"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"

By:

DR. TAMEEM IMRAN

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA,

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE

IN

GENERAL MEDICINE

Under the Guidance Of

Dr. B.N.RAGHAVENDRA PRASAD M.B.B.S., M.D.

Professor

DEPARTMENT OF GENERAL MEDICINE

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA,

KOLAR, KARNATAKA.

2019

SRI DEVARAJ URS MEDICAL COLLEGE,
TAMAKA, KOLAR-563101

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled

"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"

is a bonafide and genuine research work carried out by me
under the guidance of
Dr. B.N.RAGHVENDRA PRASAD
Professor
Department of General Medicine,
Sri Devaraj Urs Medical College & Research centre,
Tamaka, Kolar.

Date:
Place: Kolar

Signature of the candidate
Dr. TAMEEM IMRAN

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION,
TAMAKA, KOLAR, KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled

"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"

is a bonafide research work done by **Dr. TAMEEM IMRAN** *under my guidance and supervision in partial fulfillment of the requirement for the Degree of*

M.D. in GENERAL MEDICINE

Signature of the Guide
Dr. B.N.RAGHAVENDRA PRASAD,
Professor,
Department of General medicine,
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place : Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND

RESEARCH CENTRE, TAMAKA, KOLAR, KARNATAKA

ENDORSEMENT BY THE HOD,
PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled

"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"
is a bonafide and genuine research work carried out by

Dr. TAMEEM IMRAN

under the guidance of

Dr. B.N.RAGHAVENDRAPRASAD

Professor,

Department Of General Medicine.

Dr. RAVEESHA.A

Professor & HOD

Department of General Medicine,

Sri Devaraj Urs Medical College,

Tamaka, Kolar.

Date:

Place: Kolar

Dr. M. L. HARENDRA KUMAR

Principal,

Sri Devaraj Urs Medical College,

Tamaka, Kolar.

Date:

Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE, TAMAKA, KOLAR, KARNATAKA

ETHICAL COMMITTEE CERTIFICATE

This is to certify that the Ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved

Dr. TAMEEM IMRAN

Post-Graduate student in the subject of

GENERAL MEDICINE

at Sri Devaraj Urs Medical College, Kolar

to take up the Dissertation work entitled

"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"

to be submitted to

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE, TAMAKA, KOLAR, KARNATAKA.

Signature of Member Secretary,

Date:

Ethical committee,

Place: Kolar

Sri Devaraj Urs Medical College,

Tamaka, Kolar–563101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE, TAMAKA, KOLAR, KARNATAKA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research Centre, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic /research purpose.

Signature of the candidate
Dr. TAMEEM IMRAN
Post graduate student,
Department of General Medicine
Sri Devaraj Urs Medical College,
Kolar.

Date:
Place: Kolar

ACKNOWLEDGEMENT

I am highly indebted to my guide ***Dr. B.N.RAGHAVENDRA PRASAD,*** *Professor, Department of General medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar, who guided me in bringing out this work with his thought provoking ideas and constant encouragement.*

It gives me immense pleasure to express my gratitude and sincere thanks to ***Dr. RAVEESHA,*** *Professor and H.O.D., Department of General Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar, who took deep interest and gave constant support by encouraging in moulding this work.*

I also acknowledge my debt to ***Dr. LAKSHMAIAH.V, Dr. K PRABHAKAR,*** *Department of General medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar, who gave me moral support and guidance by correcting me at every step.*

I express my sincere thanks to all my teachers and Staff of Department of General Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar.

I remain thankful to all my Associate and Assistant Professors and Senior Residents for their constant support and encouragement. I acknowledge my sincere thanks to all my co-P.G's for their help and support at every step throughout my study.

I am very much thankful to my parents PARVEEN BEGUM and FIYAZ AHMED and friends for their love, blessings and invaluable help.

My heartful gratitude to all my patients who submitted themselves most gracefully and whole heartedly participated in this study. I sincerely thank my institute Sri Devaraj Urs Medical College, Tamaka , Kolar for giving me a wonderful foundation and forum of knowledge in the field of General Medicine which stands for the rest of my life. Last, but not the least, I would like to express my gratitude to the Almighty for all His blessing .

Signature of the candidate

Dr. TAMEEM IMRAN

ABSTRACT

"SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE"

I. Introduction

CKD is a global public health problem with a significant morbidity and mortality due to associated complications which patient can develop. One such complication is anemia. Anemia contributes not only to symptoms associated with reduced kidney function but also has a direct adverse cardiovascular effect, which remains the most common cause of mortality among CKD patients. Several studies have also shown that severe anemia may accelerate the progression to ESRD. Among the many causes of anemia in CKD, iron deficiency anemia is one of the easily recognizable and treatable causes. Before starting empirical treatment of anemia with ESA it is very essential that iron status/indices to be determined to avoid inadvertent complications associated with usage of ESA and also to prevent iron overload due to the usage of iron supplements. So the present study is aimed at evaluating the iron indices among CKD patients and to recognize the number of patients having absolute versus functional IDA.

II. Objectives

1. To study iron indices. [Serum iron, Serum Ferritin, transferrin saturation (TSAT), TIBC]
2. To estimate the percentage of iron-deficient patients among chronic kidney disease.
3. To estimate the percentage of patients having functional versus absolute iron deficiency.

III. Material And Methods:

Source Of Data:

The study included 70 CKD patients who presented to RLJ Hospital Kolar attached to SDUAHER during February 2017 - May 2018 who satisfied the inclusion criteria & exclusion criteria.

Sampling Procedure:

At Nephrology division of R.L.Jalappa hospital Consecutive recruitment (total enumerative sampling) of study participants was done.

The following laboratory tests were done: Serum creatinine, Blood urea, CBC, LFT, serum iron indices (Serum iron, Serum Ferritin, transferrin saturation(TSAT)TIBC), USG KUB, Urine routine.

Absolute iron deficiency is diagnosed when S.Ferritin <100ng/ml and TSAT <20%.

Functional iron deficiency is diagnosed when S.Ferritin >100ng/ml and TSAT <20%.

IV. Results

Total percentage of CKD patients who had anemia in our study were 82.8%, of whom 47.1% had iron deficiency anemia. The patients who had absolute iron deficiency were 12.8% and 32.85% of them had Functional Iron Deficiency. As stage advanced Serum iron and TSAT decreased with increasing TIBC suggesting increasing prevalence of iron deficiency anemia as CKD progresses. Mean ferritin values were relatively adequate among various stages suggesting that anemia was due to inadequate mobilization from the iron stores.

V. Conclusions

The study supports current KDOQI recommendation of routine estimation of iron status in all the patients of CKD with anemia. This is not only to avoid the in advert use of ESA which has its own complications but also to avoid iron overload in patients with adequate iron stores. In a vast majority of patients, inadequate endogenous EPO production(normal iron status) and defective iron supply for erythropoiesis in the form of functional iron deficiency seems to be the cause of anemia. A reduction of 70% in ESA dosage has been noted in patients receiving IV iron. ESAs are the main treatment of anemia of CKD and for this adequate iron stores are necessary to permit an optimal response. An early management of anemia is needed in CKD patients as anemia leads to CKD progression and accelerates cardiovascular mortality among these patients.

LIST OF ABBREVIATIONS

ACEI - Angiotensin-converting enzyme inhibitors

ADEs - adverse drug events

AID – Absolute Iron Deficiency

ALT - Alanine transaminase

ANOVA - Analysis of Variance

ARB - Angiotensin receptor blockers

AST - aspartate transaminase

BFU-E - Burst Forming Unit-Erythroid

BMI - Body mass index

CAD - Coronary artery disease

CBC- Complete blood count

CFU-E - Colony Forming Unit-Erythroid

CGN - Chronic glomerular nephritis

CHr - Reticulocyte hemoglobin content

CIN - Chronic interstitial disease

CKD - Chronic kidney disease

CKD-EPI CKD epidemiology collaboration

CVD - Cardiovascular disease

DD - dialysis-dependent

dL - Decilitre

DMT 1 - Divalent metal transporter1

eGFR - estimated Glomerular filtration rate

EPC - erythropoietin-producing cell

EPO – erythropoietin

ESRD - End-stage renal disease

ESA - Erythropoiesis-stimulating agents

FID - Functional Iron Deficiency

g - Gram

GFR glomerular filtration rate

GGT- Gamma-glutamyl transferase

GIT - Gastro intestinal tract

Hb - Hemoglobin

HD hemodialysis

IDA - Iron deficiency anemia

IV - Intravenous

KDIGO - Kidney Disease Improving Global Outcomes

K/DOQI - Kidney Diseases Outcome Quality Initiative

LDH - lactate dehydrogenase

LFT – liver function test

LVH - Left ventricular hypertrophy

MDRD - Modification of Diet in Renal Disease

mcg- Microgram

mg - Milli gram

mGFR - measured glomerular filtration rate

mL - milli litre

MRD - Medical renal disease

NDD - non-dialysis dependent

ng - Nano gram

NGAL - Plasma neutrophil gelatinase-associated lipocalin

NHANES - National Health and Nutrition Examination Survey

NKF -DOQI - National Kidney Foundation–Dialysis Outcomes Quality Initiative

nm- Nanometre

PAERI - Prevalence of Anemia in Early Renal Insufficiency

PHRC - Percentage of hypochromic red cells

pmp - Per million populations

PTH - Parathormone

RAAS - renin-angiotensin-aldosterone system

RBC - Red blood cell

rHuEpo - Recombinant human erythropoietin

RLJH – R L Jalappa Hospital

RRT - Renal replacement therapy

S-Cr - Serum creatinine

SLE – Systemic lupus erythamatosis

SPSS - Statistical Package for the Social Sciences

sTfR - Soluble transferrin receptor

T2DM – type 2 diabetes mellitus

TB - total bilirubin

TIBC - Total iron binding capacity

TSAT - transferrin saturation.

USG KUB - ultrasound kidney ureter bladder

^{o}C – degree Celsius

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

1. INTRODUCTION

The Kidney Diseases Outcome Quality Initiative (K/DOQI) & Kidney Disease Improving Global Outcomes (KDIGO) defines chronic kidney disease (CKD) as damage for ≥3 months, defined by structural or functional abnormalities of the kidney, with or without decreased GFR

OR

GFR <60ml/min/1.73m2 for > 3 months with or without kidney damage. The persistence of the damage or decreased function for the duration of three months or longer is necessary to distinguish CKD from acute kidney disease. (1)

Markers of kidney damage include albuminuria >30mg/day, urine sediment abnormalities (e.g.: hematuria, red cell casts etc.), electrolyte or other abnormalities due to tubular disorders, structural abnormalities detected by imaging, abnormalities detected by histology, history of kidney transplantation.

GFR of <60ml/min/1.73m^2, which is commonly estimated (eGFR) using serum creatinine.

CKD is a universal health problem, with a greater burden and very high cost of care especially in developing countries like India. The National Kidney Foundation in India states that kidney diseases rank 3rd amongst the life-threatening diseases after cancer and heart disease. (2)

A worldwide increase in the prevalence of diabetes mellitus, hypertension, obesity, and aging also increases the incidence and prevalence of CKD. (3)

The Patients enrolled in the end-stage renal disease (ESRD) Medicare-funded

program has increased from approximately 10,000 beneficiaries in 1973 to 661,648 as of 2013 [4]

ESRD consume a substantial share of health care resources.

Despite the huge proportion of resources used in the treatment of ESRD and substantial improvement in the quality of dialysis, still a notable mortality and morbidity is seen due to associated co-morbidities. One such complication is anemia.

Anemia is a universal public health problem affecting social and economic development of both developed and developing countries with major consequences on human health.

Anemia develops early in the course of CKD and severity increases as the disease progresses. [5] An appropriate balance between stimulating the generation of erythroblasts (erythropoiesis) and maintaining sufficient iron levels for optimum hemoglobin (Hb) production is the keystone for anemia management in CKD. [6]

Anemia contributes to many of the symptoms associated with reduced kidney function. These include fatigue, depression, reduced exercise tolerance, and dyspnea.

In addition, anemia has a direct adverse cardiovascular disease (CVD) consequences which includes left ventricular hypertrophy (LVH), left ventricular systolic dysfunction, coronary artery disease, and stroke. CVD is the most common cause of mortality among CKD patients. [7-8]

Therefore, CKD patients with anemia are at a higher risk of increased hospitalization, increased duration of hospital stay, reduction in quality of life and increased mortality.

Among non-dialysis CKD patients, severe anemia may accelerate the progression to end-stage renal disease (ESRD).

Anemia in CKD is due to many causes. Erythropoiesis and iron homeostasis are impeded due to the relative deficiency of erythropoietin, chronic inflammation, blood loss, decreased iron absorption and utilization, exogenous iron and erythropoietin acquisition via biologically unregulated mechanisms (treatment with ESA, blood transfusions, and iron administration). [9-11]

The advent of erythropoiesis-stimulating agents (ESA) and various intravenous iron preparations has resulted in an effective management of anemia of CKD, by maintaining optimal hemoglobin levels and by effectively treating iron deficiency. The newer challenges are the risks associated with administering ESA and iron therapy, which comprises from as simple as iron overload, hypertension, thrombosis to serious complications like pure red cell aplasia, even though the latter is rare. [12].

Iron deficiency remains an important cause for suboptimal response to erythropoietin in dialysis patients. [13] Maintenance of iron supplementation is required to successfully treat anemia with intravenous iron compounds especially in patients who have functional iron deficiency.

Recombinant human erythropoietin (rHuEpo) for treatment of renal anemia may results in functional iron deficiency due to insufficient iron stores for the accelerated erythropoiesis. [14-15]

The National Kidney Foundation–Dialysis Outcomes Quality Initiative (NKF-DOQI) guidelines recommend aggressive detection and management of FID. The FID can be confirmed by the response to a course of parenteral iron, which produces either an

increase in hemoglobin at the same dose of erythropoietin or the dose of EPO required for maintaining the target hematocrit level decreases.

For adequate treatment, one should ensure adequate iron is available and also iron being mobilized from the stores.

Thus, assessing iron status is integral to both iron deficiency and anemia management in CKD patients, as it not only avoids the unnecessary use of iron supplements in CKD patients with anemia but also prevents the adverse complications associated with the use of erythropoietin stimulating agents. [16]

There is a shortage of data regarding iron status among patients with CKD. Early evaluation and treatment of iron deficiency anemia in CKD will prevent an adverse impact on their quality of life and significantly decreases morbidity and mortality.

Iron indices are non-invasive and cost-effective tools for iron assessment in CKD patients with iron deficiency anemia and to estimate the optimal levels required for successful management of iron deficiency anemia in CKD patients.

2. AIM AND OBJECTIVES

2.1. Aim

The study is aimed at evaluating iron status amongst the chronic kidney disease patients

2.2. Objectives

1. To study the iron indices. [Serum Iron, Serum Ferritin, transferrin saturation (TSAT), TIBC]

2. To estimate the percentage of iron-deficient patients among chronic kidney disease.

3. To estimate the percentage of patients having functional versus absolute iron deficiency.

3. REVIEW OF THE LITERATURE

3.1 The Kidney

The two kidneys are retroperitoneal structures that lie on the posterior wall of the abdomen. Each kidney weighs about 150 grams and is typically 10-12 cm in length, 5-7 cm in width, and 2-3 cm in thickness. The hilum is an indented region on the medial side of each kidney through which passes the renal artery and vein, lymphatics, nerve supply, and ureter. Urine passes from kidney to the bladder through the ureter, where it is stored until emptied. A tough, fibrous capsule surrounds the kidneys and protects its delicate inner structures. (17)

On coronal section, of the kidney, the two major regions that can be visualized are the outer cortex and the inner medulla regions. The medulla has 8 to 10 cone-shaped tissue called renal pyramids. The junction of cortex and medulla is the base of the pyramid and the apex is the papilla, which ends in the renal pelvis, a funnel-shaped continuation of the upper ureter. The pelvis divides into major calyces and minor calyces, which collect urine from the tubules of each papilla. The contractile elements in walls of the calyces, pelvis, and ureter help to propel urine toward the bladder, where urine is stored until it is expelled through micturition. (17)

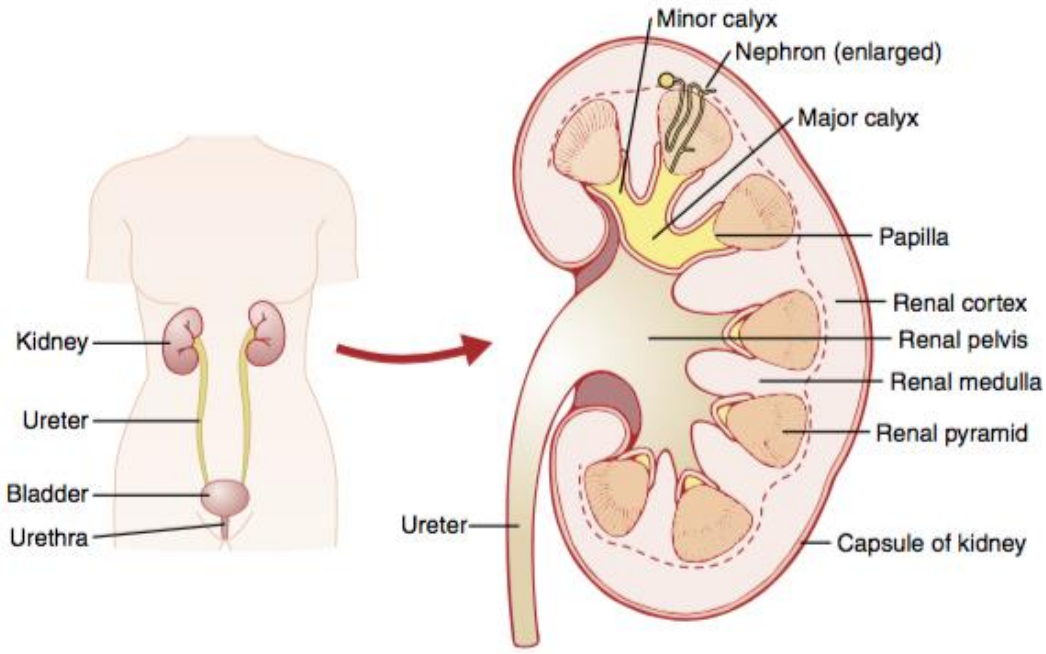


Figure 1 General organization of the kidneys and the urinary system. [17]

3.2 Renal Blood Supply

Renal Blood flow constitutes to about 22 percent of the cardiac output. Each renal artery branches, further dividing into the interlobar arteries, arcuate arteries, interlobular arteries (also called radial arteries) and afferent arterioles and the glomerular capillaries. The distal ends of each glomerular capillaries coalesce to form the efferent arteriole, and a second capillary network is the peritubular capillaries, that surrounds the renal tubules.

