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Evaluation of Homocysteine level and some Hemotological Parameters in End Stage Renal Failure Patients

تقييم مستوى الهوموسيستين وبعض المعلمات الدموية في نهاية مرحلة مرضى الفشل الكلوي

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

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الايه

قال تعالي

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ (35))

سوره النور (ايه35)

DEDICATION

"Now thank I all My almighty Allah"

I dedicate this project to My parent whom supported, guided and help me with prayers in any situation since the beginning of My life.

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First of all, thanks to Almighty **Allah** for giving me power and health throughout this study.

I wish to express my tremendous gratitude to my supervisor, **Dr. Munsoor Mahmmoud Munsoor** Department of Hematology, Sudan University of Science and Technology who has initiated this work, encouraged and supported me unlimitedly.

Also I would like to offer my special thanks to Hematology staff in Sudan University of Science and Technology and to lab staff in Sudanese Kidney Transplanted Association.

Abstract

Background: Chronic Renal Failure is a major health problem and affects the economic and social status of patients. In Sudan, according to ministry of health records, the prevalence of renal failure is increasing approximately 70 to 140 new patients undergo dialysis each year. Although Homocystin ratio has been strongly linked to end stage renal disease, biochemical test is restricted to monitoring kidney function. Therefore, introducing homocysteine as a biomarker of ESRD in Sudan hospitals is recommended.

Objective: To assess homocysteine and hematological indices in hemodialysis patients at. Sudanese Kidney Transplanted Association.

Methods: This case-control study was conducted in Sudanese Kidney Transplanted Association 2018, compared plasma homcysteine and some hemoatological parameters in 30 renal failure patients as case group and 30 helathy Sudanese people at matched age as a control group. data of pations was collected from record saved in the computer

Automated hematological analyzer (Sysmex 21N KX-) was used measure some hematological parameters (HB, RBCs, PCV, MCV, MCH, MCHC, TWBC, platelates). An auto,ated chemistry analyzer (DRIRUS, CS-T240) used to measure the plasma hemocysteine level.

Results were analyzed using statistical package for social science (SPSS version 16) computer program. Independent T test was used for data analysis and person's correlation test was used for correlation.

Results: The Study show that that there was significant decrease in mean of HCY (µmol/L), HB(g/dl), RBCs(c/ µL), PCV(%), Platelets (L) in case group when compared with control (8.3 versus 10.8 p-value 0.000), (9.5 versus 12.3, P-value 0.000), (3.6 versus 4.9, P-value 0.000), (30.1 versus 40.9, P-value 0.000), (218 versus 301, P-value 0.000) respectively.

while the mean level of MCV (ft), MCH(pg),MCHC(%), TWBC(Cmm) exhibited insifnificant defference in case compared with control (83.1 versus 86.0, P-value 0.125),(26.0 versus 25.7, P-value 0.643), 30.8 versus 30.4, P-value 0.433),(14.2 versus 6.6 P-value 0.230) respectively.

There was correlation between value of homocysteine and HB, RBCs, PCV, Platelets (r= 0.276 probability value 0.033), (r = 0.332 probability value 0.010), (r = 0.332 probability value 0.010) respectively.

While there was no correlation between plasma homocysteine and MCV,MCH, MCHC, TWB Count (r - 0.062 probability value 0.637) , (r = 0.134 probability value 0.545) respectively

Conclusions: homocysteine, hemoglobin, red blood cells, hematocrite and platelets was significantly decrease and there was non significant different in mean cell volume, mean cell Hemoglobin, mean cell Hemoglobin concentration, total white blood cel in renal failure paients.

Homocysteine was positively correlated with hemoglobin, red blood cells, hematocrite and platelets. There is no relation between homocysteine and other hematological parameter.

ملخص الدراسه

مقدمة: الفشل الكلوي المزمن هومشكلة صحية كبيرة ويؤثر على الوضع الاقتصادي والاجتماعي للمرضى. في السودان، وفقا لسجلات وزارة الصحة، فإن انتشار الفشل الكلوي يزداد من 70 إلى 140 مريضا جديدا يخضعون لغسيل الكلى كل عام وعلى الرغم من ان مستوى الحامض الأميني الهوموسستين مرتبط بقوة بمرضى الغسيل الكلوي ،إلى أن الفحوصات البيوكيميائية مقتصرة لرصد وظائف الكلى فقط، لذلك يوصى بإدخال فحص الهوموسستين كعلامة بيولوجية للمرحلة الأخيرة لمرضى الكلى في مستشفيات السودان .

الهدف: لتقييم الهوموسيستين وبعض من المؤشرات الدموية عند مرضى الفشل الكلوي في المرحلة النهائية

الطريقة:هذه دراسة حالة وحالة مشابهة في جمعية زارعي الكلي السودانية 2018، لمقارنة مستوى الهوموسيستين وبعض المؤشرات الدموية في البلازما في 30 مريض مصاب بالفشل الكلوي كمجموعة حالة 30 شخص اصحاء متطابقة العمر. تم جمع بيانات المرضى من السجلات المحفوظة في الكمبيوتر. تم استخدام محلل الدم الالي (Sysmex 21N -KX) لقياس بعض المؤشرات الدموية (الهيموقلوبين، كريات الدم الحمراء، حجم الخلية المعباة، متوسط حجم الخلية، متوسط هيموقلوبين الخلية، متوسط تركيز الهيموقلوبين الخلوي، مجموع خلايا الدم البيضاء والصفائح الدموية). تم استخدام المحلل الكيميائي الالي (DIRUIcs-1240) قياس مستوى الهوموسيستين في البلازما.

تم تحليل البيانات عن طريقة برنامج الحاسوب الاحصائي للعلوم الاجتماعية (SPSS VE16) وذلك باستخدام اختبار T المستقيم لتحليل البيانات واختبار الارتباط ومعرفة نوعه.

النتائج: .

اوضحت الدراسة ان هناك انخفاض معنوي في متوسط الخوموسيستن (µmol/1) وخلايا الدم، الحمراء (c/µl)، حجم الخلية المعباة (%)، والصفائح الدموية (1)، في الحالات مقارنة مع الضوابط (8.3 مقابل 10.8 قيمة الاحتمال 0.000)، (9.5 مقابل 12.3 قيمة الاحتمال 0.000)، (3.6 مقابل 4.9 قيمة الاحتمال 0.000)، (30.1 مقابل 40.9 قيمة الاحتمال 0.000)، (218 مقابل 301 قيمة الاحتمال 0.000) على التوالي

بينما متوسط حجم الخلية ومتوسط هيموقلوبين الخلية متوسط تركيز الهيموقلوبين الخلوية ومجموعة خلايا الدم البيضاء اظهرت اختلاف ضئيل في الحالات المقارنة بالضوابط (83.1 مقابل 86.0 قيمة الاحتمال 0.125)، 26.7 مقابل 25.7 قيمة الاحتمال 0.643)

(30.8 مقابل 30.4 قيمة الاحتمال 0.433)، (4.2 مقابل 6.6 قيمة الاحتمال 0.230) على التوالي. وكان هنالك ارتباط بين قيمة الهيموسيستين والهيموقلوبين، خلايا الدم الحمراء، حجم الخلية المعباة، والصفائح الدموية (r = 0.276 قيمة الاحتمال 0.033)، (r = 0.332 قيمة الاحتمال 0.637)، (r = 0.134 قيمة الاحتمال 0.277)، (r = 0.152 قيمة الاحتمال 0.2469

(r = 0.080 قيمة الاحتمال 0.545) على التوالي

الاستنتاجات:

كان هناك انخفاض كبير في قيمة الهيموسيستين، الهيموقلوبين، خلايا الدم البيضاء، حجم الخلية المعباة، الصفائح الدموية)

وكان هناك اختلاف ض~يل في متوسط حجم الخلية، متوسط هيموقلوبين الخلية، متوسط تركيز هيموقلوبين الخلوي ومجموع خلايا الدم البيضاء عند مرضى الفشل الكلوي.

