PREVALENCE OF ANEMIA AND ITS ASSOCIATED FACTORS IN PATIENTS WITH CHRONIC KIDNEY DISEASE AT MUHIMBILI NATIONAL HOSPITAL DAR ES SALAAM

Abdu Juma, MD

MMed (Haematology and Blood Transfusion) Dissertation

Muhimbili University of Health and Allied Sciences

October, 2012

PREVALENCE OF ANEMIA AND ITS ASSOCIATED FACTORS IN PATIENTS WITH CHRONIC KIDNEY DISEASE AT MUHIMBILI NATIONAL HOSPITAL DAR ES SALAAM

By

Abdu Juma

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine (Hematology and Blood Transfusion) of the Muhimbili University of Health and Allied Sciences (MUHAS)

Muhimbili University of Health and Allied Sciences

October, 2012

CERTIFICATION

The undersigned certify that they have read and hereby recommended for acceptance by Muhimbili University of Health and Allied Sciences entitled: **"Prevalence of anemia and its associated factors in Chronic Kidney Disease Patients at Muhimbili National Hospital, Dar es Salaam"** in partial fulfillment of the requirements for the degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences.

_____

Dr. M. Lyimo

(**Supervisor)**

Date _____

_____

Prof. E.Maro

(**Second Supervisor)**

Date _____

_____

Dr. Linda M.K. Ezekiel

(**Co-Supervisor)**

Date _____

DECLARATION AND COPYRIGHT

I, Dr. Abdu Juma declare that to the best of my knowledge this dissertation is my own original work, and has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature …… Date …….

ACKNOWLEDGEMENT

This is a great occasion to convey my respect to Dr. M.Lyimo and Prof. E.Maro for their guidance, understanding and patience, as my supervisor despite their many other academic and professional commitments and without them this study would not have been completed.

I wish to convey my gratefully acknowledgement to the head of department, Dr P. M. Magesa on behalf of all members of the department of Hematology and Blood Transfusion (MUHAS) for accepting my dissertation title and offering me various necessary support throughout my tenure as Mmed student in the department.

I owe my deepest gratitude to the entire MNH Hematologists (Dr A. Makubi, Dr, A.Magesa, Dr E.Meda and Dr Stella.R), entire Laboratory staff led by Mrs Juliana Mlalasi and entire staff of Nephrology Unit who gave me various forms of support to this dissertation.

Special thanks should go to Mr. Adarsha Krishnan (Manager-Laboratory & scientific product) of Kas Medics Limited and Mrs Doris. A. Njelekela (Laboratory scientist) who facilitated the laboratory aspect of this study on Erythropoietin measurement.

I wish to thank my patients who consented to take part in my study

I wish also to thank Mr. Cyprian Makwaya from the department of Biostatistics at MUHAS for helping me out in methodology of this study.

Finally, but most important I would like to thank Dr Linda Ezekiel Head of Nephrology Unit for making funds available for this study and critical reading of the manuscript and providing me quality mentorship during this dissertation work.

Last but not least, I wish to thank my Wife, Bahati Mfaki and my son Nurdin Abdu for their absolute love, constant encouragement, inspiration and tolerance during my three years of study.

DEDICATION

To my beautiful wife **Bahati Mfaki** and my beloved son **Nurdin Abdu** for their patience and understanding as precious family time devoted to the completion of this work.

To all my Lectures who made my study time success at MUHAS

ABSTRACT

Background: Chronic Kidney Disease (CKD) is a worldwide public health problem, the incidence and prevalence of which has increased in recent years in both developed and developing countries including Tanzania. Anemia, being a major health problem in Tanzania, is also a major co-morbidity of CKD patients and is common in all stages but becomes more pronounced at the latter stages of kidney failure. The causes of anemia are multifactorial ranging from erythropoietin deficiency to nutritional anemia due to iron deficiency, vitamin B12 and folate deficiency. However, erythropoietin deficiency is the most significant cause of anemia in CKD. Anemia has direct adverse effects on cardiovascular disease (CVD) consequences, such as left ventricular hypertrophy (LVH), and accelerates progression of CKD. As a result, patients with anemia due to CKD are at increased risk of hospitalization with increased length of hospital stay, reduced quality of life and increased mortality.

Objective: To determine the prevalence of anemia and its associated factors among CKD patients attending at MNH Nephrology unit in Dar-es-Salaam.

Methodology: A hospital based cross sectional study was carried out among CKD patients aged 18 years and above at Muhimbili National Hospital. Consecutive recruitment was adopted and 100 CKD patients were recruited out of 1476 patients with different renal diseases who were attended from May, 2011 to October 2012 at Nephrology unit. MDRD equation was used to determine GFR and abdominal ultrasound was used to determine evidence of Kidneys damage. Endogenous Erythropoietin (EPO) measurement was determined in serum using an Enzyme linked immunosorbent assay EPO ELISA EIA-3646 (DRG Diagnostic GmbH Germany) and Iron status was established using transferrin concentration, serum iron and serum ferritin levels . A questionnaire with structured interviews was used during data collection. Pre-coded data were entered into computer using Epi Info software version 3.5.1 and then data were transferred to SPSS (Statistical Package for Social Sciences) version 17.0 for further cleaning, categorizing of continuous variables and eventually analysis.

Results: One hundred (100) patients with chronic kidney disease were consecutively sampled from a total of 1476 of patients with various forms of Kidney diseases who attended at the Nephrology unit during the period of data collection. All were of African origin with mean age 44.4±14.6 years and 61% were males. Majority of study subjects (91%) were in advanced CKD stages (stage 4 and 5) and overall prevalence of anemia was (97%) defined using WHO criteria.

Of 82 study participants who were evaluated for EPO level, (87.8%) had low EPO production as response to a given hemoglobin level and there was no correlation between EPO level and hemoglobin value (r=0.012, p value=0.913)

Fifty four (54%) study participants had iron deficiency whereby majority (37%) had functional iron deficiency and (17%) had absolute iron deficiency.

Conclusion: Prevalence of anemia among CKD patients attending at Nephrology unit at MNH is high. Most of CKD patients showed evidence of inadequate endogenous EPO production and defective iron supply for erythropoiesis.

TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS

ACD	-	Anemia of Chronic Disease
CFU-E	-	Colony Forming Unit-Erythroid
CKD	-	Chronic Kidney Disease
CVS	-	Cardiovascular Disease
DRG	-	Diagnosis-related group
EGFR	-	Estimated Glomerular Filtration rate
EPO	-	Erythropoietin
ESA	-	Erythropoietin stimulating agent
ESRD	-	End stage renal disease
ELISA	-	Enzyme-Linked Immunosorbent Assay
HIV	-	Human Immunodeficiency Virus
MDRD	-	Modification of Diet in the Renal Disease
MNH	-	Muhimbili National Hospital
MUHAS	-	Muhimbili University of Health and Allied Sciences
MGFR	-	Measured Gromerular filtration rate
KDOQI	-	Kidney Foundation’s Kidney Dialysis Outcomes Quality Initiative.
LVH	-	Left ventricular hypertrophy
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Chronic Kidney Disease (CKD) is a worldwide public health problem[1]. Indeed, the incidence and prevalence of CKD has increased in recent years in both developed and developing countries [2], including Sub-Saharan Africa (SSA)[3]. In the US alone, over 30 million people are afflicted with CKD and the number of patients eventually reaching end stage renal disease (ESRD) is projected to rapidly increase from 450,000 in 2003 to 661,330 by the year 2010 (US RDS 2005).

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. Anemia occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children majority from developing countries [4].

In CKD, erythropoietin deficiency is the most significant cause of anemia in CKD and has been demonstrated to occur at each stage of kidney failure. Because the kidney is the sole source of erythropoietin (EPO) synthesis in adults, reduction in kidney mass as occurs in progressive CKD often results in impairment of EPO production, resulting in anemia[5].

1.1.1 Impact of Anemia on health of CKD

Anemia is a contributing factor in many of the symptoms associated with reduced kidney function. These include fatigue, depression, reduced exercise tolerance and dyspnoea.
In addition, anemia has direct adverse cardiovascular disease (CVD) consequences[6], has left ventricular hypertrophy (LVH) and left ventricular systolic dysfunction, coronary artery disease, accelerate progression of CKD to end stage renal disease and stroke[7]. As a result,

patients with anemia due to CKD are at increased risk of hospitalization, increased length of hospital stay, reduced quality of life and increased mortality [8-9].

1.2. Literature Review

1.2.1. Overview of Chronic Kidney Disease

The US National Kidney Foundation's Kidney Dialysis Outcomes Quality Initiative (K/DOQI) guideline defines CKD as kidney damage or estimated glomerular filtration rate (eGFR) of < 60 ml/min/1.73 m^2 for $\geq$ 3 months. Kidney damage is defined as pathological abnormalities or markers of damage including blood, urine or imaging tests[10].

Grading of CKD is achieved by calculating either the creatinine clearance using the modified Cockcroft-Gault equation or the estimated glomerular filtration rate (eGFR) by use of the Modification of Diet in the Renal Disease(MDRD) equation[10].

NKF-K/DOQI guidelines defines CKD stages as follows [10].

Stages	**Description**	**GFR (ml/min/1.73 m^2)**
1	Kidney damage with normal or Elevated GFR	$\geq$ 90
2	Kidney damage with mild decrease GFR	60-89
3	Moderate decrease GFR	30-59
4	Severe decrease GFR	15-29
5	Kidney Failure	< 15(or dialysis)

The guideline also classifies CKD based on severity and therapeutic intervention required at each stage for slowing progression of the disease and treatment of complications.

Stage 1-3 mild to moderate eGFR $\geq$30mL/min per 1.73m^2 their patients require therapeutic intervention such as screening for CKD risk factors, diagnosis and treatment of complications

conditions such as anemia and metabolic abnormalities. Stage 4 is severe impairment with severe decreased eGFR 30-15 mL/min per 1.73m^2 in which the focus of care is appropriate preparation for renal replacement therapy and stage 5 defined as established renal failure with eGFR <15 which is accompanied in most cases by signs and symptoms of uremia, such patients require renal replacement therapy (dialysis or renal transplantation)[10].

The incidence and prevalence of CKD has increased in recent years in both developed and developing countries including Sub-Saharan Africa (SSA)[11].

Sub Sahara Africa has higher prevalence of CKD as compared to USA and other some parts of Africa countries whereby prevalence of CKD is 12.4% in Kinshasa among adults in the general population and 20% reported in Uganda among HIV/AIDS patients [12-13] as compared to prevalence of 2% in the USA and 0.7% in Ethiopia and the reason for this low prevalence of CKD among Ethiopian is that, Ethiopian being mostly Nilotic have a different genetic make from the Bantu and probably this genetic lineage is protective. For example hypertension and diabetic nephropathy is more prevalent and severe among Bantu than Nilotic, but also HIV nephropathy seen among Nilotic is non-collapsing focal segmental glomerulonephritis which is more prevalent and common pathology among Bantu [14-15].

In Tanzania CKD prevalence has been reported to be 14% among adults in the hospital based general population irrespective of the cause [16].

The main causes of death in patients with CKD are kidney failure and cardiovascular related complications, which are increased in those patients with CKD [17]. Other major contributory factors for this ominous picture include severe anemia, late referral to hospital, limited renal replacement therapy (RRT), limited capacity of health workers for CKD detection and prevention, and poor awareness of kidney disease in the community[18].

1.3.0 Anemia in CKD

Definition of Anemia in CKD

In 1836 Richard Bright for the first time described the association of chronic kidney disease and anemia, when he observed pallor in the development of Brigit's disease[19].

NKF/KDOQI guideline (2006) defines anemia in Chronic Kidney Disease when the hemoglobin level is < 13.5 g/dl in adult males and 12.0g/dl in adult females, conversely the current European best practice guideline define anemia when hemoglobin is < 11.5g/dl in premenopausal women and when the hemoglobin is less than 13.0 g/dl in adult men and postmenopausal women, but adult male > 70 years old anemia is defined when hemoglobin < 12.0 g/dl [20].

Anemia being major co-morbidity of CKD, it occurs in all stages but becomes more pronounced at the latter stages of kidney failure [21]. A significant increase in the prevalence of anemia develops as the creatinine clearances (eGFR) falls to 70 ml/min or lower among males and to 50 ml/min or lower among females [22].

