EVALUATION OF IRON STATUS IN CHRONIC KIDNEY DISEASE PATIENTS IN ABUTH, ZARIA

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MAY, 2015

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BY

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MSc/Med/0615/09-10

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OFTHE REQUIREMENTS FOR THE AWARD
OF A
MASTER DEGREE IN CHEMICAL PATHOLOGY

DEPARTMENT OF CHEMICAL PATHOLOGY,
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MAY, 2015

DECLARATION

I declare that the work in this dissertationentitled;Evaluation of iron status in chronic kidney disease patients in ABUTH Zaria,has been carriedout by me in Department of Chemical Pathology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertationentitled; EVALUATION OF IRON STATUS IN CHRONIC KIDNEY DISEASE PATIENTS IN ABUTH, ZARIA by AminaMOHAMMED meets the regulations governing the award of the degree of MSc Chemical Pathology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literacy presentation.

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ACKNOWLEDGEMENT

All praises and gratitude be to Almighty God who has given me wisdom, good health and strength to carry out this study.

My sincere appreciation goes to my supervisors, Prof I S Aliyu,Dr A Ibrahim and Dr Yusuf Rasheed whose constructive criticisms, constant advice, guidance and patience saw me through this work. Appreciation also goes toDr Mohammed El-bashir for his professional advice, encouragement and assistance.

Special appreciation goes to my parents Alhaji Mohammed Usman (late) and HajiaAishatu Mohammed for my proper upbringing and prayers. My warmest appreciation also goes to my beloved husband DrUsmanAbubakar, my children Mohammed TukurUsman, Mohammad khalifaUsman,AbubakarHussainiUsman and ZaynabHassanaUsman for their understanding and support during the period of the research work.

I also wish to acknowledge the immense support and prayers by my brothers and sisters especially mama Gambo a special thank you from the kids and myself. May the almighty God bless you and your families.

To my colleagues, resident doctors of Chemical Pathology department, other doctors and nurses who assisted me during the study especially Dr (Mrs) H M Suleiman,DrAdamuRabiu and other residents and friends too numerous to mention, I say thank you all. I also want to extend my sincere appreciation to Mrs. Fatima Folorunsho, MrsFatimaAliyu,MallamTaofiq,MallamAuwaland Mr.Ochai for their technical support.

ABSTRACT

The burden of chronic kidney disease (CKD) is increasing rapidly worldwide and has become a major health issue. Iron deficiency anaemia is one contributory aspect of renal anaemia with increasing morbidity and mortality. Use of iron status markers is integral to assessment of deficiency, and to setting treatment goals in the successful management of anaemia and iron deficiency in CKD patients.Most data on anaemia of CKD in Nigeria revealed dearth of information on indices of iron status, which makes treatment interventions difficult in this setting. Therefore, this study was designed to address this gap.

The objective of the study is to evaluate iron status among CKD patients in ABUTH,Zaria

The study was a descriptive cross sectional study that involved 125 CKD patients and 125 age- and sex-matched controls. The patients were recruited consecutively from nephrology clinic while controls were sourced from hospital staff, patient's relatives and general population of Zaria. Ferritin was analyzed using ELISA; colorimetric method was used to analyze iron, Total iron binding capacity andcreatinine.Quality Control material was obtained from pooled sera for ferritin and creatinine. Commercially prepared quality control (multi control) was used for iron and Total iron binding capacity. Transferrin saturation was calculated from iron and TIBC. SPSS version 17.0 was used for data analysis and p-value of <0.05 was taken as statistically significant.The three leading diseases causing CKD found in this study were hypertensive nephropathy, diabetic nephropathy and adult polycystic kidney disease (APKD). CKDpatients had significantly higher serum ferritin, TIBC, creatinine and iron when compared with controls ($p<0.05$), with mean±SD of210.85±138.28 ng/ml, 71.27±28.23µmol/l, 623.88±681.54µmol/l and 20.21±10.23µmol/l respectively. Serum ferritin significantly correlated positively with creatinine and negatively with eCrcl.When correlated with stages of CKD,serum ferritin was found to be

significant between stages 1 and 4, 1 and 5, 2 and 5, stages 3 and 4,stages 3 and 5.Serum ironcorrelated positively with % TSAT while TIBC correlated negatively with %TSAT.The reference intervals of iron, TIBC, ferritin (males and females) and TSAT were found to be 6.8-40 µmol/l, 33-113µmol/l,18-304ng/ml,24-232ng/ml and 8.3-71% respectively in controls.

In conclusion, this study revealed high incidence of APKD, in addition to the other well known causes of CKD, such as hypertension and diabetes.A significant percentage of CKD patients in this study had iron deficiency anaemia (IDA). Therefore, iron status should be incorporated in the armamentarium of tests for patients with CKD in order to properly correct IDA that is so highly prevalent in this population. These will help in attending to the challenges of iron deficiency anaemia management and poor response to erythropoietin.

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ABBREVIATIONS /SYMBOLS

A	Absorbance
A_{std}	Absorbance of standard
ABUTH	Ahmadu Bello University Teaching Hospital
C	Concentration
APKD	Adult polycystic kidney disease
BP;	Blood pressure
CKD	Chronic kidney disease
DBP	Diastolic blood pressure
eCrCl	Estimated creatinine clearance
ELISA	Enzyme linked immunosorbent assay
ESA	Erythropoietin stimulation agent
ESRD	Endstage renal disease
Epo	Erythropoietin
GFR	Glomerular Filtration Rate
Hb	Haemoglobin
hS-CRP	High sensitive C - reactive protein
IDA	Iron deficiency anaemia
ISN	International society of Nephrologist
KDOQI	Kidney disease outcome quality initiative
kg	Kilogram

kg/m^2	kilogram per metre square
µL	Micro litre
µmol	Micromole
µmol/l	Micromole per litre
ml	Milliliter
mmol/L	Milli mole per litre
min	Minute
ng	Nanograms
ng/ml	Nanogram per millilitre
P	Prevalence
pg/mL	Picogram per millilitre
rpm	Revolution per minute
SPSS	Statistical Package for Social Sciences
US	United States
WHO	World Health Organisation
NKF	National kidney foundation
SD	Standard deviation
SBP	Systolic blood pressure.
TSAT	Transferrin saturation
TIBC	Total iron binding capacity
USRD	United States renal data

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

The Kidney Diseases Outcome Quality Initiative (K/DOQI) defines chronic kidney disease(CKD) as either damage or a decreased glomerular filtration rate (GFR) of less than 60ml/min/1.73m^2 for 3 or more months. Whatever the underlying aetiology, the destruction of renal mass with irreversible sclerosis and loss of nephron leads to a progressive decline in GFR. The different stages of kidney disease form a continuum in time.

In Stage 1 CKD, the GFR is ≥ 90 ml/min/1.73 m^2. Stages 2, 3, and 4 CKD are defined by a GFR of 60–89 ml/min/1.73 m^2, 30–59 ml/min/1.73 m^2, and 15–29 ml/min/1.73 m^2, respectively. The final stage, Stage 5, occurs when the GFR is < 15 ml/min/1.73 m^2 or when patients require dialysis (KDOQI, 2002).

The magnitude of the problem of chronic renal disease is enormous as the prevalence of kidney failure is rising. Currently chronic kidney disease (CKD) is emerging as a worldwide public health problem (Chinwuba *et al*, 2010). CKD is associated with increased cardiovascular mortality and a loss of disability-adjusted life years. Diseases of the genitourinary system were responsible for 928,000 deaths and 14,754,000 disability-adjusted life years in 2006(WHO, 2006).

However, the absence of kidney registries in most of the low- and middle-income countries has made it difficult to ascertain the true burden of CKD in most countries. The global increase in the incidence and prevalence of CKD is being driven by the global increase in the prevalence of

diabetes mellitus, hypertension, obesity, and aging (Olugbenga and Alebiosu,2010) . Globally CKD represent the 12th cause of death and 17th cause of disability (WHO, 2006 ;Chinwuba *et al*, 2010).

In the United States, there is a rising incidence and prevalence of kidney failure, with poor outcomes and high cost. Kidney disease is the ninth leading cause of death in the United States (USRDS,2009;Pradeep and Vecihi, 2012).

The Third National Health and Examination Survey (NHANES III) estimated that the prevalence of chronic kidney disease in adults in the United States was 11% (19.2 million): 3.3% (5.9 million) had stage 1, 3% (5.3 million) had stage 2, 4.3% (7.6 million) had stage 3, 0.2% (400,000) had stage 4, and 0.2% (300,000) had stage 5 CKD(mcCulloch &Curhan;2002, USRDS 2009; Pradeep and Vecihi, 2012).

The prevalence of chronic kidney disease stages 1-4 increased from 10% in 1988-1994 to 13.1% in 1999-2004. This increase correlates with increase in the prevalence of diabetes and hypertension, the two most common causes of chronic kidney disease (Pradeepand Vecihi, 2012). Data from the United States Renal Data System (USRDS) indicated that the prevalence of chronic renal failure increased 104% between the years 1990-2001.

The incidence rates of end-stage renal disease (ESRD) have increased steadily internationally since 1989(USRDS, 2009). The United States has the highest incident rate of ESRD, followed by Japan. Japan has the highest prevalence per million populations, with the United States taking second place (USRDS, 2009).

In Nigeria, the actual prevalence rate of this disease is not known, but hospital based studies show that it accounts for 2-10% of all admissions (Akinsola *et al,* 1989; Kadiri *et al,* 1999).

The prevalence of CKD has been shown in various parts of Nigeria to be 1.6% in Ibadan by Oyediran *et al*,(1970). In Lagos it was found to be 10% by Mabayoje *et al*, (1992). In Ile Ife, Akinsola *et al*, (1989)found a prevalence of 8%.In Ibadan, Kadiri *et al*,(1999) reported 6.7%, and in Sagamu Alebiosu *et al*,(2006) found 3.6% . Akinsola *et al*, (1989) suggested that the wide variation in the values from various studies appears to be due to variations in the sources of the data consulted.

It is well known that CKD is under recognized and under diagnosed; patients with end-stage renal failure (ESRD) are thought to represent the tip of the iceberg of the entire burden of CKD (Afolabi *et al*, 2009; Chinwuba *et al*, 2010).

Anaemia is a common complication of chronic kidney disease (CKD) that develops early in the course of CKD, and becomes increasingly severe as the disease progresses. The management of anaemia in CKD patients requires an appropriate balance between stimulating the generation of erythroblasts (erythropoiesis) and maintaining sufficient iron levels for optimum haemoglobin (Hb) production.

Anaemia one of the major complications in patients with CKD is due to many factors. Erythropoiesis and iron homeostasis are impaired as a result of a complex chain of events, including the relative deficiency of erythropoietin, chronic inflammation, blood loss, decreased iron absorption and utilization, exogenous iron and erythropoietin acquisition via biologically unregulated mechanisms(blood transfusions and medicinal erythropoietin and iron administration) (Weiss, 2009;Guidi and Santonastaso, 2010; Lankhorst and Wish, 2010).The

advent of erythropoiesis stimulating agents (ESA) and various intravenous iron preparations has resulted in a much more effective management of anaemia of CKD, allowing the maintenance of haemoglobin levels in certain desired ranges and to effectively treat iron deficiency. Among the emerging challenges are the risks associated with administering high ESA and iron doses, leading to elevated haemoglobin levels and iron overload (Zager*et al*, 2002).