The resistance in the afferent and efferent arterioles regulates the hydrostatic pressure in the capillaries, in turn regulating the GFR and tubular reabsorption and maintaining the homeostasis.

The peritubular capillaries empty into the venous system, which progressively forms the interlobular vein → arcuate vein →interlobar vein, which emptied into the renal vein. Renal vein exits the kidney besides the renal artery and ureter.

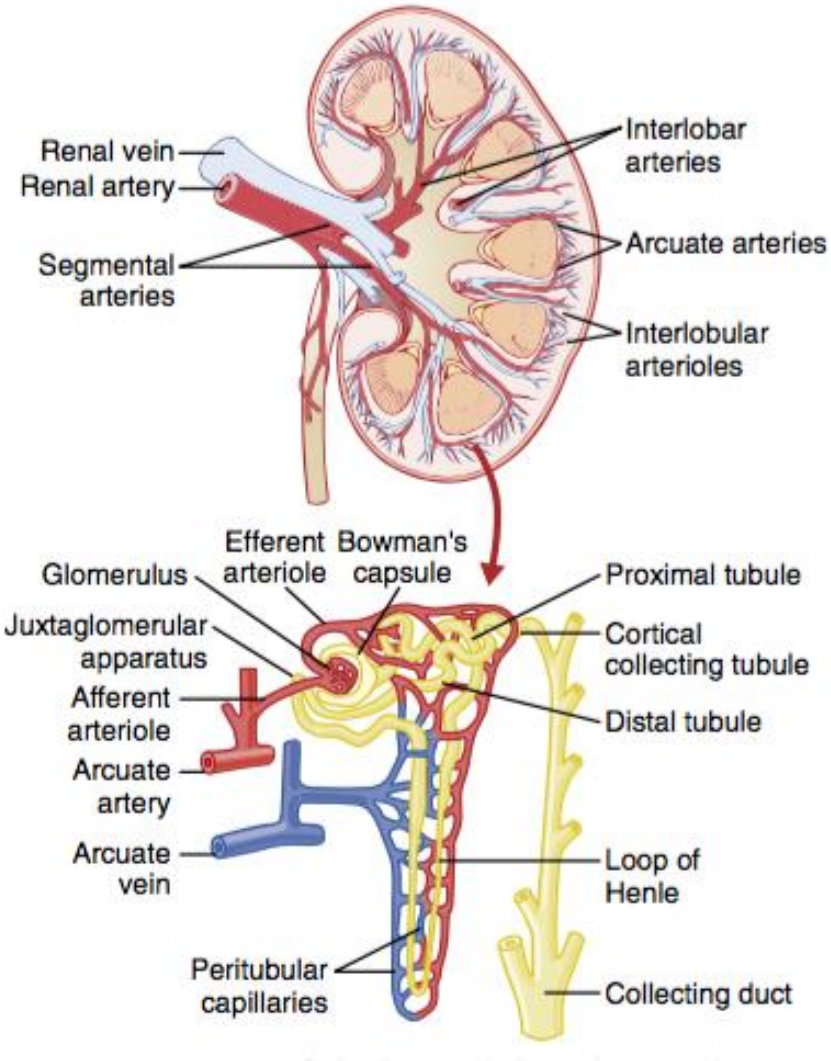


Figure 2 - Section of the human kidney showing the major vessels that supply the blood flow to the kidney and schematic of the microcirculation of each nephron

(17)

3.3 Functional Unit of the Kidney: Nephron

Each kidney in the human contains about 800,000 to 1,000,000 nephrons, each capable of forming urine. The kidney is incapable of regenerating new nephrons. Therefore, the number of nephrons gradually decreases with age, renal injury, or disease. 10 percent of functioning nephrons decrease every 10 years after 40.

Adaptive changes in the remaining nephrons that help the kidney in its functions, hence the loss is not life-threatening.

Each nephron contains a tuft of capillaries called the glomerulus, which filters large amounts of fluid from the blood and a long tubule in which converts the filtered fluid into the urine.

The renal corpuscle functions as the filtration unit made up of Bowman's capsule with a Bowman's space that collects the filtrate, glomerular capillaries and the mesangial cells supporting these capillaries. Endothelial cells, a basement membrane, and podocytes (foot processes) make up the glomerular filtration barrier.

Filtered fluid flows into the proximal tubule in the cortex of the kidney. From the proximal tubule, which has a convoluted section followed by a straight section the fluid then flows into the loop of Henle (a descending and an ascending limb), which passes into the medulla and then enters the distal tubule in the renal cortex. Fluid then empties into the collecting ducts.

There are two nephrons, which differ in the length and urine concentrating capacity: long juxtamedullary nephrons and short cortical nephrons. [17]

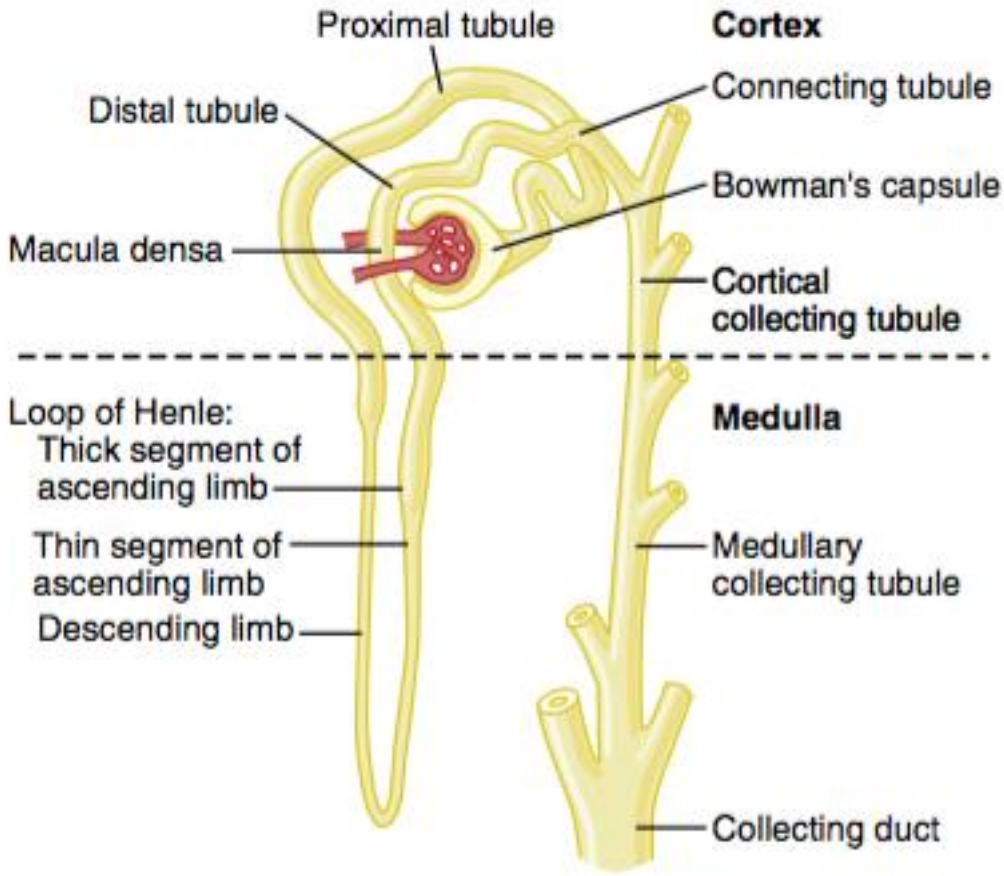


Figure 3 - Basic tubular segments of the nephron. The relative lengths of the different tubular segments are not drawn to scale [17]

3.4 Functions of the Kidney

The functional unit of the kidney → Nephron. The kidney regulates extracellular fluid volume and electrolytes composition to compensate for wide variation in water and electrolytes intake. The formed urine has potentially toxic waste metabolites, which are excreted. The kidney functions are: [18]

1. Arterial blood pressure regulation

Sodium and water balance is maintained by the kidney through an enzyme renin, produced by the granular cells of the juxtaglomerular apparatus that catalyzes the

formation of angiotensin from a plasma globulin, angiotensinogen. Angiotensin is a potent vasoconstrictor peptide that significantly contributes to salt balance and blood pressure regulation.

2. Maintenance of body composition

The kidney regulates the volume of fluid in the body.It achieves this regulationby varying amounts of water and ions excreted in the urine.Electrolytes regulated by changes in urinary excretion include sodium,potassium,chloride,calcium,magnesium and phosphate.

3. Removal of waste products and chemicals:

Waste products include urea, creatinine, uric acid (from protein and nucleic acid metabolism) and waste products generated from the breakdown of hemoglobin that gives the urine it's color.

These products generated are potentially harmful and should be excreted. The kidneys help in the removal of these waste products so that homeostasis is maintained.

4. Erythropoietin production

Erythropoietin, glycoprotein containing 165 amino acid residues and four oligosaccharide chains. Its blood levels are markedly increased in anemia. In adults 85% of the erythropoietin comes from kidneys and 15% from the liver. Erythropoietin is produced by interstitial cells in the peritubular capillary bed of the kidneys and by the perivenous hepatocytes in the liver. When renal mass is reduced in adults by renal disease or nephrectomy, the liver cannot compensate and the anemia develops. The

principal site of inactivation of erythropoietin is the liver and the hormone has a half-life in the circulation of about 5hours. However, the increase in circulating red cells takes 2-3 days to occur , since the RBC maturation is a slow process**.**

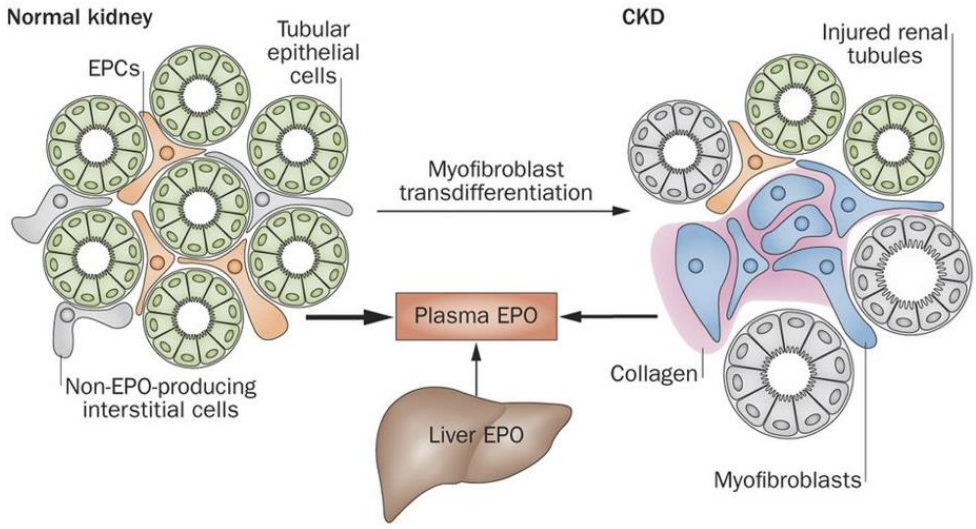


Figure 4 - Cellular basis of deficiency of erythropoietin in CKD. [19]

Erythropoietin producing cell recruitment is impaired in CKD, this results in reduced EPO output and the development of anemia.

5. Activation of vitamin D

The inactive form of vitamin D is activated in the kidney.1,25-Dihydroxyvitamin D_3,the most active form of vitamin D_3,is formed by proximal tubule cells.This steroid hormone plays an important role in the regulation of body calcium and phosphate balance.

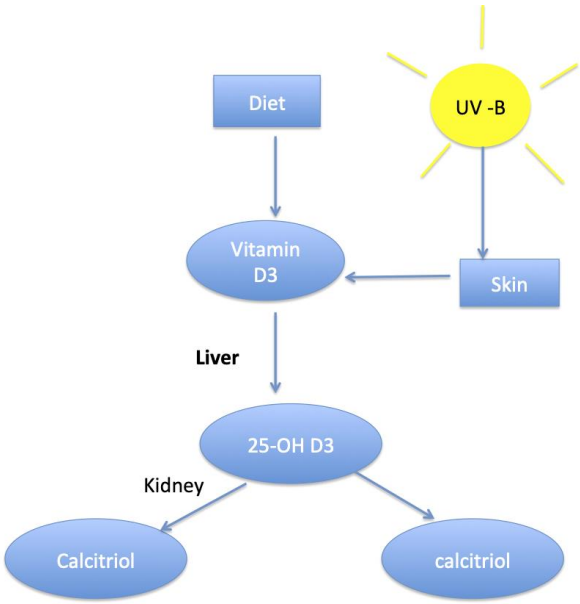


Figure 5 – activation of vitamin D

6. Acid-base balance regulation.

Two very important roles of the kidneys are in maintaining the acid-base balance: they reabsorb bicarbonate from urine. They excrete hydrogen ions into the urine .

7. Regulation of fluid osmolality.

3.5 STAGES

The national kidney foundation and kidney disease outcomes quality initiative (NKF/KDOQI) published their guidelines with the purpose to unify the classification and definition of CKD. [1]

Stage	Description	GFR (mL/min/1.73 m^2)
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with a mild decrease in GFR	60 – 89
3	Kidney damage with a moderate decrease in GFR	30 – 59
4	Kidney damage with a severe decrease in GFR	15 – 29
5	Kidney failure	<15 or dialysis

Table 1: CKD stages (classification and definition of CKD)

3.6 Etiology of CKD

Common causes of CKD are [20]

- Diabetic nephropathy (30–40%)
- Hypertension (14–22%)
- Chronic glomerular nephritis (16–20%)
- Chronic interstitial disease (5.4–12.7%)
- Hereditary, familial disease (8.4%)
- Obstruction including calculus (2.9%)

3.7 Epidemiology

In the absence of a renal registry, the exact disease burden of CKD/ESRD in the Indian population cannot be assessed accurately.

The incidence of ESRD in India is approximately 150–200 pmp annually. [21]

Because of challenges in access to health care, around half of the patients are first seen by health care providers with advanced CKD in stage 5, when the eGFR is <15 ml/min per 1.73 m2. [22]

This alarming number highlights the need for intense screening programs for those at risk for CKD. The prevalence of CKD in different regions ranges from <1% to 13%.

International Society of Nephrology's Kidney Disease Data Center Study data recently reported a prevalence of 17%. The true burden of ESRD in India is unknown as there are only few centers for care, there is a deficiency of access to RRT, and absence of a registry.

An estimation of 120,000 patients on HD is reported in India. [23]

3.8 Estimation of Glomerular Filtration Rate

Estimation of GFR and measurement of renal function is a cornerstone in the classification of kidney diseases. As a physiological measurement, it has proved to be the most specific and sensitive marker for changes in overall renal function. [24]

The fructose polysaccharide inulin is used as a gold standard for GFR measurement. [24]

Methods used for inulin estimation are inconvenient and expensive for day to day clinical practice. Instead, endogenous substances like urea, creatinine, and cystatin C are measured in urine or blood. [25] Most widely used marker is serum creatinine as it is cheap and included in routine blood sampling.

Creatine phosphate is the breakdown product of serum creatinine which is produced at a constant rate in muscle under steady-state conditions. It is freely filtered and partly secreted and this proportion becomes more important if the filtration rate decreases. The S-Cr level also assesses the nutritional status of the patient. Individual's muscle mass, recently ingested meat and the patient's fluid balance can also affect S-Cr.

