنوية للجميع=0.00) ، متوسط خضاب الدمg/dL (قبل الغسيل8.68/بعد الغسيل 9.970) ، متوسط حجم الخلايا الحمراء المكدسة% (قبل الغسيل8.68/بعد الغسيل 30,56)، متوسط الخلايا الدم الحمراء(قبل الغسيل3.03/بعد الغسيل$3,59\times10^{6/}ml$)ومتوسط خلايا الدم البيضاء$\times10^{3/}ml$ (قبل الغسيل5.12 /بعد الغسيل6,11).

لا توجد فروقات ذات دلاله إحصائية لحجم الخلايا الحمراء المكدسة الى عددخلايا الدم الحمراء (=0,62) ،و مستوى خضاب الدم الى عدد خلايا الدم الحمراء (= 0,96) ،ومستوى خضاب الدم الى حجم الخلايا الحمراء المكدسة (=34. 0) في مرحلة ما قبل وبعد الغسيل الكلوي .

أيضا هناك انخفاض في متوسط الصفائح الدموية بعد الغسيل الكلوي (مستوى المعنوية = 0.00) و المتوسط(قبل الغسيل $\times10^{3/}ml$ 218.04/ بعد الغسيل $194.86\times10^{3/}ml$) . وفي الصورة الدموية المحيطية ليس هناك أي اختلاف في الصورة الشكلية في مرحلة ما قبل وبعد الغسيل الكلوي في خلايا الدم الحمراء السوي الكريات سوي الصباغ ، خلايا الدم البيضاء و الصفائح الدموية في الشكل الطبيعي. أيضا هناك انخفاض كبير في الوزن من المرضى بعدغسيل الكلى(مستوى المنوية=0,00).

Abbreviations

AKIAcute kidney injury

ARFAcute renal failure

BFU-EBurst-forming unit, erythroid

CKDChronic kidney disease

CRFChronic renal failure

CFU-EColony-forming unit, erythroid

EDTAEthylene diamine tetra aceticacid

eGFRestimated GFR

ESRDEnd stage renal disease

GFRGlomerular filtration rate

HDHemodialysis

HbHemoglobin

MCHCMean cell hemoglobin concentration

MCHMean cell hemoglobin

MCVMean cell volume

PBPperipheral blood picture

PCVpacket cell volume

PDperitoneal dialysis

PltPlatelet

RBC Red Blood Cell

WBCwhite blood cell

List of Tables

Contents

Chapter One

Introduction and Literature Review

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction:

The incidence and prevalence of CRF and consequently end stage renal disease (ESRD) had steadily increased in the last two decades. Global ESRD population was estimated to be 1.6 million at the end of year 2002. These patients require renal replacement therapy and other supportive treatment modalities for their survival, the absence of which inevitably result in death from advanced ureamia or its recognized complications (Arogundade*et al.*, 2005).In Sudan according to ministry of health the prevalence of renal failure is increasing through the few past years approximately 70 – 140 individual per million population per year(Abboud*et al.*, 1989). This study is aimed at providing essential data concerning evaluation the changes of complete blood count that occur in renal failure patients before and after HD to take the ideal precaution before and after dialysis session in order to reduce patient morbidity and motility.

The both type of dialysis are known to have side effects on the variable blood component. These effect vary with several physiological and non-physiological factor such as age,gender,race,muscular activity, position of patient during dialysis as well as the duration and type of fdialysis.it has been reported that dialysis lowers the Hb level and RBC count; this is more pronounced in females than males, and in patient in advanced age because of the reduced erythropoietin concentration in these patient.Data concentration platelet count before and after dialysis ,suggest that there may be other factor affecting platelets during dialysis . (Ali M S,2008)

Other study was primarily conducted to investigate changes in various hematological parameters that may occur in renal failure patients after HD, to determine the differences between various hematological parameters that occur in renal failure patients before and after–HD as compared to the control group. The possible effect of the duration of dialysis on hematological parameters that may occur in renal failure patients was also investigated. Results from this study may enable us to determine whether precautions need be taken before and after HD sessions to avoid unnecessary complications. This study was therefore carried out to investigate the hematological changes before and after hemodialysis in renal failure patients compared to controls in Riyadh, Saudi Arabia (Alghythan A K,2012).
Other study was conducted in Sudan University of Science and Technology to investigate the effect of renal failure and hemodialysis on patients' blood counts in order to reduce patients morbidity and motility(Ali E A,2011).
Renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8%(Ahmed J. 2013).

1.2 Literature review

1.2.1 Blood Function:

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- 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, the response to a broken blood vessel, the conversion of blood from a liquid to a semi-solid gel to stop bleeding.
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature(Alberts, 2012).