Recombinant human erythropoietin (rHuEpo) has been available for treatment of renal anaemia since 1989. However, rHuEpo therapy results in functional iron deficiency due to insufficient iron stores for the accelerated erythropoiesis. Iron deficiency is the main cause of suboptimal response to erythropoietin in dialysis patients (Cavill and Macdougall, 1993).Maintenance of iron supplementation is required to successfully treat anaemia; intravenous iron compounds are used to treat dialysis patients who become iron deficient.

Monitoring erythropoietin treated patients for iron status is important to detect iron deficiency and avoid the adverse effects of iron medication. Anaemia management can be optimized only if functional iron deficiency can be avoided (Steven*et al*, 1996; Anatole, 2001)

The National Kidney Foundation–Dialysis Outcomes Quality Initiative (NKF-DOQI) guidelines advocate aggressive detection and management of functional iron deficiency. The presence of functional iron deficiency is confirmed by the response to a course of parenteral iron that produces either a decrease in dose of EPO needed to maintain the target haematocrit level or an increase in haemoglobin at the same dose of epoetin.

Iron deficiency is seen in majority of patients with CKD (Omara *et al*, 2008;Kalantar –Zadeh *et al*, 2004). More than 50% of adult CKD patients presenting with anaemia in the U.S.A may be iron deficient (Hsu*et al*, 2002), especially those receiving epoietin. Iron deficiency is common in

patients with ESRD on haemodialysis than on non-dialysis patients (Van wyck *etal*, 1989; Fishbane,2007).Okundaye *et al*, (2010) in New York had a 40% prevalence figure of iron deficiency in non dialysis CKD.The differences between the genders were not significant.

Iron deficiency is very common among patients with CKD than was previously thought. In a study in Israel, Gotloib *et al*,(2006)stained 47 bone marrow biopsies and found out that 46 did not have stainable iron in the bone marrow biopsies.

Thus, assessing iron status is integral to both iron deficiency and anaemia management in CKD patients, as iron is essential for haemoglobin formation (as is erythropoietin)(Chung*et al*, 2012).

1.2STATEMENT OF THE PROBLEM

Chronic kidney disease management is consuming a huge proportion of health care finances in developed countries; it is contributing significantly to morbidity, mortality and decreased life expectancy in developing ones. Anaemia is a common and early complication of chronic kidney disease. One contributing factor is iron deficiency, which may be particularly problematic during erythropoietin replacement therapy.In patients with renal impairment, iron deficiency anaemia reduces the quality of life and is an additional risk factor for early death. Untreated prolonged anaemia leads to a number of physiologic disorders, including: cardiovascular complications and increased morbidity andmortality. Irondeficiency anaemia management can only be optimized if functional iron deficiency can be avoided hence the need to monitor iron in CKD patients.

1.3 JUSTIFICATION OF THE STUDY

There is paucity of data regarding iron status among patients in early stages of chronic kidney disease (CKD), except for those with stage 5 disease on haemodialysis. It therefore, seems rational that proper evaluation and treatment of iron deficiency anaemia in patients with CKD, early in the course of the disease, will prevent adverse impact on their quality of life. Consequently the use of iron status markers have become established as non-invasive and cost effective tools for assessment of iron deficiency in CKD patients, and to setting treatment goals in the successful management of anaemia and iron deficiency in CKD patients. Monitoring erythropoietin treated patients for iron status is important to detect iron deficiency and avoid the adverse effects of iron medication and improve their quality of life.

1.4 AIM AND OBJECTIVES

1.4.1 **Aim**

The study is aimed at evaluating iron status among chronic kidney disease patients in ABUTH, Zaria.

1.4.2 **Objectives**

1) To determine the serum levels of iron, transferrin (Total Iron Binding Capacity (TIBC)), transferrin saturation and ferritin in chronic kidney disease patients and apparently healthy controls.

2) To compare the serum levels of iron, transferrin (TIBC), transferrin saturation and ferritin in chronic kidney disease patients with those of control subjects.

3) To correlate the serum levels of iron, transferrin (TIBC), transferrin saturation and ferritin with stages of chronic kidney disease.

4) To correlate the serum levels of iron, transferrin (TIBC), transferrin saturation, ferritin and serum creatinine concentration as a measure of renal impairment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE KIDNEY

Grossly, the kidneys are bean-shaped structures and weigh about 150 g in the adult male and about 135 g in the adult female. They are typically 10-12 cm in length, 5-7 cm in width, and 2-3 cm in thickness (Davidand Thomas, 2011). The functional renal unit is the nephron, which is composed of the following components: The renal corpuscle: glomerulus and bowman capsule, proximal convoluted tubules (PCT) located in the renal cortex, descending loop of henle (LOH), ascending limb, (which resides in the renal medulla, leading to the thick ascending limb) thick ascending limb, distal convoluted tubule and collecting duct (which opens into the renal papilla)

The afferent arteriole carries blood from the renal artery into the glomerulus, where it divides to form a circulatory network at the distal end of the glomerulus, the capillaries rejoin to form the efferent arteriole through which blood leaves the glomerulus.Fluid filtered from the glomerular capillaries flows into Bowman's and then into the proximal tubule, which lies in the cortex of the kidney (Arthur and John, 2006).

From the proximal tubule, filtered fluid flows into the loop of Henle, which dips into the renal medulla. Each loop consists of a descending and ascending limb. The walls of the descending limb and the lower end of the ascending limb are very thin and therefore are called the thin segment of the loop of Henle. After the ascending limb of the loop has returned partway back to the cortex, its wall becomes much thicker, and it is referred to as the thick segment of the ascending limb.

At the end of the thick ascending limb is a short segment which is actually a plaque in its wall, known as macula densa .Beyond the macula densa, fluid enters the distal tubule, which like the proximal tubule lies in the renal cortex. This is followed by the connecting tubule and the cortical collecting tubule, which leads to the cortical collecting duct. The initial parts of 8 to 10 cortical collecting ducts join to form a single larger collecting duct that runs downward into the medulla and becomes the medullary collecting duct (Arthur and John, 2006).

A large amount of circulating blood flows through the kidneys. Approximately 25% of the cardiac output or 1200ml of blood per minute is received by the kidneys. One litre of urine is the end product of more than 1000litres of circulating blood processed through the kidneys. Urine formation begins in the glomerular capillaries, with dissolved substances passing into the proximal tubule as a result of the force of blood pressure in the large afferent arteriole and pressure in Bowman's capsule (Arthur and John, 2006).

2.1.1 Functions of the Kidney

The functional unit in the kidney is the nephron. The kidney regulates extracellular fluid volume and electrolytes composition to compensate for wide variation in water and electrolytes intake. They form urine in which the potentially toxic waste products of metabolism are excreted. The functions of the kidney include;

1) Excretion of waste product; which include protein and nucleic acid metabolism: urea, creatinine, creatine, uric acid, sulphate, phosphate.

2) Homeostasis; the kidney is one of the major organs involved in whole body homeostasis. Among its homeostatic functions are acid-base balance, regulation of electrolyte concentration, control of blood volume and regulation of blood pressure.

3) Hormone secretion; the kidney secrete a variety of hormones, including erythropoietin, urodilatin, renin and 1, 25 dihydroxy cholecalciferol (the most active form of vitamin D)

2.1.2ClinicalFeatures of Chronic Kidney Disease

Patients with chronic kidney disease stages 1-3 (Glomerular Filtration Rate) [GFR] >30 ml/min/1.73m^2) are generally asymptomatic; they do not experience clinically evident disturbances in water or electrolyte balance or endocrine/metabolic derangements. Generally, these disturbances become clinically manifest with chronic kidney disease stages 4-5 (GFR < 30 ml/min/1.73m^2)(Pradeepand Vecihi,2012).

Uraemic manifestations in patients with chronic kidney disease stage 5 are believed to be primarily secondary to an accumulation of toxins, the identity of which is generally not known. Metabolic acidosis in stage 5 may manifest as protein-energy malnutrition, loss of lean body mass, and muscle weakness. Altered sodium and water handling by the kidney in chronic kidney disease can cause peripheral oedema and, not uncommonly, pulmonary oedema and hypertension. (Pradeep and Vecihi,2012).

Anaemia is associated with fatigue, reduced exercise capacity, impaired cognitive and immune function and reduced quality of life. Anaemia is also associated with the development of

cardiovascular disease, the new onset of heart failure, or the development of more severe heart failure. It is associated with increased cardiovascular mortality.

Other manifestations of uraemia in ESRD, many of which are more likely in patients who are inadequately dialyzed, include the following:

Pericarditis - can be complicated by cardiac tamponade, possibly resulting in death

Encephalopathy - can progress to coma and death

Peripheral neuropathy

Restless leg syndrome

GI symptoms - anorexia, nausea, vomiting, diarrhoea

Skin manifestations - dry skin, pruritus, ecchymosis

Fatigue, increased somnolence, failure to thrive

Malnutrition

Erectile dysfunction, decreased libido, amenorrhea

Platelet dysfunction with tendency to bleeding

2.2 EPIDEMIOLOGY OF CKD

In the United States, there is a rising incidence and prevalence of kidney failure, with poor outcomes and high cost. Kidney disease is the ninth leading cause of death in the United States (Pradeepand Vecihi 2012).

The Third National Health and Examination Survey (NHANES III) estimated that the prevalence of chronic kidney disease in adults in the United States was 11% (19.2 million): 3.3% (5.9 million) had stage 1, 3% (5.3 million) had stage 2, 4.3% (7.6 million) had stage 3, 0.2% (400,000) had stage 4, and 0.2% (300,000) had stage 5 CKD (Pradeep& Vecihi 2012).

The prevalence of chronic kidney disease stages 1-4 increased from 10% in 1988-1994 to 13.1% in 1999-2004. This increase correlates with increase in the prevalence of diabetes and hypertension, the two most common causes of chronic kidney disease.(USRDS, 2009; Pradeep andVecihi, 2012).

The incidence rates of end-stage renal disease (ESRD) have increased steadily internationally since 1989(Arthurand John, 2006).The United States has the highest incident rate of ESRD, followed by Japan. Japan has the highest prevalence per million populations, with the United States taking second place (PradeepandVecihi 2012).

Chronic kidney disease affects all races, but in the United States, a significantly higher incidence of ESRD exists in blacks than in whites; the incidence rate for blacks is nearly 4 times that for whites(Norrisand Agodoa, 2005).

Choi *et al*, (2009) found that rates of ESRD among black patients exceeded those among white patients at all levels of baseline estimated GFR (eGFR).Risk of ESRD among black patients was highest at an eGFR of 45-59 mL/min/1.73 m^2.

In NHANES III, the distribution of estimated GFRs for the chronic kidney disease stages was similar in both sexes. Nonetheless, the USRDS [United States Renal Data System] (2004) annual Data Report reveals that the incident rate of ESRD cases is higher for males, with 409 per million population in 2002 compared with 276 for females.