The most widely used formulas used to estimate renal function are

1. The Cockcroft-Gault formula [26]

Creatinine clearance = {((140–age) x weight(kg))/(72xSCrmg/dL)}x 0.85 (if female)

2. Modification of Diet in Renal Disease (MDRD) formula [26] eGFR (mL/min/1.73 m^2) = 175 × (Scr mg/dL)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American) (conventional units)

Review of the literature revealed that CG and MDRD formulae correctly assigned overall only 62% and 64%, respectively, of the subjects to their actual K/DOQI-CKD classification's GFR groups as determined by measured GFR (mGFR). [1] Normal plasma constituents can also interfere with creatinine measurement. But the sensitivity of serum creatinine is low because it fails to identify more than 50% of the patients with early stage CKD prior to the onset of stage 3 CKD (GFR of 30–60mL/min/1.73m2). [27]

The MDRD equation is extensively used in research as it gives a more accurate estimate of the GFR than the Cockcroft-Gault formula, especially for older and obese individuals. [27]

The MDRD equation's accuracy was originally proved in CKD patients but its use in healthy people or in patients with eGFR ≥90 mL/ min/1.73m2 is unclear.

At higher levels of kidney function, MDRD tends to underestimate true GFR in most studies. CKD-EPI and Cystatin C (a proteinase inhibitor) are also used for GFR estimation.

The CKD-EPI creatinine equation is based on the principle of 4 variables as the MDRD Study equation, instead uses a 2-slope spline to correlate the relationship between eGFR and creatinine, and a different relationship for sex, age, and race. The equation was reported to perform better and with less bias than the MDRD equation, especially in patients in early stages with a higher GFR. Thus this also reduces the misclassification of CKD. [26]

$$eGFR = 141 * \min\left(\frac{sCR}{\kappa}, 1\right)^{\alpha} * \max\left(\frac{sCR}{\kappa}, 1\right)^{-1.209} * 0.993^{AGE} * 1.018 * F * 1.159 * B$$

where:

sCR = serum creatinine in mg/dL
κ = 0.7 if female, 0.9 if male
α = -0.329 if female, -0.411 if male
F = 1 if female, 0 if male
B = 1 if Black/African American, 0 otherwise
AGE is measured in years

Figure 6 – CKD-EPI equation [28]

3.9 Risk Factors For CKD And Decline In Renal Function

The GFR deteriorates after the age of 40 years, with a normal rate of on average 1 ml/min per year. [29]

1. Susceptibility factors (these increase the sensitivity to kidney damage)

- Old age
- A family history of CKD

- Reduction in renal mass
- Low birth weight
- Low income or education

2. Initiation Factors – they directly initiate renal damage

- Diabetes
- Hypertension
- Autoimmune diseases
- Systemic infections
- Urinary stones
- Lower urinary tract obstruction
- Drug toxicity

3. Progression factors – worsens renal damage and leads to a faster decline in the kidney function after initiation of the damage.

- High BP
- Severe Proteinuria
- Poor glycemic control
- Smoking

4. ESRD – morbidity and mortality increases with renal failure

- Lowe dialysis dose (KT/V)
- Temporary vascular access
- Low serum albumin levels
- Late referrals

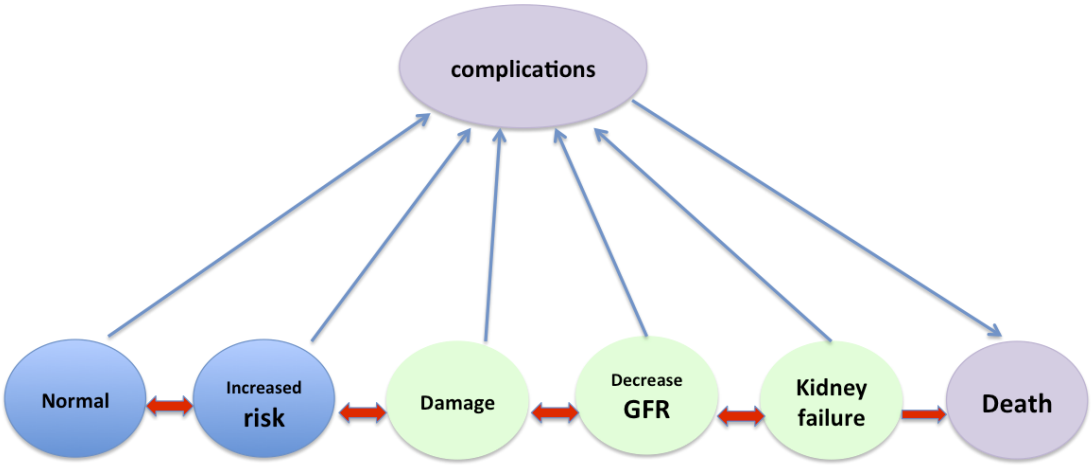


Figure 7 – Evolution of CKD.

3.10 Clinical Features of Chronic Kidney Disease

Patients with CKD stages 1-3 are usually asymptomatic. Generally, disturbances in water or electrolyte balance or endocrine/metabolic derangements become clinically evident in CKD stages 4-5.

Uremic manifestations are secondary to an accumulation of toxins in CKD stage 5.

Protein-energy malnutrition, loss of lean body mass, and muscle weakness are manifestations of Metabolic acidosis in stage 5. (30)

Hypertension, Peripheral edema and pulmonary edema are due to an imbalance in sodium and water.

Fatigue reduced exercise capacity, impaired cognitive and immune function and reduced quality of life are seen with anemia.

Anemia is associated with cardiovascular disease, new onset of heart failure, or progression into a more severe heart failure, which increases cardiovascular mortality. (30)

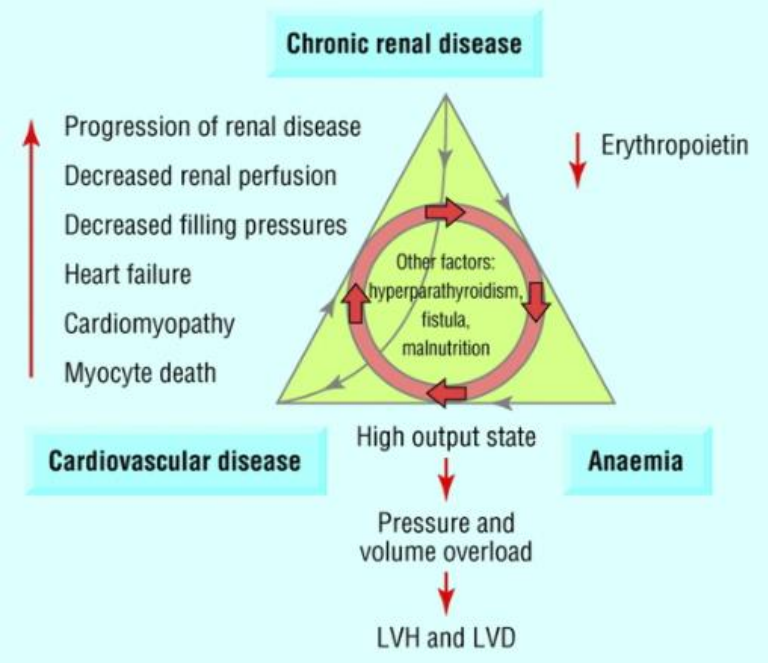


Figure 8 – The perpetuating triad of CKD, Anemia and CVD [31]

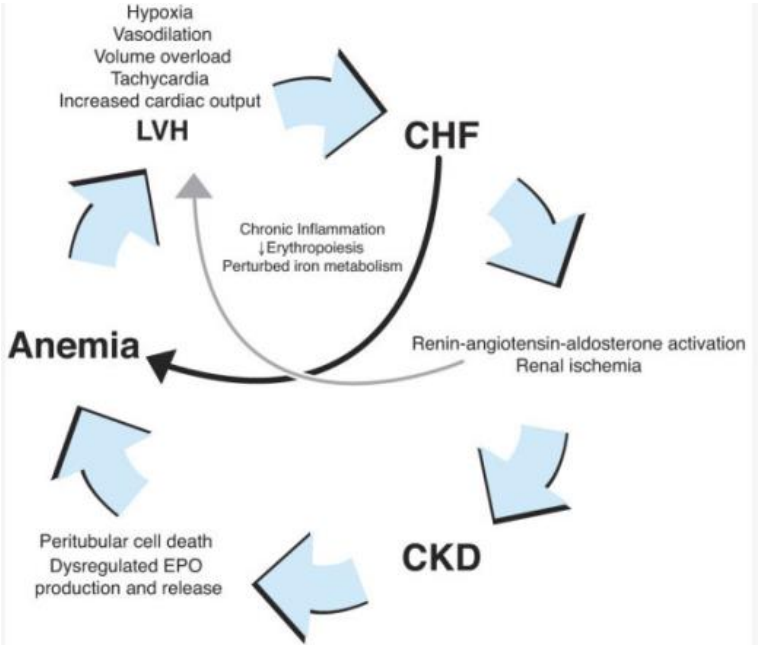


Figure 9 – The Cardiorenal anemia syndrome [32]

Congestive heart failure (CHF) is both a cause and consequence of CKD. First, heart failure is a chronic inflammatory state, which leads to an influx of cytokines that depresses erythropoiesis and disturbs iron metabolism. Second, CHF directly causes kidney damage, and GFR deteriorates. In response to the reduced cardiac output, blood pressure (and renal perfusion) is maintained by activation of the RAAS. Angiotensin II-mediated renal vasoconstriction results in renal ischemia and tubular cell death. Renal cell death hastens anemia through loss of endocrine function. Aldosterone-induced salt and water retention increases pre-load, which increases the heart rate to increase output. [32]

Other uremic manifestations in ESRD, which are more likely due to inadequate dialysis are:

- Pericarditis complicated by cardiac tamponade, which may also result in death.
- Encephalopathy can progress to coma and death.
- Peripheral neuropathy
- Restless leg syndrome
- Gastrointestinal symptoms - anorexia, nausea, vomiting, diarrhea
- Skin manifestations - dry skin, pruritus, ecchymosis. The excess urea may manifest as whitish flakes on the skin known as uremic frost.
- Fatigue, increased sleep, failure to thrive, Malnutrition
- Erectile dysfunction, decreased libido, amenorrhea.
- Platelet dysfunction with a tendency to bleed.

3.11 Biochemical Changes In CKD

The biochemical alterations in CKD patients are due to altered kidney function, where it is unable to maintain its filtration, excretion, regulatory and endocrine functions. Increased plasma concentrations of nitrogenous waste products mainly urea, creatinine and uric acid results from decreased GFR and tubular function. Metabolic acidosis [33]

- Hyperkalemia
- Hypocalcemia
- Hyperphosphatemia
- Secondary hyperparathyroidism
- Anemia

3.12 Iron Homeostasis

Iron is the most abundant transition metal in the human body and the fourth most common metal in the crust of the earth. Iron plays an important role in the function of all cells. The important role of iron is to carry oxygen as a part of hemoglobin. Normal daily Indian diet contains about 14 to 18mg of iron, mostly in the form of heme contained in the animal products, with the remainder being inorganic iron in vegetables. The total body iron content is normally about 2.5g in women & as high as 6g in men and is divided into functional and storage compartments.

Majority of the functional iron is found in hemoglobin, myoglobin and iron-containing enzymes such as catalase and the cytochromes contain the rest. Storage pools are represented by hemosiderin and ferritin contains about 12% to 20% of total body iron. Major storage sites are the liver and the phagocytes.

Healthy young females have smaller iron stores than males, mainly due to menstrual blood loss and also develop iron deficiency due to excessive losses or increased demands associated with menstruation and pregnancy, respectively.

Body Iron is recycled between the functional and storage pool. It is transported in plasma by the iron-binding glycoprotein called transferrin, which is synthesized in the liver. In normal individuals, transferrin is about one third saturated with iron, therefore serum iron levels are about 120mcg/dl. Plasma transferrin helps in delivering iron to cells, including erythroid precursors that require iron to synthesize hemoglobin. [34] Erythroid precursors possess high-affinity receptors for transferrin that mediate iron transport through receptor-mediated endocytosis.

Free iron is toxic; hence the iron should be sequestered for storage. Binding iron in the storage pool to either ferritin or hemosiderin does this. Ferritin is a protein iron complex present in large amounts in the liver, spleen, skeletal muscles and bone marrow. In the liver mainly its stored intraparenchymally: in other tissues, it is found mainly in the macrophages. Hepatocyte iron is derived from plasma transferrin, whereas storage iron in macrophages is derived from the breakdown of RBC. [35] Ferritin is situated in the cytosol intracellularly, in these partially degraded protein shells of ferritin aggregate into hemosiderin granules. [35] Hemosiderin is chemically reactive and converts into blue-black when exposed to potassium ferrocyanide, the principle behind the Prussian blue stain. With normal iron stores only trace amount of hemosiderin are found in the body, most being stored as ferritin. Iron overload state is different, where hemosiderin is the stored iron.

Total iron stores must be regulated meticulously because iron in excess is highly toxic. Iron balance is maintained by regulating the absorption of dietary iron in the duodenum. [34] No regulated pathway exists for iron excretion, which is limited to 1to

2 mg lost each day in the form of shredding of mucosal and skin epithelial cells. As body iron stores increase, absorption falls and vice versa.

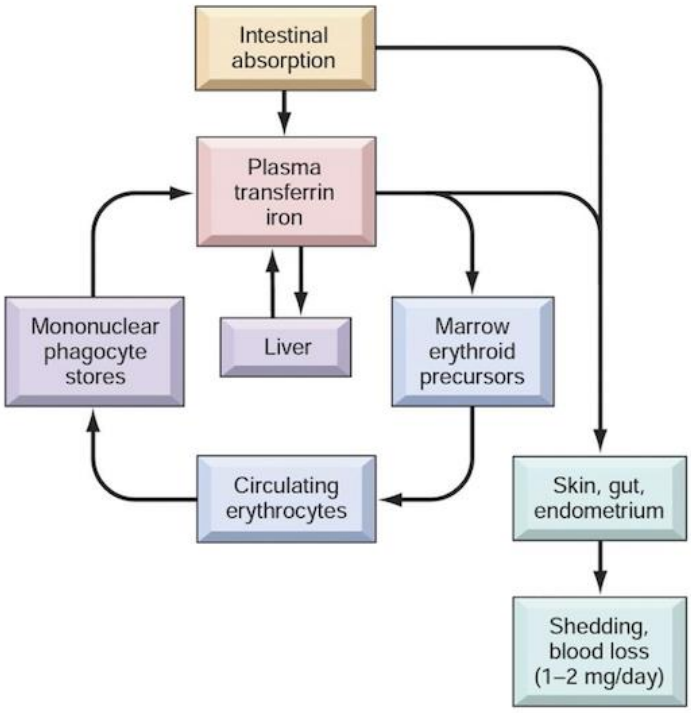


Figure 10 – Iron metabolism. [36]

The pathways responsible for non-heme and heme iron differ partially. Luminal non-heme iron is mostly in the ferric state and must first be reduced to ferrous iron ferric reductases. Fe^{+2} is transported through the membrane by divalent metal transporter1 (DMT 1). The absorption of non-heme iron is variable and often inefficient. Less than 5% of dietary non-heme is absorbed, a contrast to which 25% of the heme iron is absorbed. [36]

Iron which enters the duodenal cells is either stored as mucosal iron or transported to the blood, the distribution of which is influenced by body iron stores.

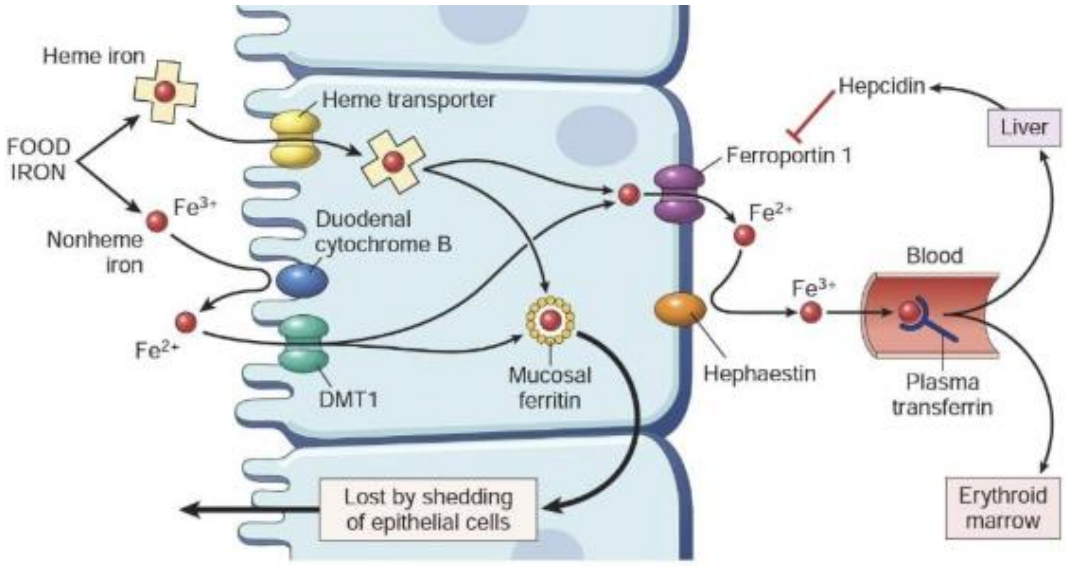


Figure 11 – Regulation of iron absorption. [36]

Ferrous iron is transported into the circulation from the cytoplasm across the basolateral enterocyte membrane by ferroportin. Then oxidation of the Fe^{+2} to the Fe^{+3} form occurs, which is carried out by the iron oxidases hephaestin and ceruloplasmin. The absorbed ferric iron binds rapidly to the plasma protein transferrin, which delivers iron to RBC progenitors in the marrow. [35]

Transferrin which carries iron exists in two forms :

- monoferric (one iron atom)
- diferric (two iron atoms).