Prevalence of Anemia among CKD patients

Anemia is common in CKD patients as it was noted in a large cross sectional survey conducted in many CKD patients centers in USA, where the prevalence was found to be 47.7% among 5222 CKD patients[21].

Annear et al, at St Georges Hospital in United Kingdom revealed prevalence of anemia to be 9.2%(99) of 1075 CKD patients (Stage 3-5) who were found among all 6073 acute medical admissions [22].

In trying to explore the comparison of anemia among CKD patients who are on dialysis and not on dialysis at Sanglash Hospital in Indonesia, the prevalence of anemia using WHO criteria was found to 84.5%(45) of 52 CKD patients studied[23].

Magnitude of Anemia in CKD has been also studied in Africa evidenced by a cross sectional study by Akinsola et al on hematological profile in chronic renal failure in Nigeria, revealed prevalence of anemia to be 87 % (34) of 39 CKD patients studied[24].

There was no great difference from another study which was a retrospective in Enugu Nigeria by Chinwuba et al which revealed magnitude of anemia to be 77.5% (171) of 221 CKD patients as compared to a control group who had risks for CKD such as Diabetes and Hypertension but had no CKD, the prevalence of anemia in this control group was 11.9% (17) of 143 studied subjects [25.]

Several studies have shown the progression of anemia as the severity of renal dysfunction increases. In a retrospective study by Chinwuba et al in Enugu Nigeria revealed the progressive increase in severity of anemia from 26.7% to 75.5% in CKD stage 3 to 5 respectively and mean hemoglobin concentration decreased progressively with declining eGFR 12.91±1.35 g/dl, 12.14±1.96 g/dl, 10.57±2.42g/dl, 8.84±2.19g/dl and 7.33±1.74g/dl for CKD stages 1 to 5, respectively, indicating that the degree of anemia was proportional to degree of renal damage[25].

Unpublished data from a dissertation study on Chronic Renal Failure and Associated Risk Factors among Medical Admission at Kilimanjaro Christian Medical Centre in Tanzania, 2009/2010 by Kajiru reported prevalence of anemia 50/54 (92.4%) as defined by WHO criteria [16].

1.3.1. Pathology of Anemia in CKD patients

Erythropoietin deficiency

The cause of anemia in patients with CKD is multifactorial. However the major cause is lack of EPO synthesis in the diseased kidneys. Kidney is a major site for EPO production contributing 80-90% as compared to liver which contribute 10-15% of EPO in circulation.
As renal disease progresses specialized peritubular cells that produce EPO are partially or completely depleted or injured resulting inappropriately low EPO comparative to the degree of anemia of normocytic normochromic type[26].

In November 2011, Lucile M et al. Measured endogenous EPO levels in 336 CKD patients both with and without anemia for assessment of response of EPO to the levels of hemoglobin concentration in CKD patients.

It was noted that the endogenous EPO response to hemoglobin concentration varied according to mean glomerular filtration rate (mGFR). In anemic group, the predicted levels of EPO at every stage of mean glomerular filtration rate (mGFR) were consistently higher compared to the measured EPO level.

It is well known that, in patient with anemia and without CKD, their serum EPO is expected to inversely correlating with hemoglobin concentration as a result of feedback process, but in CKD this feedback is deranged.

In patient with mean glomerular filtration rate (mGFR) >30ml/min per $1.73m^2$ despite presence of EPO deficiency in the very early stage of the course of CKD, physiological response to anemia was persevered hence EPO and Hb levels were negatively correlated P = 0.04, but there was no correlation between EPO level and hemoglobin in patient who had mean glomerular filtration rate (mGFR) < 30ml/min per $1.73m^2$ P value =3 and had severe EPO deficiency than patient without anemia explaining major part of CKD anemia.

Factors associated with higher level of EPO were studied and it was noted to be high in patient high with inflammatory maker c reactive protein CRP > 8mg/L P =0.06[27].

In 2007 Ferruh and Teut retrospectively analysed the relationship between hemoglobin and serum EPO concentrations routinely measured among 167 Non CKD and 333 CKD patients of the university hospital in German.

From patients without CKD (n = 167) there was a strong parametric correlation between severity of anaemia and increase in EPO, Linear regression (r^2= 0.65) while in patients with CKD it was noted that, with increasing stages of CKD the correlation between hemoglobin and EPO concentrations was gradually attenuated and was completely lost in CKD stage four and five.

In anemic patients with Hb < 11 g/dl, relative EPO deficiency defined as EPO concentrations below the 25th percentile as low level of EPO production which was 38%, 67%, 93% and 100% of the patients with CKD stages 1–5, respectively[28].

Inhibition of Erythropoiesis

Literature shows that, abnormal metabolite or substances retained in patients with CKD interfere with bone marrow functioning. A number of patients with CKD have remained anemic despite the presence of elevated EPO level on bioassay, suggesting that the marrow has decreased sensitivity to circulating EPO in these patients.

Inhibition of erythropoiesis has been detected in the presence of sera from uremic patients in several tissue culture systems employing both human and animal bone marrow cells and also in fetal mouse liver culture. Uremic sera inhibit proliferation of Burst Forming Unit-Erythroid (BFU-E) and Colony Forming Unit-Erythroid (CFU-E) hemosynthesis[26].

In non dialysis patients with declining renal function, progressive anemia is noted despite no decrease in serum EPO level**,** suggesting that erythropoietin tissues may be less sensitive to EPO in CKD [28-29] and studies have shown that patients placed on hemodialysis, manifest

with improvement in hematocrit in the absence of significant changes in plasma EPO levels, suggesting that an inhibitor was removed by dialysis[30].

Presence of inflammatory mediators (cytokines) such as tumor necrosis factor alpha, interleukin- 6 and 1 are elevated in CKD and are associated with rheumatoid disease (arthritis, lupus), chronic infections and dysmetabolic state seen in late in CKD disease. These mediators interfere maturation of RBC precursors[31-32].

Yukitaka et al in Japan studied the serum EPO and inhibitors of erythropoiesis in anemic CKD patients (*n*= 54) compared to 26 normal control group. In this study it was noted that the level of erythropoietin was significantly higher in both pre and dialysis patients (*n*=35) as compared to control subjects.

In dialysis patients level of EPO was 141.2$\pm$ 109 mU/ml significantly higher $p<0.01$ and In 7 predialysis pts 99.9$\pm$ 45.0mU/ml significantly higher than normal (42.0±25.8 mU/ml) $p< 0.05$ and it was not correlating to level of hemoglobin and serum creatinine level[33].

In another study by Deborah et all, found that 13 of the 27 Diabetic Nephropathy patients were anemic (Hb 10.6 $\pm$ 6 0.9 g/dl) in marked contrast to none of the GN patients (Hb 13.7 $\pm$ 6 1.4 g/dl, $P < 0.005$). In the Diabetic Nephropathy group, serum EPO concentrations failed to increase in response to anemia compared with the response seen in patients with microcytic anemia. Thus, the anemia of the DN group was associated with EPO deficiency[34].

Iron deficiency

Iron deficiency has been also considered as important cause of anemia in CKD patients and these patients manifest iron deficiency as ‘‘absolute’’ or ‘‘functional’’ iron deficiency.

In CKD patients iron stores (absolute) are depleted as a result of decreased intake due to malnutrition, decreased appetite associated with uremia and increased loss through chronic GIT bleeding due to blood vessel fragility associated with uremia , platelet dysfuctionatial related to uremia, chronic blood retention in the dialysis circuit [35].

Functional iron deficiency occurs when there is a need for a greater amount of iron to support hemoglobin synthesis than can be released from iron store. In CKD there is an impaired release of stored iron from macrophages and hepatocytes to transferrin[35].

In 2002,Hsu et al analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) (n=15,837) where 569 (Male191, Female 378) had anemia as per National Kidney Foundation and of 569 anemic CKD patients found to have low iron indices at all levels of reduced creatinine clearance (CrCl).

Hsu et al reported 62.6% of CKD patients with anemia were iron deficient, as indicated by serum ferritin <100 ng/mL and transferrin saturation (TSAT) <20%. Where by 25.8% had functional iron deficiency anemia as indicated serum ferritin $\geq$ 100ng/ml and < 20% TSAT [21].

In India, Talwar et al studied hematological profile in 27 chronic renal failure patients and the prevalence of anemia was 94% of which 60% had microcytic hypochromic anemia with Serum ferritin low in 62%, serum iron below in 74% of the patients and bone marrow study revealed 57% of cases had negative bone marrow iron store.[36]

James et al studying iron deficiency anemia and the role of intravenous iron in CKD patients in 2006 noted that of 102 CKD patients 68% were anemic and 29/102 (28.4%) had iron deficiency by the criteria of serum ferritin <100ng/ml and TSAT < 20% and functional iron deficiency was 41% of 102 CKD patients serum ferritin≥100ng/ml and TSAT <20%. James concluded that iron deficiency was common in CKD patient and therefore replenishing iron stores in anemic patients with CKD should be considered as an integral part of the therapy for treating anemia in CKD population[37].

Malyszko reported the prevalence of functional iron deficiency to be 21% of 200 studied hemodialysis patient as indicated by ferritin above 200ng/ml with transferrin saturation below 20%. This was also found to be associated with high hepcidin levels and inflammatory markers [38].

Findings of low levels of iron tests in the majority of CKD patients were further supported in a study by Gotloib et al although used different method of analysing iron deficiency state. In 2006, Gotloib and colleagues studying the effects of intravenous iron administration (Ferric gluconate) on hemoglobin, evaluated 47 CKD patients with hemoglobin <12 g/dl who also showed evidence of low iron. Patients underwent sternal bone marrow biopsy and 98%, or 46 of 47 patients, had no evidence of iron deposits in bone marrow, indicating severe iron deficiency[39].

Vitamin B12 and Folate deficiency among CKD patients

There is paucity of data on the relationship of vitamin deficiency and anemia in CKD. Most patients with chronic kidney disease take a multivitamin daily, although there is no strong evidence that this is beneficial. Therefore, even the prevalence of vitamin deficiencies in chronic kidney disease has been hard to establish[40].
However it is reported that dialysis and decreased intake due to uremia related poor appetite contribute to deficiency of these important vitamins[41].

In Indonesia, Ketut et al studied 52 CKD patients of which 64.5% were anemic. The morphology of 7 cases (21.3%) of anemic patients was macrocytic anemia. Ketut also reported low level of serum folic acid among two anemic CKD patients, one patient being among those with macrocytic anemia and the other normocytic normochromic type of anemia. In this study the serum B12 level was found to be normal in all cases[23].

Secondary hyperparathyroidism

This is primarily due to deficiency in 1–25 vitD3 and elevated serum inorganic phosphate level both of which stimulate parathyroid hyperplasia and increased parathyroid hormone synthesis. This increases bone turnover, leading to development of bone cysts and marrow fibrosis, impairing bone marrow function and subsequently anemia[26].

Hemolysis

Hemolysis contribute to anemia in CKD patients as the breakdown of red blood cells tend to occur in association with various conditions in CKD patients including systemic lupus erythematosus, microangiopathic hemolytic anemia and red blood cell fragility due to uraemic effect. Tests of hemolysis such as total bilirubin, lactate dehyrogenase, urine hemosiderin, urine hemoglobin, haptoglobin, and a peripheral smear should be performed to diagnose the presence of hemolysis[26].

Pure red cell aplasia (PRCA)

Pure red cell aplasia **(**PRCA) is an isolated disorder of erythropoiesis that leads to a progressively developing, severe, isolated anemia with sudden onset. Some factors are known to be associated with PRCA, but in approximately half of the cases, PRCA does not have an identifiable cause and is classified as idiopathic. The mechanisms that induce PRCA under these conditions have been shown to be mainly of autoimmune origin.

The use of Erythropoietin Stimulating Agents as it is prepared as recombinant human erythropoietin (EPO), may result in formation of anti-erythropoietin antibodies thereby leading to pure red cell aplasia and erythropoietin resistance hence the hemoglobin level suddenly starts to decline, despite continued therapy with EPO at the same or even increased doses [26].

Angiotensin converting enzyme inhibitors and receptor blockers

Angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) can contribute to anemia in CKD patients. A circulating natural inhibitor of bone marrow

(N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) has been implicated in the pathogenesis of ACEI-induced anemia. The ACE enzyme is responsible for the degradation of AcSDKP, thus blockade of ACE with ACEI leads to increased circulating levels of AcSDKP and therefore bone marrow inhibition[26].