Chronic kidney disease is found in persons of all ages. However, in the United States, the highest incidence rate of ESRD occurs in patients older than 65 years. As per NHANES III data, the prevalence of chronic kidney disease was 37.8% among patients older than 70 years. Besides diabetes mellitus and hypertension, age is an independent major predictor of chronic kidney disease. The geriatric population is the most rapidly growing kidney failure (chronic kidney disease stage 5) population in the United States (Pradeep andVecihi 2012).

Several studies from Nigeria have shown that the peak incidence of ESRD in Nigeria is between the third and fifth decades of life (Ojogwu and Anah, 1983; Akinsola*et al* 1989;Ojo*et al*, 1990; Salako*et al*, 1995; Arije*et al*, 2000;Alebiosu *et a,l* 2006).

The prevalence of CKD is higher in developing countries than in the developed world (Akinsola *et al*, 1989, Arije *et al*, 2000, Naicker, 2003). The most common causes of CKD in the developing countries and indeed Nigeria are chronic glomerulonephritis and systemic hypertension(Naicker, 2003, Arogundade*et al*, 2011). But in Europe, United state and Japan with the rise in the prevalence of diabetic nephropathy it is the most common cause. Factors contributing to the regional differences in the aetiology and prevalence of CKD are race, ethnicity, genetic predisposition, increasing prevalence of type 2 diabetes, obesity, and possibly cigarette smoking (Alebiosu and Ayodele,2005).

In Nigeria, the actual prevalence rate of this disease is not known, but hospital based studies show that it accounts for 2-10% of all admissions (Akinsola*et al*, 1989, Kadiri and Arije, 1999)

The burden of renal disease in Nigeria is probably significantly higher than any previous study on end-stage renal disease (ESRD) has documented, as most studies are hospital-based and fail to include the many patients who do not have access to hospital care(Alebiosu and Ayodele,2005).The increased prevalence of ESRD among blacks in the United States and South Africa compared with other races also suggests that ESRD may be more prevalent in Africa than in the United States and other developed nations (Norris and Agodoa,2005).

In another study the leading cause of end stage renal disease is hypertension and diabetes in sub-Saharan Africa and indeed Nigeria (Kadiri *et al*, 1999). Access to renal replacement therapy (RRT) in Nigeria is limited, and mortality rates are very high, ranging between 40 and 50% (Odubanjo *et al*, 2011). Poverty, inadequate facilities and lack of donors are major problems facing kidney transplant in our society (Badmus *et al*, 2005).

It therefore underscores the pressing need for aggressive and comprehensive strategies for early detection and treatment of causes of CKD at the primordial and primary prevention levels, as advocated by International Society of Nephrology, to reduce the impact of CKD in the society (K/DOQI, 2002).

2.3 **AETIOLOGY AND STAGING OF CKD**

Causesofchronickidneydiseaseincludethefollowing:

Diabetic kidney disease,Hypertension,Vascular disease,Glomerular disease (primary or secondary), Cystic kidney diseases,Tubulointerstitial disease,Urinary tract obstruction or

dysfunction,Recurrent kidney stone disease,Congenital (birth) defects of the kidney or bladder and unrecovered acute kidney injury.

In 2002, the National Kidney Foundation of the United States called upon an international group of experts to develop guidelines, which provided a clear and simple definition of CKD and a 5-stage classification system developed through an evidence-based process and supported by existing literature (see Figure 2.1 and Table 2.1). CKD is defined by the occurrence of kidney damage or GFR <60 mL/min/1.73 m^2 for ≥3 months. Disease staging is based on the GFR (KDOQI, 2002).

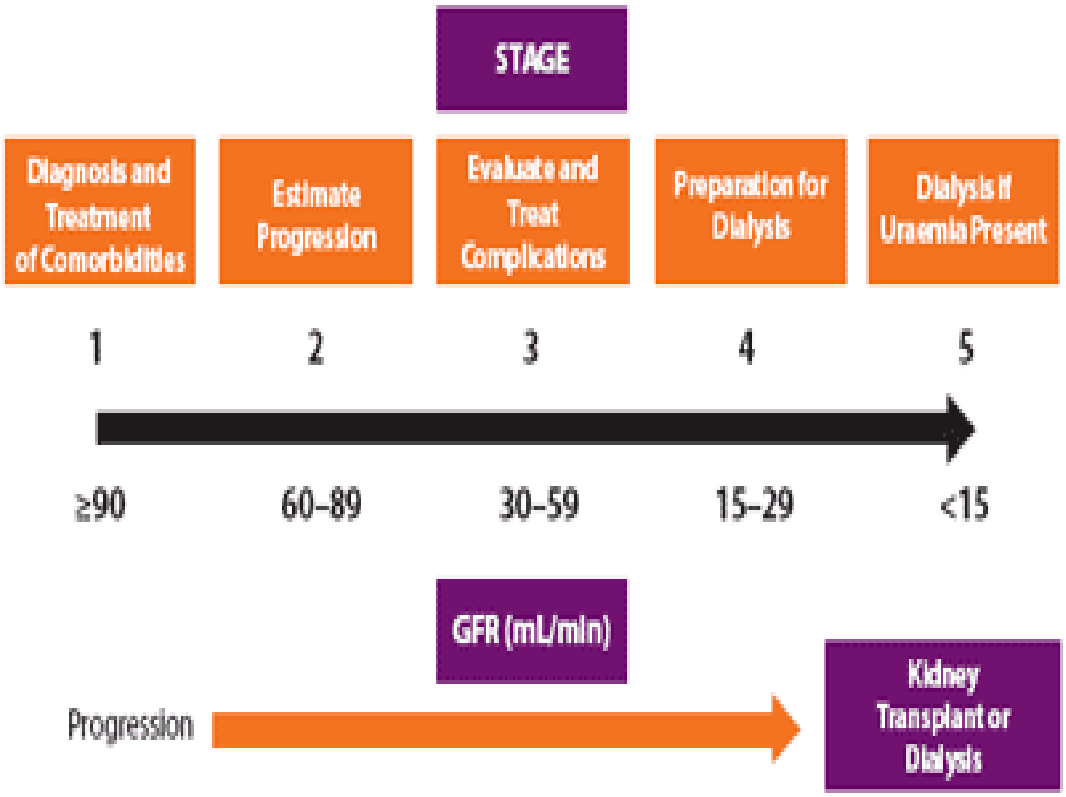

Figure 2.1 The CKD continuum **(www.epgonline.**org) accessed Dec 2013

Table 2.1 Stages of CKD by GFR (KDOQI, 2002)

	Description	GFR (ml/min/1.73 m^2)
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60–89
3	Moderate ↓ GFR	30–59
4	Severe ↓ GFR	15–29
5	Kidney failure	<15 or dialysis or transplant

2.4 BIOCHEMICAL CHANGES IN CKD

The features of CRF indicate the inability of the kidney to maintain its filtrative, excretory, regulatory and endocrine functions. Consequently, the following biochemical features are seen in patient with CRF (Isah, 2007)

Increased plasma concentrations of nitrogenous waste products mainly urea, creatinine and uric acid.This result from decreased GFR and tubular function. The excess urea may manifest as whitish flakes on the skin known as uraemic frost.

Metabolic acidosis

Hyperkalaemia

Hypocalcaemia

Hyperphosphataemia

Secondary hyperparathyroidism

Anaemia

2.5 IRON HOMEOSTASIS

The normal western diet contains 15–20 mg iron in haem (10%) and non-haem (ionic, 90%)

forms. Only 1–2 mg of iron is absorbed and lost every day. Importantly, the total amount of

iron in the body can be regulated only by absorption, whereas iron loss occurs only passively

from sloughing of skin and mucosal cells as well as from blood loss. Iron absorption is balanced against iron loss so daily iron absorption may increase in response to increased iron demand (eg, growth, pregnancy or blood loss) (Conrad and Umbeit,2002; Miret *et al*, 2003). Nearly all absorption of dietary iron occurs in the duodenum. Several steps are involved, including the reduction of iron to a ferrous state, apical uptake, intracellular storage or transcellular trafficking, and basolateral release. Molecular participants in each of these processes have been identified.

The non-haem iron mainly exists in the ferric (Fe^{3+}) state. The ferric iron is reduced to ferrous iron (Fe^{2+})before it is transported across the intestinal epithelium. The reduction of iron from the ferric to the ferrous state occurs at the enterocyte brush border by means of a duodenal ferric reductase . Once the insoluble Fe^{3+} is converted to Fe^{2+}.Ferrous iron is then transported across the apical plasma membrane of the enterocyte by divalent metal transporter 1 (DMT1). DMT1 is expressed at the duodenal brush border where it controls uptake of dietary iron, and also traffics other metal ions such as zinc, copper and cobalt by a proton-coupled mechanism (Conrad and Umbeit, 2002).

Iron taken up by the enterocyte may be stored intracellularly as ferritin and excreted in the faeces when the senescent enterocyte is sloughed or transferred across the basolateral membrane to the plasma. This iron is transferred out of the enterocyte by the basolateral transporter ferroportin; this process is facilitated by the ferroxidase activity of the

ceruloplasmin homologue hephaestin (Fleming and Bacon, 2005).

Iron homeostasis is dependent on regulatory feedback between body iron needs and intestinaliron absorption.Iron stores, erythropoietic activity, haemoglobin, oxygen content, and inflammationmodulate the dietary iron absorption (Nemeth *et al*, 2004).

Essentially all circulating plasma iron normally is bound to transferrin. The liver synthesizes transferrin and secretes it into the plasma. The chelation of ferric iron serves three purposes: it renders iron soluble under physiologic conditions, it prevents iron-mediated free radical toxicity and it facilitates transport into cells. Transferrin is the most important physiological source of iron for red cells (Ponka *et al*, 1998).

Although transferrin was characterized fifty years ago, its receptor eluded investigators until the early 1980s.The molecule is a transmembrane homodimer linked by disulfide bonds. This disulfide linked homodimer has subunits containing 760 amino acids each (Beaumont and Delaby, 2009). Oligosaccharides account for about 5% of the 90 kDa subunit molecular mass. A broad body of literature nowsupports the concept that the iron-transferrin complex is internalized by receptor-mediatedendocytosis (Beaumont and Delaby, 2009).

Most of the body iron is associated to haemoglobin in circulating erythrocytes. Erythropoiesis is a very active process that takes place in the bone marrow and leads to the dailyproduction of 200 billion new erythrocytes to compensate for the destruction of senescentred cells by tissue macrophages. The control of erythropoiesis depends mostly onerythropoietin production by the kidney and on the availability of iron.

Macrophages play a central role in the organism as they recycle iron after phagocytosis of senescent erythrocytes. This mechanism mainly occurs in the spleen and bone marrow and to a lesser extent in the Kupffer cells of the liver. During aging, erythrocytes accumulate multiple modifications (cell shrinkage, externalization of phosphatidyl-serine, peroxydation of the membrane). The fixation andingestion of red cells by macrophages are triggered by cellular receptor-mediatedphagocytosis through recognition of externalized phosphatidyl-serine or neoantigens ofsenescence red cells.