The turnover (half-clearance time) of transferrin-bound iron is typically 60–90 min. The iron-transferrin complex remains in the plasma until it comes across the transferrin receptor on the surface of marrow erythroid cells. Diferric transferrin has more affinity for transferrin receptors than apotransferrin (not carrying iron) which has a low affinity. [37]

Transferrin receptors are found on most of the cells within the body and all cells during development will display transferrin receptors—the cell having the greatest number of receptors (300,000–400,000/cell) is the developing erythroblast. [38] Both

DMT1 and ferroportin are distributed widely and are involved in transportation of iron in the other tissues as well. When iron-transferrin complex interacts with the receptor, the complex is internalized and transported to an acidic endosome, where the iron is released at a low pH. The iron is then utilized for heme synthesis and transferrin-receptor complex is sent back to the cell surface, where the transferrin is released back into the circulation and the transferrin receptor than re-anchors onto the cell membrane. Here some amount of the transferrin receptor protein is released into the circulation that can be measured as soluble transferrin receptor protein. [36]

Iron absorption is regulated by hepcidin, a small peptide that is synthesized in the liver in response to an increase in intrahepatic iron levels. The main function of the hepcidin is to inhibit iron transfer from the enterocyte to plasma by binding to ferroportin and causing endocytosis with subsequent degradation. [34]

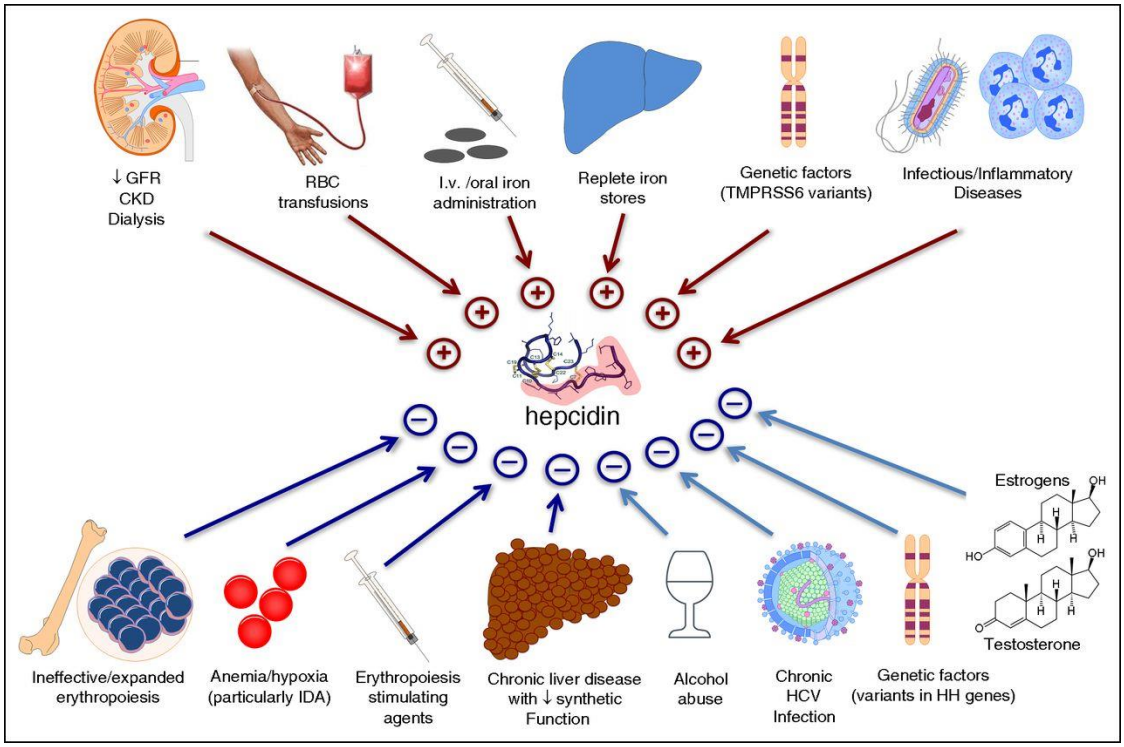


Figure 12 – Clinical conditions that influence hepcidin levels. [39]

As hepcidin levels increase iron becomes trapped within the duodenal cells in the form of mucosal ferritin and is lost as these cells are sloughed. Thus when the body has enough iron stores, high hepcidin levels inhibits its absorption into the blood. Conversely, with low body stores of iron, hepcidin synthesis falls and in turn facilitates iron absorption. By inhibiting ferroportin, hepcidin not only reduces iron uptake from enterocytes but also suppresses iron release from macrophages, which is the main source of iron for erythroid precursors to make hemoglobin.

3.13 Erythropoietin and Iron dependence in erythropoiesis

EPO and iron are both important in erythropoiesis. They are involved at different stages of the process of differentiation and maturation i.e. pluripotent stem cell to erythrocyte.

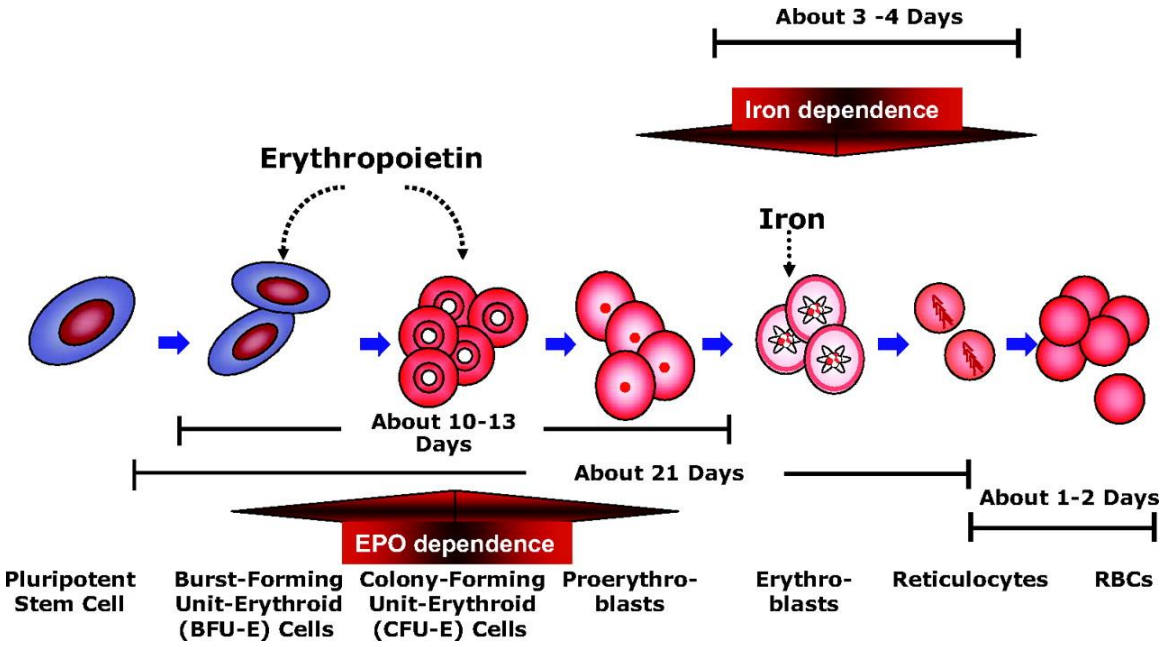


Figure 13 - Erythropoietin and Iron dependence

EPO is crucial in first 10- to 13-day period when BFU-E transforms into CFU-E that differentiates into proerythroblasts. In absence of EPO, apoptosis within the CFU-E

and BFU-E stages occurs. During this period, very little iron is incorporated into Hb. In contrast, iron incorporation into Hb synthesis is evident during the later shorter (3–4 days) stage when erythroblasts develop into reticulocytes. At this time, a lack of iron can impair full hemoglobinization of the RBCs, leading to both absolute and functional iron deficiency.

3.14 Anemia in CKD

Anemia is a common accompaniment in CKD patients, usually associated with poor outcomes. The association between the CKD and anemia was linked about 170 years back by Richard bright. Anemia is especially common in advanced stages of CKD, anemia prevalence is significantly higher in eGFR < 60 ml/min amongst the males and <50 ml/min in females. Population studies such as the National Health and Nutrition Examination Survey (NHANES) by the National Institutes of Health and the Prevalence of Anemia in Early Renal Insufficiency (PAERI) study reports an incidence of anemia of <10% in CKD stages 1 and 2, 20% - 40% in CKD stage 3, 50%- 60% in CKD stage 4, and ≥ 70% in CKD stage 5. [40]

Anemia leads to reduced quality of life in CKD patients and also increased cardiovascular diseases, hospitalizations, cognitive impairment, and mortality. [41]

KDIGO guideline defines anemia in CKD when the hemoglobin level is < 13.0 g/dl in adult males and 12.0g/dl in adult females. [1]

Pathology of Anemia in CKD patients

Anemia in CKD is typically normocytic, normochromic, and is due to many factors.

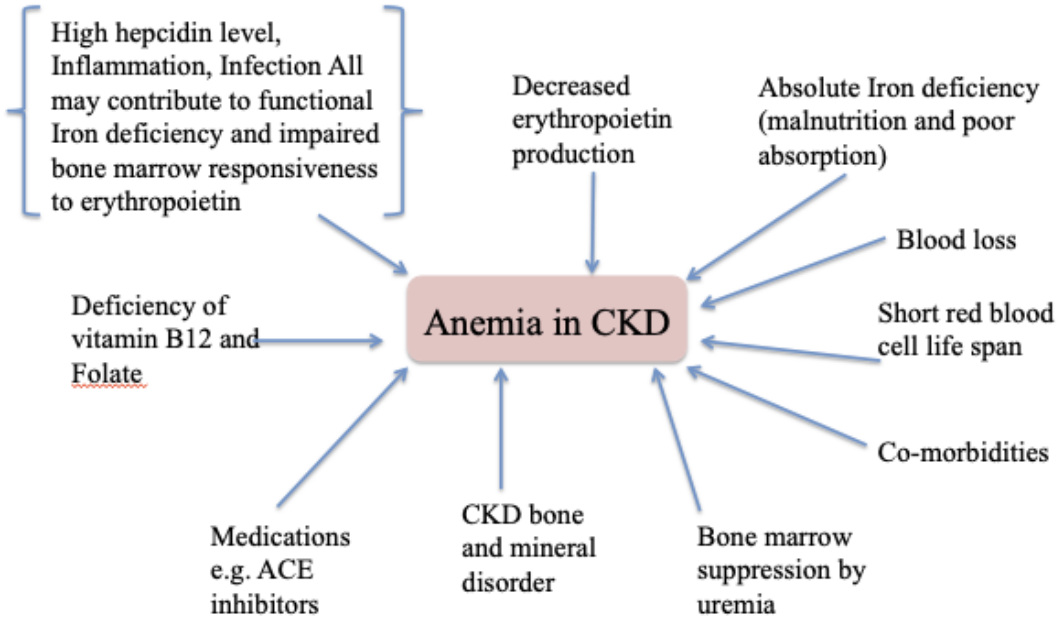


Figure 14 – causes of anemia in CKD

1. Lack of EPO synthesis in the diseased kidneys – most common.

The kidney is the main site for EPO production contributing to 80-90% and the liver contributes only 10-15% of EPO in circulation. [41]

As the 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.

2. Inhibition of Erythropoiesis.

It is shown that abnormal metabolite or substances are not adequately excreted in CKD patients, which interfere with bone marrow functioning. A vast number of CKD patients have remained anemic despite the presence of elevated levels of EPO, suggesting that the marrow has decreased responsiveness and sensitivity to circulating EPO in these patients. [42]

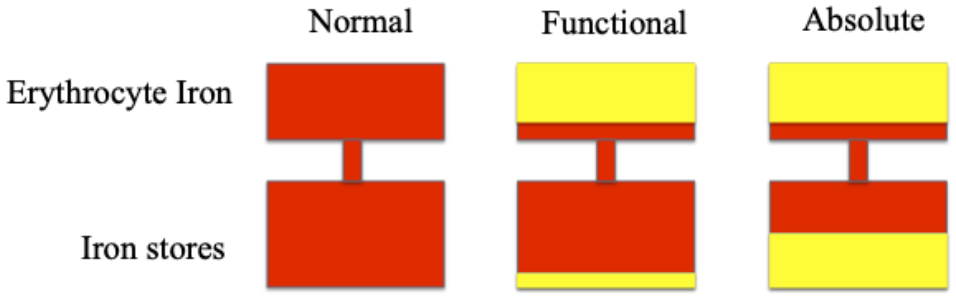
Uremic sera inhibit proliferation of Burst Forming Unit-Erythroid (BFU-E) and Colony Forming Unit-Erythroid (CFU-E) hemosynthesis.

Patients with anemia on dialysis showed an improvement in hematocrit in the absence of significant changes in plasma EPO levels, suggesting that an inhibitor was removed by dialysis.[30] Inflammatory mediators (cytokines) such as tumor necrosis factor alpha, interleukin- 1 and 6 are elevated in CKD and are associated with dysmetabolic state seen in late in CKD disease. These mediators interfere erythroblast maturation.

3. Iron deficiency anemia

Iron deficiency is an important cause of anemia in CKD patients

- Absolute iron deficiency
- Functional iron deficiency



Serum Ferritin	>300mg/dl	100-300mg/dl	<100mg/dl
Transferrin saturation	>20%	<20%	<20%

Figure 15 – Defining Iron deficiency

Causes of depleted iron stores in CKD patients include 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 dysfunction related to uremia, chronic blood retention in the dialysis circuit. [38]

Absolute iron deficiency is due to severely reduced or an absent iron store in the liver, spleen, and bone marrow, which diminishes the erythropoiesis and leads to the

development of anemia. Functional iron deficiency describes a state where the total iron content is normal or even elevated, despite adequate iron stores, iron is not available for incorporation into erythroblast. There is an imbalance between the iron supply and the iron required for erythropoiesis, with iron supply not maintained at a sufficient rate for adequate hemoglobinization of reticulocytes and RBC [13].

In AID, there is decrease in the quantity of storage form of iron in the body, where as functional iron deficiency there is reduced mobilization of iron from the iron stores.

Diagnosing an absolute or a FID anemia particularly becomes challenging in patients who have associated inflammatory condition(acute/chronic) because the iron indices/markers are affected by acute phase reaction. This is the cause of anemia in chronic disease. [43]

The main cause of suboptimal response to erythropoietin in dialysis patients is iron deficiency. For adequate treatment, one should ensure adequate iron is available and also iron being mobilized from the stores. [38]

Long-term oral iron therapy has disadvantages such as poor compliance due to dyspepsia, drug interactions, and inadequate absorption. In CKD patients on dialysis who become iron deficient intravenous iron compounds are preferred.

Anemia is highly prevalent in CKD both in dialysis-dependent and non-dependant patients. Iron deficiency is not adequately treated and unrecognized among CKD patients. Majority of anemia in CKD are iron deficient indicating IDA remains under recognized and undertreated.

As already mentioned two major types of iron deficiency anemia are

AID is diagnosed when S.Ferritin <100ng/ml and TSAT <20%.

FID is diagnosed when S.Ferritin >100ng/ml and TSAT <20%.

Absolute Iron Deficiency	Depleted body iron stores • Low serum ferritin <100 ng/ml • TSAT < 20% • Low hepcidin
Functional Iron Deficiency	Inadequate iron supply to meet demand despite normal or abundant iron stores • Normal or high serum ferritin levels • TSAT < 20% • High hepcidin

Figure 16 – Diagnosing Absolute iron deficiency and Functional iron deficiency.

Assessment of iron status remains an integral part of the management of patients with anemia in CKD.