1.2.2 Hemopoiesis:

Hemopoiesis means the formation of blood cells which is determined by the interaction of multiple genes and involves cytokines and other protein factors.

During the first few weeks of embryonic life, the formation of blood cells takes place in the yolk sac. Later, until the sixth or seventh month of fetal development, the liver and spleen are the major hematopoietic organs. By the time of birth, more than 90% of all new blood cells are formed in the bone marrow.During infancy and childhood, the marrow of all bones contributes to hematopoiesis. During adult life, hematopoietic marrow is restricted to certain bones (e.g., pelvic bones, vertebral column, proximal ends of the femur, skull, ribs, and sternum). Even in these areas, a proportion of the marrow cavity consists of fat. During periods of hematopoietic stress (e.g., in severe hemolytic anemias and in some myeloproliferative disorders), the fatty marrow as well as the spleen and liver can resume the production of blood cells. This situation is called extramedullary Hematopoiesis (Munker et al., 2007).

1.2.2.1Erythropoiesis:

Red blood cells are specialized cells that deliver oxygen to tissues and remove carbon dioxide from the human body. Erythropoiesis, “making of

red cells," involves many different genes and gene products that lead to the production of the mature cell. Erythropoiesis begins at the level of the multipotent stem cell, which then undergoes commitment and differentiation. Listed as follows are the stages of erythroid differentiation:

1.Stem cell.

2. Burst-forming unit, erythroid (BFU-E); immature erythroid progenitor.

3.Colony-formingunit,erythroid(CFU-E);more mature erythroid progenitor.

4.Proerythroblasts,erythroblasts,normoblasts(morphologically recognizable red cell precursors, they still have a nucleus, multiply by cell division, and progressively decrease in size as hemoglobin content increases).

5. Reticulocytes; mature red blood cells (Munker*et al.*, 2007).

1.2.2.2Granulopoiesis:

The blood granulocytes and monocytes are formed in the bone marrow from a common precursor cell. In the granulopoietic series progenitor cells, myeloblasts, promyelocytes and myelocytes form a proliferative or mitotic pool of cells while the metamyelocytes, band and segmented granulocytes make up a post-mitotic maturation compartment. Large numbers of band and segmented neutrophils are held in the marrow as a 'reserve pool' or storage compartment. The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2 : 1 to 12 : 1, the largest proportion being neutrophils and metamyelocytes. In the stable or normal state, the bone marrow storage compartment contains 10-15 times the number of granulocytes found in the peripheral blood. Following their release from the bone marrow, granulocytes spend only 6-10 hours in the circulation before moving into the tissues where they perform their phagocytic function. In the bloodstream there are two pools usually of about equal size: the circulating pool (included in the blood count) and the marginating pool (not included in

the blood count). It has been estimated that they spend on average 4-5 days in the tissues before they are destroyed during defensive action or as the result of senescence (Hoffbrand*et al.*, 2006).

1.2.2.3 Platelet production (Thrombopoiesis):

Platelets are produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoietic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin. Once released from the bone marrow young platelets are trapped in the spleen for up to 36 hours before entering the circulation, where they have a primary hemostatic role (Provan, 2003).

1.2.3 The Red Blood Cell:

Red blood cells are specialized cells that deliver oxygen to tissues and remove carbon dioxide from the human body. The normal erythrocyte has a diameter of about 8 μm and a biconcave disc form that provides the red cell with a maximum surface-for-gas exchange as well as optimal deformability. The bipolar lipid layer of the red cell membrane is stabilized on the inner side by the attachment of the structural proteins actin and spectrin. Defects of these proteins lead to hemolytic anemia. The outer layer is covered with mucopolysaccharides that form part of the structure of blood group antigens. The *N*-acetylneuraminic acid found in these glycoproteins results in a negative charge of the cell surface.

Because red cells have lost their nuclei, they are no longer capable of synthesizing proteins, including enzymes. Red cells remain viable and functional for an average of 120 days. The necessary energy for red cell metabolism is supplied by the Embden-Meyerhof pathway, which generates

adenosine triphosphate by metabolizing glucose to lactate. This anaerobic process also results in the formation of nicotinamide-adenine dinucleotide, which is essential for the reduction of methemoglobin to functionally active hemoglobin (Munker*et al.*, 2007).

1.2.3.1 Hemoglobin:

Hemoglobin is the molecule responsible for the transport of oxygen. Under physiological conditions, three types of hemoglobins exist:

• Hemoglobin A (α□ β□): major adult hemoglobin (96–98%).

• Hemoglobin F (α□ γ□): predominant during fetal development, 60–80% at birth, 0.5–0.8% during adult life.