Iron can be stored in the macrophages associated to ferritin or hemosiderin or exported tothe plasma. Iron export from macrophages to transferrin is accomplished by ferroportin, thesame iron-export protein as expressed in the duodenal enterocyte and reoxydized byceruloplasmin (Knutson *et al*, 2005).

Metabolically inactive iron is stored in ferritin and hemosiderin. Normally, 95% of thestored iron in liver tissue is found in hepatocytes as ferritin. The level of serum ferritinparallels the concentration of storage iron within the body; regardless of the cell type inwhich it is stored. The control of iron homeostasis acts at both the cellular and the systemic level and involvesa complex system of different cell types, transporters, and signals. To maintain systemic ironhomeostasis, communication between cells that absorb iron from the diet (duodenalenterocytes), consume iron (mainly erythroid precursors) and store iron (hepatocytes andtissue macrophages) must be tightly regulated (Swinkels *et al*, 2006).

In the last 10 years, understanding of the regulation of iron homeostasis has changedsubstantially. A small peptide hormone, hepcidin, emerged as the central regulator of iron

absorption, plasma iron levels, and iron distribution. Hepcidin is secreted mainly by hepatocytes, and to a lesser extent by macrophages and adipocytes. The hormone inhibits iron flows into plasma from macrophages involved in recycling of senescent erythrocytes, duodenal enterocytes engaged in the absorption of dietary iron, and hepatocytes that store iron. (Ganz and Nemeth, 2009).

Hepcidin expression is controlled by various stimuli: iron, inflammation, erythropoiesis and hypoxia. Iron and inflammation induce hepcidin production, while iron deficiency, hypoxia and stimulation of erythropoiesis completely inhibit its production. Hepcidin is secreted into the circulation, where it down-regulates the ferroportin-mediated release of iron from enterocytes, macrophages and hepatocytes and is the key for the regulation of systemic iron homeostasis (Fleming and Bacon,2005).It reduces the quantity of circulating iron bylimiting the egress of the metal from both intestinal and macrophage cells; the cellular process by which hepcidin acts, through its binding to ferroportin, thereby inducing internalization and subsequent degradation of the exporter (Bergamaschi and Villani, 2009).

In the intestine, delivery of dietary iron to plasma transferrin is inhibited by increasing concentrations of hepcidin, and iron is subsequently removed from the body, through the elimination of enterocytes (desquamation process). In macrophages, degradation of ferroportin by hepcidin results in the trapping of iron inside the cells, thereby limiting the

acquisition of iron by erythroid cells (Nemeth *et al*, 2004).

Iron is absorbed from the diet by duodenal enterocytes and then bound to plasma transferrin (Tf). Fe-Tf is distributed to the bone marrow for erythropoiesis. At the end of their lifespan, senescent erythrocytes are phagocytosed by tissue macrophages and heme iron is recycled back to plasma transferrin.

Hepcidin regulates the systemic iron homeostasis; synthesized by the liver is secreted into the circulation, where it down-regulates the ferroportin-mediated release of iron from enterocytes, macrophages, and hepatocytes. (Swinkels *et al*, 2006) (See figure 2.2)

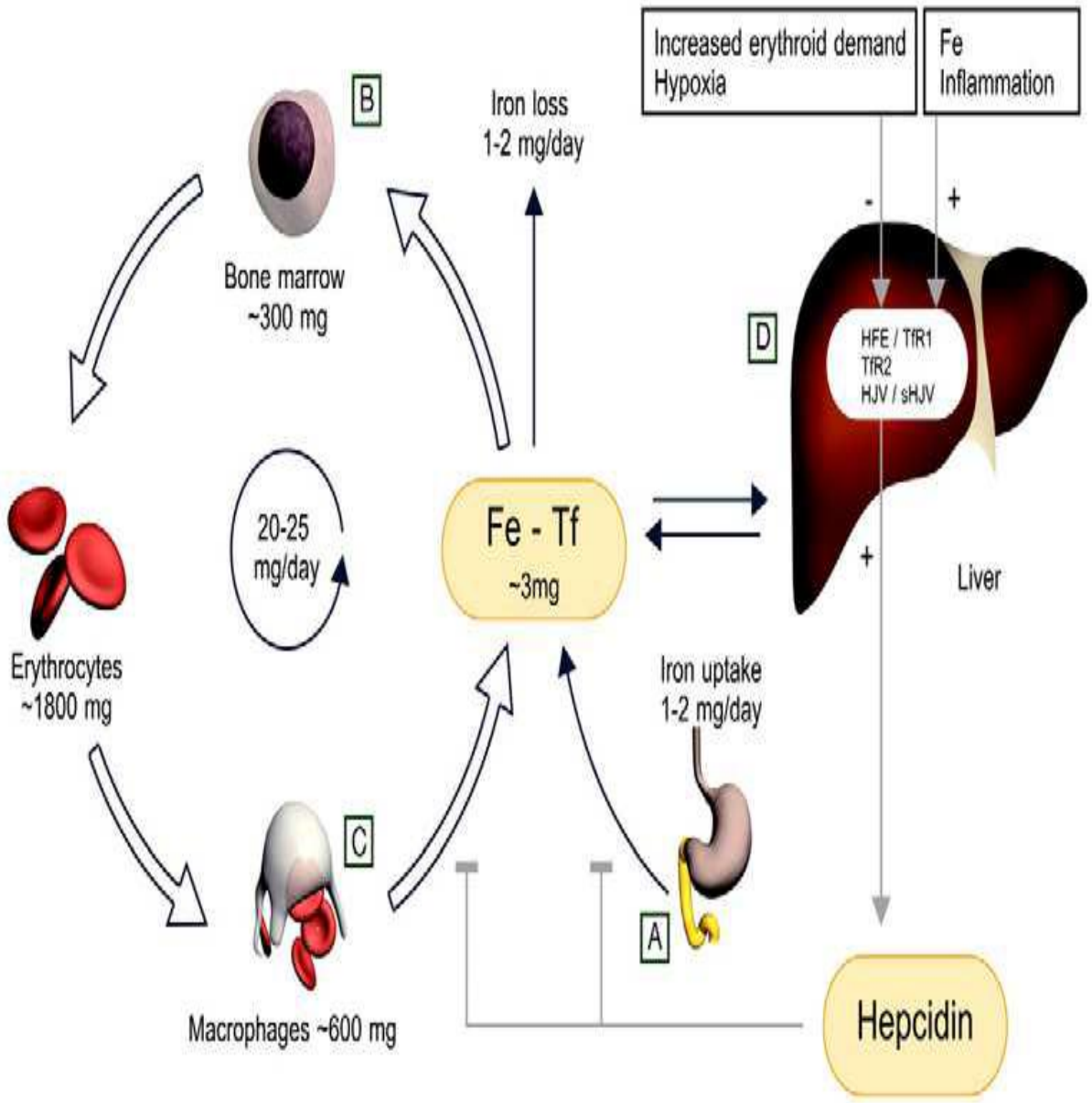

Figure 2.2 Iron metabolism (Swinkels *et al*, 2006; Eloisa *et al*, 2012).

2.5.1 Importance of Iron

Iron regulation is critical to red blood cell production. Absorption of iron is regulated by 3 primary mechanisms: a dietary regulator that responds to iron that is consumed a storage regulator that responds to the total amount of iron in the body and an erythropoietic regulator that responds to the requirements of red blood cell (RBC) production (Andrew,1999).

More than two-thirds of the body's iron is incorporated into haemoglobin (Hgb) for use in mature and developing RBCs (Andrew, 1999).

Most of the remaining iron is stored in reticuloendothelial cells of the liver, spleen, and lymph nodes. Transferrin in blood plasma transports iron between storage pools and erythroid precursors, with minimal daily losses being replaced through absorption of iron from the diet (Andrew,1999).

2.6 ANAEMIA IN CKD

Anaemia, a common observation in CKD, can develop in the early phases of the disease. It isassociated with poor outcomes and contributes to a reduced quality of life, with symptoms includingdyspnoea, headache, light-headedness, and fatigue. Anaemia in patients with CKDis due to many factors. The most well-known cause is inadequate production oferythropoietin. As renal failure progresses, the contribution of erythropoietin deficiency toanaemia increases (Lankhorst and Wish, 2010).Other causes of impaired erythropoiesiswhich contribute to anaemia include; diversionof iron traffic, diminished erythropoiesis, blunted response to erythropoietin,erythrophagocytosis, reduced proliferative activity of erythroid precursors in bone marrow,reduced survival of red cells, the decreased iron availability lead to impaired erythropoiesis(Weiss, 2009).

Absolute iron deficiency is defined as decreased total body iron content. Iron deficiencyanaemia (IDA) occurs when iron deficiency is sufficiently severe to diminish erythropoiesisand cause the development of anaemia. Functional iron deficiency describes a state where thetotal iron content of the body is normal or even elevated, but the iron is “locked away” andunavailable for the production of red blood cells. This condition is observed mainly inpatients with chronic renal failure who are on haemodialysis.

Functional iron deficiency is defined as an imbalance between the iron needs forerythropoiesis and the iron supply, with the latternot maintained at sufficient rate foradequate haemoglobinization of reticulocytes and mature erythrocytes (Cavil and Macdougall, (1993).

In iron deficiency anaemia (IDA), iron supply depends on the quantity of iron storage in thebody, while in functional iron deficiency (iron restricted erythropoiesis) supply depends on

the rate of mobilization of iron from the stores. The diagnosis of iron deficiency or functionaliron deficiency is particularly challenging in patients with acute or chronic inflammatoryconditions because most of the biochemical markers for iron metabolism are affected byacute phase reaction. This is the case of the anaemia of chronic disease (ACD) and the anaemia associated with chronic renal disease (CKD)(Cavil and Macdougall, 1993).Recombinant human erythropoietin (rHuEpo) has been available for treatment of renal anaemia since 1989 (Eschbach*et al*, 1989). However, rHuEpo therapy results infunctional iron deficiency due to insufficient iron stores for the accelerated erythropoiesis.

Iron deficiency is the main cause of suboptimal response to erythropoietin in dialysis patients. Maintenance of iron supplementation is required to successfully treat anaemia. Long term orally administered iron therapy is limited by noncompliance, gastrointestinal sideeffects, insufficient absorption and drug interaction; intravenous iron compounds are usedto treat dialysis patients who become iron deficient.

Monitoring erythropoietin treated patients' iron status is important to detect iron deficiencyand avoid the adverse effects of iron medication (Sunder-Plassmann *et al*, 1997;Kletzmayr *et al*, 2002; Zager*et al*, 2002).

Iron deficiency anaemia (IDA) is a condition frequently diagnosed in patients with chronic kidney disease (CKD)—one that has proven to be much more prevalent than previously thought(Mc Farlane *et al*, 2008). Increasing awareness about current issues in the diagnosis and treatment of IDA is important in improving anaemia management practices.

Anaemia is prevalent in both non-dialysis dependent CKD (NDD-CKD) patients and dialysis dependent CKD (DD-CKD) patients across all stages of CKD (Hsu*et al*, 2002).