وجد ان الهيموسيستين يرتبط طرديا الهيموسيستين، الهيموقلوبين، خلايا الدم البيضاء، حجم الخلية المعباة، صفائح الدموية) ولايوجد ارتباط بين الهيموسيستين وباقي المؤشرات الدموية)

Table of Contents

Item	Page
Declaration	I
Dedication	II
Acknowledgment	III
Abstract	IV
Arabic abstract	VI
Table of Contents	VII
List of Tables	XI
Abbreviations	XII

Chapter 1 Introduction

1.1 introduction	1
1.2 Rational	3
1.3General objective	3
1.4Specific objectives	3

Chapter 2: Literature Review

2.1 Blood	7
2.1.1 Constituents of human blood	4
2.1.1.1Fluid portion (Plasma)	4
2.1.1.2 Solid portion (Cells)	4
2.1.2 Blood functions	5
2.1.3 Formation of blood cells (Haematopoiesis)	5
2.1,3.1 Postnatal Haematopoiesis	5
2.1.3.2 Hematopoietic growth factors	6
2.1.3.3 Erythrocyte Production (Erythropoiesis)	6
2.1.3.4 Granulocyte Production (Granulocytopoiesis)	7
2.1.3.5 Monocyte Maturation	8
2.1.3.6 Lymphocyte Maturation	9
2.1.3.6.1 B lymphocytes	9
2.1.3.6.2T lymphocytes	9
2.1.3.7 Platelet (Thrombocyte) Production (Thrombopoiesis)	10
2.2 Complete blood count14	10
2.2.1 Principle of automated blood count	10
2.2.2 Red blood cells	10
2.2.3 White blood cells	11
2.2.4 Platelets	11

2.3 The kidneys	12
2.3.1 Location and structure	12
2.3.2 Functions of the Kidney	12
2.4 Kidney disease	13
2.4.1 Definition chronic kidney disease	13
2.4.2 Epidemiology of chronic kidney disease	14
2.4.3 Etiology and symptoms of chronic kidney disease	15
2.4.4 Causes of end stage renal disease	16
2.4.5 Treatment of end stage renal disease	16
2.5 Hemodialysis	16
2.6 Renal dialysis Principle	17
2.6.1 Peritonel dialysis	17
2.6.2 Kidney transplantation	17
2.6.3 Hematological complications of ESRD	18
2.7 Homocysteine	18
2.7.1 Definition and structure	18
2.7.2 Biosynthesis of homocysteine	18
2.7.3 Homocysteine species	19
2.7.4 Metabolism of homocysteine	19
2.7.5 Homocysteine values	19
2.7.6 Hyperhomocycteinemia	19
2.8 Homocysteine studies in chronic kidney disease	20

Chapter 3: Materials and Methods

3.1 Study design	21
3.2. Study area	21
3.3. Study duration	21
3.4. Study population	21
3.5. Ethical considerations	21
3.6 Sampling	21
3.6.1. Sample type	21
3.6.2. Sample size	21
3.7. Data collection	22
3.8. Laboratory Methods	22
3.8.1 Blood sampling and processing	22
3.8.2. Complete blood count	22
3.8.3. Determination of homocysteine	22
3.8.3.1. Principle	22
3.8.3.2. Reagents	23
3.8.3.3. Analytical procedure	23
3.8.3.4. Calculation of results	23
3.8.3.5. Reference value	24

3.9. Data analysis and presentation	24

Chapter 4: Results

4.1. Homocysteine levels of the study population	25
4.2. Hematological parameters of the study population	25
4.2.1. White blood cell count of the study population	25
4.2.2 Red blood cell/mass indices of the study population	26
4.2.3. Red blood cell indices of the study population	27
4.2.4 Blood platelet count of the study population	28
4.3. Hemocysteine relations	29
4.3.1. Homosycteine level in relation to WBC count of the Study population	29
4.3.2. Homosycteine level in relation to RBC mass of the study population	29
4.3.3 Homosycteine level in relation to RBC indices of the study population	30
4.3.4 Homosycteine level in relation to blood platelet count of the study population	31

Chapter 5: Discussion

5.1 Discussion	33
5.2 Conclusion	36
5.3 Recommendations	36

References	37
Appendix : Questionnaire	

List of Tables

Tables	Page
Table 2-1Erythropoiesis	6
Table 2-2Classification of the stages of chronic kidney disease	14
Table 4-1Homocysteine levels of the study population	25
Table4-2White blood cell (WBC) count of the study population	25
Table4-3 RBC count, hemoglobin (Hb), and hematocrit (Hct) of the study population	26
Table4-4 Mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and mean corpuscular hemoglobin concentration (MCHC) of thestudy population	27
Table4-5Platelets count of the study population	28
Table4-6Homocysteine level in relation to WBC count of the study	29
Table4-7Homocysteine levels in relation to RBC count, HB, and HT of thestudy population	32

List of Abbreviations

Abbreviation	Full Name
AKD	Acute Kidney Disease
ATP	Adenosine Triphosphate
ADP	Adensine Diphosphate
BASO	Basophil
B-cell	Bursa-Derived-Lymphocyte
BFU	Burst-Forming Unit
BFU-E	Burst-Forming Unit - Erythroid
DNA	Dexoyribonucliec Acid
CKD	Chronic Kidney Disease
CKF	Chronic Kidney Failure
CBC	Complete Blood Count
CLL	Chronic Lymphocytic Leukaemia
CFU	Clony-Forming Unit
CFU-GEMM	Granulocytes-Erythrocytes Monocytes/Macrophages-Megakaryocytes
CFU-GM	Colony-Forming Unit-Granulocyte Macrophage
CFU-L	Colony-Forming Unit- Lymphoid
CFU-MK	Colony-Forming Unit- Megakaryocyte
CRF	Chronic Renal Failure
ESRD	End Stage Renal Disease
GPX	Glutathione Peroxidase
GFR	Glomerular Filtration Rate
Hct	Hematocrit
Hcy	Homocysteine

HIV	Human Immunideficiency Virus
HSC	Hematopoietic Stem Cell
MCH	Mean Corpuscular Hemoglobin Concentration
MCHC	Mean Corpuscular hemoglobin Concentation
MCV	Mean Corpuscular Volume
MDRD	Modification of Diet in Renal Disease
MPV	Mean Platelet Volume
NK	Natural Killer
NADH	Nicotinemide Adenine Dincleotide Hydrite
NIST	National Institute of Standard and Technology
PDW	Platelet Distribution Width
PMNs	Polymorph Nuclear Neutrophil Width
RBC	Red Blood Cell
RDW	Red Blood Cell Distribution Width
RNA	Ribonucleic Acid
SAM	S-Adenosyl Methionine
SPSS	Statistical Package for Social Science
TCEP	Tris (2-Carboxyethly) Phosphine Hydrochloride Powder
T-cell	Thymus-derived Lymphocyte
THF	Tetrahydrofolate
WBC	White Blood Cell

CHAPTER ONE
INTRODUCTION

CHAPTER ONE
INTRODUCTION

1.1 Introduction

The kidneys are responsible for filtering and excreting wastes from the blood. Without proper functioning, toxic waste products will accumulate and the patient will die. Therefore, the kidneys are vital to maintain life**.** Kidney diseases were classified into acute kidney disease (AKD) and chronic kidney disease (CKD). Acute kidney disease is a syndrome characterized by rapid decline (hours to days) in glomerular filtration rate (GFR), retention of nitrogenous waste products, and perturbation of extracellular fluid volume and electrolyte and acid-base homeostasis **(Dennis *et al.*, 2005).** Chronic kidney disease is a progressive loss in renal function over a period of months or years, and it may lead to one of its recognized complications such as cardiovascular disease, anemia or pericarditis **(Nurko 2006, Herzog *et al.*, 2011).** Chronic kidney disease (CKD) is a well-known risk factor for end stage renal disease, ESRD **(Iseki *et al.*, 2004).**

Hemodialysis is the most common treatment option for ESRD patients. This treatment involves blood being taken from the body and circulated through a machine with an artificial kidney called a dialyzer, which performs ultra filtration and diffusion through a semi permeable membrane **(KFC 2004).** Under such circumstances, kidney dialysis is typically administered using a fixed schedule of three times per week **(Abo Shamala 2006).** Among the common complications seen in persons with ESRD, are anemia mainly due to loss of erythropoietin production **(Nurko 2006, Jonathan 2010, Lovcić *et al.*, 2011, Portoles *et al.*, 2013),** abnormalities in WBC, platelets functions **(Kaw and Malhotra 2006, Turkmen *et al.*, 2010),** and uremia **(Depner 2005, Locatelli and Canaud 2012).** The number of patients being treated for ESRD globally was estimated to be 2,786,000 at the end of 2011 and, with a 6-7% growth rate, continues to increase at

a significantly higher rate than the world population. Of these 2,786,000 ESRD patients, approximately 2,164,000 were undergoing dialysis treatment (hemodialysis or peritoneal dialysis) and around 622,000 people were living with kidney transplants. In the USA, Japan and the European Union, dialysis patient population growth rates between 2010 and 2011 were in a range of 1– 4% and, as such, were significantly lower than growth rates in regions such as Asia, Latin America, the Middle East and Africa **(FMC 2011).**

Homocysteine is a sulphur containing intermediary amino acid which is derived by the demethylation of methionine **(Shipchandler and Moore 1995).** The primary source of methionine is animal protein **(**Hankey and Eikelboom 1999**).** The normal range of homocysteine is 5 to15 μmol/L **(Ueland *et al*., 1993, Graham *et al*., 1997).** Elevated serum homocysteine beyond the normal range (>15 μmol/L) is traditionally referred to as hyperhomocysteinemia. Recently, hyperhomocysteinemia has been linked to different stages of CKD including ESRD **(Guldener 2006, Vieira *et al.*, 2010, Paterson 2011).**

Homocysteine is a non-protein α-amino acid. It is a homologue of the amino acid cysteine, differing by an additional methylene bridge (-CH_2-). It is biosynthesized from methionine by the removal of its terminal Co methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins **(Dietzen 2018).**

1.2 Rationale

End-stage renal disease (end-stage renal disease) is an emerging health problem in Sudan with limited treatment facilities. In developing countries, where rare complications can be observed with regular medical examination, this study was conducted in Sudan to study the level of homocysteine plasma and changes in blood parameters in patients with chronic renal failure, hemodialysis in Armed Forces Medical Services Hospital, Omdurman (2018).