Other causes of anemia

Other rare causes of anemia in CKD patients include Haemoglobinopathies, malignancy, and malnutrition. These causes should be routinely investigated and treated[26].

Morphological Characteristics of Anemia in CKD patients

Despite erythropoietin deficiency being major cause of anemia in CKD patients, which mainly is normocytic normochromic morphologically, but studies has shown great variation on morphological type, indicating that the cause of anemia in CKD is multifactorial.

Annear et al conducted study at St Georges Hospital in UK in which their interest was to find out the prevalence of CKD stage 3-5 among all acute medical emergencies, they noted that the prevalence of normocytic normochromic anemia which is of characteristic in EPO deficiency, was higher in CKD stages 3-5 group was 9.2% than in stage 1-2 group which was 2.7%. In the same study the prevalence of normocytic anemia was associated with increased renal disease as indicated by stages, where in Stage 1, 2.7% (4998), Stage 2 , stage 3, 8.2%(950), stage 4 13.0% (100) and stage 5 32%(25)[22].

In 2005, Ketut et al conducted a study in Indonesia, The morphology of 33 anemic CKD patients was reported to be normochromic normocytic in 26 (78.8%) cases, slightly macrocytic in 7 (21.2%) cases, and no hypochromic anemia was found[23].

Reza et al reported normochromic normocytic being 80%, hypochromic microcytic 15% and macrocytic anemia 5% among 100 CKD patients studied in Iran[42]. This was different from study conducted in New Delhi India by Tawlar et al, who reported 60% of the patients had microcytic hypochromic anemia, 5 % had macrocytic anemia while 30% had normocytic normochromic anemia[36].

1.3.2. Diagnostic approach of Anemia in CKD

The anemia of CKD is typically morphologically a normochromic normocytic, the corrected absolute reticulocyte count and serum erythropoietin levels are inappropriately low when compared to values seen in patients without CKD but other parameter such as Leukocyte and platelets count are normal[43].

The bone marrow examination is usually normal and not usually necessary for hematological evaluation but the peripheral smear may show burr cells and their frequency is roughly proportional to the severity of uremia[43].

Anemia due to EPO deficiency is defined in CKD when eGFR is less or equal 60 ml/min/1.73m^2 and when the eGFR is greater than or equal to 60 ml/min/1.73m^2 the anemia is more likely to be related to other causes[43].

The diagnosis of the anemia of chronic renal failure therefore rests on the presence of normochromic, normocytic anemia with, normal leukocyte and platelet counts, coupled with, iron sufficiency (transferrin saturation > 20% and serum ferritin level >100 ng/ml),Low absolute reticulocyte count and rapid response to epoetin therapy within 4 weeks confirms the diagnosis [47].

Iron status test results reflect either the level of iron in tissue stores or the adequacy of iron for erythropoiesis. Functional iron deficiencies reflect the adequacy of iron for erythropoiesis and absolute iron reflects the level of iron in tissue stores.

The diagnosis of the absolute Iron deficiency anemia rests on the presence of microcytic, hypochromic anemia with, serum ferritin level < 100 ng/ml, transferrin saturation <20%, low mean cell volume and percentage of hypochromic red cells >6%.

The diagnosis of functional iron deficiency anemia rest on the presence of microcytic, hypochromic anemia with, serum Ferritin level > 100ng/ml, TSAT (Transferrin saturation) <20%, normal mean cell volume and mean cell hemoglobin concentration and percentage of Hypochromic red cells (HRC) >6% [47].

2.0. PROBLEM STATEMENT

Over the past few decades, there have been major advances in the knowledge of epidemiology, causes and natural history of anemia in CKD patients. In addition, the availability of effective ESAs therapy has resulted in major advances in the management of anemia in these patients.

The mainstay of treatment of anemia secondary to CKD has become ESAs but the use of ESAs is expensive and there is considerable biological and epidemiological evidence that ESAs does carry risks such as thromboembolic events and red cell aplasia.

The prevalence of iron deficiency and its contribution to anemia in CKD patients has been extensively studied and studies shows up 10% of patients with renal disease receiving recombinant human erythropoietin (rHuEPO) therapy show poor responsive to the drug, major factor being iron deficiency as adequate iron stores are necessary to permit an optimal response to ESA, therefore correction of iron deficiency can improve anemia and ESA response[37].

Despite all of these advances on understanding of renal anemia and associated treatment challenges but still in our setting the treatment of anemia in CKD is treated presumptively without establishing the type and cause of anemia. This approach of treatment carries the risk of providing inadequate treatment and consequently leading to complications related to anemia in CKD patients such as cardiovascular disease, increased rate of progression to end stage renal disease and decreased quality of life[44-46].

It is important for clinicians to be aware of the magnitude, morphological type and factors contributing to anemia in CKD patients in our set up. Therefore this study will provide data that will influence the provision of comprehensive and effective management of anemia in CKD patients.

3.0. RATIONALE

Anemia is a common and debilitating condition in patients with chronic kidney disease, where its related complications in CKD patients can be avoided if patients receive optimal and quality care of anemia[21].

As patients with CKD can have anemia for many reasons, it is recommended that in order to improve management of anemia in these patients require a thorough evaluation to identify type and causes of anemia other than erythropoietin deficiency which is a primary cause of anemia in CKD. Therefore treatment of renal anemia should not be started until other treatable causes of anemia for instance, iron deficiency, folate and B12 deficiency have been evaluated and treated, with further investigation of the underlying cause[47].

Despite the fact that ESAs has been recognized as the mainstay treatment of anemia in CKD but it has been observed that 10% of patients on ESAs therapy show poor responsive to the drug and major factor being iron deficiency[37], yet no data that describe types of anemia among CKD patients in Tanzania which would be used as guidance during the management renal anemia.

It is therefore justifiable to have this study, which describe the prevalence and associated factors of anemia in CKD patients as the information that will be obtained will help in designing the appropriate treatment measures of anemia, so as to reduce complications and morbidity among patients with CKD.

4.0. OBJECTIVES

4.1. Broad Objective

To determine the prevalence of anemia and its associated factors among CKD patients at Muhimbili National Hospital

4.2. Specific Objectives

1. To determine the prevalence of anemia among CKD patients by stages and sex.
2. To determine the prevalence of (Iron, Vitamin B12 and Folate deficiency) among CKD patients by sex.
3. To determine the association between EPO level and hemoglobin level among CKD patients.
4. To describe the morphology of RBC among anemic CKD patients.

CHAPTER TWO

2.0. RESEARCH METHODOLOGY

2.1. Study site.

The study was conducted at Nephrology clinic which is in the new outpatient building and medical ward at the Muhimbili National Hospital.

The Muhimbili National Hospital is a national referral hospital for both inpatient and outpatient from municipals hospital and other referral hospitals across the country.

2.2. Study design.

Hospital based descriptive cross- sectional study

2.2 Study Population.

Study participants included all patients 18 years and above with diagnosis of CKD as per USA NKF- K/DOQI criteria regardless of its primary cause at Muhimbili National Hospital.

2.3. Inclusion Criteria

1. Patients with 18 years of age and above
2. Patients who meets definition criteria of CKD
3. Patients consented to take part in the study

2.4. Exclusion criteria

1. Pregnant women.

2.5. Sample size

The minimum sample size was determined using the following statistical formula.

$$N = \frac{Z^2 P (1-P)}{d^2}$$

N = Minimum sample size

Z = Standard normal deviate corresponding to two sided specified significant level will be 1.96 (at 95%c confidence interval)

d = Margin of error (precision) will be 0.05

P = Proportional of patients

A study done in Nigeria [24] involving similar cohort reports 87% prevalence of anemia among CKD patients, therefore p=87% was adopted in this present.

Putting P= 87%, d=0.05

$$N = \frac{(1.96)^2 \text{ X } 0.87 (1-0.87)}{(0.05)^2}$$

Therefore the calculated sample size was 173 patients.

However following pilot study which was conducted at the Nephrology unit revealed CKD study population to be 240 CKD patients per year and 173 calculated sample size was 72% of the study population.

Because of the small population of CKD patients at Muhimbili National Hospital a Finite population correction formula was used to adjust sample size so that it provide proportionately more information for small population.

Therefore final sample size was given by $n = \frac{n_o}{1 + (n_o - 1)\frac{}{N}}$

Where

n = Final sample size

n_o = The estimated sample size using the above formula 173 which assume large population

N = Estimated CKD patients population attending at MNH per year, a pilot study revealed 240 CKD patients

Therefore the calculated **sample size was 100 patients.**

2.6. Sampling Procedure and Data Collection

Muhimbili National Hospital Nephrology Unit is a specialized newly inaugurated unit which offer Renal Replacement Therapy (RRT) in the form of hemodialysis and Clinical management expertise for CKD patients attending outpatient clinic and admitted in the medical ward. Nephrology unit operates clinic every Wednesday per week and all patients admitted in Medical ward with different diagnosis of renal disease including CKD, are allocated in renal unit.

A survey was conducted and the investigator gathered information before embarking on the current study and determined that on average about 20 patients attended MNH nephrology unit per week, of these at least 5 patients had Chronic Kidney disease. The investigator targeted to deal with at most all eligible sampled CKD patients per day.

Consecutive recruitment of study participants was used. The investigator attended Nephrology clinic and medical wards on respective day targeting to recruit all eligible sampled CKD patients per day because of small CKD patient population. In the clinic and wards investigator reviewed registration sheet of patients followed with review of their case notes.

Using serum creatinine value documented in the case notes of the study participants within three months or more and electronic calculator for Modification of diet in Renal Disease (MDRD) approved by NKF-KDQOI clinical practice guidelines downloadable from this web site http://nkdep.nih.gov/professionals/gfr_calculators/idms_si.htm

Two eGFR were calculated using atleast any two serum creatinine values and study participants with two eGFR < 60 ml/min/1.73 m^2 within three months interval or more apart were recruited as they fulfilled CKD diagnosis and were considered to be in stage 3, 4 or 5 based on the level of eGFR.

The validated MDRD formula is expressed as eGFR (mL/min/1.73m2) = 175 X $(S_{cr}/88.4)^{-1.154}$ X $(Age)^{-0.203}$ X 0.742 (if female) X 1.212 (if African American) where Scr denote, serum creatinine, 88.4, -1.154 & -0.203 are mathematical constants and Age – age of the patient[10].

The formula was designed for male Caucasians, however my study population was assumed to be genetically closer to black American so the race factor (1.212) was tailored to all study participants due to the fact that the formula has not been validated in black African despite its wide use in many studies.

For study participants who had eGFR > 60ml/min and did not fulfill the above criteria, documented abdominal renal ultrasound with evidence of kidney damage defined by contracted small size, increased cortico echogenicity and/or loss of corticomedullary differentiation and proteinuria taken on two different occasions within 3 months were all consecutively recruited and considered to be in stage 1 or 2 based on level of eGFR.

Sampled patients who consented to take part in the study were interviewed on their social-demographic details such as age, sex, marital status, occupation, level of education, dialysis status, treatment of anemia (if any) e.t.c. Physical assessment included vital signs, signs of anemia and CKD e.t.c.

After conducting interview and physical assessment for each eligible study participants, venopacture were done on every recruited study participants and aseptically collected blood samples approximately 4ml was collected into a sterile vacutainer with EDTA anticoagulants and analyzed for hematological parameter i.e. Full blood count (Cell DYN 350) and Peripheral blood film.

Blood samples for renal functional tests (Comprehensive Chemistry), Serum ferritin, serum B12 and folate were collected aseptically in 5ml red top vacutainer and analyzed using Abbott

automated AxSym^{TM} Chemistry analyzer system (Abbott, USA) whilst Serum iron and transferrin concentration were analyzed using architect c8000 analyzer system (Abbott, USA).

Endogenous Erythropoietin (EPO) measurement was determined in serum using an Enzyme linked immunosorbent assay EPO ELISA EIA-3646 (DRG Diagnostic GmbH Germany) at the Muhimbili National Hospital Microbiology Laboratory. The prediction of the erythropoietin value for given hemoglobin level was done according to the following a validated equation from non CKD patient 4.460 - (0.274 x Hemoglobin) = logEPO[62].

To assess the response of endogenous EPO to hemoglobin levels, The ratio (observed/predicted) was obtained by dividing the log of observed EPO by the log of predicted EPO from the equation.