Iron deficiency is under-recognized and undertreated among NDD-CKD patients with anaemia.Studies have demonstrated that high proportions of NDD-CKD patients with anaemia are iron deficient, indicating that IDA remains significantly under recognized and undertreated (Gotloib *et al*, 2006;NKF 2006;Post *et al* ,2006, Dowling, 2007).

Evidence shows that iron deficiency (transferrin saturation [TSAT] <20% or serum ferritin <100 ng/ml) can occur as early as in CKD Stage 2(Weiss *et al,* 2005; Gotloib *et al*, 2006).

Two major types of iron deficiency anaemia (IDA) may occur in chronic kidney disease (CKD) patients:

1) Absolute iron deficiencyrefers to the depletion of iron stores and the absence of stainable iron in the bone marrow (Wish 2006; Fishbane2007).Absolute IDA may be considered as serum ferritin <100 ng/ml and transferrin saturation (TSAT) <20 %(NKF 2006; Wish, 2006).Dialysis dependent CKD (DD-CKD) patients are at a greater risk due to increased blood loss (Wish, 2006).It occurs when there is insufficient iron available for haemoglobin production (Fishbane, 2007).

2) Functional iron deficiency is a clinical condition where stored iron is sufficient but circulating iron is deficient. It can occur when erythropoiesis stimulating agent (ESA) therapy stimulates red blood cell (RBC) production beyond the available supply of iron necessary for haemoglobin synthesis; it can also be caused by chronic inflammation(Wish, 2006).

Elevated serum ferritin and TSAT <20% may indicate functional iron deficiency (Fishbane,2007).

2.7IRON STATUS IN CKD

Assessing iron status is integral to both iron and anaemia managements in CKD patients. Bone marrow iron stores are often regarded as the best indicator of iron status,although this is not universally accepted. (National Clinical Guidelines Centre, 2011) However, taking a bone marrow sample is invasive and involves risks of infection or bleeding at the biopsy site (Bain, 2005). Other classical iron status tests, of which ferritin and transferrin saturation (TSAT) are the most widely used, reflect either the level of iron in tissue stores or the adequacy of iron for erythropoiesis. Serum ferritin reflects storage iron—iron that is stored in liver, spleen, and bone marrow reticuloendothelial cells. The TSAT percentage value reflects iron that is readily available for erythropoiesis. Guidelines on monitoring iron status stipulate that haemodialysis (HD) patients receiving erythropoietin should have their iron status monitored every 3 months, and maintain a transferrin saturation (TSAT) >20 percent and a serum ferritin level >100 ng/mL (>200 ng/mL for CKD patients on haemodialysis) (NKF-KDOQI 2000; KDOQI 2006). The National Kidney Foundation guidelines have been widely adopted in dialysis centres across the United States. Though widely used, classical laboratory biomarkers of iron status are not without drawbacks when used in CKD patients: CKD is a pro-inflammatory state, and the biological variability of serum iron, transferrin saturation, and ferritin is known to be large in the context of underlying inflammation (Wish, 2006).

2.7.1Assessment of Iron Status

2.7.1.1Serum Iron

The serum iron concentration is likely to be a poor index of total body iron content, as only a very small amount is found in this compartment.But in combination with total iron binding

capacity (TIBC), which reflects the amount of unsaturated iron and the serum iron concentration, it provides the current iron supply to the tissues. This is reflected in the transferrin saturation, which provides the current iron supply to the tissues. Transferrin saturation is expressed as serum iron as a percentage of TIBC i.e Fe/TIBC x 100.The level of serum iron is affected by both physiological(e.g. diurnal variation, sex, age and pregnancy) and pathological(e.g. acute illness, haemolytic anaemia, acute liver disease) conditions. Diagnosing iron deficiency in CKD is rendered difficult in the presence of uraemia. The inflammatory state complicates the issue (Wish, 2006).

2.7.1.2Serum Ferritin

The usefulness of serum ferritin as an iron marker for the management of anaemia in chronic kidney disease (CKD) is currently the subject of debate. When evaluating iron status tests, consider the following:Serum ferritin is an acute-phase reactant (Kalantar –Zadeh *et al*, 2004)

A low serum ferritin level (<200 ng/mL in dialysis dependent CKD [DD-CKD] patients or <100 ng/mL in non-dialysis dependent CKD [NDD-CKD] patients) can be a reliable indicator of iron deficiency (Kalantar –Zadeh *et al*, 2006)

Reduced levels of serum ferritin or transferrin saturation (TSAT) are present in most patients with CKD. A normal to moderately high serum ferritin does not rule out iron deficiency or indicate adequate or excessive iron levels (Kalantar –Zadeh *et al*, 2006)

Recent updates to the National Comprehensive Cancer Network® (NCCN®) Guidelines recognize the potential for iron deficient patients with CKD to present with elevated serum ferritin levels.

2.7.1.4Serum Total Iron Binding Capacity (TIBC)

The measurement of the blood's capacity to bind iron with transferrin is the iron binding capacity. TIBC is an indirect method assessment of transferrin levels. The unsaturated iron binding capacity is the residual after substraction of serum iron from TIBC.

Transferrin is a glycoprotein synthesised in the liver and is responsible for the transportation of iron (Fe^{3+}) in serum. This glycoprotein has 2 iron binding domains and is normally 30% saturated with iron. In iron deficiency, reduced transferrin saturation leads to iron deprivation for erythroblasts. In iron overload raised transferrin saturation leads to parenchymal iron overload(Wish, 2006).

In iron deficiency anaemia the serum iron level falls. As a result the liver is stimulated to synthesize more transferrin and the transferrin saturation falls (usually <15%). Serum iron concentration is a measurement of circulating iron (Fe^{3+}) bound to transferrin. Only 0.1% of total body iron is bound to transferrin at any one time. In addition, the transferrin iron pool turns over 10-20 times each day which is reflected in varying serum iron levels. Serum iron levels can fluctuate throughout the day so that measurement of serum iron concentration alone provides little useful clinical information(Wish, 2006).

2.8 TREATMENT OF IRON DEFICIENCY ANAEMIA

The 2006 and 2007 updates to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines for anaemia of CKD recommend that HD patients on ESA therapy receive IV iron supplementation to maintain a target Hgb of 11 g/dL to 12 g/dL (not to exceed 13 g/dL). The guidelines also recommend that non–dialysis-dependent and peritoneal dialysis–dependent patientsreceive iron either orally or IV.

Iron repletion should begin before initiation of ESA therapy. Iron levels should initially be monitored monthly until iron stores are replenished, then monitored quarterly. Smaller maintenance doses may be necessary after repletion, because Hgb optimization can occur inpatients whose ferritin is >500 ng/mL. Overall clinical status, degree of ESA responsiveness, and other causes of increased ferritin (infection, inflammation) should be considered. Data are lacking regarding cessation of IV iron therapy. An increase in Hgb >1 g/dL over 1 month indicates a therapeutic response to iron therapy when IDA diagnosis in complex situations is unclear. Monitoring of iron levels also is important to avoid iron overload or toxicity and to prevent haemochromatosis from developing.(KDOQI: NKF, 2006).

Despite its benefits, ESA therapy is associated with an increased risk of adverse drug events (ADEs) such as cardiovascular (CV) complications, hypertension, RBC aplasia, and development of antierythropoietin antibodies. IV iron as an adjunct to ESA therapy has become standard treatment for optimizing Hgb status and reducing ESA dosing requirements. Up to a 70% reduction in required ESA dosage has been noted in patients receiving IV iron. (Coyne, 2008; Coyne,2010). However, iron underutilization has been reported in anaemic CKD patients receiving ESA therapy (Horl, 2007).

HD-dependent patients, who often have limited absorption of oral iron, IV supplementation is preferred. (Besarab and Coyne 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1BACKGROUND OF STUDY AREA

Zaria is the reference location for this study.It has a cosmopolitan mix, comprising most of the ethnic groups in Nigeria. This study was carried out in ABUTH, Zaria which is located on the high plains of Northern Nigeria, 652.6 metres above sea level. It's a metropolitan city that is internationally known as a centre of learning.

3.2STUDY POPULATION (SUBJECTS)

For the purpose of this study, 125 consecutive adult patients in various stages of chronic kidney disease (CKD) who presented at the nephrology clinic of ABUTH, Shika- Zaria were enrolled into the study. Another (125) apparently healthy subjects, matched for sex and age were selected from University population and neighbouring villages to serve as controls.

3.2.1 Inclusion Criteria for Patients

i) Age 18 years and above

ii) Confirmed CKD patients

iii) Patients who consented to participate in the study

iv)Apparently healthy individuals as controls who have consented to participate in the study

v) Lack of verifiable evidence of infection/inflammation either by history or physical examination.

3.2.2 Exclusion Criteria

i) Those with clinical evidence of infection

ii) Those that had blood transfusion

iii) Those that are pregnant among patients and controls

iv) Patients who have not consented

v) Severely moribund patients.

vi) Those with clinical evidence of liver disease.

3.2.3 Consent

Prior to enrolment into the study, the aims, objectives, procedures, risks and benefits of the research was explained to each individual patient and control subjects in their best understood language. Concerns and clarifications which arose were addressed after which they were freely allowed to make their decisions to opt in or opt out of the study, with assurances that the outcome of such decisions will not prejudice against their relationship with the institution. Consents obtained were individually signed by each patient and control subject (see appendix I), and witnessed.

3.2.4 Sample Size Determination

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Oyejide, 1992; Singha, 1996).

Estimated sample size was calculated using the formula below:

$n=z^2pq/d^2$

n=sample size, z=confidence interval (1.96), d=precision (0.05), p=prevalence in target populationIle Ife (8%) (Akinsola *et al*), q=1-p

$n= (1.96)^2\ 0.080 \times 0.920/ (0.05)^2 =113.1$

Minimum number of chronic kidney disease patients to be recruited in this study =115, but because of attrition rate (10%) we recruited one hundred and twenty five (125) chronic kidney disease patients and another 125 apparently healthy age matched controls.

3.2.5 Ethical Approval

This was sought and obtained from Ethical and Scientific Research Committee of ABUTH Zaria before embarking on the study.

3.3SAMPLING TECHNIQUES

A questionnaire attached to this dissertation was administered to all participants. This contained biodata including family history of hypertension, diabetes mellitus, renal diseaseandclinical examination for blood pressure, weight and height.

The weight of each patient was recorded to the nearest 0.5kg using Harsons standard bathroom (standing) weighing scale with subjects wearing light clothing. Blood pressure was taken on the dominant arm of the patient using Accusons mercury sphygmomanometer with an appropriate standard cuff of 12-13cm by 55cm size. The following biochemical investigation was performed on all study subjects viz: iron, ferritin,iron binding globulin(transferrin), Transferrin saturation (TSAT) was calculated by dividing serum iron by transferrin and multiplying by 100. Serum

creatinine was measured and estimated creatinine clearance (ecrcl) was calculated by the use of prediction equation using Cockcroft and Gault formula(Cockcroft and Gault, 1976).

ecrcl= 140- age (yr) x wt (kg) x 1.2/cr(µmol/l) for male

= 140- age (yr) x wt (kg) x 1.2 x 0.85/cr (µmol/l) for female

3.4 SPECIMEN COLLECTION AND PROCESSING

Blood specimen 5mls was collected from a peripheral vein (antecubital venopuncture). In this case, the antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was applied a few centimeters above the antecubital fossa to distend veins. Blood was taken using a sterilized 5ml syringe and 21 G needle. The blood sample was transferred into a plain bottle and allowed to stand for about 30 minutes to clot and then centrifuged at 4000rpm for 10 min. The serum was separated and transfered into a Bijou (sample) bottle. Specimen that would not be assayed within 24hours due to logistic problems was frozen at -20^{o}C until time for analysis.