The majority of patients who undergo dialysis have anemia, and this condition was a risk factor for death. However, iron stores were adequate in many patients. The main causes of anemia can be a lack of erythropoietin and insufficient dialysis. Also, hyperhomocysteinemia is present in the majority of patients.

1.3. General objective

To asses Evaluation of Homocysteine level and some Hemotological Parameters in end Stage Renal Failure Patients

1.4. Specific objectives

1. To determine Evaluation of Homocysteine level and some Hemotological Parameters in end Stage Renal Failure Patients

2. To measure complete blood count (CBC) in hemodialysis patients and controls using Sysmex.

3. To verify possible relations between homocystein with the studied parameters.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1 Blood

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

2.1.1 Constituents of human blood

2.1.1.1 Fluid portion (Plasma)

About 55% of blood is plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 litters in an average human. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood **(Blood 2015).**

It also includes a group of other ingredients such as serum albumin, blood clotting factors (to facilitate clotting), immunoglobulin (antibodies), lipoprotein molecules, various other proteins, various electrolytes (mainly sodium and chloride) and blood serum which refers to the plasma that has been removed. Including coagulation proteins. **(Blood 2015).**

2.1.1.2 Solid portion (Cells):

One microlitter of blood contains:

4.7 to 6.1 million (male), 4.2 to 5.4 million (female) Erythrocytes: Red blood cells contain the blood's haemoglobin and distribute oxygen, They carry oxygen from

the lungs to the tissues and return carbon dioxide (CO_2) from the tissues to the lungs to exhaled. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. RBCs have life span 120 days. **(Martini *et al*., 2006).**

2.1.2 Blood functions

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

Supply of oxygen to tissues (bound to haemoglobin).

Hydraulic functions: the restriction of blood flow can also be used in specialized tissues to cause engorgement, resulting in an erection of that tissue; examples are the erectile tissue in the penis and clitoris **(Jones 1998).**

2.1.3 Formation of blood cells (Haematopoiesis)

The term comes from the Greek haima (blood) and poiein (to make). For the average adult, the bone marrow produces ~5 x 1011 cells per day. The Haemopoietic system includes the bone marrow, liver, lymph nodes and thymus. Haematopoiesis begins in the yolk sac during the first month of embryogenesis but gradually shifts to the liver and, to a lesser extent, the spleen. Haematopoiesis may resume in the liver and spleen after birth in conditions associated with fibrosis of the bone marrow (extramedullary haematopoiesis) **(Ciesla 2007).**

2.1.3.1 Postnatal Haematopoiesis

During infancy and childhood, there is active haematopoiesis in the medullar cavity of virtually bone. The yellow marrow can resume active haematopoiesis under conditions of chronic hematologic stress (chronic bleeding or haemolytic anaemia) **(Ciesla 2007).**

All blood cells derive from pluripotent hematopoietic stem cells (HSC). These stem cells are supported by stromal cells which has two properties the first is self renewal, and the second is its proliferation and differentiation in to progenitor

cells, committed to one specific cell line **(Hoffbrand *et al*., 2006).** Each committed progenitor cell gives rise to a thousand or more mature blood cells **(Ciesla 2007).**

2.1.3.2 Hematopoietic growth factors

Hematopoietic growth factors are proteins or glycoproteins that regulate the production and differentiation of hematopoietic precursors. The majority are produced within the marrow and act locally. Erythropoietin and thrombopoietin are produced outside the marrow and reach the marrow through the blood **(Ciesla 2007).**

2.1.3.3 Erythrocyte Production (Erythropoiesis)

The erythron is the sum of all erythroid cells, including circulating red blood cells (RBCs) and marrow erythroid precursors. The earliest recognizable RBC precursor is the proerythroblast, which is characterized by fine nuclear chromatin and intensely blue cytoplasm (Table1). However, under intense stimulation by erythropoietin, reticulocyte may be released into the blood early where they may contain RNA for 2.0 to 2.5 days (shift reticulocyte) **(Ciesla 2007).**

Table 1 Erythropoiesis (Ciesla 2007).

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

2.1.3.4 Granulocyte Production (Granulocytopoiesis)

Neutrophils, eosinophils, and basophiles go through similar and parallel maturation processes. The earliest two stages of the three pathways are not distinctive (myeloblast and promyelocyte); the appearance of specific (secondary) granules at the myelocyte stage differentiates the three cell types Neutrophil, eosinophil, and basophil maturation follows parallel pathways; they can be differentiated at the myelocyte stage, when secondary (specific) granules appear. The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2 : 1 to 12 : 1 **(Beck 2009).**

Neutrophils:

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

* Secondary neutrophils (also called polymorph nuclear leukocytes (PMNs) have a nucleus divided into multiple distince lobes connected by thin strands of chromatin. The cytoplasm has fine granules that stain lightly with the usual blood stains, normally comprise - 50 to 70% of total WBCs.

* Band neutrophils ("band," or "stabs") have a horse-shoe-shaped nucleus, without the distinct lobes of PMNs. They are can carlier stage than segmented neutrophils but are fully functional, Bands normally represent - 2 to 6% of all WBCs

The primary function of neutrophils is phagocytosis, predominatly of, neutrophil granules. Neutrophils circulate in the blood for -10 hours and may live 1 to 4 days in the extravascular space. The number of Neutrophils increase with acute stress or infection (Ciesla 2007).

Eosiophils ("Eos"):

Eosnophils contain large granules that stain reddish-orange (eosinophilic) with usuall blood smear stain. The nucleus is segmented (often bilobed). Functions of

eosinphils include phagocytosis of antigen-antibody complexes and defence against parasitic infection. The normal eosinophil count is -2 to 4% of total WBC. The number of eosinophils increases with allergic reactions and parasitic infections (Ciesla 2007)

Basophils ("Baso"):

Basophils contain large dark blue purple (basophilic) granules, which often obsure the nucleus. The nucleus is segmented. Basophils are the least common type of leukocytes, normally <1% of total WBCs. The basophil granules contain heparin (an anticoagulant), histamine (a fast vasodilator), the compounds. Basophils appear to be involved in immediate hypersensitivity reactions related to immunolobulin class E (IgE) (Beck 2009).

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

The primary function of neutrophils is phagocytosis, predominantly of Bacteria:

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

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

2.1.3.5 Monocyte Maturation

Monocytes normally comprise ~3 to 8% of leukocytes, derived from the CFU-GEMM. Monoblast developed into promonocyte which develop into mature monocyte. Monocytes are large cells, with abundant light gray to light blue finely granular cytoplasm. Monocytes circulate through the blood and then after 8 to 14 hours enter the tissues to become either phagocytes (macrophages also called

histiocytes) or professional antigen presenting cells (Langerhans' cells and dendritic reticulum cells) **(Ciesla 2007).**

2.1.3.6 Lymphocyte Maturation

lymphoblast developed into prolymphocyte which develop into mature lymphocyte. Lymphocyte maturation begins in the bone marrow; B cells complete initial development in the marrow and then circulate to peripheral lymphoid tissues (lymph node, spleen, and mucosal surfaces) to await antigen exposure and final maturation into plasma cells. Differentiation into T helper and T suppressor subsets occurs in the thymus **(Ciesla 2007).**

2.1.3.6.1 B lymphocytes:

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

2.1.3.6.2 T lymphocytes:

The main effectors of cell-mediated immunity. T cell precursors originate in the bone marrow but develop and mature in the thymus (T = thymic dependent).T cells are divided into two main subtypes: **(Ciesla 2007).**

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

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

Unlike other leukocytes, which make a one-way trip between blood and tissues, lymphocytes can recirculate between blood, tissue, and lymph fluid. **(Ciesla 2007).**

2.1.3.7 Platelet (Thrombocyte) Production (Thrombopoiesis)

Platelets are derived from bone marrow megakaryocytes, which are large cells with multilobated nuclei and abundant finely granular light gray-blue cytoplasm. Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys **(Ciesla 2007).**

2.2 Complete blood count

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

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

2.2.1 Principle of automated blood count

The blood is well mixed (not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated haematology analyzers also measure the amount of haemoglobin in the blood and within each red blood cell **(CBCWD 2015).**

2.2.2 Red blood cells

- Total red blood cells count: given as an absolute number per litre.
- Haemoglobin: expressed in grams per decilitre.
- Reticulocyte count: which is a measurement of the absolute count or percentage of young red blood cells in blood.