4. Secondary hyperparathyroidism

Occurs due to deficiency of Vitamin D3 and increased levels of inorganic phosphate both of which causes parathyroid hyperplasia and increased production of PTH hormones. There is an increased bone turnover, which leads to the occurrence of bone cysts and fibrosis of the bone marrow which leads to the impairment of the bone marrow and subsequent development of anemia. [44]

5. Hemolysis

Hemolysis can be in association with microangiopathic hemolytic anemia and fragility of RBC due to uremic effect, CKD secondary to SLE. Various tests of hemolysis(TB, LDH, haptoglobin, urine Hb)should be done to diagnose the same. [45]

6. Pure Red cell aplasia

Pure red cell aplasia is a disorder characterized by the maturation arrest of the erythrocytes. Erythroblasts are almost absent in bone marrow; however, it doesn't affect the white blood cells and platelets. The cause remains unknown and is hypothesized to be autoimmune in origin.

Usage of Erythropoietin Stimulating Agents can result in the formation of anti-erythropoietin antibodies leading to pure red cell aplasia and erythropoietin resistance which causes a decline in the hemoglobin, despite continued therapy with EPO at the same or even increased doses. [45]

7. Angiotensin-converting enzyme inhibitors and receptor blockers

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) The reason why Angiotensin-converting enzyme inhibitors lower erythropoietin production or increase its degradation is not known. One hypothesis suggests that ACEIs may lower EPO production by increasing renal blood flow. [46]

Another possibility put forth is angiotensin II regulates sodium absorption in the proximal tubules which determine renal oxygen consumption. As sodium absorption is decreased by Angiotensin-converting enzyme inhibitors, there is a reduction in renal oxygen consumption which may lead to an increased oxygen availability for the EPO producing cells.

Other causes of anemia include

8. Malnutrition,

9. Associated malignancy

10. Hemoglobinopathies.

3.15 Morphological Characteristic Of Anemia

Erythropoietin deficiency, a major cause of anemia in CKD is normochromic and normocytic morphologically. There are different morphological types, indicating that the cause of anemia in CKD is multifactorial. [41]

A study done in India reported that patients with microcytic hypochromic anemia were 60%, 30% had normocytic normochromic anemia while 5 % had macrocytic anemia.

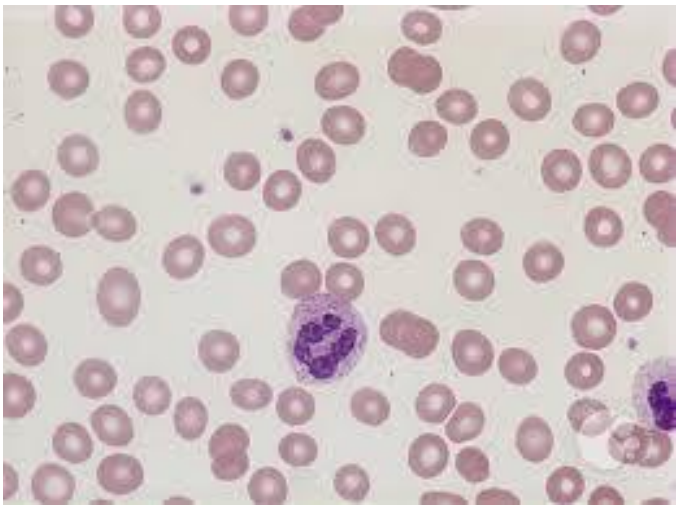


Figure 17 – Normocytic Normochromic Anemia

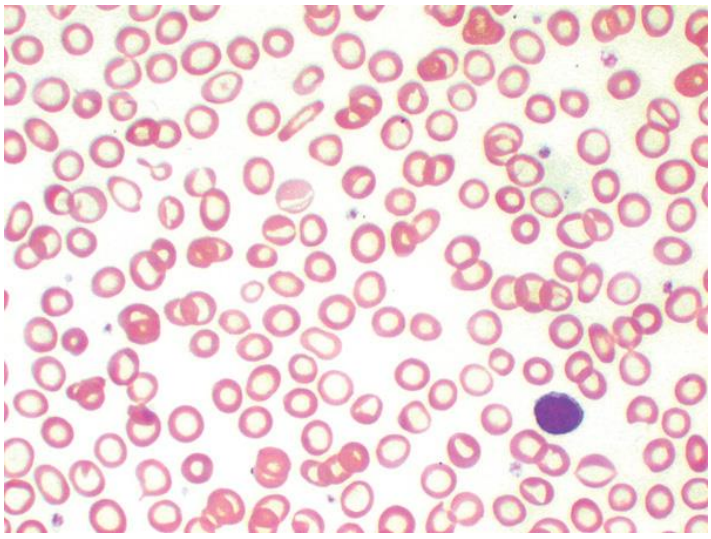


Figure 18 – Microcytic Hypochromic Anemia

3.16 Iron Status Assessment

The amount of iron in the plasma or serum can be assessed in three ways:

1. Total iron content per unit volume in µg/dl
2. Total iron binding capacity, in µg/dl
3. By estimating the percentage of transferrin saturation.

3.16.1 Serum iron

The plasma or serum iron is that part of the iron pool that is bound primarily to transferrin. Transferrin has abundant binding sites so that 100% saturation does not occur, and the typical range of saturation is 35–45%. Methods like ultrafiltration give an estimate of the concentration of non-transferrin bound iron in the plasma pool. About 80% of the iron in plasma is taken up by developing bone marrow erythroblasts. Therefore a change in the rate of red cell synthesis might alter both the rate of turnover of plasma iron and the iron concentration in the plasma. [47]

Iron in the plasma pool exists for a brief period of 40–50 minutes. Therefore the iron concentration changes quickly with every dynamic move of iron from tissue (e.g. enterocytes, reticuloendothelial cells, hepatocytes, others) into the plasma pool as well as the movement of iron out of the plasma pool into tissue (e.g. bone marrow, myocytes, blood-brain barrier, etc.).

Infection and inflammation significantly alter the plasma iron concentration.

The normal plasma iron concentration is 50–120 µg/dl [48,49]. The range for iron deficiency is typically from 50– 60 µg/dl

The amount of plasma iron can be easily measured by a number of ways that are reliable, sensitive, reproducible, and require very small amounts of sample [48,49]. Determinations of plasma or serum iron are based on either colorimetric principles or are made by direct measurement using an instrument such as an atomic absorption spectrophotometer. These methods have been fundamentally unchanged for more than two decades [48,49].

3.16.2 Transferrin, total iron binding capacity, transferrin saturation

Transferrin is a globular, specific transport protein for iron in the plasma pool, and each molecule binds two molecules of iron with similar affinity. It is a carrier, which delivers iron to the cells through the transferrin receptor pathway [49]. The concentration of transferrin varies it increases during iron deficiency and decreases with protein deficiency, so it is sensitive to several factors. The concentration of transport protein will reflect iron status only when iron stores are worn out and the plasma iron concentration is <40–60 µg/dl, so it does not diagnose iron deficiency prior to ineffective erythropoiesis.

A proxy measure of transferrin is the measurement of the total iron binding capacity (TIBC) which applies the assay for plasma iron with one additional step to measure the iron saturation of transferrin. [47]

TIBC is indirect measure of the circulating transferrin.

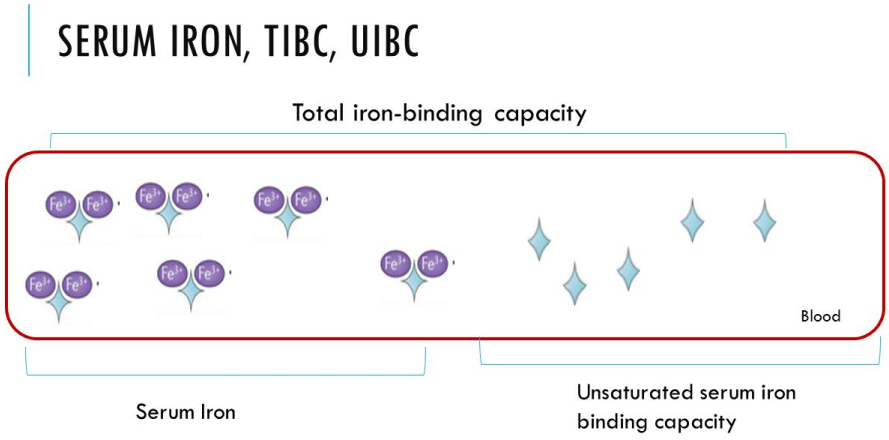


Figure 19 : Serum iron, TIBC, UIBC

The concentration of transferrin is often estimated by measuring the total iron binding capacity, it is susceptible to the same problems as measuring the serum iron concentration.

The assay is really a measure of the total number of transferrin binding sites per unit volume of serum or plasma and is performed much like the serum iron assay.

The TIBC is not subjective to rapid changes in concentration as the plasma iron concentration, so it is more stable as an indicator of iron status than serum Iron. The TIBC by itself is not often used as a measure of iron status because it appears not to change until iron stores are depleted [(49).] Normal range of TIBC is 300-360mcg/dl.

Transferrin saturation reflects the current iron supply to the tissues.

Transferrin saturation is expressed as a percentage of serum iron by TIBC i.e serum iron/TIBC x 100. Transferrin saturation (TSAT) of <15% is insufficient to meet normal daily requirements for erythropoiesis [(49).] A prolonged period of time with a TSAT below 15% results in iron-deficient erythropoiesis .

The TSAT itself shows a very little variation either within subjects or from day to day. Anything that alters the plasma iron concentration will alter the TSAT, thus there is the same lack of specificity for TSAT as there is for plasma iron concentration.

3.16.3 Serum Ferritin

The important function of ferritin is to provide a store of iron which may be used for haem synthesis when required. Iron uptake in vitro requires an oxidizing agent, and iron release requires a reducing agent [38].

Body iron is primarily stored in the form of ferritin, which carries about 4000–4500 iron atoms. Serum ferritin concentration positively correlates with total body iron levels if acute inflammation is absent. Its measurement specifically represents body iron level.

The usefulness of serum ferritin as an iron marker in the management of anemia of CKD is currently the subject of debate. When evaluating iron status tests, consider the following:

Serum ferritin is an acute-phase reactant [50]

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 iron deficiency indicator. [50]

Reduced levels of serum ferritin or transferrin saturation (TSAT) are present in most patients with CKD. A normal or high serum ferritin will not rule out iron deficiency or indicate adequate or excessive iron levels [50]

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.

3.17 Alternative and novel iron biomarkers

TSAT and serum ferritin are commonly used iron biomarkers, other tests may provide more accurate assessments of iron status. These include reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR) and percentage of hypochromic red cells (PHRC).

CHr measures hemoglobin content of reticulocytes (newly formed red blood cells (RBCs)). In a review article, Jay B. Wish explains that CHr provides a "snapshot of how much iron was available for RBC production in a clinically relevant timeframe."[43]

PHRC estimates the hemoglobin concentration rather than hemoglobin content in erythrocytes. RBCs size and absolute amount of hemoglobin are taken into account as well. The disadvantage in using this test is that erythrocytes tend to expand during storage. Most of the hospitals store blood samples for a considerable time until they are analyzed. [43]

The **sTfR** test is based on the principle that when there is insufficient iron for erythropoiesis and it is being stimulated by an ESA, then increased transferrin receptors will become expressed on the erythroblasts, which comes off partly and is detectable in the circulation. [43]

Hepcidin

Hepcidin may be a novel biomarker of erythropoietin resistance and iron status, it may contribute to abnormal iron regulation and erythropoiesis.

Even in the absence of anemia, it's a sensitive indicator of iron deficiency. When compared to hematocrit or hemoglobin, reduction in hepcidin is an early marker of iron deficiency along with transferrin saturation and reduced ferritin."[51]

But at present, the measurement of hepcidin is erratic and does not give any diagnostic value over ferritin and other available iron studies." [52]

NGAL

Plasma neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of acute kidney injury has a potential use to assess iron status in CKD patients. Plasma NGAL is independently associated with TSAT and is superior to serum ferritin in both sensitivity and specificity in identifying an iron deficiency in CKD patients. [53]

3.18 According to KDIGO frequency of testing for anemia is:

1. For CKD patients without anemia, measure Hb concentration: [1]

- at least annually in patients with CKD 3
- at least 6 monthly in patients with CKD 4–5
- at least once in 3 months in patients with CKD 5 hemodialysis and CKD 5 peritoneal dialysis.

2. For CKD patients with anemia not being treated with an ESA, measure Hb concentration: (1)

- at least every 3 monthly in patients with CKD 3–5ND and CKD 5PD

- at least monthly in patients with CKD 5HD

3. Measurement of Hemoglobin concentration in patients being treated with ESA. [1]

- During the initiation phase of ESA therapy, measure Hb concentration at least monthly.
- For CKD ND (non dialysis) patients, during the maintenance phase of ESA therapy measure Hb concentration at least every 3 months.
- For CKD 5D patients, during the maintenance phase of ESA therapy measure Hb concentration at least monthly.

3.19 Investigations for anemia

In patients with CKD and anemia (regardless of age and CKD stage), the following tests are recommended by KDIGO for initial evaluation of the anemia: [1]

- Complete blood count (CBC), which should include Hb concentration, red cell indices, white blood cell count and differential, and platelet count
- Absolute reticulocyte count
- Serum ferritin level
- Serum transferrin saturation (TSAT)
- Serum vitamin B12 and folate levels

3.20 Treatment Of Iron Deficiency Anemia

While on treatment with iron therapy, a balance of potential benefits (avoiding or minimizing ESA therapy, blood transfusions, and anemia related symptoms) versus

the risks of harm in individual patients. (e.g., anaphylactoid and other acute reactions, unknown long-term risks) and exclusion of active infection. [1]

1. When the patient isn't on any ESA or iron therapy, a 1-3 month trial of IV iron (CKD dialysis) or oral iron therapy(CKD - ND) is recommended. An increase in Hb concentration without starting ESA treatment, TSAT is ≤30% and ferritin is ≤500 ng/ml is desired.

2. CKD patients already on ESA therapy not receiving iron supplementation, a 1-3 month trial of IV iron (CKD dialysis) or oral iron therapy (CKD - ND) is recommended.

   Again an increase in Hb concentration or a decrease in ESA dose is desired along with TSAT of ≤30% and ferritin of ≤500 ng/ml.

3. CKD patients not on dialysis who require iron supplementation route of iron administration are selected based on the severity of iron deficiency anemia, prior response to oral iron therapy, availability of venous access, patient compliance, cost, and side effects with prior oral or IV iron therapy.

Subsequent iron administration in CKD patients is based on Hb responses to recent iron therapy, ongoing blood losses, TSAT, serum ferritin, Hb concentration, ESA responsiveness and dose in ESA treated patients and the patient's clinical status.

Iron monitoring monthly is advised initially until iron stores are replenished, then monitored quarterly.

Despite its benefits, ESA therapy is associated with an increased risk of adverse effects such as hypertension, RBC aplasia, cardiovascular (CV) complications, and development of anti-erythropoietin antibodies.

IV iron as an adjunct to ESA therapy has become standard treatment for optimizing Hb status and reducing ESA dosing requirements.

A reduction of 70% in ESA dosage has been noted in patients receiving IV iron. [54, 55].