2.7. Definitions of terms used in this study

Definitions of CKD stages from 1-5, CKD stages were defined as per NKF-KDOQI guidelines

Patients with $\geq$ 90 eGFR (ml/min/1.73 m^2) and evidence of kidney damage were categorized as stage 1, 60-89 eGFR (ml/min/1.73 m^2) and evidence of kidney damage were categorized as stage 2, 30-59 eGFR (ml/min/1.73 m^2) stage 3, 15-29 eGFR (ml/min/1.73 m^2) stage 4 and patients who had eGFR (ml/min/1.73 m^2) < 15 or on dialysis were categorized as stage 5[10].

However for the purpose of this study CKD stage were categorized into three main categories based on therapeutic intervention required for each category.

1. Early CKD stage 1-3 defined as eGFR $\geq$30, CKD patients category where factors and complications need to be identified and managed accordingly to slow progression of disease.

2. CKD stage 4 defined as eGFR 15-29, CKD patients category who requires preparation for dialysis/transplantation.

3. CKD stage 5 defined eGFR < 15, CKD patients for renal replacement therapy (Dialysis/Transplantation)[10].

Definition of anemia, in this study anemia was defined using The World Health Organization (WHO) criteria, hemoglobin concentration lower than 13.0 g/dl in men and lower than 12.0 g/dl in non pregnant women defined anemia and hemoglobin10 - 12.9 g/dl for men and 10 - 11.9 g/dl for women was used to define mild anemia, hemoglobin 7 - 9.9 g/dl for both genders defined moderate anemia and hemoglobin < 7 g/dl for both genders defined severe anemia[53].

Transferrin saturation (TSAT), the following formula TSAT = SI/TIBC x 100, where TIBC was calculated by multiplying transferrin concentration (g/l) by 25(mathematical constant) was used to obtain percentage transferrin saturation[49].

Iron deficiency status, in this study, absolute iron deficiency was defined with serum ferritin of <100ng/ml and transferrin saturation of < 20%, Functional iron deficiency was defined with serum ferritin of $\geq$ 100ng/ml and transferrin saturation < 20%, Adequate iron store was defined with serum ferritin of $\geq$ 100-800ng/ml and transferrin saturation of 20-50% and Iron overload was considered when patient had serum ferritin of > 800ng/ml transferrin saturation of > 50% [47-59.]

Serum folate, in this study, serum folate level was as per Axysm folate test kit package insert, where serum folate of 7.2 to 15.4ng/ml was defined as normal range, serum folate of 3-7.2ng/ml was defined recent inadequate dietary intake of folate and serum folate < 3ng/ml was defined serum folate deficiency[60.]

Serum vitamin B12, in this study, serum B12 level will be interpreted as per Axysm B12 test kit package insert where serum B12 level 19.1 – 119.3pmol/L was defined as normal range, serum B12 level <19.1pmol/L below normal range and was defined as serum B12 deficiency and serum B12 level >119.3pmol/L above normal range and was considered as over supplementation of B12[61].

Erythropoietin level, in this study, appropriateness response of Kidney to produce erythropoietin the ratio of Observed log erythropoietin /Predicted log erythropoietin (O/P) was used. O/P ratio of < 0.916 was used to define lower than expected erythropoietin production, O/P ratio >1.087 was used to define higher than expected erythropoietin production and O/P ratio 0.916 -1.087 defined normal response of EPO production[62].

3.1. Data Management and Statistical analysis

All questionnaires were checked daily for completeness by the investigator and pre-coded data were entered into computer using Epi Info software version 3.5.1 and then data were transferred to SPSS (Statistical Package for Social Sciences) version 17.0 for further data cleaning so that to allow consistence and eliminate discrepancies, categorizing of continuous variable and finally analysis.

The investigator performed descriptive statistics for social-demographic parameters, CKD stages, morphological pattern of RBC and dialysis status and two way tables was used to summaries the prevalence of anemia by sex. Fisher's exact test was used to determine association between independent and dependent categorical variable through which a level of was set at 5% to be statistically significant.

EPO values were not normally distributed, thus were long transformed in regression analysis. The relationship between EPO, hemoglobin and CKD stages were studied, investigator analysed EPO response to decreased hemoglobin according CKD stages. For this purpose

investigator assessed correlation between hemoglobin and log EPO and P value of < 0.05 was considered statistically significant.

3.2. Ethical Considerations and Confidentiality

The investigator sought ethical clearance from Muhimbili University Health and Allied Sciences research and publication committee and permission to conduct this study was sought from director of clinical services of Muhimbili National Hospital.

Patients were only included in this study after giving verbal and written consent. Patient confidentiality was maintained using codes instead of names during filling information in the data base and database with patient information was protected by password under investigator. Patient were informed on the purpose of the study, procedures to be performed on patients and related risks, benefits of the study and relevant authority to be contacted in case of questions or concerns (refer to consent form annexed).

Physical examinations were conducted in privacy and whenever needed in presence of a nurse, those who declined to consent received medical attention and advice. Patients who were found to have low EPO production were referred to the nephrologists and head of the renal unit for arrangement of starting ESAs while patients who had absolute iron deficiency and serum B12 deficiency appropriate treatment was prescribed and dietary advice was given.

3.0. RESULTS

Investigator recruited consecutively 100 CKD patients out of 1476 patients with different renal diseases, who attended at nephrology unit as from May, 2011 to October, 2011.

Demographic characteristics

All 100 CKD patients recruited were of African origin with mean age (±SD) of 44.4(±14.6) years and (61%) were male.

The majority of the study population (50%) had completed primary level of education and seven participants (7%) had no formal education. Most of participants (72%) had employment ranging from civil servants, private company and business and thirty eight participants (38%) had no formal employment. (Table 1)

Table 1. Social demographic characteristics of the study population (n = 100)

Variable	Attribute	Frequency	Percentage
Age years	18-29	17	17%
	30-59	64	64%
	60-89	19	19%
Sex	Male	61	61%
	Female	39	39%
Occupation	Unemployed	38	38%
	Employed	72	72%
Marital status	Married	77	77%
	Single	17	17%
	Divorced/Separated	6	6%
	Widowed	0	0
Education	No formal education	7	7%
	Primary school	50	50%
	Secondary school	30	30%
	College/University	13	13%

Clinical interventions among CKD patients

All 78 CKD patients in stage 5 had criteria for dialysis, but only 17% were able to access dialysis compared to (61%) that did not access dialysis and of 97 CKD patients who were anemic by WHO criteria, (61%) of them received clinical intervention for anemia while (36%) did not receive any form of treatment for anemia.

Among 61 who received clinical interventions (17%) were on ESAs in less than four weeks duration and (15%) received blood transfusion in less than 3 months at time of recruitment and (36%) were on haematenics. (Table 2)

Table 2. Clinical interventions among CKD patients in study population (n=100)

Intervention	Status	Frequency	Percentage
Dialysis status	On dialysis	17	17%
	Not on dialysis	83	83%
ESAs Treatment	<4 weeks	17	17%
	>4 weeks	15	15%
Transfusion	<3 months	15	15%
	>3 months	8	8%
Haematenics	Folic acid and Ferrous Sulphate	36	36%
No treatment of anemia	No Treatment	36	36%

Risk factors for CKD

The two main medical disease conditions, hypertension (61%) and diabetes mellitus (26%) were identified as commonest underlying cause for CKD. Human Immunodeficiency Virus (HIV) 4% was as third close underlying disease for CKD in this study whilst multiple myeloma and sickle cell disease as hematological disorders were documented as underlying cause for CKD (Table 3).

Table 3. Risk factors for CKD in study population (n=100)

Risk factor for CKD	Frequency	Percentage
Hypertension	61	61%
Diabetes Mellitus	26	26%
HIV	4	4%
Multiple Myeloma	1	1%
Sickle Cell Disease	1	1%
Unknown	7	7%

Distribution of CKD stages

The majority of patients were in advanced stages of renal disease, (78%) stage 5 and (13%) stage 4, both accounting for (91%) of the study population. Few patients were in early CKD (stage 1-3) accounted for (9%). (Figure 1)

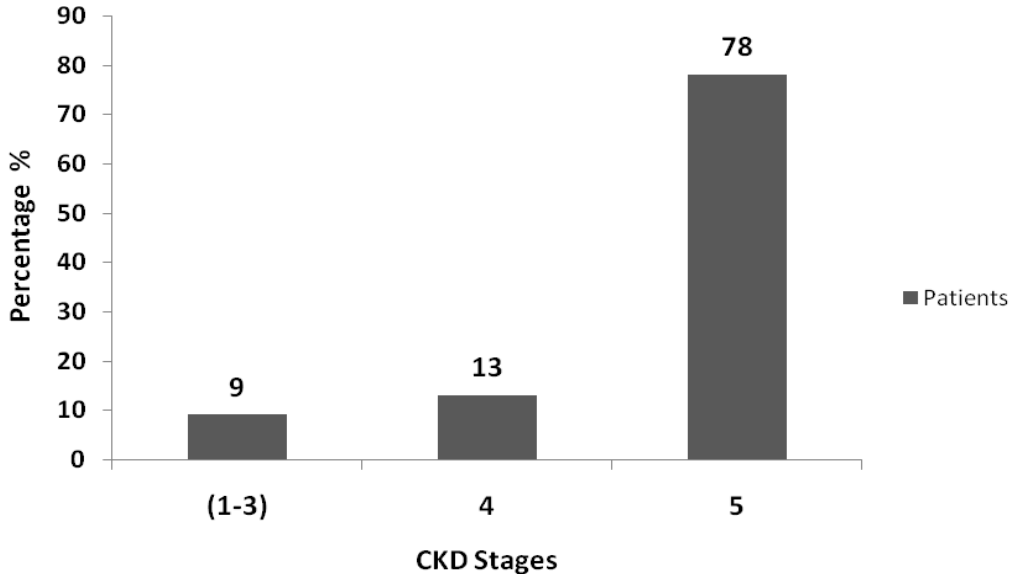

Figure 1. Distribution of study participants among stages of CKD stage (n=100).

Prevalence of anemia

Ninety seven participants (97.0%) had anemia as defined by WHO criteria, male being (58.0%) and female (39.0%). Majority of anemic CKD patients had moderate anemia (40.0%). (Figure 2) Female had severe anemia (41.0%) compared to male (24.6%) and the gender difference between severe and moderate anemia was not statistically significant p-value=0.867 and 0.083 respectively (Figure 3)

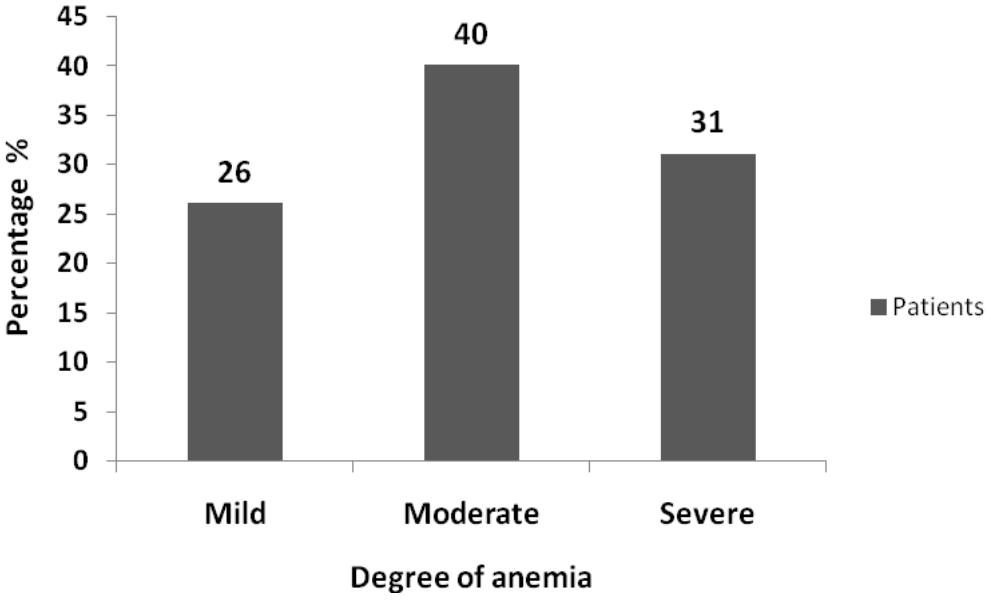

Figure 2. Overall prevalence of anemia among CKD patients (n=100)