• Hematocrit (Hct) or packed cell volume (PCV): This is the fraction of whole blood volume that consists of red blood cells.

• Red blood cell indices:

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

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

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

- Red blood cell distribution width (RDW): the variation in cellular volume of the RBC population **(CBCWD 2015).**

2.2.3 White blood cells

All the white cell types are given as a percentage and as an absolute number per litre. A complete blood count with differential will also include:

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

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

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

-Eosinophil granulocytes: Increased in parasitic infections, asthma, or allergic reaction.

-Basophil granulocytes: May be increased in bone marrow related conditions such as leukaemia or lymphoma**. (CBCWD 2015).**

2.2.4 Platelets

Platelet numbers are given, as well as information about their size and the range of sizes in the blood.

- Mean platelet volume (MPV): It is a measurement of the Platelate average size.
- Platelet distribution width (PDW): It is a measurement of the variation of platelet size. **(CBCWD 2015).**

2.3 The kidneys

2.3.1 Location and structure

The kidneys are small, dark red organs lie against the dorsal body wall beneath the parietal peritoneum in superior lumbar region where they receive some protection from the lower part of the rib cage. An adult kidney (about 12 cm long, 6 cm wide, and 3 cm thick) has a medial indentation (the hilus) in which there is two renal arteries, renal vein, and ureter **(Marieb 2003).** The kidney has three regions, outer granulated layer called renal cortex, renal medulla that consists of cone shaped tissue masses called medullary pyramids, and renal pelvis which is a central space or cavity that is continuous with the ureter **(Mader 2004).**

Urine from many nephrons is collected in the collecting ducts, which deliver the final urine product into the calyces and pelvis of the kidney **(Mader 2004, Thibodeau and Patton 1999, Guyton and Hall 2011).**

2.3.2 Functions of the Kidney

The kidneys excrete natural waste products, including urea and creatinine, as well as foreign substances like alcohol and drugs, from the body. The kidneys also regulate the water and electrolyte (dissolved salts) balance and the acidbase balance **(Barrett et al., 2010).** The kidneys also produce and secrete important hormones, including renin, erythropoietin, and vitamin D. Renin is involved in regulating blood pressure, erythropoietin is used to stimulate the bone marrow to produce red blood cells, and vitamin D is needed to absorb the calcium from food in the intestine **(Faratro et al., 2004, Barrett et al., 2010)**.

2.4 Kidney disease

Renal disease may be acute or chronic. Acute renal failure occurs when the kidney fails suddenly, but this may be a temporary problem, and after a short period of treatment the patient may recover. Chronic renal failure results from an abnormal loss of renal function over months to years. Chronic renal disease is rarely reversible and leads to progressive decline in renal function **(Faratro et al., 2004).** Reduction in renal mass leads to hypertrophy of the remaining nephrons with hyperfiltration, and the GFR in these nephrons are transiently at supranormal levels, that may worsen renal function **(Lawrence et al., 2003).**

2.4.1 Definition of chronic kidney disease

The National Kidney Foundation **(NKF 2002)** defines CKD as ″kidney damage for ≥ 3 months, as confirmed by kidney biopsy or markers of kidney damage, with or without a decrease in GFR or GFR < 60 ml/min/1.73 m2 for ≥ 3 months, with or without kidney damage″. Kidney damage is ascertained by either kidney biopsy or markers of kidney damage, such as urine abnormalities (proteinuria), blood abnormalities or abnormalities on imaging studies. Levey et al. **(Levey *et al.*, 2005)** defined CKD as kidney damage or GFR<60 ml/min/1.73 m2 for 3 months or more, irrespective of cause.

Chronic kidney disease has been classified into various stages for the purpose of prevention, early identification of renal damage and institution of preventive measures for progression of the primary damage and appropriate guidelines for instituting management for prevention of complications in severe CKD **(Vijayakumar *et al.*, 2007)**. National kidney foundation classified CKD into 5 stages according to the level of GFR (Table 2). For stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio **(NKF 2002)**

Table 2 Classification of the stages of chronic kidney disease.

CKD Stage	Description	GFR (ml/min/1.73 m2)
1	Kidney damage with normal or increased	≥ 90
2	Kidney damage with mild reduction of GFR	60-89
3	Moderate reduction of GFR	30-59
4	Severe reduction of GFR	15-29
5	Kidney failure (ESRD)	<15 or dialysis

GFR: Glomerular filtration rate. Adopted from National Kidney Foundation **(NKF 2002).**

2.4.2 Epidemiology of chronic kidney disease

Most epidemiological information on CKD originates from data available on ESRD; the terminal stage of CKD when treatment with renal replacement therapy (dialysis or transplantation) becomes necessary to sustain life. Little information is available on the prevalence of earlier stages of CKD, as patients are often asymptomatic **(Warady and Chadha 2007).**

In USA, kidney disease was the ninth leading cause of death in 2004 **(Minino *et al*., 2004).** Chronic kidney disease in USA affected an estimated 16.8% of adults aged 20 years and older, during 1999 to 2004 (Centers for Disease Control and Prevention) **(CDC 2007).** The National Health and Nutrition Examination Surveys (NHANES) in 1999-2004 estimates the prevalence of CKD stages as 1.8 % for stage one, 3.2 % for stage two, 7.7 % for stage three, and 0.35 % for stage four. The prevalence in all stages of CKD was higher than the prevalence of the survey in 1988-1994 **(Coresh *et al*., 2007).** In Canada 1.9-2.3 million people have CKD **(Levin 2008).** United Kingdom (UK) estimates suggest that 8.8% of the population of Great Britain and Northern Ireland have symptomatic CKD **(APHO 2007)**. The annual incidence of ESRD in United Kingdom, is around 10 per 100000

population, and it is expected to rise 5-8% annually. The European average is around 13.5 per 100000 population **(Hamer and Nahas 2006).**

The reported prevalence of chronic renal failure is 80 to 120 per million population (pmp) in the Kingdom of Saudi Arabia and 225 pmp in Egypt **(Shaheen and khader 2005).** In Saudi Arabia Mohamed et al. **(Mohamed *et al*., 2004)** indicated an increase in the incidence of ESRD from 6.52 per 100000 populations in the 1988 to 13.75 per 100000 populations in the 2001. The Palestinian reports showed that the incidence of renal failure was 10.8 per 100000 populations, and the prevalence was 4%, distributed as 1.1% in Gaza strip and 2.9% in the West Bank **(MH 2005).**

2.4.3 Etiology and symptoms of chronic kidney disease

In adults, diabetes is the leading cause of kidney disease, 48.5% of renal failure is due to diabetes, 16.5% hypertension, 17.7% glomerulonephritis and 20.3% is due to other causes such as kidney inflammation, genetic diseases, autoimmune diseases, and birth defects **(Kinchen *et al*., 2002)**. Certain food stuff was linked to CKD. However, data regarding such association is conflicting. It was suggested that frequent consumption of meat is associated with the development of CKD **(NIH 2006, Odermatt 2011)**. In addition, there is an evidence that consumption of cooked meat, in particular, may affect CKD categorization based on estimated GFR **(Preiss *et al*., 2007)**. Recently, patients with CKD, especially ESRD, exhibit many abnormalities in protein and amino acid metabolism. One of these alterations involves an increased plasma concentration of the sulphurcontaining amino acid homocysteine. Therefore, hyperhomocysteinemia has been linked to CKD **(Khajuria, and Houston 2001, Vieira *et al*., 2010).** The most common symptoms of CKD included pericarditis, encephalopathy, malaise cardiac arrhythmias, anemia, fatigue and edema **(NKF 2002).**

2.4.4 Causes of end stage renal disease.

End stage renal disease has many causes that vary from one patient to another. The most common causes include **(Hyman 2006, Soyibo and Barton 2007, Hartmann et al., 2009, Herzog *et al*., 2011):**

• Uncontrolled hypertension.

• Glomerulonephritis.

• Atherosclerosis.

• Obstruction of the urinary tract by stones or cancer.