Despite that, iron underutilization has been reported in anemic CKD patients receiving ESA therapy [56]. IV supplementation is preferred in HD-dependent patients as they have limited absorption of oral iron. [57]

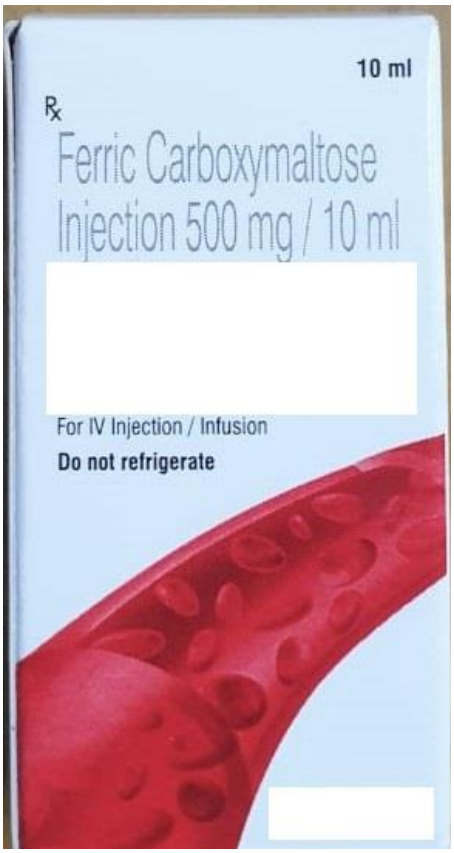


Figure 20 – IV iron supplementation

Figure 21 – patient receiving IV iron preparation

Figure 22 – erythropoietin stimulating agents - Darbepoetin

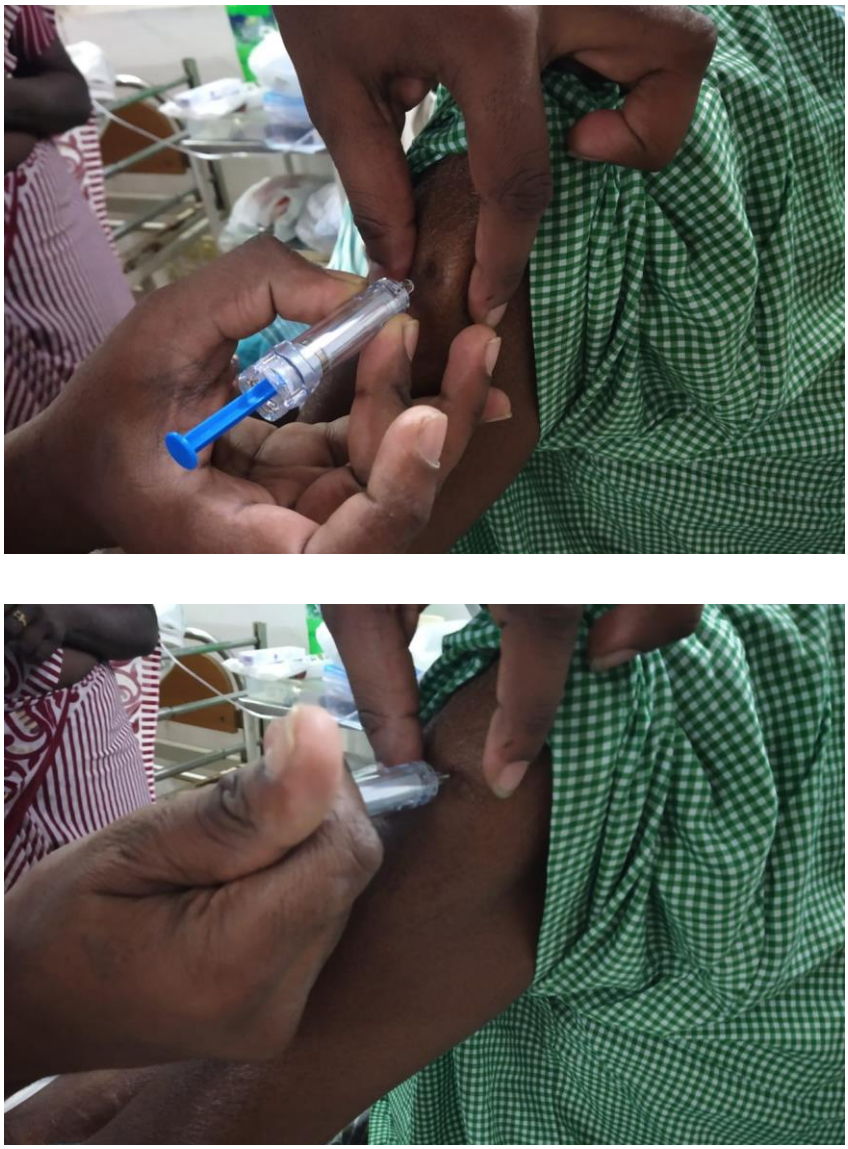


Figure 23 – patient receiving ESAs - darbepoetin

4. MATERIAL AND METHODS:

4.1 Source Of Data:

The study included 70 CKD patients who presented to RLJ Hospital Kolar attached to SDUAHER during February 2017 - may 2018.

4.2 Inclusion Criteria:

Patients aged 18 years and above who met the definition criteria of CKD were included in the study.

4.3 Exclusion Criteria:

- Clinical evidence of infection
- Previous history of blood transfusion
- Pregnant women
- Acute kidney injury.
- Severely moribund patients.
- Clinical evidence of liver disease.

4.4 Methods:

Patients were only included in this study after giving verbal and written consent. Patient confidentiality was maintained. And were informed about the study, procedures involved, relative risks, and benefits of the study.

A detailed history from the study participants was taken. A meticulous examination was done. 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 be iron deficient received appropriate treatment and dietary advice were given and were referred to a nephrologist for further care if required.

4.5 Sampling Procedure

Nephrology division at R.L.Jalappa hospital offers clinical expertise for management of CKD patients and renal replacement therapy in form of hemodialysis to patients attending OPD and who are admitted in wards. Nephrology OPD operated clinic every Thursday per week. On an average 10-15 patients attend the nephrology unit per week, of those at least 2-5 patients had CKD.

Consecutive recruitment (total enumerative sampling) of study participants was used. Investigator attended nephrology clinic and wards on respective days targeting to recruit sampled CKD patients per day because of small CKD patient population.

4.6 Specimen Collection And Processing

Blood specimen 10ml was collected from a peripheral vein (antecubital venipuncture). The area 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 10 ml 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 transferred into a Bijou (sample) bottle. The specimen that would not be assayed within 24hours due to logistic problems was frozen at -20oC until time for analysis.

The following laboratory tests were done:

1. Serum creatinine
2. Blood urea
3. CBC
4. LFT
5. SERUM IRON INDICES. (Serum iron, Serum Ferritin, transferrin saturation(TSAT), TIBC
6. USG KUB
7. Urine routine

Serum iron indices i.e. especially Ferritin and transferrin saturation (TSAT) were estimated amongst these patients.

4.7 Analytical Methods

1. Measurement of Serum Iron

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

2. Measurement of Serum Total Iron Binding capacity (TIBC)

Serum transferrin is saturated with an excess of Fe^{3+} and the unbound portion is precipitated with magnesium carbonate. The total amount of iron is then determined. Unsaturated iron-binding capacity is a difference in total iron binding capacity (TIBC) and serum iron.

Figure 24 – Estimation of serum iron and TIBC

3. Measurement of Serum Ferritin

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. At the same time, the sample is allowed to react with the antibodies which result in the ferritin being sandwiched between the solid phase and enzyme-linked antibodies. The color is yellow, which is measured spectrophotometrically at 450 nm. The intensity of the color in the test sample gives the concentration of ferritin.

Figure 25 – Estimation of serum Ferritin.

4. TSAT

TSAT = serum iron ÷ TIBC × 100

5. Creatinine

Creatinine in alkaline medium reacts with picric acid (jaffes reaction), to give a red color (creatinine picrate), whose intensity is measured using spectrophotometry at 520nm.

For reliable test results, regardless of the assay:

- Contamination with iron from needles and plastic-ware was prevented during the collection of the samples.
- Blood sampling protocols were standardized to avoid post-prandial effects.
- Appropriate laboratory techniques were used to minimize contamination with iron from equipment and the environment;

- A high level of competency was required to ensure good laboratory practices and reproducible results.

Total Percentage of patients having iron deficiency was determined.

Further, the percentage of patients having functional, absolute iron deficiency and normal stores was estimated.

4.8 Study Design:

It is an observational Prospective study, in which 70 patients were included.

4.9 Sample size:

The minimum sample size using the formula was calculated.

$N = Z^2P(1-P) / d^2$

N = Minimum sample size

Z = standard normal deviate corresponding to two sided specified significant level will be 2.58(at 99% confidence interval)

D=margin of error (precision) will be 15 %(0.15)

P=proportional of patients

Considering 10% non response a sample size of 60+10=70

4.10 Statistical analysis:

All questionnaires were checked daily for completeness by the investigator and pre-coded data were entered into the computer.

Data were entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Frequencies and proportions were represented in the form of Categorical data. Chi-square test was used for the test of significance for qualitative data. Mean and the standard deviation was represented as continuous data. ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups for quantitative data.

Graphical representation of data: MS Excel and MS Word were used to obtain various types of graphs such as bar diagram, Pie diagram.

p-value (Probability that the result is true) of <0.05 was considered statistically significant after assuming all the rules of statistical tests.

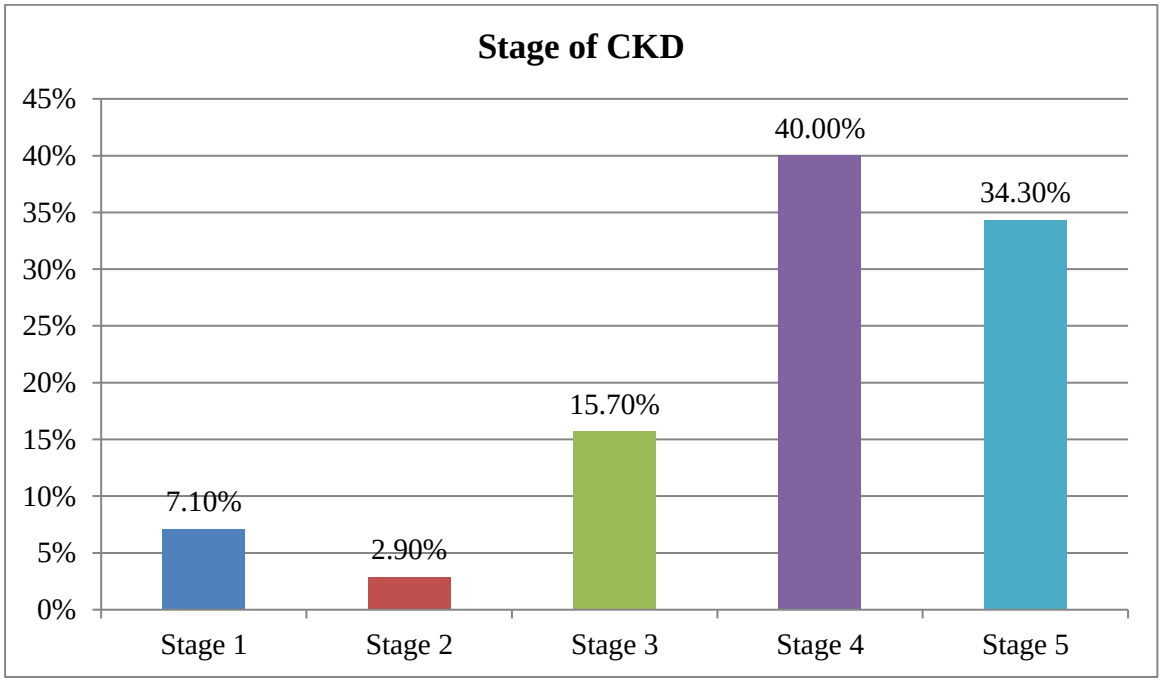
Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data. [58-61]

5. RESULTS

Table 2: CKD Stages in the study

		Count	%
Stage	Stage 1	5	7.1%
	Stage 2	2	2.9%
	Stage 3	11	15.7%
	Stage 4	28	40.0%
	Stage 5	24	34.3%

In our study, 7.1% had Stage 1 CKD, 2.9% had Stage 2 CKD, 15.7% had Stage 3 CKD, 40% had Stage 4 CKD and 34.3% had Stage 5 or ESKD.

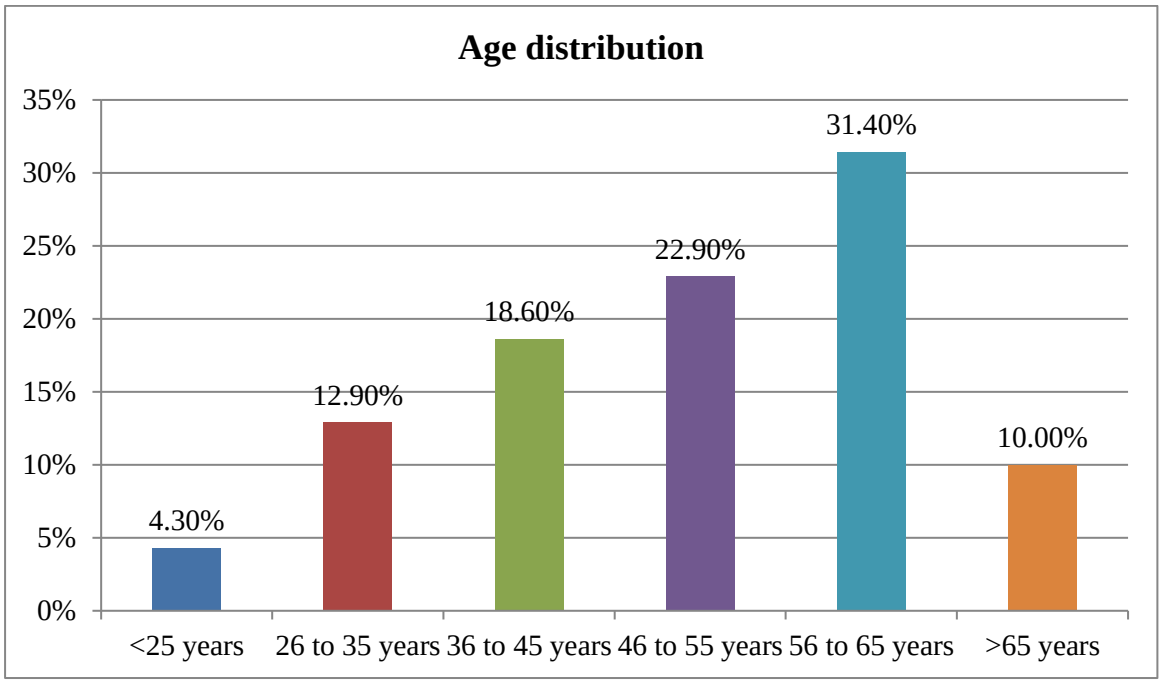


Graph 1: Bar diagram showing CKD Stages in the study

Table 3: Age distribution in the study

		Count	%
Age	<25 years	3	4.3%
	26 to 35 years	9	12.9%
	36 to 45 years	13	18.6%
	46 to 55 years	16	22.9%
	56 to 65 years	22	31.4%
	>65 years	7	10.0%

In this study, 4.3% were in the age group of <25 years, 12.9% were in the age group of 26 to 35 years, 18.6% were in the age group of 36 to 45 years, 22.9% were in the age group of 46 to 55 years, 31.4% were in the age group of 56 to 65 years and 10% were in the age group of >65 years.

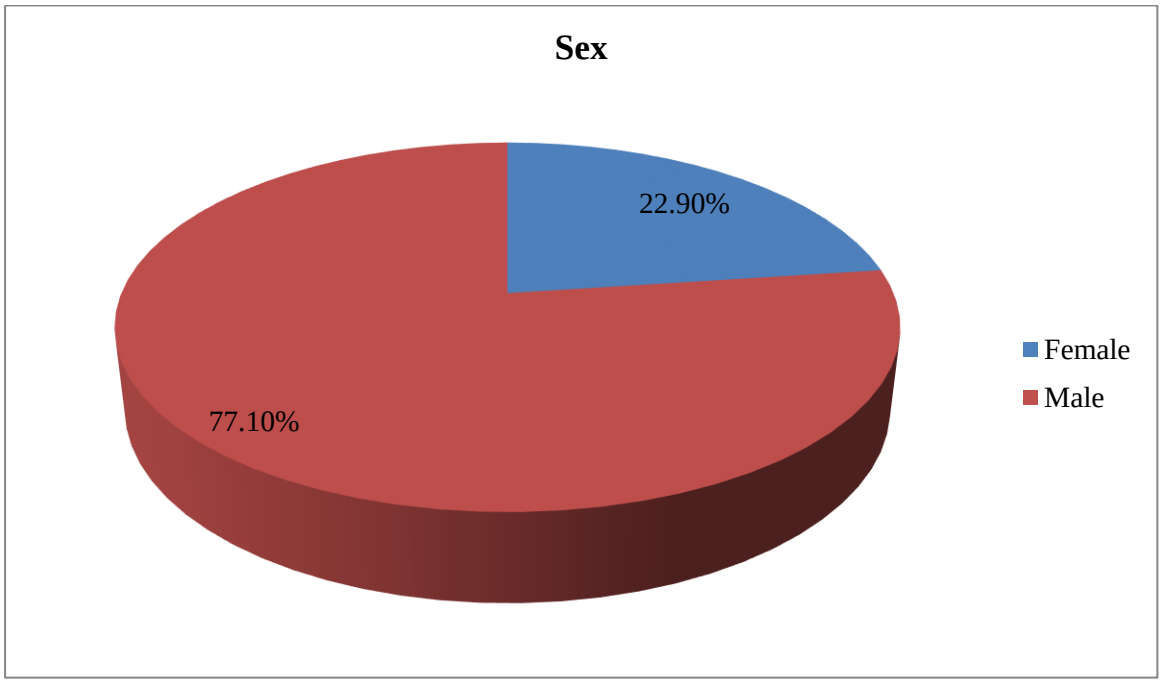


Graph 2: Bar diagram showing Age distribution in the study

Table 4: Sex distribution in the study

		Count	%
Sex	Female	16	22.9%
	Male	54	77.1%

In our study, 77.1% were males and 22.9% were females.