3.5 CHEMICALS

The chemicals and kits used for the measurement of serum iron, transferrin (TIBC) and creatinine were procured commercially from labkit Barcelona, Spain and ferritin was procured from Perfermed diagnostic system San francisco USA. All chemicals and kits were of analytical grade.

3.6 EQUIPMENT

Hettich universal 32 centrifuge (Germany) were used to spin the blood specimens. Beckman coulter Du-20 UV/visit spectrophotometer (Germany) was used for the measurement of

absorbance of the Iron, TIBC andbiorad pr -5100 Vamed engineering Nig limited L10006-ZR-002 microplate reader was used for ferritin.

3.7 ANALYTICAL METHODS

3.7.1 Measurement of Serum Iron (Stookey, 1970)

3.7.1.1Principle

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions with ferrozine give a coloured complex. The intensity of the colour, measured at 562nm, is directly proportional to the iron concentration in the sample.

3.7.1.2 Procedure

Workingreagent1000µl was pipetted into 4 test tubes labelled working reagent(WR) blank, standard, sample blank and test.One drop of colour reagent was added into WR blank, test and standard. Distilled water 200µl was added into WR,200µl of standard into standard tube.Sample 200µl was added into test and test blank. It was mixed and incubated for 10min at room temp. Absorbance of standard and sample was read against working reagent blank.

3.7.1.3 Calculation

$$\text{Iron (µg/dl)} = \frac{\text{(A) sample} - \text{(A) sample Blank}}{\text{(A) standard}} \times 100(\text{standard conc.})$$

A = Absorbance

3.7.2 Measurement of Serum Total Iron Binding capacity (TIBC) (Wilson and Lahey 1963)

3.7.2.1 Principle

Serum transferrin is saturated with an excess of Fe^{3+} and the unbound portion is precipitated with magnesium carbonate. The total amount of iron isthen determined. The difference between the total iron binding capacity (TIBC) and initial serum iron yields the unsaturated iron-binding capacity.

3.7.2.2 Procedure

Sample 0.5ml, 1ml of iron solution was mixed well and incubated for 10 min at room temperature. Three spoonful of magnesium carbonate was added and mixedwell. This was incubated for 10 min at room temp. Centrifuged for 15min at 3000rpm. The supernatant was collected and iron concentration was measured.viz 1000ul of working reagent was added into 4 test tubes labelled working reagent (WR) blank, standard, and sample blank and test. 1 drop of colour reagent was added into WR blank, test and standard. 200ul of distilled water was added into WR, 200ul of standard into standard tube. 200ul of sample into test and test blank. Mixed and incubated for 10 min at room temp.Absorbance of standard and sample against working reagent blank was read at 562nm.

3.7.2.3 Calculation

$$\text{Iron } (\mu g/dl) = \frac{(A)\ \text{sample} - (A)\ \text{sample Blank}}{(A)\ \text{standard}} \times 100$$

A = Absorbance 100 = standard conc.

TIBC = iron concentration in the supernatant x 3 (dilution factor)

3.7.3 Measurement of Serum Ferritin (Perlmann and Engvall , 1971)

3.7.3.1 Principle

The assay system utilizes one anti-ferritin antibody for solid phase enzyme (microtiter wells) immobilization and another mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. The colour change to yellow is measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the colour intensity of the test sample.

3.7.3.2 Procedure

Twenty 20µl of standard, specimens, and controls was dispensed into appropriate wells. 100µl of Enzyme Conjugate Reagent was added into each well. Thoroughly mix for 30 seconds. It is very important to have completed mixing in this setup. Incubate at room temperature (18-22 ^{0}C) for 60 minutes.Incubation mixture was removed by flicking plate content into a waste container. The microtiter wells were rinsed and flicked 5 times with washing buffer (1X). The wells were striked sharply onto absorbent paper or paper towels to remove all residual water droplets. 100µl of TMB solution was dispensed into each well and gently mixed for 5 seconds. This was incubated at room temperature in the dark for 20 minute. The reaction was stopped by adding 100µl of Stop Solution to each well. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour completely.Optical density was read at 450nm with a microtiter reader within 15 minute.

3.7.3.3 Calculation

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples a standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis.The mean absorbance values for each specimen was used to determine the corresponding concentration of Ferritin in ng/ml from the standard curve.

3.7.4 Creatinine (Max Jaffe1886)

3.7.4.1 Principle

Creatinine in alkaline medium reacts with picric acid (jaffes reaction), to give a red tautomer of creatinine picrate the intensity of which is measured spectrophotometrically at 520nm.

3.7.4.2 Procedure

Into a centrifuge tube the following was added 1ml serum,3ml distilled water,1 ml 10% sodium tungstate, 1 ml 2/3 sulphuric acid,centrifuged for 10 min and supernatant collected. 3mls supernatant, 3mls standard 3mls distilled water was added to a test, standard and blank test tubes respectively. An addition of 1ml each of picric acid and 0.75N sodium hydroxide followed to each test tube, with incubation for 15min at room temperature. The reading was at 520nm.

3.7.4.3 Calculation

Creatinine (mmol/L) = A test/A std x Cstd.

Where A= absorbance, C= concentration, std= standard.

3.8QUALITY CONTROL

Adequate quality control was observed when carrying out the analysis of the samples to sensure that the results obtained are reliable. This was done by analyzing the samples in batches together with quality control sera for specific analytes purchased from labkit for Iron, TIBC. Pooled sera were used for ferritin and creatinine.

3.9 STATISTICAL ANALYSIS

The data were analyzed using Statistical Package for the Social Sciences 17.0 (SPSS 17.0) for windows (SPSS inc. Chicago, 17).Results were expressed as absolute numbers,percentages, means with standard deviations and ranges as appropriate. Serum iron, total iron binding capacity, ferritin, creatinine obtained from the patients were compared with those of the controls using the two tailed student's t-test. Also the correlation of analytes with eGFR was carried out using Pearson's linear correlation analysis. A p-value of equal to or less than 0.05 ($P \leq 0.05$) was considered statistically significant. Categorical data were summarized as frequencies and percentages while continues data were summarized as mean $\pm$ standard deviation. Discrete variables were analyzed using X^2. Students-t-test was used to analyze continues normally distributed variables.

CHAPTER FOUR

4.0 RESULTS

4.1SOCIODEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION

One hundred and twenty five (125) chronic kidney disease patients (CKD)aged 18 to 85 years were recruited for the study with a mean age of 48 years. These were made up of 67 (53.6%) males, and 58 females (46.4%).Their ethnic groups were as follows; 76 (70.4%) of the patients were Hausa, 19 (7.6%) Yoruba, Igbo 3.2% while the minority comprising of Nupe, Tiv etc, were made up of 47(18.8%).Their level of educational attainment showed that 35.6% of them had tertiary education, 33% had secondary education while13.2% had primary education.

Similarly one hundred and twenty five (125) apparently healthy aged and sex matched controls were recruited with a mean age of 48 years and made up of 53.6% males and 46.4% female.These findings are presented in Table 4.1.

4.2STAGES OF CKD IN STUDIED POPULATION

Table 4.2 shows all CKD patients in different stages 8.8% of the CKD patients were in stage 1,1 6% in stage 2, 21.6% in stage 3, 12% in stage 4 and a greater proportion in end stage renal disease 41.6%

4.3CLINICAL AND LABORATORY CHARACTERISTIC OF HEALTHY CONTROLSUBJECTS AND PATIENTS WITH CKD

Table 4.3 shows the mean age, systolic blood pressure, diastolic blood pressure, and body mass index of both controls and patients in CKD.Higher systolic blood pressure as well as diastolic blood pressure and BMI were recorded in patients compared with controls. There was

statistically significantdifference ($p<0.05$) between the mean systolic blood pressure and diastolic blood pressure in both patients and controls.The mean values ofbody mass index in both patients and in controlswere insignificant. The table also showed mean serum levels of creatinine, iron, TIBC and TSAT in patients and controls with mean values of serum creatinine significantly higher in the patients ($p=0.000$). There was no significant difference in the mean values of iron ($p=0.32$) and TIBC ($p=1.29$) in both study groups. The patients had a significantly ($p=0.000$) higher mean value for ferritin than the control group.

4.4AETIOLOGICAL CAUSES OF CKD IN THE STUDIED PATIENTS

The summary of the aetiological causes of CKD is presented in table 4.3. Hypertensive nephropathy is the leading cause of CKD with 45.6%, followed by Diabetic nephropathy and Adult polycystic kidney disease occurring with similar frequency of 16.8% each, while Chronic glomerulonephrities accounted for the fourth cause with a frequency of 12%. Other sundry causes comprising sickle cell nephropathy, etc accounted for 5.6%.

4.5TREATMENT MODALITIES OF CKD PATIENTS

Treatment modality of CKD patients is presented in table 4.4. It showed that majority of the CKD patients, 108 (86.4%) were on conservative treatment; while 16 (12.8%) patients were on haemodialysis and only 1(0.8%) had a kidney transplant.

4.6 SERUM CREATININE, IRON, TIBC, FERRITIN CONCENTRATIONS ANDSTAGES OF CKD

The mean values and SD of serum creatinine, iron, ferritin, TIBC and TSAT were shown in table 4.7. A statistically significant difference was observed between the stages of CKD and

ferritin (p=0.000). These statistically significant difference in mean values of serum ferritin was noted between stage 1 and 4(p=0.019), and stage 1 and 5 (p=0.000). There is also a statistically significant difference between the stage 2 and stage 5 (p=0.000) as well as stage 3 and 4 p= (0.012), and stage 3 and 5 p= (0.000). A statistically significant difference was demonstrated between the stages of CKD and creatinine (p=0.000). This statistical significant difference in the levels of creatinine was noted throughout the five stages of CKD. There was no statistical significance in the serum levels of iron, TIBC and TSAT.