• Diabetes mellitus.

• Obesity.

• Polycystic kidney disease.

• Medications such as the use of some analgesics regularly over long durations of time.

2.4.5 Treatment of end stage renal disease

The most important treatment alternatives for ESRD include hemodialysis, peritoneal dialysis and kidney transplantation. The populations of ESRD patients, dialysis patients and patients living with a transplanted kidney have increased steadily over the past years, whereby consistently more than three quarters of all ESRD patients were treated by dialysis **(FMC 2011).**

2.5 Hemodialysis

In haemodialysis, the patient's blood is pumped through the blood compartment of a dialyzer. exposing it to a partially permeable membrane. Blood flows through the fibers, dialysis solution flows around the outside of the fibers, and water and wastes move between these two solutions **(Ahmad *et al*., 2006).** These frequent long treatments are often done at home while sleeping, but home dialysis is a flexible modality and schedules can be changed day to day, week to week. In

general, studies have shown that both increased treatment length and frequency are clinically beneficial. **(Ronco and Levin 2007).**

2.6 Renal dialysis Principle

Dialysis works on the principles of the diffusion of solutes and ultrafiltration of fluid across a semi-permeable membrane. Wastes and excess water move from the blood, across the peritoneal membrane, and into a special dialysis solution, called dialysate, in the abdominal cavity which has a composition similar to the fluid portion of blood. **(Pendse *et al*., 2008).**

2.6.1 Peritoneal dialysis

Compared to hemodialysis, peritoneal dialysis offers lower risk of death across all subgroups for the first 1–2 years of dialysis and is now recommended for use as the initial modality of dialysis in the majority of ESRD patients due to the lower prevalence of infections and better preservation of residual renal function **(Chung et al., 2009).** The two common choices for peritoneal dialysis are continuous ambulatory peritoneal dialysis and automated continuous cycling peritoneal dialysis, both of which function by infusing peritoneal dialysis fluid in the peritoneal cavity and draining it 4–6 hours later with the number of exchanges varying according to patient size, peritoneal membrane permeability, and residual kidney function **(Crawford and Lerma 2008).**

2.6.2 Kidney transplantation

Is the surgical procedure of placing a fully functioning kidney into a person with ESRD. The transplanted kidney may originate from a deceased donor or from a related or unrelated person **(Cueto and Rojas 2007).** Several recent studies have demonstrated significantly improved patient and allograft survival as well as lower rates of delayed graft function or acute rejection episodes in those with preemptive

transplants versus those who were on dialysis for a period of time before transplantation **(Gill *et al*., 2004, Baura 2012).**

2.6.3 Hematological complications of ESRD

End stage renal disease is associated with a variety of hemopoietic changes. Anemia is common among ESRD patients, with more than 95% of individuals on dialysis receiving some form of anemia treatment **(Zadeh and Aronoff 2009, Anees *et al*., 2010).** The life span of red blood cells is reduced by approximately one third in hemodialysis patients **(Marticorena *et al*., 2004).** Other factors include suppression of bone marrow erythropoiesis, hematuria, and gastrointestinal blood loss **(Suresh *et al*., 2012).** Patients with ESRD develop increased bleeding tendency, which is characterized by defective interaction of platelets with damaged sub endothelium due to impaired platelet functions In this context, conventional hemostasis parameters PT, APTT and INR were disturbed and thrombotic complications and bleeding abnormalities are common among patients undergoing hemodialysis **(Mohsin *et al*., 2010, Holley 1999, Rios *et al*., 2010, Alghythan and Alsaeed 2012).**

2.7 Homocysteine

2.7.1 Definition and structure

Homocysteine is an amino acid with the formula $HSCH_2CH_2CH(NH_2)$ CO_2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH_2-) group. Homocysteine exists at neutral pH values as a zwitterion: Betatine form of (S)-Homocysteine and (R)-Homocysteine.

2.7.2 Biosynthesis of homocysteine

Homocysteine is not obtained from the diet **(Selhub 1999).** The adenosine is then hydrolyzed to yield L-homocysteine. L-homocysteine has two primary fates:

conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine **(Champe and Harvey2008).**

2.7.3 Homocysteine species

Several homocysteine species have been identified in human plasma including albumin-(protein)-bound, free circulating disulfides and sulfhydryl forms **(Maron and Loscalzo 2009).**

2.7.4 Metabolism of homocysteine

The metabolism of homocysteine can be divided into three distinct pathways the remethylation of homocysteine to methionine by the vitamin B12 dependent methionine synthase; the transsulfuration pathway, converting homocysteine to cystathionine and then cysteine via vitamin B6 dependent cystathionine β-synthase enzyme; in the liver and kidneys, homocysteine can be remethylated back to methionine by betaine-homocysteine methyltransferase **(Maron and Loscalzo 2009).**

2.7.5 Homocysteine values

The American Heart Association released an advisory statement classifying total homocysteine plasma concentrations as follows: 5-15 μmol/L homocysteine as normal, 16-30 μmol/L homocysteine as moderate, 31-100 μmol/L homocysteine as intermediately elevated and total homocysteine levels above 100 μmol/L as severely elevated concentrations **(Malinow *et al*., 1999).**

2.7.6 Hyperhomocycteinemia

Hyperhomocysteinemia, is defined as total homocyteine concentrations elevated above 15 μmol/L. Plasma homocysteine concentration exhibits a strong relationship with (indices of) renal function. Hyperhomocysteinemia has been implicated in patients with CKD **(Khajuria and Houston 2001, Vieira *et al*., 2010).** Hyperhomocysteinemia has been also associated with the pathogenesis of

cardiovascular disease **(Al-Obaidi *et al*., 2001, Lubos *et al*., 2007, Coldea *et al*., 2011).** Deficiencies of the vitamins folic acid (B9), pyridoxine (B6), or B12 (cyanocobalamin) can lead to hyperhomocysteinemia **(Brosnan 2004, Abraham *et al*., 2010)**. Hyperhomocysteinemia also occur in the rare hereditary disease homocystinuria and in the methylenetetrahydrofolate reductase polymorphism genetic traits **(Qi et al., 2003).**

2.8 Homocysteine studies in chronic kidney disease

(Van Guldener *et al*. 2005) conducted a stable isotope study on homocysteine and methionine metabolism in ESRD. Decreased remethylation may explain hyperhomocysteinemia in ESRD. In addition, **(Chou *et al*. 2000)** found that the mean concentrations of plasma total homocysteine in maintenance hemodialysis (21.28±4.32 microM) were statistically higher than in age-matched normal subjects (11.02±2.85 microM). **(Sobki *et al*., 1994).** A significant correlation was observed between tHcy and serum urea, creatinine, vitamin B12 and potassium in renal transplant patients. In addition, Bayés et al. **(Bayés *et al*., 2005)** confirmed that the patients undergoing dialysis demonstrated hyperhomocysteinemia, an increased inflammatory status, and an increase of the lipid peroxidation markers.

(Kotb 2010) investigated the levels of homocysteine, nitric oxide (NO) and glutathione peroxidase (GPX) activity in the plasma of chronic renal failure (CRF) patients and compared the results with that of normal controls and find if homocysteine level is related to the level of NO and/or glutathione peroxidase activity. At the same time, a non significantly positive correlation was noted between total homocysteine and Gpx activity in CRF patients. On the other hand, a non significantly positive correlation was noted between NO and Gpx activity in CRF patients. **(Kotb 2010).**

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

The present study is a case-control design.

3.2. Study area

This study was conducted in Khartoum State

3.3. Study duration

The study was conducted during the period from September to December 2018.

3.4. Study population

The study population comprised Evaluation of Homocysteine level and some Hemotological Parameters in end Stage Renal Failure Patients in Sudanese Kidney Transplanted Association 2018

3.5. Ethical considerations

This research is ethically commited to ask the involved institutions for permission to conduct the data among it and from the participants.

3.6 Sampling

3.6.1. Sample type

Non- probability convenience sampling technique.

3.6.2. Sample size

A total of 30 patients (15 males and 15 females) maintained before hemo dialysiswere included in the present study. Thirty healthy individuals (15 males and 15females) were served as controls. Controls and patients were age and sex matched.

3.7. Data collection

Primary Data was collected using data collecting sheets

Secondary Data was collected from Books and previous researches

3.8. Laboratory Methods

3.8.1 Blood sampling and processing

Blood samples were collected by the researcher herself from both patients (before hemodialysis sessions) and controls. 2.5 ml of blood were obtained from each subject into EDTA tube for CBC analysis. Then, plasma samples were obtained by centrifugation at 3000 rpm for 15 minutes for homocysteine estimation.

3.8.2. Complete blood count

Blood samples were processed by an automatic counter for hemoglobin concentration and other whole blood component concentrations (Sysmex KX-21N).