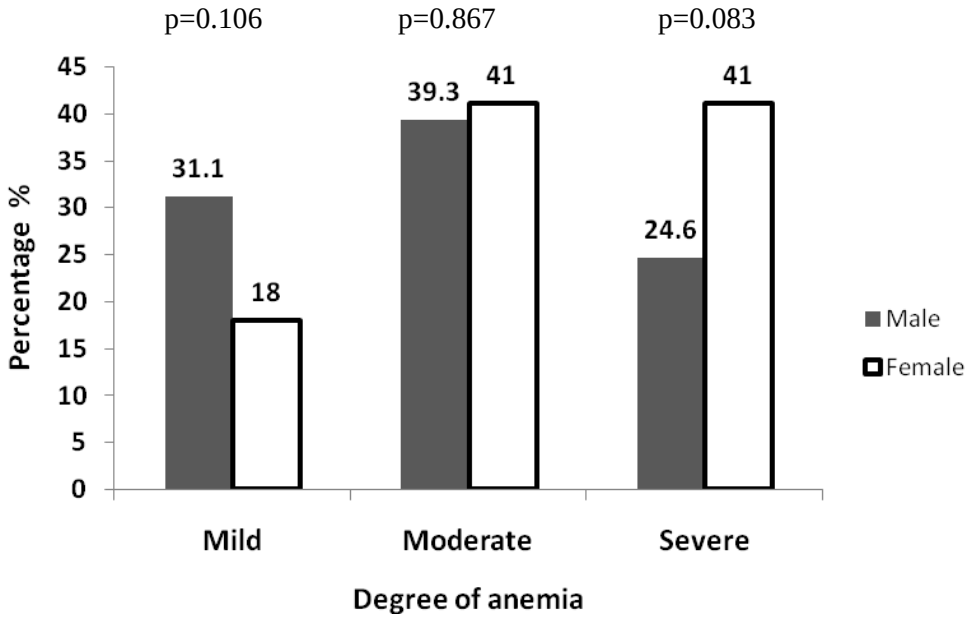

Figure 3. Prevalence of anemia among CKD patients by sex (n=100).

Prevalence of anemia among CKD stages

The proportion of CKD patients with anemia was varying among CKD stages with 88.9% in categorical early stage (1-3), 100% in stage 4 and 97.4% in stage 5. Difference observed between stages was not statistically significant p=0.420. (Table 4)

Table 4. Prevalence of anemia among categories of CKD stages (n = 100)

CKD Stage	N. of Patients	N.of anemic patients	Prevalence of anemia	p-value
Stage1-3	9	8	88.9%	0.420
Stage 4	13	13	100%	
Stage 5	78	76	97.4%	

Serum EPO levels among study participants

Among 82 CKD study participants who were evaluated for EPO levels, (87.8%) had low EPO production and (4.9%) had normal level of EPO as response to a given level of hemoglobin. Majority of CKD patients with low EPO production were among CKD patients in stage 5 (93.8%) compared to early CKD stages (1-3) 66.7% and stage 4 (66.7%) respectively. The difference observed between the EPO levels among CKD stages was statistically significant p-value=0.01. (Table 5)

Table 5. The serum levels of EPO among CKD stages (n = 82)

EPO levels	Stage (1-3) n=9	Stage 4 n=9	Stage 5 n=64	Total	p-value*
Normal EPO	1(11.1%)	2(22.2%)	1 (1.6%)	4(4.9%)	0.01
Low EPO	6(66.7%)	6(66.7%)	60 (93.8%)	72(87.8%)	
High EPO	2(22.2%)	1(11.1%)	3(4.7%)	6(7.3%)	

Key: p-value *= Fisher' exact test

Correlation between Hemoglobin and serum EPO levels among study participants

Overall scatter plots shows no evidence on correlation between serum EPO level and hemoglobin levels (r = 0.01, p value=0.913) from CKD stage 1 to 5. (Figure 5A)KD, whereas with increasing CKD stages, the correlation existed only between hemoglobin and serum EPO levels in early CKD stages 1-3 (r = -0.061, p value = 0.037). (Figure 5B) but was completely lost in stage four and five (r = 0.002, p value 0.750 and r=0.001, p value =0.906 respectively). (5C and 5D) (Figure 4).

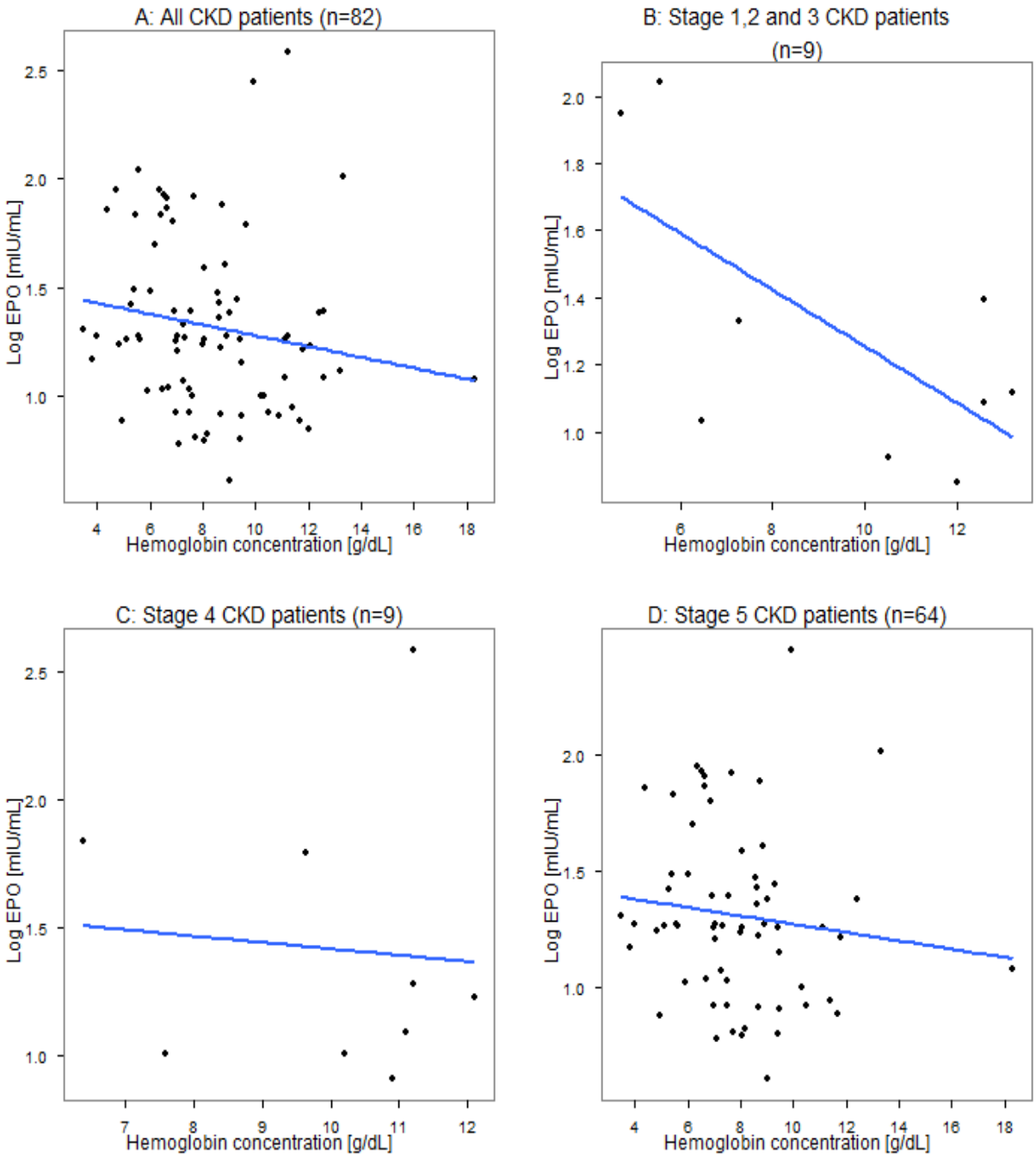

Figure 4. Scatter plots showing correlation between EPO level and Hemoglobin level (n=82)

Magnitude of Iron deficiency, Vitamin B12 and Folate deficiency among CKD patients

Seventeen study participants (17%) had absolute iron deficiency and female had high proportion (23.1%) whilst functional iron deficiency was seen in (37%) of study participants. The gender difference on iron status was not statistically significant, p-value >0.05 respectively in all categories of iron status. (Table 6)

Table 6. Iron Status among CKD patients (n = 100)

Iron Status	Male n=61	Female n=39	Total	p-value
Normal iron status	26 (42.6%)	17(43.6%)	43 (43 %)	0.9241
Absolute Iron deficiency	8(13.1%)	9(23.1%)	17 (17 %)	0.1961
Functional Iron deficiency	24(39.3%)	13(33.3%)	37 (37 %)	0.5437
Iron overload	3(4.9 %)	0	3 (3 %)	0.4451*

Key: p-value* = Fisher' exact test

Only (1.0%) of study participants had serum B12 below normal range while (47.0%) had normal range and (52.0%) had serum B12 above normal range (Figure 5). The gender difference on levels of Serum B12 was not statistically significant p-value >0.05 (Figure 6).

Among study participants (2.0%) had serum folate below normal range, 43.0% had normal range and 25.0% had intermediate range of serum folate (Figure 7). The gender difference was not statistically significant p-value=0.199. (Figure 8)

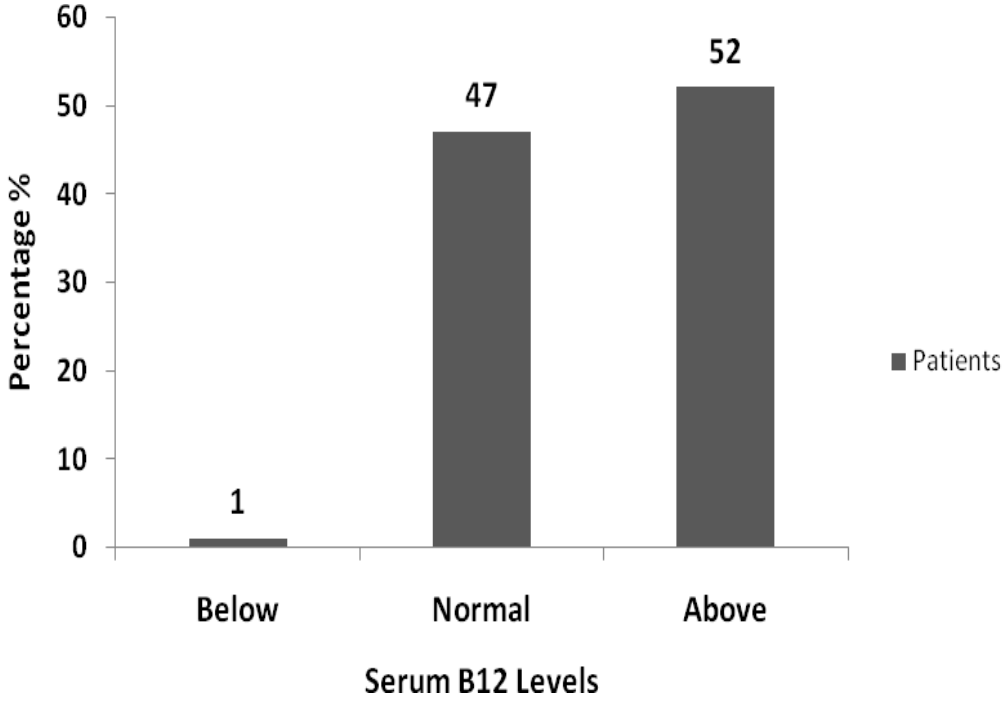

Figure 5. Serum B12 levels among CKD patients (n=100)