Table 4.1Sociodemographic characteristics of the study population

Characteristics	Healthy Controls (n =125)		CKD Patients(n=125)	
Age categorized (years)	N	%	n	%
Less than or equal 25	11	8.8	12	9.6
26-35	21	16.8	20	16.0
36-45	22	17.6	22	17.6
46-55	32	25.6	32	25.6
56-65	24	19.2	25	20.0
66+	15	12.0	14	11.2
Sex	Ratio M: F (1.15:1).		Ratio M: F (1.15:1).	
Male	67	53.6	67	53.6
Female	58	46.4	58	46.4
Ethnicity				
Hausa	96	76.8	82	65.6
Igbo	1	0.8	6	4.8
Yoruba	7	5.6	13	10.4
Others	21	16.8	24	19.2
Highest level of educationattained				
Primary	9	7.2	24	19.2
Secondary	23	18.4	35	28.0
Tertiary	56	44.8	30	24.0
None	37	29.6	36	28.8

n = frequency

CKD =Chronic Kidney Disease

% = percentage

Table 4.2 Stages of CKD in studied patients

STAGES	N	Percentage
1	11	8.8%
2	20	16%
3	27	21.6%
4	15	12%
5	52	41.6%
Total	125	100%

n = frequency

CKD =Chronic Kidney Disease

% = percentage

Table 4.3 Clinical and Laboratory characteristics of patients with CKD and healthy control subjects

Characteristics	Controls (n=125) mean (SD)	Patients (n=125) Mean(SD)	P values
Age (years)(range)	48(18-85)	48(18-85)	
Systolic BP (mmHg)	126.12(18.71)	147.45(28.21)	0.00
Diastolic BP (mmHg)	78.64(10.63)	92.61(18.12)	0.00
BMI (kg/m^2)	24.81(6.09)	26.39(6.49)	0.48
Creatinine (umol/l)	81.47(27.43)	623.88(681.54)	0.00
eCrCl($ml/min/1.73m^2$)	93.93(42.43)	36.28(35.44)	0.00
Serum iron (umol/l)	17.59(8.91)	20.21(10.23)	0.32
Serum TIBC (umol/l)	66.22(23.92)	71.27(28.23)	1.29
serum ferritin (ng/ml)	93.25(58.50)	210.85(138.28)	0.00
TSAT (%)	28.88(15.58)	31.90(20.41)	0.00

n = frequency

CKD =Chronic Kidney Disease

BMI=Body Mass Index

TSAT=Transferrin Saturation

TIBC= Total Iron Binding Capacity

eCrCl= estimated creatinine clearance

Table 4.4 Aetiological Causes of CKD in the studied Patients at ABUTH, Zaria

Aetiological factors	n	%
Hypertensive nephropathy	57	45.6
Diabetic nephropathy	21	16.8
Adult polycystic kidney disease (APKD)	21	16.8
Obstructive uropathy	04	3.2
Chronic glomerulonephritis	15	12
Others	07	5.6
Total	125	100

CKD =Chronic Kidney Disease

n =frequency

% = percentage

APKD= Adult Polycystic Kidney Disease

ABUTH= Ahmadu bello university teaching hospital

Table 4.5 Treatment modalities in CKD patients studied at ABUTH, Zaria

Treatment modalities	n	%
Conservative	108	86.4
Haemodialysis	16	12.8
Kidney transplant	1	0.8
Total	125	100

n = frequency

CKD =Chronic Kidney Disease

% = percentage

Table 4.6Serum values of the studied analytes in different stages of CKD (mean±SD)

	n	Creatinine (µmol/l)	Iron (µmol/l)	TIBC (µmol/l)	Ferritin (ng/ml)	TSAT (%)
Stage 1	11	70.64±21.08	17.53±8.30	86.21±31.99	106.27±94.61	22.35±11.48
Stage2	20	109.95±32.62	24.52±9.32	75.63±41.59	146.70±106.07	38.86±21.39
Stage3	27	141.07±39.28	16.87±6.20	70.76±19.68	127.52±91.47	26.07±13.61
Stage4	15	316.53±155.33	20.60±10.64	64.80±19.09	250.67±145.77	36.22±26.40
Stage5	52	1277.92±603.78	20.74±11.95	68.55±26.82	289.42±125.63	33.02±21.62
p-value		0.00	0.116	0.307	0.000	0.098
posthoc		*0.00			*0.019	
		**0.00			**0.00	
		***0.00			***0.00	
		****0.00			****0.012	
		*****0.00			*****0.00	

KEY	KEY
*stages 1	*stage 1&4
**stages2	**stage 1&5
***stages3	***stage2&5
****stages4	****stage 3& 4
*****stages5	*****stage3&5

n=frequency CKD= chronic kidney disease TIBC= total iron binding capacity

Tsat transferrin saturation %= percentage

4.7SERUM LEVELS OF IRON, CREATININE, TIBC, TSAT, FERRITIN AND ECRCL IN MALES AND FEMALES CKD PATIENTS.

There were higher serum creatinine and ferritin values in males than in females while higher serumTIBC, estimated creatinine clearance and iron were observed in females than males. These differences between male and female CKD patients in serum creatinine, ferritin and estimated creatinine clearance were found to be statistical significant with p value of 0.002, 0.000 and 0.028 respectively. No statistical significant differences were noted in serum levels of iron, TIBC and TSAT.

4.8RELATIONSHIP BETWEEN THE DIFFERENT MEASURED ANALYTES IN THIS STUDY

There was a statistically significant positive correlation between the level of serum creatinine and serum ferritin in the patients with CKD (r=0.503 and p=0.000). No statistical significance was noted between serum creatinine and serum iron, TSAT and TIBC.

4.9CORRELATIONS BETWEEN INDICES OF IRON STATUS IN CKD PATIENTS

There was a positive statistical significance correlation between iron and TSAT (r=0.736, p=0.000). However, a negative statistical significance correlation between TIBC and TSAT (r=-0.442, p=0.000). There was also a positivestatistical significance correlation between TIBC and eCrCl(r= 0.236, p= 0.008). There was also a negative statistical significance correlation between ferritin with eCrCl (r=-0.494, p=0.000).

4.10REFERENCE VALUES OF TIBC, IRON, FERRITIN AND % TSAT

Reference values for all analytes generated from the 125 apparently healthy controls were shown in table 4.10 using 2.5% - 97.5% percentiles of the analytes.

Table 4.7 Serum levels of iron, creatinine, TIBC, TSAT, ferritin and eCrCl in males and females CKD patients.

Analytes	Male	Female	p- value
Creatinine(µmol/l)	793.8±754	427.5±527	0.002
TIBC(µmol/l)	67.39±30.81	75.74±24.41	0.094
Iron(µmol/l)	20.14±10.27	20.28±10.27	0.0941
Tsat(%)	33.44±19.30	30.11±21.64	0.370
Ferritin(ng/ml)	250.68±134.58	164.82±128.85	0.000
eCrcl($1.73m^2$/ml/min)	29.80±30.97	43.75±38.93	0.028

Statistically significant ($p< 0.05$)

4.8Correlation of serum creatinine and serum indices of iron status in patients with CKD

Parameter	Indices of iron status	Correlation	significance
		rp	
Creatinine	Iron	0.060	Ns
	TSAT	0.008	Ns
	Ferritin	0.503	0.000
	TIBC	-0.035	Ns

r= correlation coefficient

p= degree of significance

Ns=not statistically significant

TSAT=transferrin saturation

TIBC=total iron binding capacity

CKD=chronic kidney disease

Table 4.9Correlation between the different indices of iron status in CKD patients

Variables	r-value	p-value
TSAT/iron	0.736	0.000
Ferritin/ creatinine	0.503	0.000
Ferritin/eCrCl	-0.494	0.000
TIBC/TSAT	-0.442	0.000
TIBC/eCrcl	0.236	0.008

r= correlation coefficient

p= degree of significance

TSAT=Transferrin Saturation

TIBC=Total Iron Binding Capacity

eCrCl= estimated creatinine clearance

CKD=chronic kidney disease

Table 4.10 Reference intervals of analytes in healthy control subjects

Analytes	2.5%	97.5%	Reference interval (2.5%-97.5%)
Iron (µmol/l)	6.8	40	6.8-40
TIBC (µmol/l)	33	113	33-113
ferritin(ng/ml) male	18	304	18-304
ferritin(ng/ml) female	23.8	232	24-232
TSAT(%)	8.3	71	8.3-71
Creatinine(µmol/l)	35	141	35-141

TSAT=Transferrin Saturation

TIBC=Total Iron Binding Capacity

4.11SERUM LEVELS OF FERRITIN AND TSAT IN THE STAGES OF CKD

Thirty four(27.2%) CKD patients had iron deficiency anaemia based on ferritin levels<100ng/ml, forty patients (32%) had IDA based on TSAT levels<20%, both indicating depletion of iron stores. While(17) 13.6% had IDA based on the two parameters indicating absolute iron deficiency. Functional iron deficiency was seen in 22 patient (17.6%) based on TSAT <20% and ferritin >100ng/ml. In functional IDA more patient were seen in stages 4-5 and less in stages1-3 reverse was the case in absolute iron deficiency anaemia with more in stages 1-3 and less in stages 4-5. See table 4.11

4.12IRON STATUS IN CKD PATIENTS

Normal iron status was seen in 68(54.4%) CKD patients based on TSAT >20% and ferritin >100ng/ml.as per KDOQI guidelines. Absolute iron deficiency was seen in 13.6% Functional iron deficiency was seen in 17.6%. None had iron overload (Table 4.12).

Table 4.11 Serum levels of ferritin and TSAT in thestages of CKD patients

Stages	Ferritin<100ng/ml	Ferritin>100ng/ml TSAT<20%	TSAT<20%	TSAT< 20%, Ferritin <100ng/ml	TSAT>20% Ferritin >100ng/ml
1	7	0	6	5	3
2	8	2	4	2	10
3	12	4	12	8	11
4	2	3	4	1	10
5	5	13	14	1	34
Total	34 (27.2%)	22(17.6%)	40(32%)	17(13.6%)	68(54.4%)

TSAT=Transferrin Saturation

CKD=chronic kidney disease

% = Percentage

Table 4.12 Iron status among CKD patients

Iron status	Patients n=125
Normal iron status	68(54.4%)
Absolute iron deficiency	17(13.6%)
Functional iron deficiency	22(17.6%)
Iron overload	0(0%)

CKD = Chronic Kidney Disease

% = percentage

CHAPTER FIVE

5.1 DISCUSSION

This study report a predominance of male (53.6%) with CKD, over female patients (46.4%) which is similar to the works of Alebiosu *et al,*2006 in Sagamu. This finding may not be surprising as observations in animal models and several studies in human populations have found that males generally predominate in CKD Arogundade *et al,* 2011). Health seeking behaviour due to economic factors which favour males in our communities could also play a role. The mean age of the study population was found to be 48 years which compares with the work of Chinwuba *et al,* in Enugu (2010) of 45 years in a study on prevalence of anaemia in predialysis patients and that of Ketut *et al,* (2005) in Indonesia who found the mean age to be 47 years. However, it is in contrast to the works of Alebiosu in Sagamu which showed a lower mean age of 39 years. Majority of the patients in our study presented in late stages of CKD, with over 40% in ESRD requiring modalities of kidney replacement treatment. Such trend was observed in the studies conducted in South Western Nigeria by Akinsola *et al,* in Ile-Ife (2004) and Alebiosu *et al,* in Sagamu (2006) who also indicated in their series that majority of patients were in advanced stages of CKD.

The mean values of both systolic and diastolic blood pressures were higher in study patients as compared to that of the controls. The same trend was earlier documented in the work of Chijioke *et al,* (2011) in Ilorin involving 120 CKD patients with similar age-matched controls. This may not be unexpected as hypertension was the leading cause of CKD in this study and is in accord with reports from other studies. The presence of hypertension in these patients could accelerate the progression of CKD. Hypertension leads to high intraglomerular pressure, impairing

glomerular filtration and the damaged glomeruli leads to increase in protein filtration. Microalbuminuria often is the first sign of CKD.