3.8.3. Determination of homocysteine

Homocysteine was determined by enzymatic UV method for the quantitative determination of homocysteine, using Fortress diagnostics kit, United Kingdom.

3.8.3.1. Principle

This assay is based on an assay principle that assess the co-substrate conversion product (a molecule that is not a substrate of the Hcy conversion enzyme and does not contain any element from sample Hcy) instead of assessing co-substrate or Hcy conversion product of Hcy.

3.8.3.2. Reagents

Reagent	Concentration
S-adenosylmethionine	0.1mM
NADH	>0.2mM
TCEP	>0.5mM
2-oxoglutarate	5.0 mM
Glutamate dehydrogenase	10 KU/l
SAH hydrolase	3.0 KU/l
Adenosine deamiase	5.0 KU/l
Hcy methyle transferase	5.0 KU/l

3.8.3.3. Analytical procedure

About 0.5 ml of plasma was transferred to the **CS-T240**Auto-Chemistry Analyzer to perform the test according to these parameters:

Parameter	Value
Hcy buffer (R1)	240µl
Standard/Sample	17µl
Mix and incubate for 5 minutes at 37ºC and then add	
Hcy substrate (R2)	40 µl
Reaction type	Fixed time
Wavelength (nm)	340
Reaction	Descending

3.8.3.4. Calculation of results

A two point calibrations curve is constructed between the Calibratar 1 and 2 using Δ absorbance of the calibrators. The Δ absorbance of the sample read off the graph.

3.8.3.5. Reference value

National Institute of Standards and Technology (NIST) standardized study shows 15 μmol/l as the cut-off value for normal level of homocysteine for adults.

3.9. Data analysis and presentation

The data obtained were analyzed and presented using Statistical Package for Social Science (SPSS) computer software version 16.0 for Windows. The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between cases and controls homocysteine levels. Pearson's correlation test was applied. The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1. Homocysteine levels of the study population

There was a significant decrease in the mean level of homocysteine in case compared to controls (8.3 vs. 10.8 µmol/l, P=0.000) **(Table 4.1)**.

Table 4.1. Homocysteine levels of the study population

Parameter	Case	Control	P-value
Homocysteine	8.3 SD	10.8 SD	0.000

P<0.05: significant

4.2. Hematological parameters of the study population

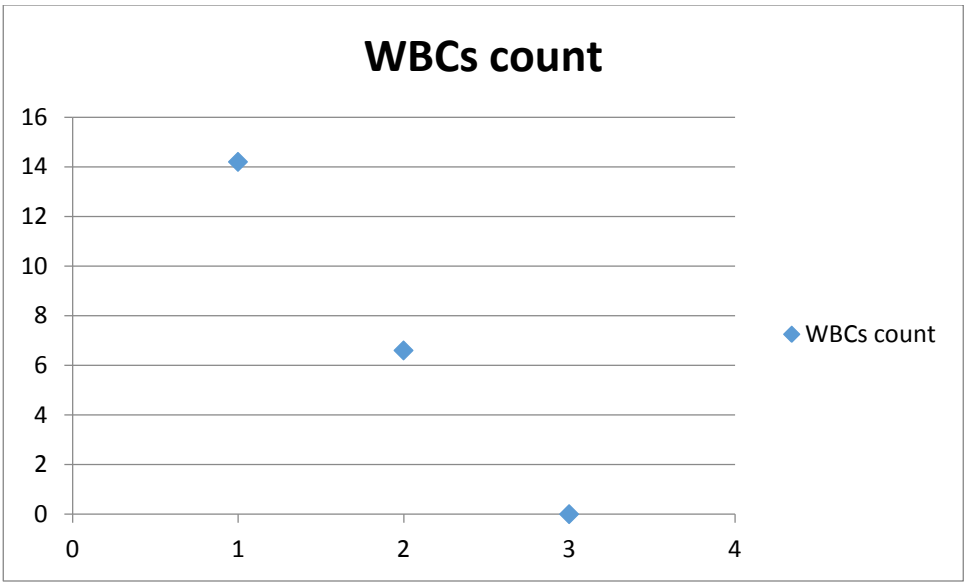
4.2.1. White blood cell count of the study population

There was a non-significant increase in the mean level of WBCs count in cases compared to controls (14.2 vs. 6.6 $x10^3$ cell/µl, P=0.230) **(Table 4.2).**

Table 4.2. White blood cell (WBC) count of the study population

Parameter	Case	Control	P-value
WBCs count ($x10^3$ cell/µl)	14.2	6.6	0.002

P<0.05: significant



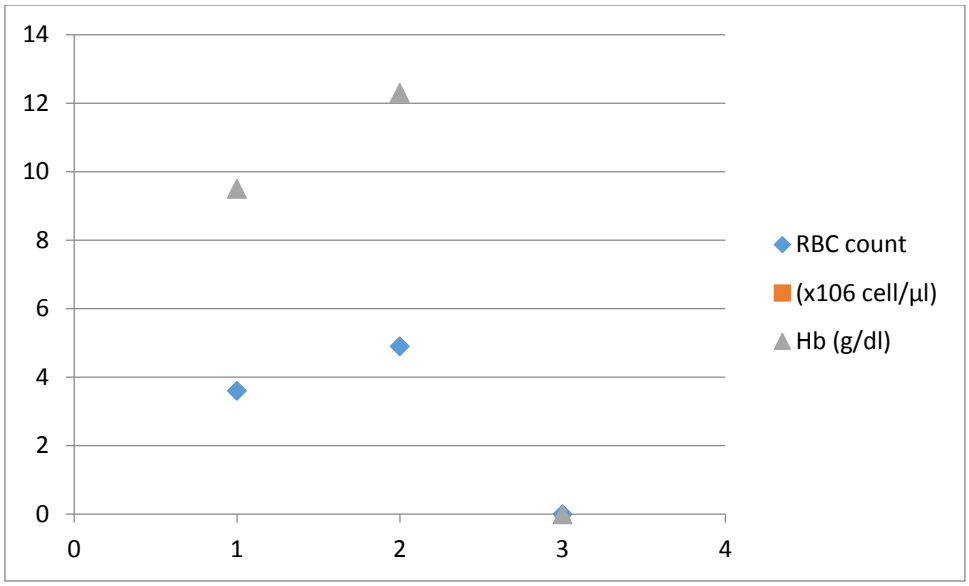
4.2.2 Red blood cell/mass indices of the study population

Red blood cell mass of the study population including red blood cell (RBC) count, hemoglobin and hematocrit of the study population are illustrated in **Table 4.3**. The means of RBC count, hemoglobin and hematocrit were found to be lower in cases (3.6 x10 cell/ml, 9.5 g/dl and 30.1 %) compared to controls (4.9 x106 cell/ml, 12.3 g/dl and 40.9 %) respectively, P=0.000.

Table 4.3. RBC count, hemoglobin (Hb), and hematocrit (Hct) of the study population

Parameter	Case	Control	P-value
RBC count (x106 cell/µl)	3.6	4.9	0.000
Hb (g/dl)	9.5	12.3	0.000
Hct (%)	30.1	40.9	0.000

P<0.05: significant



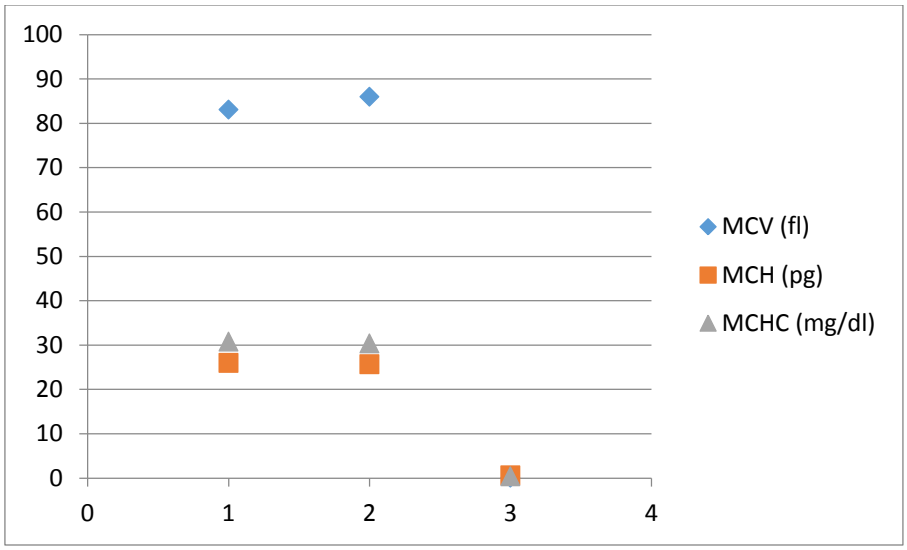
4.2.3. Red blood cell indices of the study population

Table 4.4 shows RBC indices of the study population. The average level of mean corpuscular volume (MCV) exhibited a non-significant decrease in cases compared to controls (83.1 vs. 86.0 (fl), p=0.125). However, mean corpuscular hemoglobin (MCH) displayed non-significant increase in cases (26.0 vs. 25.7 (pg),p=0.643). In addition, mean corpuscular hemoglobin concentration (MCHC) was non-significantly increase in cases compared to controls (30.8vs 30.4. p=0.433).