• Hemoglobin A□ (α□ □□): normally 1.5–3%.

The hemoglobin molecule has a molecular weight of 64,500 and consists of four polypeptide chains, each carrying a heme group. The heme synthesis starts with the amino acid glycine. Later, porphobilinogen, uroporphyrinogen, coproporphyrinogen, and protoporphyrin are formed as intermediate steps. Iron (Fe^{+2}) is supplied from serum transferrin and combines with protoporphyrin to form heme. One heme molecule then binds with one globin chain to form the hemoglobin molecule that avidly binds oxygen. The release of oxygen from red cells into tissue is strictly regulated. (Munker*et al.*, 2007).

1.2.4 white blood cells (leucocytes):

The white blood cells (leucocytes) may be divided into two broad groups: the phagocytes and the immunocytes. Granulocytes, which include three types of cell-neutrophils (polymorphs), eosinophils and basophils-together with monocytes, comprise the phagocytes. Only mature phagocytic cells and lymphocytes are found in normal peripheral blood (Table 1.1). The function

of phagocytes and immunocytes in protecting the body against infection is closely connected with two soluble protein systems of the body: immunoglobulins and complement (Hoffbrand*et al.*, 2006)

Table 1.1 White cells: normal blood counts (Hoffbrand*et al.*, 2006)

Adults	Blood count	Children	Blood count
Total leucocytes	4.00-11.0 x 10^9/L *	Total leucocytes	
Neutrophils	2.5-7.5 x 10^9/L *	Neonates	10.0-25.0 x 10^9/L
Eosinophils	0.04-0.4 x 10^9/L	1 year	6.0-18.0 x 10^9/L
Monocytes	0.2-0.8 x 10^9/L	4-7 years	6.0-15.0 x 10^9/L
Basophils	0.01-0.1 x 10^9/L	8-12 years	4.5-13.5 x 10^9/L
Lymphocytes	1.5-3.5 x 10^9/L		

* Normal black and Middle Eastern subjects may have lower counts. In normal pregnancy the upper limits are: total leucocytes 14.5 x 10^9/L /L, neutrophils 11 x 10^9/L.

1.2.5 Anemia of renal disease:

Severe renal disease is almost always accompanied by a failure of the normal erythropoietin response. As with other hypoproliferativeanemias, the erythropoietic profile is characterized by normocytic, normochromic morphology, a normal mean cell volume(MCV), a low reticulocyte count, and an absence of polychromasia on the peripheral blood smear.The severity of the anemia correlates with the severity of the renal failure. Acute loss of renal function as with acute tubular necrosis is associated with the rapid development of amoderately severe anemia,with hemoglobin levels of 7to9 g/dL. Progressive renal failure with blood urea nitrogen (BUN) in excess of 50 mg/dL and a creatinine above 3 mg/dL is associated with more severe

anemia (hemoglobin levels below 7 g/dL). This situation reflects the added impact of marked nitrogen retention on red blood cell survival. Patients with end stage renal disease show significant reductions in red blood cell lifespan that cannot be recognized andcompensated for by an increased level of erythropoietin production.(Hillman *et al.*,2005).

1.2.6 The kidneys:

Each kidney weight approximately 150 g and measures 10-12 cm in length. The kidneys are highly vascular organs, receiving 20% of the cardiac output via the renal arteries .(Moorthy*et al*. 2009).

The kidney hasa bean-shaped structure; each kidney has a convex and concavesurface. The concave surface, the renal hilum, is the point at which the renal arteryenters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal ygfascia (of Gerota) and paranephric fat.

The substance, or parenchyma, of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla. Grossly, these structures take the shape of 8 to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (Walter F and Boron 2004).). Between the renal pyramids are projections of cortex called renal columns. Nephrons are urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct.The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty

into major calyces, and major calyces empty into therenal pelvis, which becomes the ureter. At the hilum, the ureter and renal vein exit the kidney while the renal artery enters. Surrounding these structures is hilar fat and lymphatic tissue with lymph nodes. The hilar fat is contiguous with a fat-filled cavity called the renal sinus. The renal sinus collectively contains the renal pelvis and calyces and separates these structures from the renal medullary tissue(Clapp, 2009.)

1.2.6.1 Functions of the kidneys:

1. Secretion of rennin: When blood pressure decreases, the juxtaglomerular (juxta means “next to”) cells in the walls of the afferent arterioles secrete the enzyme renin. Renin then initiates the renin-angiotensin mechanism to raise blood pressure. The end product of this mechanism is angiotensin II, which causes vasoconstriction and increases the secretion of aldosterone, both of which help raise blood pressure. In these ways the kidneys help ensure that the heart has enough blood to pump to maintain cardiac output and blood pressure(Scanlon and Sanders 2007).