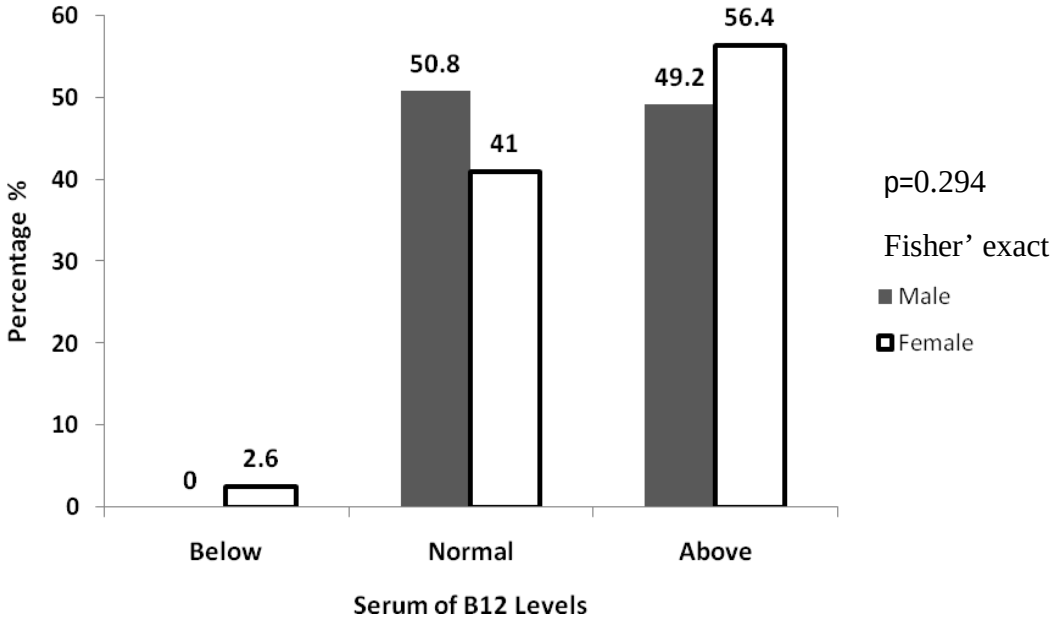

Figure 6. Serum B12 levels among CKD patients by sex (n=100)

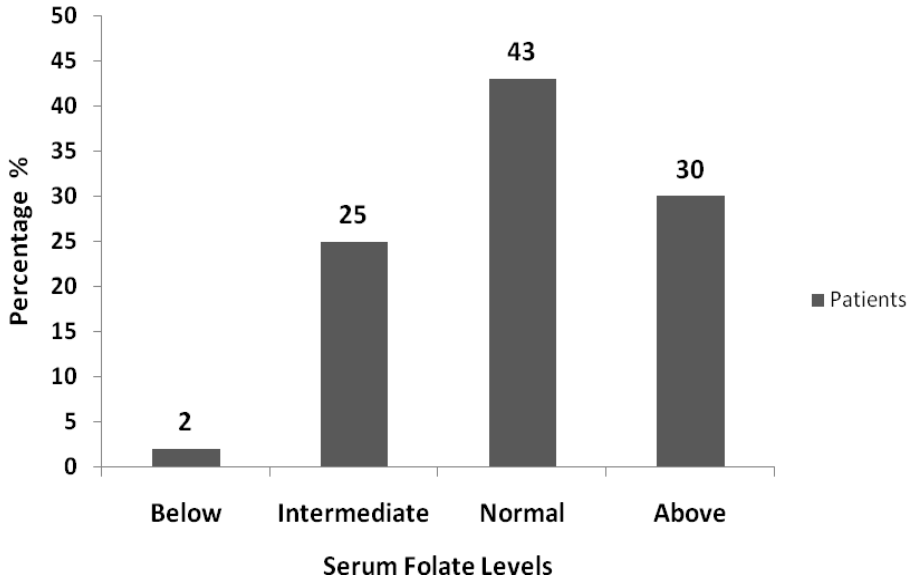

Figure 7. Serum folate levels among CKD patients (n=100)

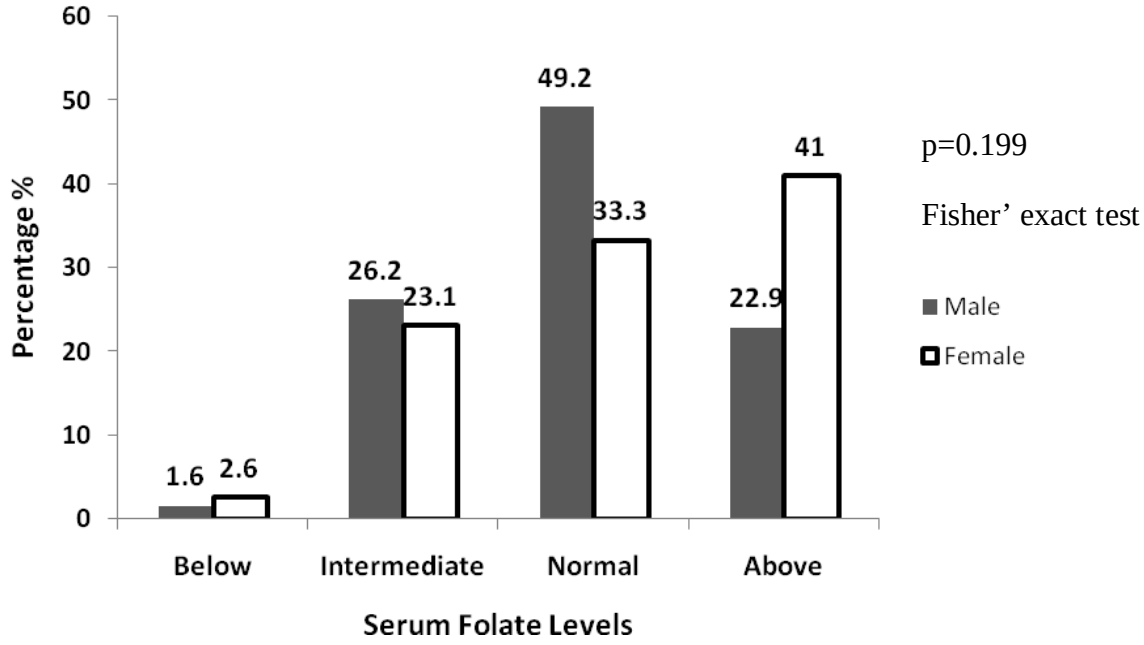

Figure 8. Serum folate levels among CKD patients by sex (n=100)

Red blood cells morphological patterns in CKD patients

The most frequent morphologic features among 97 anemic CKD patients were Normochromic -normocytic (47.4%) followed with hypochromic-microcytic by (28.9%). Five participants (5.2%) had Macrocytosis. (Table 7)

Table 7. Frequency distribution of morphological pattern among anemic CKD patients (n=97)

Morphology	Frequency	Percentage
Normocytic Normochromic	46	47.4%
Microcytic Hypochromic	28	28.9%
Dimorphic Picture (NN/MH)	16	16.5%
Macrocytic	5	5.2%
Others (Microcytic Normochromic)	2	2.0%

Key: NN-Normocytic Normochromic and MH-Microcytic Hypochromic

4.0. DISCUSSION

Anemia is a common complication among patients with CKD. Early detection and treatment of anemia would potentially improve quality of life and reduce the burden of care.

Social-demographic characteristics

This study recruited CKD patients among patients with different kidney disease seen at Muhimbili National Hospital in Dar es Salaam. The renal unit at MNH is the first and the only unit in a public hospital offering care and treatment for kidney disease patients. The unit serves patients for the whole country, thus the study is a hospital based in tertiary care setting.

The majority of study subjects were males (61.0%), in line with several other studies emulating this fact [24, 25], as men suffer CKD than females due to fact that in developing countries men tend to seek medical attention than females.

The mean age in this study was 44.4±14.6 years which was similar finding to 45.0±15.0 and 47.0±2.0 years in the Nigerian and Indonesia study respectively [23, 25]. This average age in this study is on the other hand is another reminder that, increasing age is a traditional risk factor for CKD as age increases eGFR decreases, therefore CKD is common in adult as compared to young age. Hypertension and diabetes mellitus were identified as main underlying disease for CKD in this study.

The majority of patients were in advanced renal disease stages 78 (78.0%) stage 5 and 13 (13.0%) stage 4 both accounting for 91 (91.0%) of the study population. Few patients were in early CKD stages. This finding is similar to reports from other African studies which have indicated that the majority of patients are also in advanced stages[25]. However the distribution of patients in early stages, was dissimilar in the Nigerian study which revealed (7.7%) for stage 1, 49 (13.5%) for stage 2, and 66(18.1%) stage 3. This difference can be partly explained by the fact that, Nigerian study had large sample size compared to this study and was a retrospective study which may explain the enrollment of patients who had early screening for CKD hence fair distribution of patient in early stage[25] and the present study being a hospital

based, it is only when patients are in advanced stage of renal disease get referred to MNH and thus few CKD patients in early CKD 1-3 stage were seen.

The distribution of patients among CKD stages was different from reports of studies done in developed countries USA and Indonesia [5, 23], in these studies there was almost equal distribution of patients among all stages of CKD and this can be explained by fact that in these countries prevention is given priority, as aggressive screening programme of patients especially at risk such as diabetes mellitus and hypertension is in place, thereby decreasing progression of CKD.

Prevalence of anemia among CKD patients

This study has demonstrated a very high prevalence of anemia in CKD patients at MNH with overall prevalence of 97.0%, a finding similar to this was observed in a study conducted in northern Tanzania whereby the prevalence was 92.4 % among 52 CKD patients seen Kilimanjaro Christian Medical Centre (unpublished data Mmed dissertation by Kajiru K.2009/2010).[16] compared to general population.

Compared to prevalence of anemia in general population in Tanzania, this was high as demonstrated from the demographic health survey of 2010 in Tanzania, where the prevalence of anemia among women aged 15-49years in the general population was 40%.

The prevalence of anemia in this study was higher than values obtained elsewhere. McClellan et al[5], in a large scale cross sectional USA multicenter survey study involving 5,222 patients and using 12g/dl as definition of anemia reported an overall prevalence of anemia 47.75% and progression of anemia from 26.7% in stage 3 to 75.5% in stage 5.

Suega et al in Indonesia and Afshar et al in Iran [23, 42], reported the prevalence rate of anemia in predialysis patients 73.1% and 75.0% respectively and Africa a Nigerian studies documented 77.5% and 87% prevalence of anemia in CKD patients [24, 25].

This high prevalence of anemia among CKD patients in this study may be accounted for by the disproportionately high number of patients with advanced CKD (78.0%) in whom the mean hemoglobin was 7.8 g/dl. This findings should alert clinicians that majority of patients with Chronic Kidney diseases who are referred at MNH as tertiary hospital have advanced CKD contributing to presence of severe anemia and progression to end stage renal disease.

It is also possible that the high prevalence of anemia might be explained partly by other causes peculiar to environment including poor nutrition and parasitic infestations.

In contrast to other studies[25], the prevalence and severity of anemia among CKD stages was varying as severity of kidney disease increased. In early stages 1-3 the prevalence was 88.9% with mean hemoglobin 9.4$\pm$3.4, stage 4 100% with mean hemoglobin 10.2+1.7 and stage 5 97.4% with mean hemoglobin of 7.9$\pm$2.4. This difference may be explained by small sample size in this study and perhaps patients in early CKD stages had other contributing factors to anemia than compared to stage 4 patients. Another possible explanation is improvement of hemoglobin of patients in stage 5 as had already been started on ESAs and dialysis.

Relationship between EPO, Hemoglobin and CKD stages

Majority of CKD patients evaluated for EPO level had low expected EPO production (87.8%) only (4.9%) had normal expected level EPO production as response to given hemoglobin level, the result in the present study point toward the contribution of EPO deficiency on anemia among study participants.

As renal disease progresses specialized peritubular cells that produce EPO are partially or completely depleted or injured resulting inappropriately low EPO comparative to the degree of anemia[26]. This trend of EPO deficit was demonstrated clearly in the present study by 66.7% of EPO deficit in stage 1-3, 66.7% in stage 4 and 93.8% in stage 5.

Such trend was also previously reported in a Germany study by Ferruh A and Teut R who defined EPO deficiency as EPO concentration below the 25th percentile 38%, 67%, 93%, and

100% in stage 1-5 respectively[28] and Lucile. M et al [27] reported the same trend of EPO deficit of 28% to 68% in early CKD stages (1-2) and stage 5 respectively.

In patients without CKD, EPO and hemoglobin levels are negatively correlated, In the present study, the association between hemoglobin and EPO concentration was examined and was demonstrated that no correlation existed between EPO and hemoglobin levels among 82 studied CKD patients.