Table 4.4. Mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and mean corpuscular hemoglobin concentration (MCHC) of thestudy population

Parameter	Case	Control	P-value
MCV (fl)	83.1	86.0	0.125
MCH (pg)	26.0	25.7	0.643
MCHC (mg/dl)	30.8	30.4	0.433

P<0.05: significant



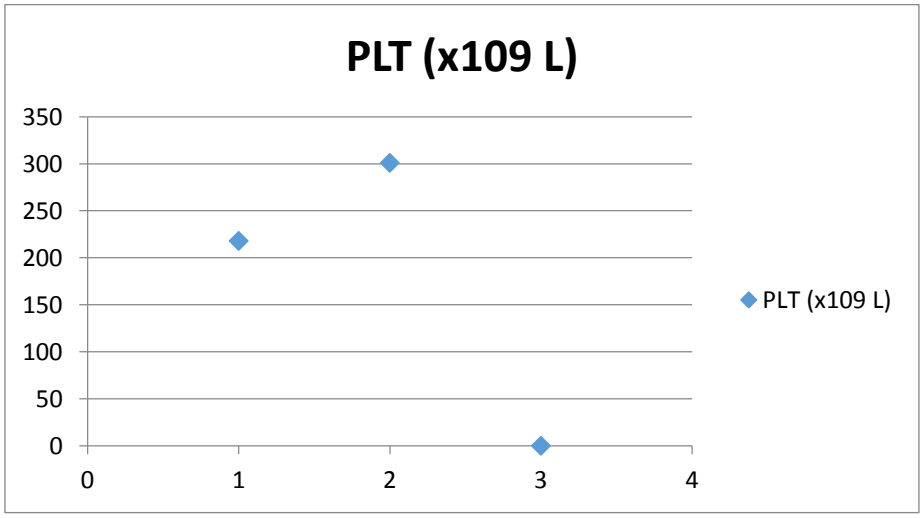
4.2.4 Blood platelet count of the study population

Table 4.5 shows blood platelet count of the study population. There was insignificant difference in the mean blood platelet count between cases and controls (218 vs 301 x109 L, p=0.000).

Table 4.5. Platelets count of the study population

Parameter	Case	Control	P-value
PLT (x109 L)	218	301	0.000

P<0.05: significant



4.3. Hemocysteine relations

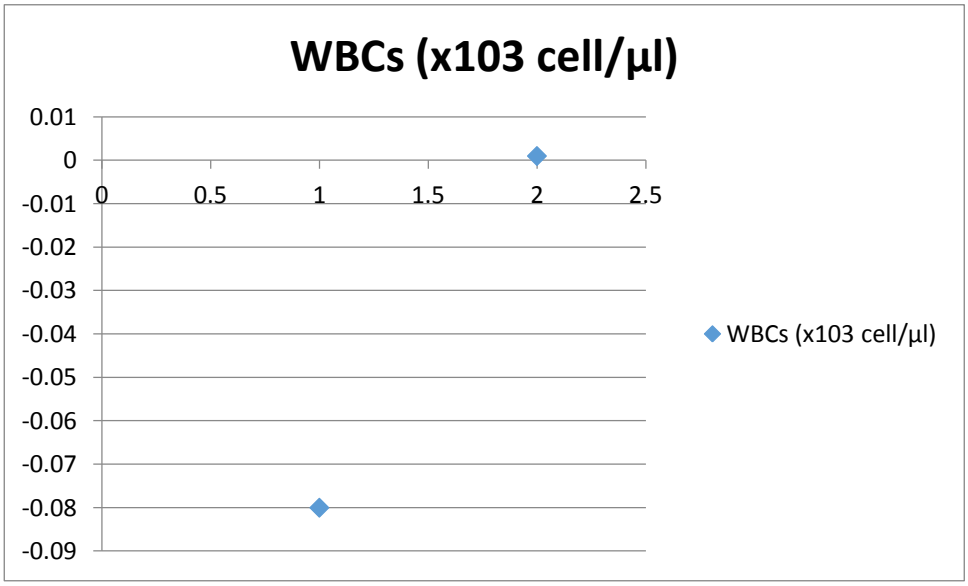
4.3.1 Homosycteine level in relation to WBC count of the Study population

Homosycteine level in relation to WBC count of the study population is illustrated in **(Table 4.6)**. The Pearson correlation test showed negative non-significant correlations of homocysteine level with WBC count (r= -0.080, P= 0.545).

Table 4.6Homocysteine level in relation to WBC count of the study

Parameter	**Homocysteine (µmol/l)**	
	Pearson correlation (r)	**P-value**
WBCs (x103 cell/µl)	-0.080	0.001

P<0.05: significant



4.3.2. Homosycteine level in relation to RBC mass of the study population

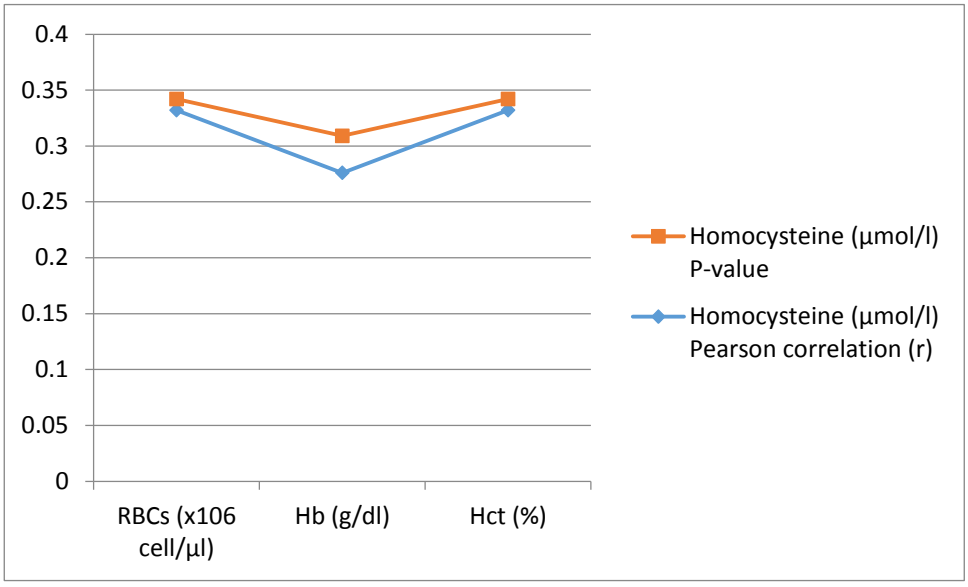
Table 4.7 shows the correlation between homocysteine level with RBC count, hemoglobin and hematocrite of the study population. The Pearson correlation test showed that the higher the homosycteine, the higher the RBC count,

hemoglobin and hematocrite. This positive correlation was statistically significant (r=0.332, P= 0.010, r=0.276, P=0.033 and r=0.332, P= 0.010 respectively).