Body mass index, as a marker of obesity, was not a discriminating factor between CKD patients and controls, despite the numerous publications by Chijioke *et al,* (2011) in Ilorin, Mshelia *et al,* (2002) in Maiduguri and Ikuo *et al,* (2009) in Japan which showed that weight increase is a risk factor of CKD. It is plausible that the similarity in BMI between CKD patients and controls may relate to high proportion of patients in ESRD whose uraemic state impedes weight gain and the fluid retention that forms part of the spectrum.Therefore it is tempting to speculate that if dry weight is measured most of the observed increase in BMI in the CKD patients may be more apparent than real.

Mean serum creatinine and estimated creatinine clearance, found in patients with CKD in this study was similar to the work Chijioke *et al,* in Ilorin (2011) which was also estimated by the validated Cockroft and Gault formula . This high mean creatinine levels may reflect the late presentation of majority of CKD patients in the study. This was also reported by Chinwuba *et al,* in Enugu in 2010 while assessing the enormity of CKD. It is pertinent to note that many of the patients with advanced CKD were managed conservatively because they could not afford the cost of renal replacement therapy (RRT).It is instructive to note that out of the 52 patients in ESRD only 16 received some form of haemodialysis treatment. This trend is similar to findings in other studies from other developing world showing that most cases of CKD present in advanced stages and cannot afford the cost of renal replacement therapy.

The prognosis of CKD is abysmal; in our centre only 1 patient had a kidney transplant(0.8%). Similar observation was made by Chinwuba*et al,* in Enugu (2010) who found that only 6 patient out of 1538 ESRD patients had kidney transplantation as a treatment option.

The mean serum Iron, TSAT, ferritin and TIBC in the patients found in this study was similar to the work of Gangadhar *et al,* (2010) in India on the predictive value of iron store markers in anaemia of CKDin 207 CKD patients. Except for TIBC which was higher in our study.The serum iron levels were higher in patients than in controls. The difference was not statistically significant. This may be because CKD patients were on iron supplement as part of their treatment protocol as recommended by KDOQI guidelines. However, routine investigation and monitoring of iron indices are not easily done in ABUTH.TSAT is an indicator of circulating iron and the positive association with serum iron found in this study reflects the availability of sufficient iron in the form of iron bound with transferrin.

Higher serum iron, TIBC, ferritin, and TSAT were noted in the patients than controls. The difference was statistically significant in TSAT and ferritin levels.

In a study of 102 patients in stage 5 CKD by Gupta *et al,* (2009) in India the mean values of serum ferritin, iron, TIBC and TSAT showed statistically significant difference between cases and controls. They recorded higher serum iron, ferritin and TSAT in the cases than controls.

Serum levels of ferritin was similar to the findings of Chijioke *et al,* in Ilorin (2011) who reported ferritin level of 194ng/ml and also closer to the findings of Gotlib *et al,*(2006) of 235 ng/ml in United States in a study on IDA in CKD patients.

The correlation between the stages of CKD and serum creatinine was statistically significant at all the stages of CKD. This is because of the increase in creatinine as the stage of CKD progresses being a continuum.

Ferritin level was found to increase with worsening of renal function rising from106ng/ml in stage1 through 285ng/ml in stage5 this finding is similar to the works of Chijioke *et al*,(2011) in Ilorin while assessing the correlation between serum ferritin and C - reactive protein in dialysis naïve CKD patients in Ilorin. Hence the positive correlation with creatinine and the negative correlation with eCrCl. There was significant gender disparity in the ferritin levels and in creatinine. The former is due to hormonal influence in females while the latter is because creatinine is mainly derived from the metabolism of creatine in muscle and its generation is proportional to muscle mass which is more in males.

The leading cause of CKD was hypertensive nephropathy, similar to the works of Kadiri *et al*,(1999) in Ibadan and Chinwuba *et al,* (2010) in Enugu. This was followed by diabetic nephropathy and adult polycystic kidney disease (APKD) occurring with similar frequency. Many of the patients with diabetic nephropathy in the population studied were managed at the diabetic clinics and were only referred to nephrology clinic where this study took place when they developed overtly impaired kidney function. It is therefore plausible that this seemingly low frequency of diabetic nephropathy may merely reflect a tip of the iceberg of the true prevalence of diabetic kidney disease in this population. The high frequency of APKD is an intriguing finding in this study, as most Nigerian studies that emanated predominantly from southern part of the country have not confirmed this observation. In fact, Chijioke *et al,* (2011) in Ilorin did not find APKD in more than 2.5% in a prospective study on Pattern of Lipid Profile in Dialysis

Naive Chronic Kidney Disease Patients. However, the same author, in a retrospective study on the prevalence of APKD in Ilorin (2010) found that 67(15.4%) out of a large cohort of 436 renal disease patients had APKD, which compares favourably with our finding. This high prevalence of APKD may point to the demographic peculiarities of the patients, who were predominantly Hausa (57.1%) whose cultural practices include high rate of consanguineous marriages. Other studies including a recent one that utilized molecular analysis on APKD conducted in an Iranian family by Vasifehman *et al,* (2013) have indicated the role of consanguinity in the aetiology of APKD. However, more study will be needed to determine the significance of this relationship.

The reference intervals of serum iron, TIBC, ferritin have been established using the 2.5 and the 97.5 percentiles the analytes being non parametric data.

The National Kidney Foundation (NKF-K/DOQI) practice guidelines recommend maintaining serum ferritin >100ng/ml and transferrin saturation TSAT >20% to ensure adequate iron supply for erythropoiesis among CKD patients whether they are dialysis dependent or not.

Analysis of the data in the current study revealed 54.4% of 125 studied CKD patients met these K/DOQI targets of adequate iron supply. While13.6% of CKD patients were iron deficient, as indicated by serum ferritin <100ng/ml and transferrin saturation (TSAT) <20%. Where by 17.6% had functional iron deficiency as indicated by serum ferritin > 100ng/ml and < 20% TSAT. In a dissertation on prevalence of anaemia and its associated factors in patients with chronic kidney disease at Muhimbili National Hospital Dar es Salaam by Abdu (2012) Tanzania reported 43% of CKD patient met K/DOQI targets of adequate iron supply. While 17% were iron deficient and 37% had functional iron deficiency. This is similar to the finding in this study.

However in contrast to the findings in this current study, Hsu *et al* (2002) in California reported 62.6% of CKD patients with anaemia were iron deficient, as indicated by serum ferritin <100 ng/ml and transferrin saturation (TSAT) <20%. Where by 25.8% had functional iron deficiency anaemia as indicated serum ferritin > 100ng/ml and < 20% TSAT. This upward trend of high Proportional of CKD patients with iron deficiency done elsewhere could be attributed to the sample size (125) investigated in the current study compared to (15,837) in the study by Hsu *et al,* (2002).

There was also a positive correlation between iron and TSAT which was statistically significant. TSAT is an indicator of circulating iron and the positive correlation with serum iron seen in this present study reflects the availability of sufficient iron in our CKD patients the form of bound iron (with transferrin).

There was, however, a negative correlation between TIBC and TSAT which was also statistically significant. This indicates an inverse relationship between the analytes across the stages of CKD. Implying that as TIBC is increasing TSAT decreases.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The findings in this study include the following

1. There is a high prevalence of iron deficiency anaemia observed in the CKD population.

2. The study has provided the basis for establishing Reference Intervals of Iron, Ferritin, TIBC and %TSAT for Zaria population.

3. A significant proportion of patients with CKD had high blood pressure levels and body mass index suggesting poor control of the CKD population.

4. The leading causes of CKD in order of importance were hypertensive nephropathy, diabetes-associated nephropathy and adult polycystic kidney disease.

5. The mean age of CKD patients was found to be 49 years in ABUTH Zaria

6. Male preponderance was observed in the CKD population

6.2 CONCLUSION

The study supports current KDOQI recommendation of replenishing iron stores in anaemic patients with chronic kidney disease. It is pertinent for clinicians to refer CKD patients as soon as diagnosis is made or suspected to nephrologists for early evaluation, prevention and treatment of CKD.

6.3 RECOMMENDATIONS

1) There is need to achieve the blood pressure target of<130/80 as recommended by KDOQI, ISN guidelines on all CKD patients.

2) Iron indices should be incorporated into the routine investigations of CKD patients in order to manage, treat and monitor iron deficient CKD patients as per KDOQI guidelines.

3) It is important that iron supplementation should only be given based on objective iron indices from the laboratories.

4) Genetic studies will be needed to determine the reason for the high incidence of APKD in this studied population.

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APPENDIX I

Patient's Information sheet

EVALUATION OF IRON STATUS AMONG CHRONIC KIDNEY DISEASE PATIENTS IN ABUTH ZARIA

My name is Dr. Mohammed Amina. Iam a senior registrar from Chemical Pathology Department of ABUTH, Zaria. I am requesting you to voluntarily participate in this study so that you can share with me your views; experiences and possibly thoughts that will help me better access and manage patients with long term kidney disease (CKD). The study will be used for planning purposes which are geared towards improving the health status of Chronic Kidney Disease patients. Blood sample about 5mls will be taken for this purpose. Your opinion is important, so feel free to express what you feel. Your contributions will be kept confidential. This process of discussion and sample collection will last only for about ten minutes or so. The investigation will be at no cost to you. If you are willing to participate, kindly sign the consent form attached.

APPENDIX II

TITLE OF PROJECT: Evaluation of iron status among Chronic Kidney disease patients in ABUTH, Zaria.

I confirm that, the purpose and importance of this study has been explained to me.

I am also aware that there will be a need for blood to be taken from me.

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected.

I understand that there may be a need for some medical intervention in the form of medicine or referral and that in the event of my withdrawing from the study before its completion, i shall not be deprived of or denied of these interventions.

I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information, if I wish.

I agree to take part in the above study.

Name of patient.....

Signature: .....

Date: .....

Witness's Name: ……

Signature.....

Date:..... The contact details of the researcher are: Dr. Mohammed Amina.Department of Chemical Pathology, ABUTH Shika. Zaria. Mobile Phone Number: +2347039648107. Email address: aminamohammed75@yahoo.com

APPENDIX III

QUESTIONNAIRE

Personal Data

Name: _____

Serial No: _____

Hospital No: _____

Age (years) _____ Gender_____

Nationality_____Ethnicity_____

Weight (Kg) _____ Systolic BP (mm Hg) _____Diastolic BP (mm Hg)

Height (m) _____BMI

Address: _____

Education: primary 1 secondary 2 tertiary 3 None 4

Diagnoses:

Duration of disease or condition (years)

Treatment modality (conservative, dialysis, kidney transplant)

Medications

LABORATORY INVESTIGATIONS

IRON (ug/dL	CREATININE (umol/L	TRANSFERRIN (mg/dl)	FERRITIN (ng/ml)	TSAT (mmol/L	eCrcl (ml/min)

BP=blood pressure eCrcl=estimated creatinine clearance TSAT=transferrin saturation.