2. Secretion of erythropoietin: This hormone is secreted whenever the blood oxygen level decreases (a state of hypoxia). Erythropoietin stimulates the red bone marrow to increase the rate of RBC production. With more RBCs in circulation, the oxygen-carrying capacity of the blood is greater, and the hypoxic state may be corrected.

3. Activation of vitamin D: This vitamin exists in several structural forms that are converted to calcitriol (D2) by the kidneys. Calcitriol is the most active form of vitamin D, which increases the absorption of calcium and phosphate in the small intestine (Scanlon and Sanders 2007).

1.2.6.2Assessment of Kidney function:

Functions of the kidney (metabolic, excretory, regulatory and endocrine) can be impaired, at different times, either separately or globally. A customary method to assess global kidney function is the measurement of glomerular filtration rate (GFR). The GFR is the amount of plasma that is filtered by functioning nephrons in a period of time. It can be measured by finding the clearance of a substance that is freely filtered by the glomerulus, and neither secreted nor reabsorbed by the tubules. Clearance is defined as the volume of plasma of a given substance that is completely cleared during a given time period.

Clearance = (UXV)/P

U=Urinary concentration of the substance (mg/dl)

V=Urine flow rate (ml/min)

P=Plasma concentration of the substance

A- Measurement of creatinine clearance:

Inulin, which is a large carbohydrate like molecule that is freely filtered by the glomerulus, was used initially for the measurement of GFR. Today creatinine, which is and endogenous substance produce from creatine that originate in the muscles, is used to calculate the GFR. However it is more appropriate to call this creatinine clearance, because renal tubule does secrete a small amount of creatinine. Radioisotopes have been used to measure GFR in patient. However, these are expensive for routine clinical use.

B- Estimation of creatinine clearance and the glomerular filtration rate:

Timed collections of urine over 24 hour can be difficult to obtain, and they are often inaccurate as a result of missed samples. Hence, several formulae have been developed to estimate kidney function from serum

creatininemeasurements alone. The oldest of these for the estimation of creatinine clearance is Cockcroft-Gault Formula. It is better than to serum creatinine measurement alone and also to the measure creatinine clearance with a 24 hour urine collection.

Cockcroft-Gault Formula:

$$\text{Men: } \frac{(140 - \text{ age in years})(\text{Weight in Kg})}{72 \text{ X Serum Cr (mg/dl)}}$$

$$\text{Women: } \frac{(140 - \text{ age in years})(\text{Weight in Kg})}{72 \text{ X Serum Cr (mg/dl)}} \text{ X } 0.85$$

The formula currently recommended by the national kidney foundation to estimate GFR is called the modification of diet in renal disease (MDRD) study formula (Moorthy*et al.,* 2009).

1.2.7 Renal failure:

Renal failure is a condition in which the kidneys are less able than normal to perform their functions of removing excess water, helping to control blood pressure and control red cell manufacture. It can be acute (ARF) or chronic (CRF), when advanced CRF called end stage renal disease (ESRD) (Moorthy*et al.*, 2009).

1.2.7.1 Causes of renal failure:

Renal failure, the inability of the kidneys to function properly, may be the result of three general

Causes, which may be called:

1- Prerenal: means that the problem is “before” the kidneys, that is, in the blood flow to the kidneys. Any condition that decreases blood flow to the kidneys may result in renal damage and failure. Examples are severe hemorrhage or very low blood pressure following a heart attack (Myocardial infarction).

2- Intrinsic renal: means that the problem is in the kidneys themselves. Diabetes mellitus and hypertension damage the blood vessels of the kidneys, and are the causes of 70% of all cases of end-stage renal failure. Bacterial infections of the kidneys or exposure to chemicals (certain antibiotics) may cause damage to the nephrons. Polycystic kidney disease is a genetic disorder in which the kidney tubules dilate and become nonfunctional. Severe damage may not be apparent until age 40 to 60 years but may then progress to renal failure (Scanlon and Sanders 2007).
3- Postrenal: means that the problem is "after" the kidneys, somewhere in the rest of the urinary tract. Obstruction of urine flow may be caused by kidney stones, a twisted ureter, or prostatic hypertrophy. Treatment of renal failure involves correcting the specific cause, if possible. If not possible, and kidney damage is permanent, the person is said to have chronic renal disease. (Scanlon and Sanders 2007).

1.2.7.2 Acute Kidney injury:

Acute kidney injury (AKI) is defined as decline in the glomerular filtration rate (GFR) that occurs during a period of less than 2 weeks. The decline in GFR is measured by an increase in serum creatinine by 0.5 or 1.0 mg/dl or by 25% or 50% (Moorthy*et al.,* 2009).