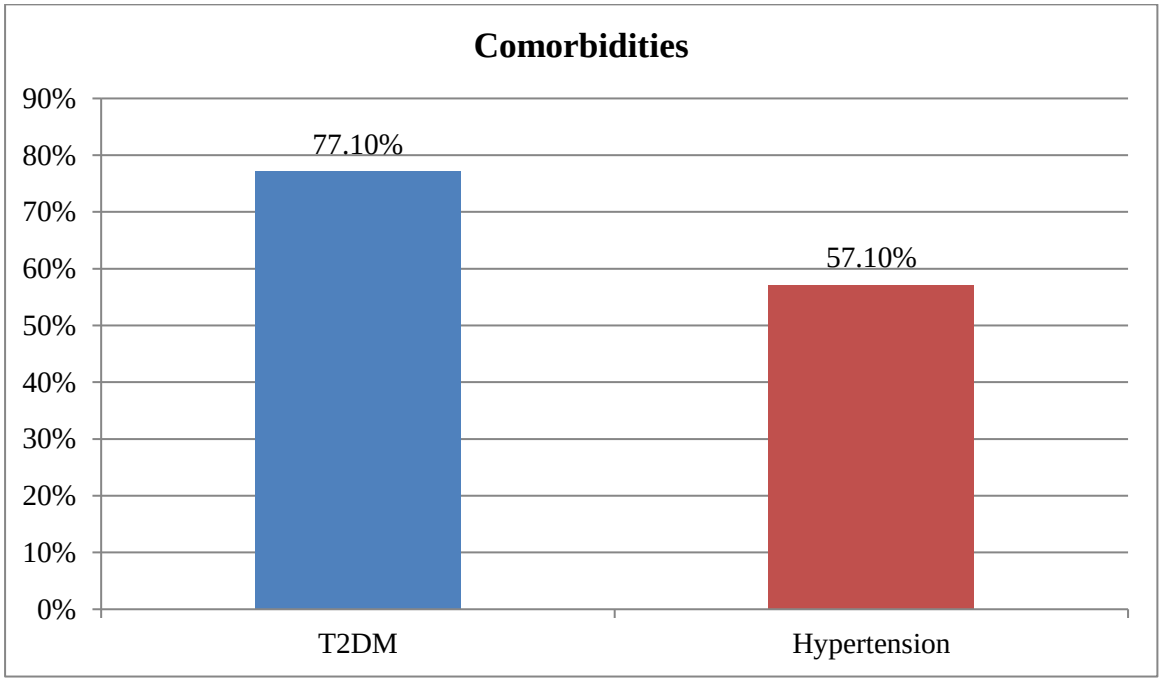


Graph 3: Pie diagram showing Sex distribution in the study

Table 5: Comorbidities amongst the subjects

		Count	%
T2DM	No	16	22.9%
	Yes	54	77.1%
Hypertension	No	30	42.9%
	Yes	40	57.1%

In the study 77.1% had Diabetes and 57.1% had HTN.

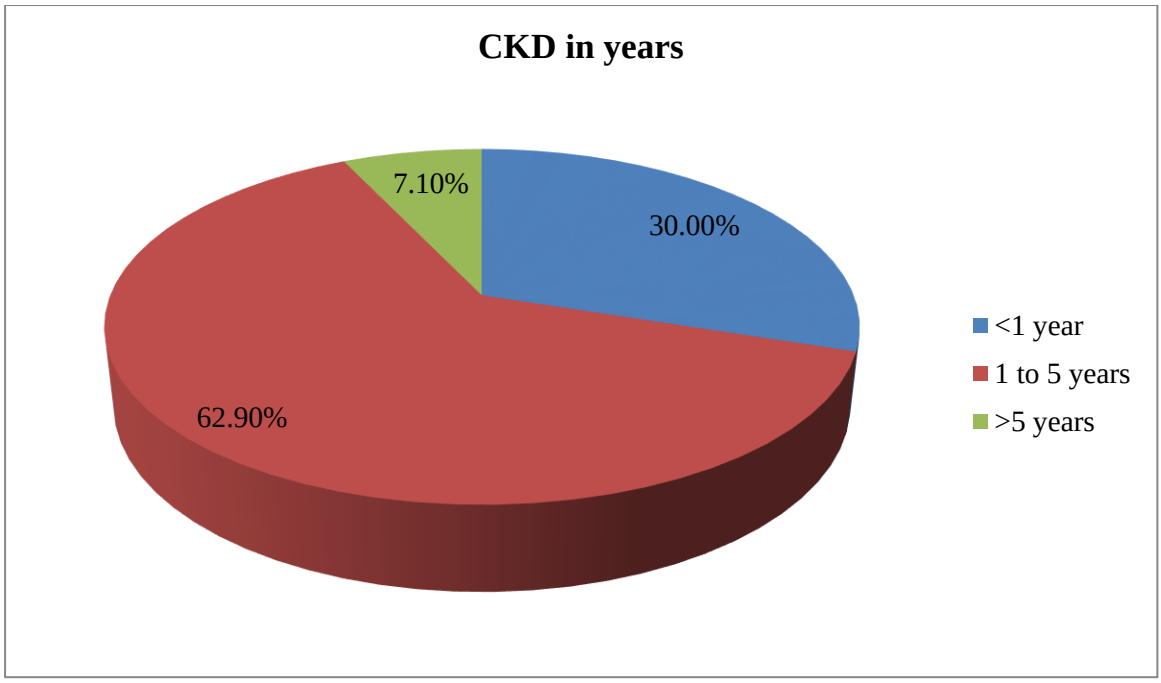


Graph 4: Bar diagram showing comorbidities amongst the subjects

Table 6: Duration of CKD among subjects

		Count	%
CKD in years	<1 year	21	30.0%
	1 to 5 years	44	62.9%
	>5 years	5	7.1%

In the study 30% had CKD for <1 year, 62.9% had CKD for 1 to 5 years and 7.1% had CKD for >5 years.

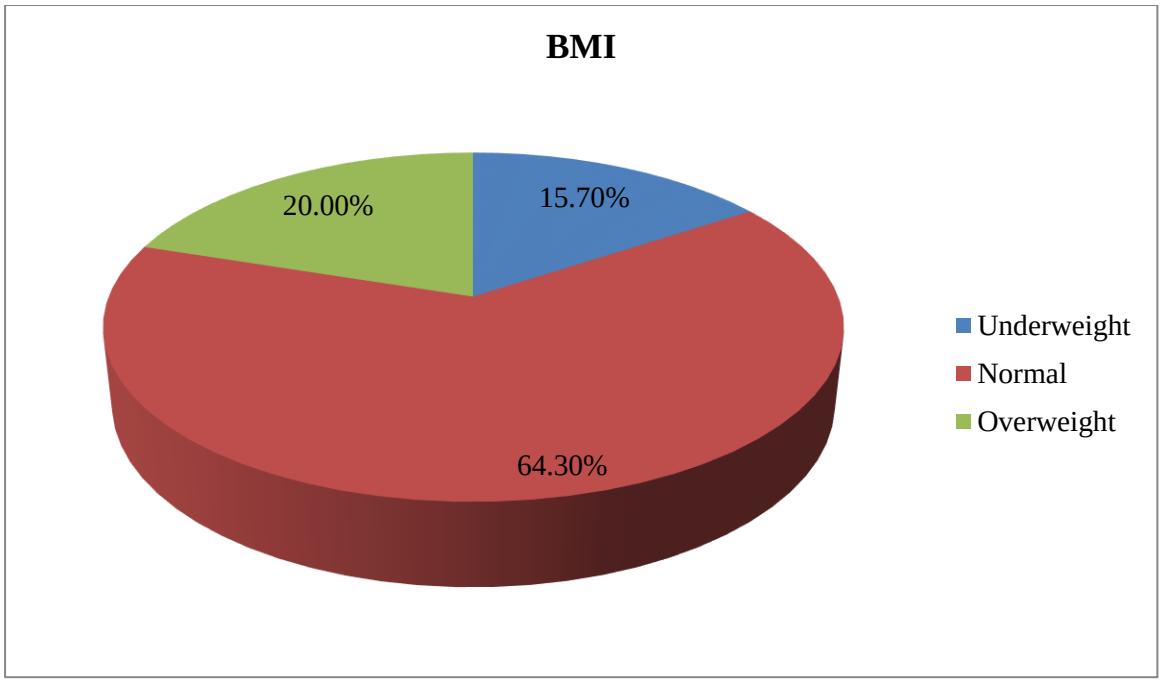


Graph 5: Pie diagram showing Duration of CKD among subjects

Table 7: BMI distribution among subjects

		Count	%
BMI	Underweight	11	15.7%
	Normal	45	64.3%
	Overweight	14	20.0%

In the study 15.7% were underweight, 64.3% had Normal BMI and 20% were overweight.



Graph 6: Pie diagram showing BMI distribution among subjects

Table 8: Hb, Urea, Creatinine and Iron Indices distribution amongst the subjects

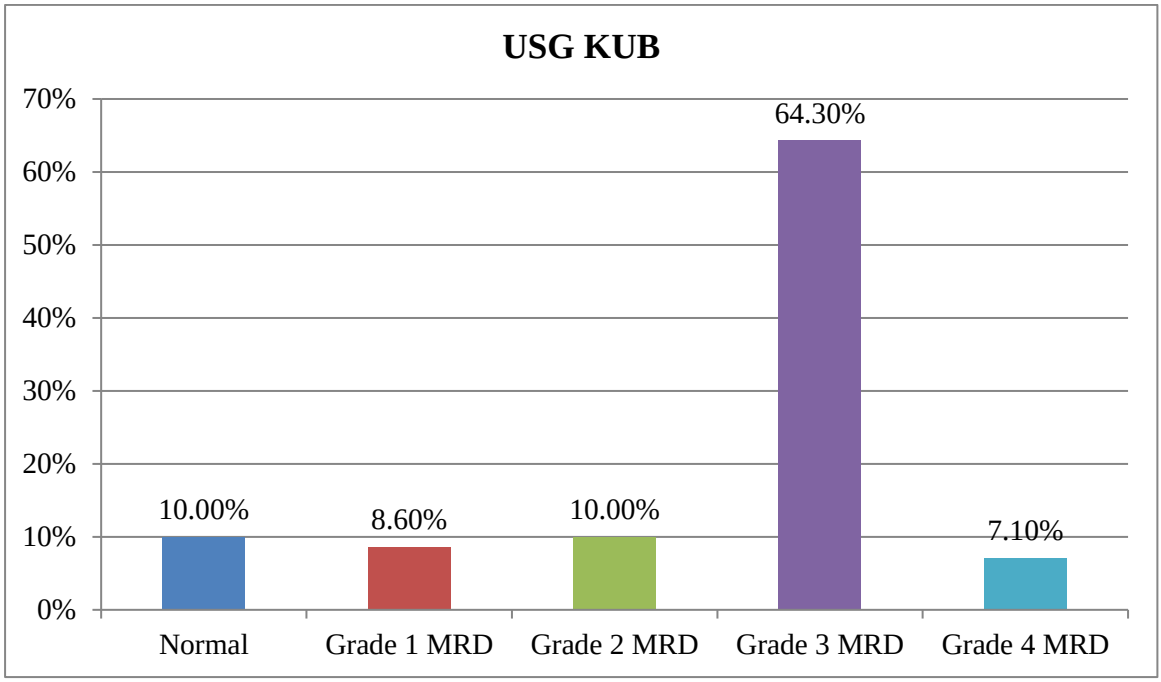
	Mean	SD	Minimum	Maximum	Median
Hb	9.36	2.53	4.60	14.50	8.90
Blood Urea	77.01	34.60	16.00	198.00	76.00
Serum Creatinine	3.37	1.53	.60	7.90	3.55
S. Iron	64.40	29.10	12.00	150.00	67.00
TIBC	309.36	43.29	254.00	421.00	299.50
S. Ferritin	142.02	71.38	13.00	345.00	124.00
Transferrin saturation	21.23	9.54	3.18	49.67	21.93

In the study mean Hb was 9.36 ± 2.53 gm%, mean blood urea was 77.01 ± 34.60, mean Serum Creatinine was 3.37 ± 1.53 mg/dl, mean Serum Iron was 64.40 ± 29.10, mean TIBC was 309.36 ± 43.29, mean Serum Ferritin was 142.02 ± 71.38 and mean Transferrin saturation was 21.23 ± 9.54.

Table 9: USG KUB findings among subjects

		Count	%
USG KUB	Normal	7	10.0%
	Grade 1 MRD	6	8.6%
	Grade 2 MRD	7	10.0%
	Grade 3 MRD	45	64.3%
	Grade 4 MRD	5	7.1%

In the study 10% were normal, 8.6% had Grade 1 MRD, 10% had Grade 2 MRD, 64.3% had Grade 3 MRD and 7.1% had Grade 4 MRD.

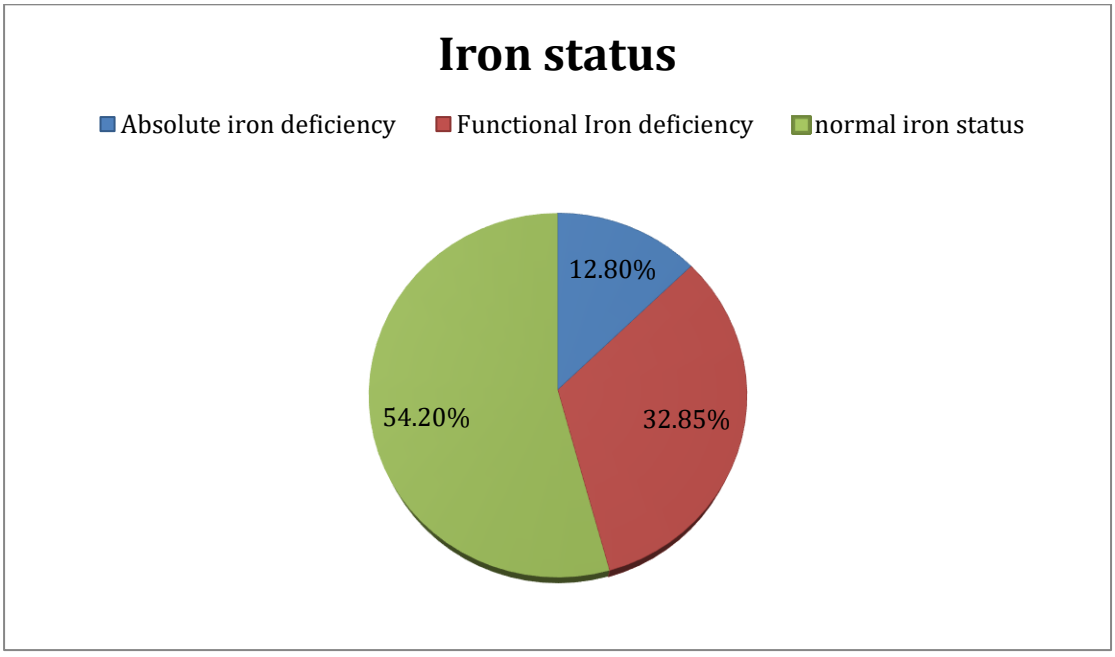


Graph 7: Bar diagram showing USG KUB findings among subjects

Table 10: Iron Status amongst the subjects

		Count	%
Iron status	Absolute iron deficiency	09	12.8%
	Functional iron deficiency	23	32.85%
	Normal iron status	38	54.2%

In the study 12.8% had an absolute iron deficiency, 32.85% had a functional iron deficiency and 54.2% had normal iron status.

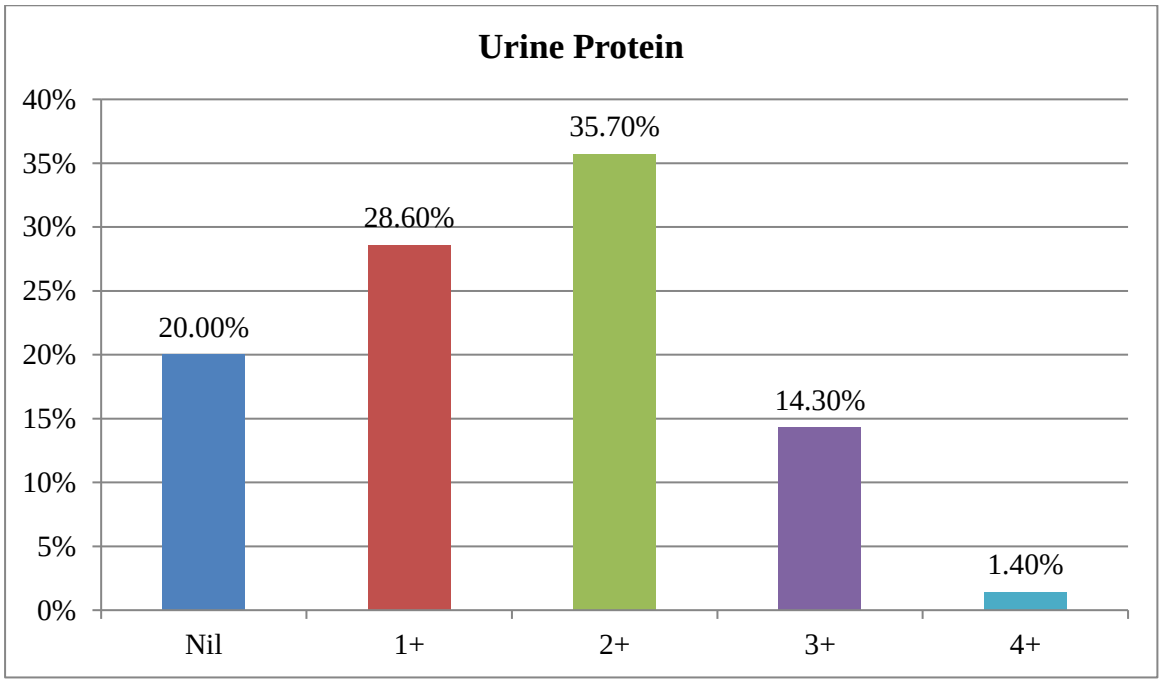


Graph 8: Pie diagram showing Iron Status amongst the subjects

Table 11: Proteinuria amongst the subjects

		Count	%
Urine Protein	Nil	14	20.0%
	1+	20	28.6%
	2+	25	35.7%
	3+	10	14.3%
	4+	1	1.4%

In the study 20% had no protein in urine, 28.6% had 1+ Proteinuria, 35.7% had 2+ Proteinuria, 14.3% had 3+ Proteinuria and 1.4% had 4+ Proteinuria.