With increasing CKD stages, despite relative EPO deficiency in early course of CKD physiological response to anemia was somewhat preserved (correlation existed between EPO and Hemoglobin for stage 1-3 r = - 0.061, P value 0.037), and correlation was completely lost in stage 4 and 5(r = 0.002, P value 0.750 for stage 4 and r = 0.001, P value 0.906 for stage 5).

This finding was in accordance with Lucile M et al and Ferruh A et al who reported negative correlation between EPO and Hb levels (r = - 0.2, P value 0.04) in patients who had mGFR >30 ml/min per 1.73m^2 but not mGFR <30 ml/min per 1.73m^2 (r = 0.07, P value 0.32) and Ferruh A et al who documented gradual attenuation of correlation between Hb and EPO levels (r = -0.61, r = -0.42,r = 0.16 and r = - 0.03 for stage 1+2, 3, 4 and 5 respectively) [27, 28]. The data in present study demonstrate again that the extent of damage to peritubular cells that produce EPO in early stages of CKD is somehow small hence slight preservation in the endogenous EPO response to anemia.

The Status of Iron, Vitamin B12 and Serum folate

The National Kidney Foundation (NKF-K/DOQI) practice guidelines recommend maintaining serum ferritin $\geq$100ng/ml and transferrin saturation TSAT $\geq$20% to ensure adequate iron supply for erythropoiesis among CKD patients whether or not they are dialysis dependent. Analysis of the data in the current study revealed only 43% of 100 studied CKD patients met these K/DOQI targets.

In this study 17% of CKD patients were iron deficient, as indicated by serum ferritin <100 ng/ml and transferrin saturation (TSAT) <20% Where by 37% had functional iron deficiency as indicated serum ferritin ≥ 100ng/ml and < 20% TSAT and all were anemic.

The findings in the current study is in agreement with James et al who, while studying iron deficiency anemia and the role of intravenous iron among CKD patients, found out that 28.4% of 102 anemic CKD patients had iron deficiency (serum ferritin <100 ng/ml and transferrin saturation (TSAT) <20%) and 41% had functional iron deficiency[37].

However in contrast to these finding in the current study, Hsu et al reported 62.6% of CKD patients with anemia were iron deficient, as indicated by serum ferritin <100 ng/mL and transferrin saturation (TSAT) <20%. Where by 25.8% had functional iron deficiency anemia as indicated serum ferritin ≥ 100ng/ml and < 20% TSAT [21]. This upward trend of high proportional of CKD patients with iron deficiency done elsewhere could be attributed to small sample size (100) investigated in the current study compared to(15,837) in the previous study [21].

In this study it was evidently noted that, fewer anemic CKD patients (2%) had low levels of serum folate and both cases were normochromic normocytic morphologically whilst only 1% of anemic CKD patient had low level of serum vitamin B12 deficiency. Ketut S et al exploring the profile of anemia among CKD patients reported similar results in which also two cases had low level of serum folic acid and all cases had normal level of serum vitamin B12[23].This result verifies that, folic acid and vitamin B12 were not important contributing factor to anemia in this study.

Morphological characteristics of CKD anemia

It is well known that the anemia of CKD is normocytic normochromic type as the loss of renal mass could be the principle mechanism[26]. In this study, the morphology of 97 anemic CKD patients was normocytic normochromic in (47.4%) cases, microcytic hypochromic in (28.9%) cases, dimorphic picture (normocytic normochromic & microcytic hypochromic) in (16.5%) cases, macrocytic in 5 (5.2%) cases and few 2% had microcytic normochromic. Apparently,

several other studies among CKD patients mirror this fact. Annear et al, Ketut et al and Reza et al [22, 23, 42], This shows that the anemia in CKD is generally due to EPO deficiency while microcytic anemia may reflect iron deficiency, aluminum excess or Haemoglobinopathies. Macrocytic anemia may be explained by B12/folate deficiency or iron excess and ESAs therapy that shift immature larger reticulocyte into circulation.

5.0. CONCLUSION

This study shows that anemia is prevalent among CKD patients by 97% where moderate degree of anemia is most frequent finding in both sexes and the degree of anemia was severe in female as compared to male. Across CKD stages, the proportion of patients with anemia was varying among CKD stages, early stage 88.9%, stage 4 100% and stage 5 97.4%.

In the vast majority of patients, this study showed evidence of inadequate endogenous EPO production and defective iron supply for erythropoiesis whilst vitamin B12 and folate deficiency were unlikely to be important factor for anemia in CKD patients.

These observations may have clinical implication as in most instances intravenous iron, combined in selected cases with subcutaneous recombinant human ESAs would represent a rational therapeutic approach to these anemic CKD patients.

Among (78.0%) of the patient in this study was seen by nephrologists in advanced kidney disease stage 5. In the light of this study, there is a need for clinicians to refer CKD patients as soon as diagnosis is made or suspected to nephrologists for early evaluation and treatment of anemia and prevention of CKD progression, therefore decreasing the burden of dialysis and transplantation.

6.0. STUDY LIMITATIONS AND RECOMMENDATIONS

Study limitations

This study being a hospital based and used small study sample size, as a results majority of study population was in advanced CKD stages which might have overestimated the prevalence of anemia among the CKD study population.

In absence of specific and sensitive test for the assessment of iron, such as content of Hb in reticulocyte (CHr), iron status was evaluated by serum ferritin and transferrin saturation (TSAT) However, ferritin is an acute-phase reactant and can be elevated for reasons other than sufficient or excessive iron stores in infection and inflammation, similarly transferrin concentration are altered in states of hypoalbuminemia and chronic disease, resulting in false TSAT values. This might have either underestimated or overestimated iron deficiency in this population.

In this study, it was not possible to measure EPO levels in 18 CKD patients primary due to financial constraints.

Recommendations

There is a need for early diagnosis and treatment of anemia in CKD patients as anemia leads to CKD progression and cardiovascular disease in these patients.

As mainstay treatment of anemia in CKD is ESAs and adequate iron store are necessary to permit an optimal response, therefore it is highly recommended to do iron studies to establish types of iron deficiency as functional iron deficiency will need intravenous iron supplement compared to absolute iron deficiency which needs oral iron.

This was a hospital based study, therefore the results doesn’t reflect true community picture, it is therefore recommended to do similar study using large CKD sample size at the community level which would ascertain all stages of CKD and more factors related to anemia as in this

present study with high prevalence of anemia, only few factors were studied and skewed advanced CKD stage.

7.0. REFERENCES

1. El Nahas M: The global challenge of chronic kidney disease. Kidney international, 2005; 68:2918-2929.

2. Sumaili EK, Cohen EP, Zinga CV et al: High prevalence of undiagnosed chronic kidney disease among at-risk population in Kinshasa, thye Democratic Republic of Cong. BMC Nephrology, 2009;10: 10.1186/1471-2369-10-18.

3. Arogundade FA, Barsoum.RS. CKD prevention in Sub-Saharan Africa: a call for governmental, non governmental and community support. Am J Kidney Dis, 2008; 51: 515-523.

4. WHO: Global data base on anemia accessed at www.who.int/vmis/anaemia/data/ database/countries/omn_ida.pdf. on 19/02/2011

5. McClellan W, Aronoff SL, Bolton WK. The prevalence of anemia in patients with chronic kidney disease. Curr Med Res Opin, 2004;20:1501–10.

6. McFarlane SI, Moro S, Makaryus J, Anemia and cardiovascular disease in diabetic nephropathy. Curr Diab Rep 6, 2006;6:213–8.

7. McClellan WM, Flanders WD, Langston RD: Anemia and renal insuffi ciency are independent risk factors for death among patients with congestive heart failure admitted to community hospitals: a population-based study. J Am Soc Nephrol, 2002;13:1928.

8. Ma JZ, Ebben J, Xia. H, Collins. AJ: Hematocrit level and associated mortality in hemodialysis patients. J Am Soc Nephrol. 1999;10:610.

9. Collins AJ, Ma JZ, Ebben J. and Impact of hematocrit on morbidity and mortality. Semin Nephrol. 2000;20:345.

10. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis, 2002;39:1-266.

11. Hosseinpanah F, Kasraei F, Nassiri AA, Azizi F, High prevalence of chronic kidney disease in Iran: a large population-based study. BMC Public Health, 2009;9:44.

12. Sumaili EK, Krzesinski JM, Zinga CV, Cohen EP, Delanaye P, Munyanga SM, Nseka NM, Prevalence of chronic kidney disease in Kinshasa: results of a pilot study from the Democratic Republic of Congo. Nephrol Dial Transplant 2009;24:117-122.

13. Peters PJ, Moore DM, Mermin J, Brooks JT, Downing R, Were W, et al. Antiretroviral therapy improves renal function among HIV-infected Ugandans. Kidney Int, 2008;74: 925-929.

14. Humphreys MH: Human immunodeficiency virus-associated nephropathy. East is east and west is west? Arch Intern Med, 1990;150:253-255.

15. Behar DM, Shlush LI, Maor C, et al. Absence of HIV-associated nephropathy in Ethiopians. Am J Kidney Dis, 2006;47:88-94.

16. Kilonzo, KG. Chronic Renal Failure and Associated Risk Factors among Medical Admission at KCMC.Dissertation study for partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine) of Tumaini University, 2009/2010.

17. Go AS, Chertow GM, Fan D,McCulloh CE,Hsu C-y: Chronic kidney disease and the risks of death, cardiovascular events,and hospitalization. N Engl J Med, 2004;351:1296-305.

18. Krzesinski JM, Sumaili EK, Cohen E: How to tackle the avalanche of chronic kidney disease in Sub-Saharan Africa; the situation in the Democratic Republic of Congo as an example. Nephrol Dial Transplant, 2007;22:332-335.

19. Remuzzi G, R.E: Hematologic consequences of renal failure. Brenner BM, Ed. The kidney. 5th ed. Philadelphia, 1995(WB Saunders Co):2170-85.

20. National Kidney Foundation's Kidney Dialysis Outcomes Quality Initiative (NKF-K/DOQI)Clinical Practice Guidelines for Anemia of Chronic Kidney Disease. Am J Kidney Dis, 2006;**47:**11–145.

21. Chiyuan Hsu, Charles E. McCulloch, Gary C.Curhan, Iron Status and Hemoglobin level in Chronic renal Insufficiency. J Am Soc Nephrol 2002;13:2783-2786.

22. Annear NMP, Josep J, Harries T.H, Rahman S, Eastwood J.B, Prevalence of chronic kidney disease stages 3–5 among acute medical admissions .Q J Med 2008;101:91–97.

23. Ketut Suega, Bakta M, Tjok Gde Dharmayudha, Profile of Anemia in Chronic Renal Failure Patients: Comparision Between Predialyzed and Dialyzed Patients at The Division of Nephrology, Department of Internal Medicine, Sanglah Hospital, Denpasar, Bali, Indonesia. Indonesia Journal of Internal Medicine, 2005;37:Original article.

24. Akinsola A, Durosinmi MO, Akinola NO, The hematological profile of Nigerians with chronic renal failure. African Journal of Medicine and Medical Sciences,2000;29:13-6.

25. Chinwuba I, Uchenna I, Ngozi I. High prevalence of anemia in predialysis patients in Enugu, Nigeria. Nephrology Reviews, 2010; 2:14.

26. McGonigle RJ, Shadduck RK, Fisher JW: Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. Kidney Int, 1984;25:437- 444.

27. Lucile M, Marie M, Nicole C et al. Timing and determinants of Erythropoietin deficiency in Chronic Kidney Disease. Clin J Am Soc Nephrol;2012;7:35-42.

28. Ferrh A, Teut R: Serum erythropoietin concentrations in respaonse to anemia in patients with Chronic Kidney Disease.Nephrol Dial Transplant 2007;22:2900-2908

29. Chandra M,Clemons GK,McVicar MI. Relation of serum erythropoietin levels to renal excretory function: Evidence for lowered set point of erythropoietin production in chronic renal failure. J Peds 1988;113:1015-1021.

30. Radtke HW, Erbes PM, Schoeppe W, Koch KM. Improving anemia by hemodialysis: effect on serum erythropoietin. Kid Intl 1980;17:382 -387.

31. Tsirpanlis G, Bagos P, Ioannou D. Exploring inflammation in hemodialysis patients: persistent and superimposed infl ammation. Kidney Blood Press Res, 2004;27:63–70.