1.2.7.3 Chronic kidney disease:

Chronic kidney disease (CKD) is defined as decline in the glomerular filtration rate (GFR) that occurs over months to years (Moorthy*et al.*, 2009).

Categories of chronic kidney disease:

CKD is categorized by the level of GFR and the presence or absence of proteinuria to:

Stage 1: Include patients with normal in GFR but with kidney abnormalities

Stage 2: Include patients with mild CKD with an estimated GFR (eGFR) of 60 to 89 ml/min/1.73 $m^2$and with kidney abnormalities.

Stage 3: Include patients with an estimated GFR (eGFR) of 30 to 59 ml/min/1.73 m^2.

Stage 4: patients have an e GFR of 15 to 29 ml/min/1.73 m^2.

Stage 5: is kidney failure; this includes patients with an eGFR of less than 15 ml/min/1.73 m^2 (Moorthy*et al.*, 2009).

1.2.8 kidney Dialysis:

In medicine, dialysis is a process for removing waste and excess water from the blood, and is used primarily as an artificial replacement for lost kidney function in people with renal failure. 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 stage 5 (previously chronic renal failure or end-stage renal disease). The latter form may develop over months or years, but in contrast 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 inappropriate(Pendse S et al,2008).

Indications for chronic dialysis are:

1- Symptomatic uremia: anorexia, nausea, vomiting, encephalopathy, pericarditis, or severe fatigue.
2- Fluid over load not responsive to diuretics
3- Hyperkalemia not controlled with diet or loop diuretics.
4- Glomerular filtration rate of less than 10 ml/min/1.73 m^2 in patients with diabetes. (Moorthy*et al.*, 2009).

1.2.8.1 Types of kidney dialysis:

1- **Hemodialysis:**

In hemodialysis, 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 wall acts as the semipermeable membrane. Blood flows through the fibers, dialysis solution flows around the outside of the fibers, and water and wastes move between these two solutions.(AhmadS 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 liters of excess fluid during a typical 4-hour treatment.(Pfuntner et al,2011)

2-Peritoneal dialysis

In peritoneal dialysis, a sterile solution containing glucose (called dialysate) is run through a tube into the peritoneal cavity, the abdominal body cavity around the intestine, where the peritoneal membrane acts as a partially permeable membrane. The peritoneal membrane or peritoneum is a layer of tissue containing blood vessels that lines and surrounds the peritoneal, or abdominal, cavity and the internal abdominal organs (stomach, spleen, liver, and intestines)(Blake et al, 2008) Diffusion and osmosis drive waste products and excess fluid through the peritoneum into the dialysate until the dialysate approaches equilibrium with the body's fluids. Then the dialysate is drained, discarded, and replaced with fresh dialysate.(Kallenbach,2005).

1.3 Rationale:

In Sudan according to ministry of health the prevalence of renal failure is increasing through the few past years approximately 70 – 140 individual per million population per year.On June 2011 the number of renal failure patients reaches 4215 according to the records of National Center for Kidney Diseases and Surgery (NCKDS) with an increase of 246 new cases since January 2011(Abboud*et al.*, 1989). This study is aimed at providing essential data concerning evaluation the changes of complete blood count that occur in renal failure patients before and after HDto take the ideal precaution before and after dialysis session in order to reduce patient morbidity and motility.

1.4Objectives:

1. 1.4General objective

To measurement of complete blood count(CBC) of renal failure patients before and after hemodialysis.

1.4.2 Specific objective:

-To measure the RBC indices(MCV,MCH,and MCHC) before and after hemodialysis.

-To detect the differences in RBC,WBC,andPLt count in pre and post hemodialysis.

-To evaluate the change in Hb and PCV .

-To show the peripheral blood picturefor morphological change.

Chapter Two

Materials and Methods

CHAPER II

MATERIAL AND METHODS

2.1 Study design:

This was an cross-sectional study was conducted to measure complete blood count (RBC.PCV.Hb.WBC.PLt,MCV,MCH and MCHC) and show the peripheral blood picture(PBP) in patients with end stage renal disease (ESRD) who attended hemodialysis sessions in Omdurman Teaching Hospital. This study was carried out during the period from March to June 2014.Renal failure patients under hemodialysis at different ages and sexes, exposed to different periods of hemodialysis were recruited for this study.

2.2 Criteria of selection

2.2.1 Inclusion criteria:

1. Patient with Renal failure disease.
2. Patient under hemodialysis.
3. Both sexes were included in the study.
4. All ages.

2.2.2 Exclusion criteria:

1. Patient without renal failure disease.
2. Hepatitis patients.

2.3 Sample size

Pre and post dialysis sampling was taken from 50 patients under hemodialysis.