Table 4.7 Homocysteine levels in relation to RBC count, HB, and HT of thestudy population

Parameter	**Homocysteine (µmol/l)**	
	Pearson correlation (r)	**P-value**
RBCs (x106 cell/µl)	0.332	0.010
Hb (g/dl)	0.276	0.033
Hct (%)	0.332	0.010

P<0.05: significant



4.3.3 Homosycteine level in relation to RBC indices of the study population

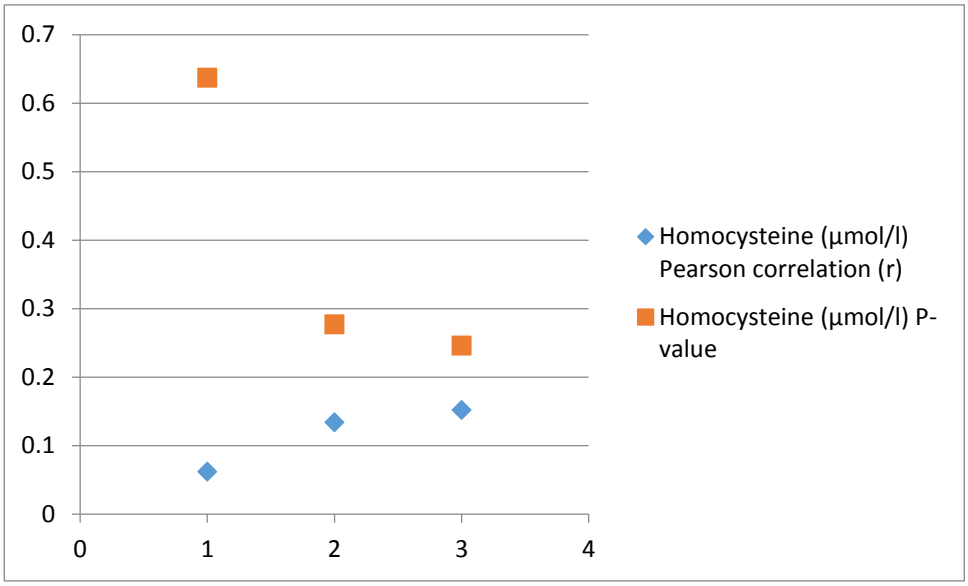
Homosycteine level in relation to MCV, MCH and MCHC of the study population is illustrated in **Table 4.8**. The Pearson correlation test showed non-significant correlations of homocysteine level with MCV, MCH and

MCHC (r=0.062, P=0.637, r= -0.134, P=0.277, r= -0.152, P=0.246 respectively).

Table 4.8. Homocysteine level in relation to MCV, MCH, and MCHC of thestudy population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
MCV (fl)	0.062	0.637
MCH (pg)	0.134	0.277
MCHC (mg/dl)	0.152	0.246

P<0.05: significant



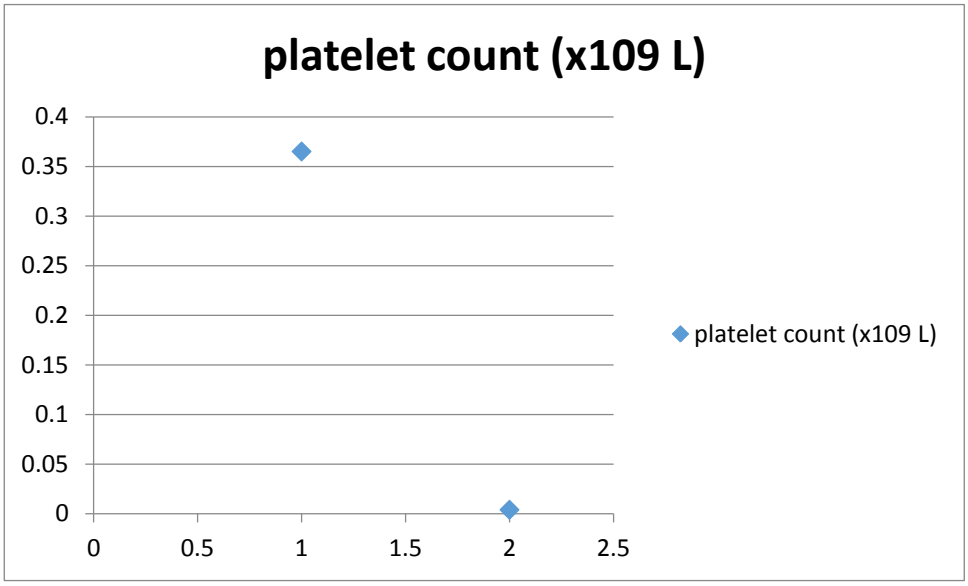
4.3.4 Homosycteine level in relation to blood platelet count of the study population

Table 4.9 points out the correlation between the homocysteine level and platelet count of the study population. Pearson correlation test showed a positive significant correlation between homocysteine level and platelet count (r=0.365, P= 0.004).

Table 4.9. Homocysteine level in relation to platelet count of the studypopulation

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	**P-value**
platelet count (x109 L)	0.365	0.004

P<0.05: significant



CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

5. Discussion:

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

As indicated in this study, there was a significant decrease in the mean level of homocysteine in cases compared to controls. This finding is in disagreement with that demonstrated by van Guldener *et al*. (2005) and Vieira *et al*. (2010). In addition, Friedman *et al*., (2001) reported that hyperhomocysteinemia is very common in patients with chronic renal insufficiency and is nearly ubiquitous in patients with end-stage renal disease; who have up to a 30 times higher risk of cardiovascular related death than the general population. Not mutually exclusive hypotheses for hyperhomocysteinemia in CKD There are: 1) homocysteine disposal in the kidneys themselves is disturbed and 2) extrarenal homocysteine metabolism is impaired (van Guldener, 2006). The first hypotheses are supported by our findings that 1) GFR was markedly decreased in CKD patients (cases) compared to controls and 2) There is a significant negative association between GFR and homocysteine. Ninomiya *et al*. (2004) has linked higher serum homocysteine levels to a greater decline of GFR.

White blood cell count and platelet count were significant increase in the mean level of WBCs count in cases in hemodialysis patients compared to controls. Leukocytosis recorded in the present study is in concurrent with that obtained by Reddan *et al*. (2003); Nasri, (2007); Wei Hsu *et al*. (2010) and Molnar *et al*. (2011).

It is known that hemodialysis patients suffer inflammation which is associated with Increase in WBCs (Nasri and Bradran, 2006 and Nasri, 2006; Afshar., 2009 and Molnar *et al*., 2011). In addition, Wei Hsu *et al*. (2010) pointed out that WBC count was closely associated with index of inflammation. When related to homosycteine level, results revealed that the higher the homosycteine, the higher the WBC counts. This positive correlation between homosycteine level and WBC count was reported by Ventura *et al*. (2004); Nasri and Baradaran, (2005) and Guerra-Shinohara *et al*. (2007).

Red blood cell count, hemoglobin, hematocrit and MCH values were non-significant lower, whereas MCHC was non-significant higher in hemodialysis patients compared to controls. This indicates that hemodialysis patients are more likely to be anemic. Such results are in agreement with that reported in earlier studies (Besarab *et al*., 1998; Afshar *et al*., 2009; Anees *et al*., 2010 Mohsin *et al*., 2010; Suresh *et al*., 2012 and Poudel *et al*., 2013). Anemia in hemodialysis patients may be due to many factors including blood loss, shortened red cell life span, vitamin deficiencies, the "uremic milieu," renal erythropoietin deficiency due to kidney failure, iron deficiency, and inflammation (Nurko, 2006; Locatelli *et al*., 2007 and Anees *et al*., 2010). In addition, Shittu *et al*. (2013) reported that hematological parameters are commonly affected in ESRD. Of all the parameters, red cell indices are the ones commonly and severely affected. This is because as high as 90% of erythropoietin is produced in the juxta glomerular apparatus of the kidney while 10% are produced in the liver and other organs. The severity of effect depends on the stage of renal failure. Person's correlation test showed negative significant correlations of homocysteine with RBC count, hemoglobin, hematocrit and MCH values whereas MCHC exhibited a positive significant correlation with homocysteine. Such correlations were previously obtained by Bachmann *et al*. (1995); Nasri and Baradaran, (2005); Anees *et al*. (2010) and Poudel *et al*. (2013),

and reinforced the idea that homocysteine is a suitable biomarker of ESRD, where most patients suffered hematological disorders.

Regarding blood platelets, there was a positive significant correlation in the mean platelet count in hemodialysis patients compared to controls. Such finding is in agreement with that demonstrated by Nasri. (2006); Molnar *et al*. (2011) and Alghythan and Alsaeed. (2012). A significant positive correlation of platelet count with serum homocysteine was found. Similar result was obtained by nasri (2006) who reported that in hemodialysis patients high homocystiene levels make the platelets more likely to clump and cause clots and contributes to the possibility of thrombotic events among these patients.

5.2 Conclusion

1. The mean levels of homocysteine were significantly decrease in hemodialysis patients compared to control
2. White blood cell count, MCHC, MCH count were non-significntly increase in cases compared to controls, whereas RBC count, hemoglobin, hematocrit and plts showed a significant decrease in cases compared to controls and MCV exhibited a non-significant decrease in cases compared to controls.
3. Homocysteine level were positively correlated with RBC count, hemoglobin and hematocrite where as negative non significant correlations were found between homocysteine and WBC count, non-significant correlations of homocysteine level with MCV, NCH and MCHC.

5.3 Recommendations:

1. Introducing of homcyteine as prognostic test for ESRD pateints in Sudan Hospital and clinics is highly recommended
2. Further research on the relation of homosysteine with kidney tranplanted ESRD with hypertension and diabetes patients with other paramete urea and creatinine, PT, APTT, clotting factors and the role homocysteine in fibriolusis is needed in ERD patients.

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