32. Macdougall IC, Cooper AC. Erythropoietin resistance: the role of inflammation and pro-inflammatory cytokines. Nephrol Dial Transplant 2002;17:39–43.

33. Yukitaka. F, Mitsuyuki. F. Serum Erythropoietin Levels and Inhibitors of Erythropoiesis in Patients with Chronic Renal Failure. Tohoku J. exp. Med, 1986;150:1-15.

34. Deborah. R, Bosman, Andrea. S. Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 2001;24:495–499.

35. Besarab A, Frinak S, Yee J. An indistinct balance: the safety and efficacy of parenteral iron therapy. J Am Soc Nephrol 1999;10:2029–2043.

36. Talwar VK, Gupta HL, Shashinarayan. Clinico-haematological profile in chronic renal failure. The Journal of Association of Physicians of India, 2002;50:228-33.

37. James B. Post, B.M., Wilkes Michael F, Iron deficiency in patients with chronic kidney disease: potential role for intravenous iron therapy independent of erythropoietin. International Urology and Nephrology 2006;38:719-723.

38. Malyszko J, Malyszko JS, Pawlak K,Mysliwiec M, Hepcidin, iron status, and renal function in chronic renal failure, kidney transplantation, and hemodialysis. Am J Hematol, 2006;81:832-7.

39. Gotloib L,Silverberg D,Fudin R et al. Iron deficiency is a common cause of anemia in Chronic Kidney Disease and can often be corrected with intraveneous iron.J Nephrol 2006;19:161 –7.

40. Descombes E,Hanck AB,Fellay G,Water soluble vitamins in chronic hemodialysis patients and need for supplementation. Kidney International journal, 1993;43:1319–1328.

41. Sevitt L.H, Hoff brand A.V, Serum folate and Vitamin B12 levels in acute and chronic renal disease. Effect on peritoneal dialysis. British Medical Journal. 1959;2:18-21

42. Reza Afshar, Suzan Sanavi, Javad Salimi, Mahnaz Ahmadzaheh. H. Hematological profile of Chronic Kidney disease in Iran, in Predialysis stages and after initiation of Haemodialysis. Saudi Journal Kidney Disease Transplant, 2009;20:368-371.

43. Chandra. M, Pathogenesis of the anemia of chronic renal failure: The role of erythropoietin. Nefrologia 1990;10.

44. Foley RN, Parfrey PS, Sarnak MJ, Clinical epidermiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis, 1998;32:112-119.

45. Baigent C, Landray M, Leaper **C**, Altmann P, Armitage J, Baxter A, et al, Premature cardiovascular disease in chronic renal failure. Lancent, 2000;356:147-152.

46. Painter P, Moore G, Carlson L, Paul S, Myll J, Phillips W, et al**,** Effects of exercise training plus normalization of hematocrit on exercise capacity and healthrelated quality of life. Am J Kidney Dis 2002;39:257–65.

47. Anaemia management in chronic kidney disease: National clinical guideline for management in adults and children. London: Royal College of Physicians, 2006 at www.guideline.gov/content.aspx?id=9817.accessed on 23.02.2011.

48. DRG EPO (Erythropoietin) ELISA EIA-3646 Revised 02.December.2010 version 6.0 accessed at http://www.drg-diagnostics.de/24-1-Immunology+Hematology.html

49. Decie and Lewis. Practical Haematology: Tenth Edition Churchill Livingstone; 2010;146-147

50. Abbott AxYSM system Ferritin REF 7A58, 34-3481/R9 manual.Accessed on 13th August, 2011 at http://promtest.am/tests/other/Ferritin.pdf.

51. Wish JB, Assessing iron status: Beyond serum ferritin and transferrin saturation, Clin J Am Soc Nephrol.2006;1:4-8.

52. The Gastroenterological Society of Australia. Iron deficiency. Guideline for general practioners: GESA 1st edition 2008.Accessed on 29th September, 2011 at www.gesa.org.au/pdf/booklets/IronDeficiancyClinical.pdf.

53. World Health Organization. Iron deficiency anemia: assessment, prevention and control;

a guide for programmer manager.Geneva:WHO 2001. Accessed on 29th September,2011 www.who.int/entity/nutrition/publications/.../GFF_References_en.pdf.

54. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood. 1997;89:1052-1057

55. Weiss G, Goodnough LT. Anemia of chronic disease. Engl J Med. 2005;352:1011-1023.

56. Jonathan O. Cullis. Diagnosis and management of anaemia of chronic disease: current status. British Journal of Haematology. 2011;154:289-300.

57. National Kidney foundation.KDOQI Clinical practice guidelines and clinical practice recommendations for anemia in Chronic Kidney Disease. Am J Kidney disease 2006;47:1-145.

58. Horl WH. Clinical aspects of iron use in the anemia of kidney disease. J Am Soc Nephrol 2007;18:382-93.

59. Sant-Rayn S Pasricha, Stephen C Flecknoe-Brown, Katrina J Allen. Diagnosis and management of iron deficiency anaemia: a clinical update. Medical Journal of Australia 2010;193:525-532

60. Abbott AxSYM SYSTEM Folate assay laboratory procedure manual REF 7K46, 49-3690/R3, B7K460. Accessed on 12th.August.2011 at http://www.ilexmedical.com/files/PDF/Folate_AXS.pdf.

61. Abbott AxSYM Active-B12 (Holotranscobalamin) manual IVD REF 1P43-20 ABOL039/R1.Accessed on 12th.August.2011 athttp://www.ilexmedical.com/files/PDF/AXS_ActiveB12Holot.pdf.

62. Anne MSB, Adriaan AV, Peter van der Meer WHG et al; Endogeneous Erythropoietin and outcome in Heart Failure, Circulation 2010;121:245-251.

APPENDIX 1; CONSENT FORM (ENGLISH VERSION)

CONSENT FORM TO PARTICIPATE IN THE STUDY.

Greetings! My name is Dr. Abdu Juma, a postgraduate student in the department of Hematology and Blood Transfusion. I would like to conduct a study mentioned above for my research project as one of the criteria to be fulfilled in postgraduate training.

Purpose of the study. The aim of the study is to determine the **magnitude and associated factors of anemia among CKD patients attending MNH.** This will help in improving future management of anemia in CKD patients.

How to participate. Participation is voluntary and every patient has a right to withdraw from the study at any time. Patients who meet the inclusion criteria will be recruited into the study, they will be interviewed using a questionnaire, which will include their social demographic characteristics, medical history, and clinical examination and undergo Laboratory investigations including **EPO levels, FBP, Iron study, serum B12 and folate level, peripheral smear and reticulocyte count**. Blood sample expected to be drawn are 4mls for FBP, peripheral smear and reticulocyte count, 4mls for serum creatinine, serum urea, iron study, vitamin B12/folate assay and erythropoietin levels.

Risks. There is a mild pain and a risk of thrombophlebitis during venopuncture.

Benefits: Patients found to have CKD will be counseled and followed up appropriately through our Nephrology Clinics.

Confidentiality: Any patient's results will not be revealed to anybody except attending doctors and patient himself.

Cost. No payment is requested from you as a fee to participate in the study.

Person to contact in questions or problems:

Dr. A.Juma (Tel: 0713446522) Student and principal investigator

Dr. E.Linda (Tel: 0754290499) Head of Renal Unit, MNH

I…… have read/been told of the contents of this form and understood its meaning. I agree to participate in this study.

Signature…….. (Participant) Date……

Signature…….. (Researcher) Date……

APPENDIX 2, QUESTIONNAIRE

PREVALENCE OF ANEMIA AND ITS ASSOCIATED FACTORS AMONG PATIENTS WITH CKD ATTENDING AT MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM

A.SOCIODEMOGRAPHIC DATA

Serial number……Date of interview…….

Hospital registration number…….

Name of patient……Contact……

1. Sex; (1) Male (2) Female

2. Age…… 3.Race…….

4. Occupation (1) Civil servant (2) Business (3) Peasant (4) Self employed (5) Other……

5. Education (1) None (2) Primary Education (3) Secondary Education

(4) College/University Education (5) Post University Education (6) Other…….

6. Marrital Status (1) Single (2) Married (3) Divorced (4) Widowed

B. DIAGNOSIS AND STAGES OF CKD

Criteria of CKD diagnosis as per eGFR <60 ml/min/1.73m^2 based on two GFR calculations in three months or more apart) or renal damage evidence (Indicate for each criteria used)

7. Baseline serum creatinine and its eGFR (ml/min/1.73m^2) in between 3 months or more

Date…….Serum Creatinine……eGFR……Stage…...

Date……..Serum creatinine…….eGFR……Stage……

8. Serial proteinuria (spot urine examination/urinalysis) in three months duration or more

A. Absent B. Present (+1, ++2, +++3, ++++4)

Record date for each…….……...

9. Serum creatinine and its eGFR (ml/min/1.73m^2) at time of recruitment

Date……Serum creatinine…..……eGFR……Stage…….

10. Documented duration since when CKD diagnosis was made……..months
11. Other Evidence of kidney damage

(1) Hematuria (2) Oliguria (3) Anuria (4) renal imaging results……

12. Documented primary cause/risk factor for CKD

(1) Diabetes Mellitus (2) Hypertension (3) HIV/AIDS (4) Gromerulonephritis

(5) Obstructive uropathy (6) Polycystic kidney disease (7) pregnancy (8) others…

13. Any history of dialysis? (a) Yes (b) No If yes duration of haemodialysis……

C. DIAGNOSIS OF ANEMIA AND OTHER HAEMATOLOGICAL PARAMETER

D. HISTORY AND PHYSICAL EXAMINATION

14. Symptoms of anemia at time of recruitment

(1) Lassitude/Fatigue
(2) Breathlessness (3) Palpitation (4) Throbbing in head and tears
(5) Dizziness (6) Tinnitus (7) Headache (8) Dimness of vision
(9) Insomnia (10) Numbness in lower limbs and upper limbs

15. Current treatment for anemia

(1) Blood transfusion, if yes state last date of receiving transfusion

(2) Haematenics (Fessolate and/or Folic acid) (3) Erythropoietin Stimulating Agents (ESA)

Indicate date of starting treatment…….

(4) No treatment

16. Documented history of malignancy or hematological disorder……

17. Documented history of acute or chronic inflammatory disease……..

18. General Examination

A. Pallor (1) Pale (2) Not pale B. Severity (1) Mild (2) Moderate (3) Severe (4) Very severe

C. Anemia stigmata signs

(1) Hyperpigmentaion on palm (2) Koilonychias (3) Brittle nails

(4) Beef tongue (5) Silk hair (6) Non of above

D. CKD stigmata

(1) Uremic frost (2) elevated JVP (3) Lower limb oedema

(4) Altered mentation (5) flapping tremor (6) Lower limb oedema (7) Anasarca

(8) Sacral oedema (9) Fascial swelling

C. Jaundice (1) Present (2) Absent

D. Temperature (1) Febrile…… (2) Not febrile……

19. Abdominal Examination

(1) Ascites (2) Hepatomegaly (3) Splenomegaly (4) Rt kidney bimanually palpable

(5) Lt Kidney bimanually palpable (6) both kidneys bimanually palpable

(7) Palpable masses (8) Combination of any of the above (mention)……

(10) None of the above (11) Renal angle tenderness (1) Present (2) Absent

20. Central Nervous System

Peripheral Neuropathy (1) Present (2) Absent

Other hematological parameters.

21. Hemoglobin Level…….g/dl as per WHO criteria

(1) Normal Hb Level (2) Anemic (3) Polycythemic

22. RBC Indices (1) MCV….. (2) MCH …… (3) MCHC……..

23. WBC level……K/uL (1) Normal (2) Leucopenia (3) Leucocytosis

24. Other differential

(1) Lymphocyte… (2) Neutrophil… (3) Monocytes…… (4) Basophil…. (5) Eosinophil…

25. Platelet Level……..K/uL (1) Normal (2) Thrombocytopenia (3) Thrombocytosis

26. Serum ferritin level……ug/L

27. Transferrin Saturation……%

28. Serum B12 level……Serum Folate level……

29. Serum Erythropoietin level……..mIU/mL

30. Peripheral smear results for those defined to have anemia

(1) Film Number…….

(2) Results RBC……..WBC…….PLTS……

31. Reticulocyte count ……