2.4Study variables

Hb, PCV, RBC, MCV, MCH, MCHC, , TWBC and PLT count.In addition the morphological picture in hemodialysis Patients,

2.5 Tools of Data collection:

A questionnaire was filled for each patient

2.6Data analysis:

Data were collected manually and analysis was performed using computerized SPSS program Version 17 to obtain the mean,SD,andP.value.

2.7 Ethical considerations

All patients were informed about the aim of the study and obtain their consent to participate in this study.

2.8. Sample collection

Three ml of venous blood samples was collected using disposable syringes and added slowly to containers K3-EDTA for CBC analysis.

2.9. Procedure

Performed by Sysmex KX-21 hematological analyzer was used to measure Hb, PCV, RBC, MCV, MCH, MCHC , TWBC,and PLT count.

A thin blood film was prepared and stained with Lieshman□s stain, and examined to show the blood picture.

2.9.1 Principle of Sysmexautoanalyzer:

The KX-21 employs three detector blocks and two kinds of reagents for blood analysis.WBC count was measured by WBC detector block using detection method.RBC and plateletscounts were taken from RBC detector block, also using the DC detection method. The hemoglobin detector block measures hemoglobin concentration using the noncyanide hemoglobin method.

A blood sample was aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are

the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizesis plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

For Hemoglobin determination the Non-cyanide hemoglobin method was used. Blood hemoglobin was converted rapidly to oxyhemoglobin. The later absorbance was measured in the Hb flow cell at 555 nm wavelength against diluent and the concentration of Hb was calculated.

Sysmex KX-21calculates RBC indices (mean corpuscular volume MCV, mean corpuscular hemoglobin MCH, and mean corpuscular Hb concentration MCHC) from red cell count (RBC), hemoglobin concentration (Hb) and packed cell volume (PCV).

2.9.2 Leishman's stain

Leishman stain used in microscopy for staining blood smears. It provides excellent stain quality. It is generally used to differentiate and identify leukocytes, malaria parasites, and trypanosomas. It is based on a metabolic mixture of methylene blue and eosin.

2.9.2.1 Staining Methods

1 Air dried blood smear was taken and covered with undiluted stains and incubated to usually 1–2 minutes, 3 minutes. The undiluted stain both acts as a fixative and also partially stains the smear.

2 Twice volume of pH 6.8 buffered water was added(i.e. If e.g. 7 drops of stain was used, then use 14 drops water) to dilute the stain. Allow to stained for 10–12 minutes.

3. Stain smear was washed with clean (or filtered) tap water. the back of the slide was cleaned and standed it in a draining rack to dry. The stained smear was grossly appear neither too pink nor too blue (the final results was showed microscopically).

Chapter Three

Results

CHAPTER III

RESULTS

This study was conducted in Khartoum state to show the change in complete blood count (CBC) and morphological picture in pre and post hemodialysis,50 Renal failure patients under hemodialysis at different ages, from different tribes and sexes(31from male and 19 from female)from Omdurman Teaching Hospital in Khartoum state,,between March toApril 2014. In this study there areincrease in the mean of Hb,RBC,PCV,and WBC after hemodialysis ,Hb=(pre dialysis 8.68g/dL/ post dialysis 9.97g/dL), PCV=(pre dialysis 27.55%/post dialysis 30.56%),RBC=(pre dialysis3.03×10^{6}/ml/post dialysis 3.59×10^{6}/ml)and WBC=(pre dialysis5.12×10^{3}/ml / post dialysis6.11×10^{3}/ml).

and there are no different in the mean of MCV, MCH and MCHC in pre and post hemodialysis, also there is decrease in the mean of Plt after hemodialysis(predialysis218.04×10^{3}/ml/postdialysis194.86×10^{3}/ml).There is decrease in weight of patients after dialysis compare to pre dialysis. Inperipheral blood picture(PBP)there is no different in the morphological picture in pre and post hemodialysis the RBC normocytic normochromic,WBC and Pltare normal picture.

Table (3-1):Distribution of study group according to age:

age group	Frequency	Percent
20-29	7	14.0
30-39	10	20.0
40-49	10	20.0
50-59	9	18.0
60-69	9	18.0
70-79	4	8.0
80-89	1	2.0
Total	50	100.0

Table(3-2):Distribution of study group according togender:

Gender	Frequency	Percent
Male	31	62.0
Female	19	38.0
Total	50	100.0

Table(3-3) Comparison of study group pre hemodialsis(pre HD) and post hemodialysis(post HD) on TWBC,RBC,Hb,PCV,MCH,MCHCMCV,PLTandWeight:

Groups	Mean ± SD	P.Value
TWBC×10^3/ml preHD	5,12 ± 1.52	0.00
PostHD	6.12 ± 1.85	
RBC×10^6/mlpreHD	3.03 ± 0.69	0.00
PostHD	3.59 ± 0.81	
Hbg/dL preHD	8.68 ± 1.96	0.00
PostHD	9.97 ± 2.49	
PCV% preHD	27.54 ± 6.33	0.00
PostHD	30.56 ± 7.53	
MCV fL preHD	90.98 ± 6.04	0.62
PostHD	91.24 ± 5.81	
MCH pg preHD	26.41 ± 3.25	0.96
PostHD	26.39 ± 3.14	
MCHC g/dL preHD	29.02 ± 3.14	0.34
PostHD	28.64 ± 2.95	
PLT×10^3/ml preHD	218.04±90.09	0.00
PostHD	194.86±79.71	
Weight Kg pre HD	58.80 ± 12.03	0.00
Post HD	55.74 ± 12.13	

Chapter four

Discussion ,Conclusion, and Recommendations

CHAPTER IV

DISCUSSION,CONCLUSION,AND RECOMINDATION

4.1 Discussion:

Chronic kidney disease globally resulted in 735,000 deaths in 2010 up from 400,000 deaths in 1990(Lozano etal,2012*)*.Dialysis is a treatment method that replicates the function of the kidneys when they are failing. Various hematological parameter should be monitoring to prevent the complication and consequently, the mortality rate(Ali et al,2008).

Fifty patients of renal failure under hemodialysis were recruited in thisstudy.The sample were collected from Omdurman Teaching Hospital in Khartoum state. Practical work done also at Omdurman Teaching Hospital .

In this study there were increase in the mean of Hb,PCVand,RBC after hemodialysis when compare with the mean before hemodialysis and these due to the west product and hypervolemia effect on Hb,PCVand RBC so after hemodialysis causes hypovolemic due to excreted of waste product and water lead to concentrate the Hb,PCV,and RBC and then increase after HD,In addition to that the patients take erythropoietin treatment help the patients to produce RBC. This was similar to the result reported by Ali et al(2008) Who reported increase in the mean of Hb,PCV.and RBC. And agree with the study carried out by Alghythan (2012) who found the mean of each RBCs count, Hb and, PCV increase in renal failure patients' post-HD when compared to pre-HD levels. As well as agree with result reported by Ali E (2011) who reported increase in the mean of Hb,PCVand WBC .On other hand our study disagree with study carried out by Ahmed (2013) who reorted there were lower in the mean of RBC,Hb and HCT.

This study found increase in the mean of WBC after hemodialysis session and thatdue to the west product and hypervolemia effect on WBC count so after hemodialysis hypovolemic occur due to excreted of waste product and water lead to concentrate WBC this was agree with study carried out by Ali etal(2008),Ahmed(2013)andAlghythan(2012)there were foundstatistically significant increase in the mean of WBC in renal failure patients' post-HD when compared to pre-HD levels.

In these study there were no differences between means of MCV, MCHC, and MCHC levels before and after HD session because have no thing effect in RBC indices in the pre and post dialysis, this is agree with Ali E (2011) who said the same results but this is contrast to Ali et al(2008) who reportedthere were decrease in the mean of MCH and MCHC after hemodialysis. Also disagree with result of Alghythan (2012) which found statistically increase in the mean of MCH and MCHC in post-HD when compared to pre-HD levels. Also it contrast to reported by Ahmed (2013) who found lower in the mean of MCH and was higher in the mean of MCHC .

In these study there was decrease in the mean of PLT after hemodialysis when compare with the mean of PLT before HD and that due to One factor associated with poor outcomes in hemodialysis patients is exposure to a foreign membrane. Older membranes are very bio incompatible causes to activates platelets and brake down of itRecently, newer membranes have been developed that were designed to be more biocompatible,this is contrast to result reported byAhmed (2013) who foundhigher in the MeanofPLT.

4.2 Conclusion:

In base of these research It can be concluded that:

- The mean ofHb,RBC,PCV,andWBCswere significantly increased after hemodialysis compared to before it, whereas the mean of PLTs was significant decrease after hemodialysis.
- There were no differences between means of MCV, MCHC, and MCHC levels in renal failure patients before and after HD.
- Majority of patient is anemic due to failure of kidney to produce the erythropoietin.
- The majority of patients were Normocytic normochromic. And PLt and WBC were normal morphology.
- Decrease in toxic substance after HD lead to increase in mean of Hb,PCV,RBC and WBC and that due to the hypervolimic that occur after HD.

4.3 Recommendations

- Patients under hemodialysis should be follow up and routine hematological CBC should be done regulatory.
- In order to get more informative data, the sample size should be increased in related subsequent researches.
- Make deferential White blood cell count in related subsequent researches.
- A further study about erythropoietin level in CKD patient.
- A further study about the Platlate and the effect of renal failure patients and hemodialysis on it.

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Appendix I

Questionnaire:

Sudan University of Science and Technology

Colleges of Graduate Studies

Questionnaire

Title: Effect of Hemodialysis in CBC before and after Hemodialysis

NO----------DATE----/------/2014

Name;----------Age;----------

Phone;----------Address;----------

-Weight;pre:---------post:------*The date of appears of disease-----*

Treatment taken;----------Response to treatment; yes-------No----

Date of first time to dialysis----------

How many time dialysis / weak----------

RESULT;

CBC;

Hb----------PCV----------RBCs count----------PLT count--------

MCV----------MCH----------MCHC----------

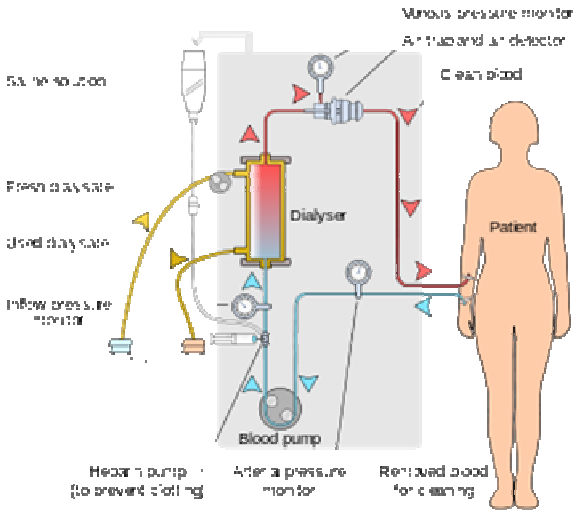
TWBCs-------

Thin blood film;----------

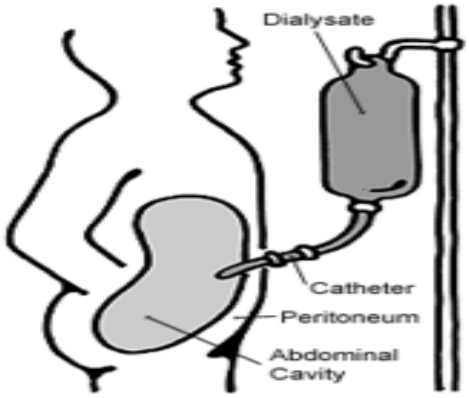
Appendix II

Color photos

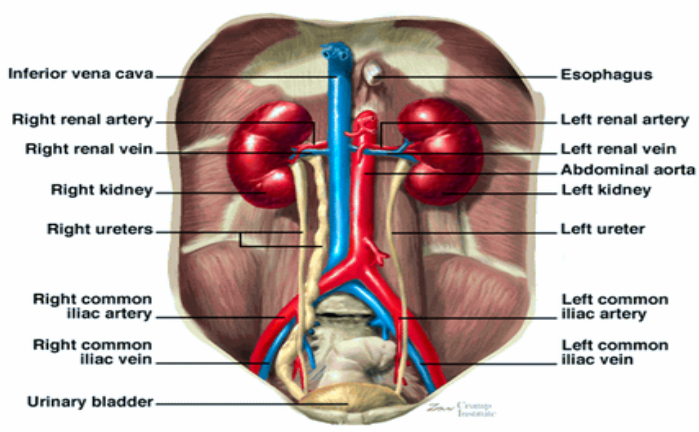
Automated hematology analyzer (sysmex KX-21N)



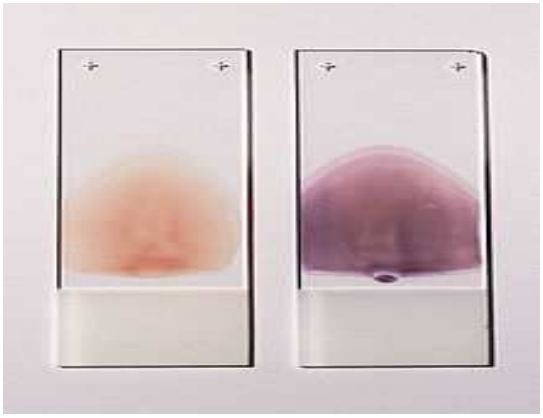
Hemodialysis schematic



Schematic diagram of peritoneal dialysis



The urinary system shown in anterior view



Two push-type peripheral blood smears suitable for characterization of cellular blood elements. Left smear is unstained, right smear is stained with Wright-Giemsa stain.