Graph 9: Bar diagram showing the degree of Proteinuria in the study

Table 12: LFT findings in the study

	Mean	SD
Total Bilirubin	0.60	0.32
Direct Bilirubin	0.09	0.07
AST	28.06	9.89
ALT	43.39	12.94
Alkaline Phosphatase	75.99	28.07
Total Protein	6.85	0.43
Albumin	3.97	0.35
Globulin	2.88	0.61
Albumin Globulin Ratio	1.48	0.49
GGT	63.00	17.30

In the study mean Total Bilirubin was 0.60 ± 0.32,

Mean Direct bilirubin was 0.09 ± 0.07,

Mean AST was 28.06 ± 9.89

Mean ALT was 43.39 ± 12.94

Mean Alkaline Phosphatase was 75.99 ± 28.07

Mean Total Protein was 6.85 ± 0.43

Mean Albumin was 3.97 ± 0.35

Mean Globulin was 2.88 ± 0.61

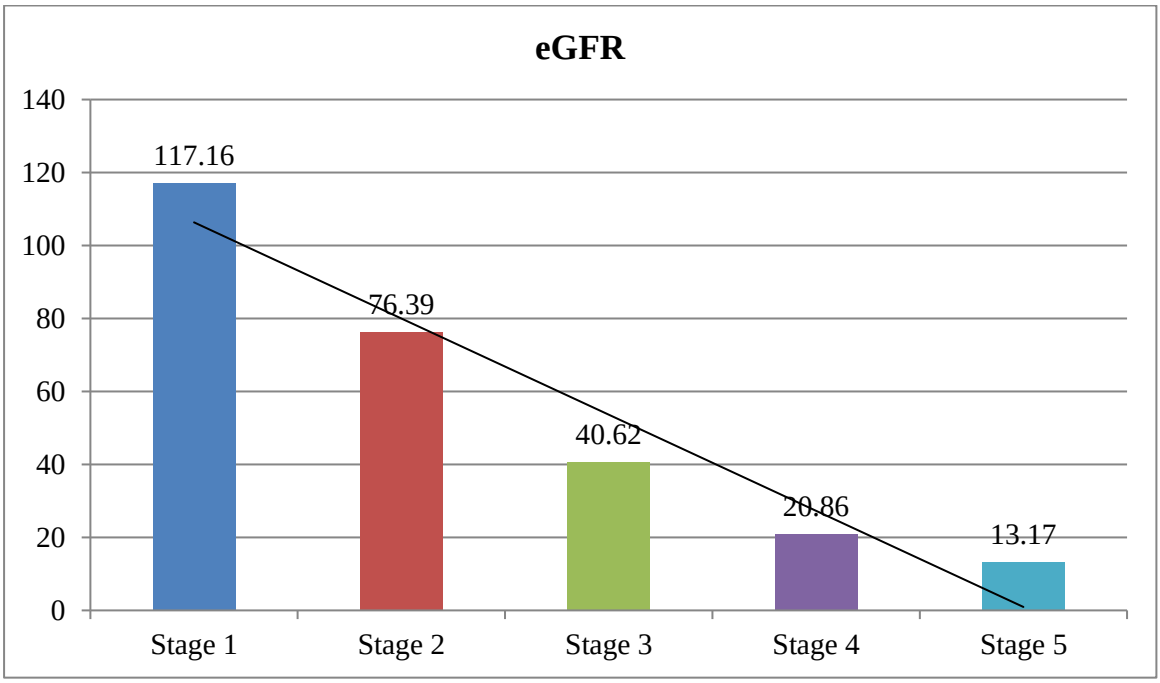
Mean Albumin Globulin Ratio was 1.48 ± 0.49

Mean GGT was 63.00 ± 17.30

Table 13: eGFR in comparison to CKD Stage

		e GFR		P value
		Mean	SD	
Stage	Stage 1	117.16	21.97	<0.001*
	Stage 2	76.39	15.81	
	Stage 3	40.62	5.23	
	Stage 4	20.86	4.80	
	Stage 5	13.17	2.63	

In the study mean eGFR in stage 1 was 117.16 ± 21.97, in Stage 2 was 76.39 ± 15.81, in Stage 3 was 40.62 ± 5.23, in Stage 4 was 20.86 ± 4.80 and in Stage 5 was 13.17 ± 2.63. There was a significant difference in mean eGFR with the stage of CKD. With the increase in Stage of CKD, there was a decrease in eGFR.

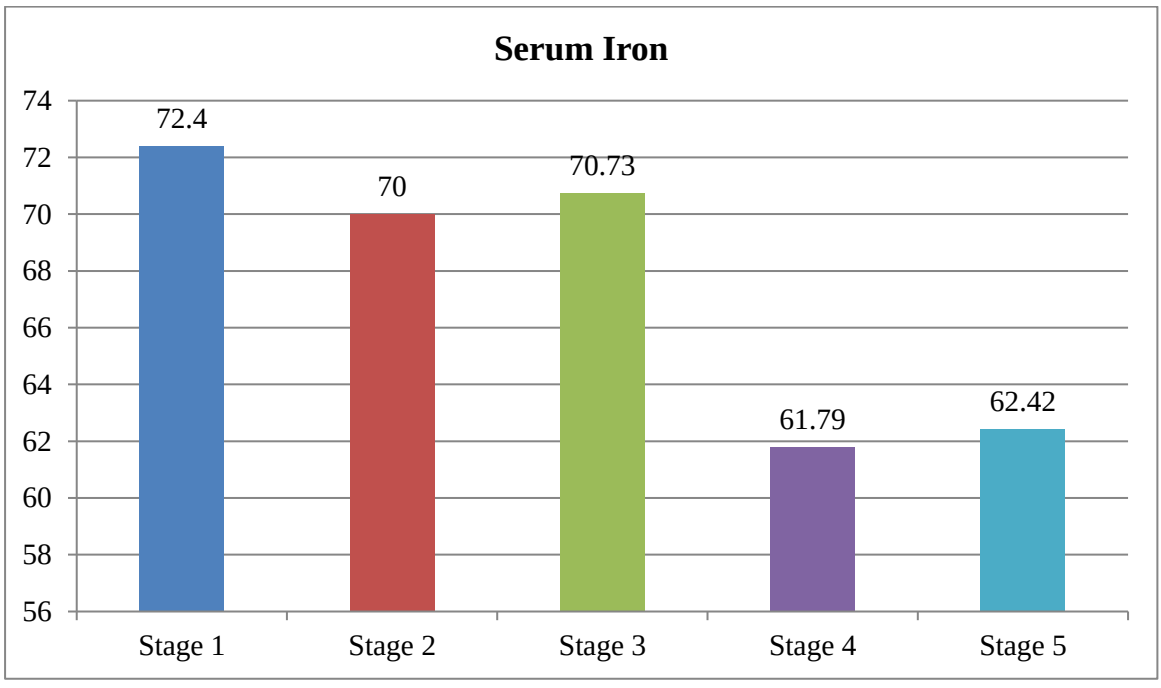


Graph 10: Bar diagram showing eGFR comparison with respect to Stage of CKD

Table 14: Serum Iron comparison with respect to Stage of CKD

		S. Iron		P value
		Mean	SD	
Stage	Stage 1	72.40	9.32	0.867
	Stage 2	70.00	.00	
	Stage 3	70.73	25.45	
	Stage 4	61.79	33.47	
	Stage 5	62.42	29.67	

In the study, there was no significant difference in mean Serum Iron with respect to stage of CKD.

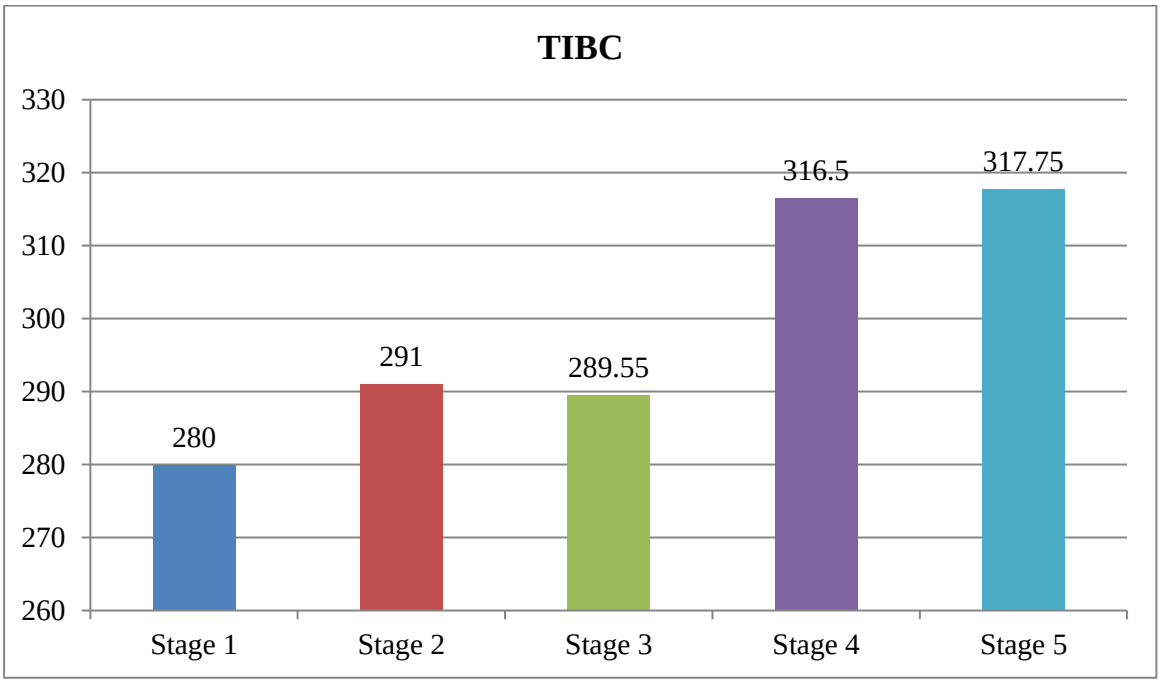


Graph 11: Bar diagram showing Serum Iron comparison with respect to Stage of CKD

Table 15: TIBC comparison with respect to Stage of CKD

		TIBC		P value
		Mean	SD	
Stage	Stage 1	280.00	17.96	0.155
	Stage 2	291.00	15.56	
	Stage 3	289.55	25.21	
	Stage 4	316.50	49.17	
	Stage 5	317.75	43.60	

In the study there was no significant difference in mean TIBC with respect to stage of CKD.

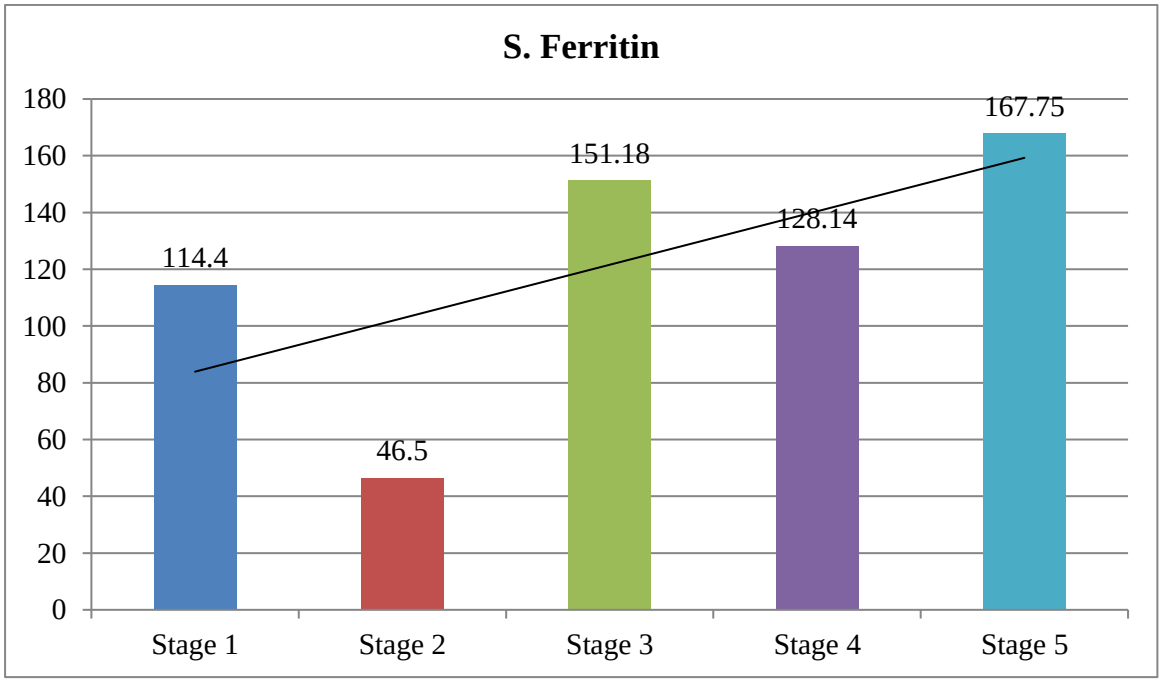


Graph 12: Bar diagram showing TIBC comparison with respect to Stage of CKD

Table 16: Serum Ferritin comparison with respect to Stage of CKD

		S. Ferritin		P value
		Mean	SD	
Stage	Stage 1	114.40	34.53	0.002*
	Stage 2	46.50	2.12	
	Stage 3	151.18	63.07	
	Stage 4	128.14	69.11	
	Stage 5	167.75	76.28	

In the study mean **Serum Ferritin** in stage 1 was 114.40 ± 34.53, in Stage 2 was 46.50 ± 2.12, in Stage 3 was 151.18 ± 63.07, in Stage 4 was 128.14 ± 69.11 and in Stage 5 was 167.75 ± 76.28. There was a significant difference in mean Serum Ferritin with the stage of CKD.

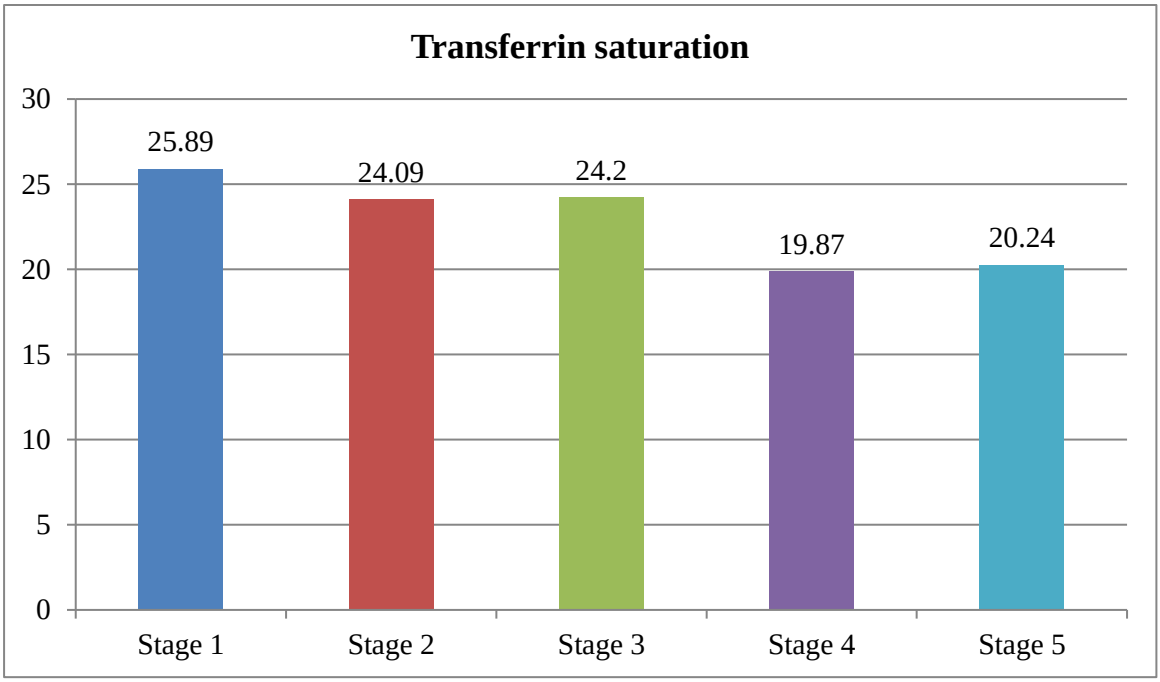


Graph 13: Bar diagram showing Serum Ferritin comparison with respect to Stage of CKD

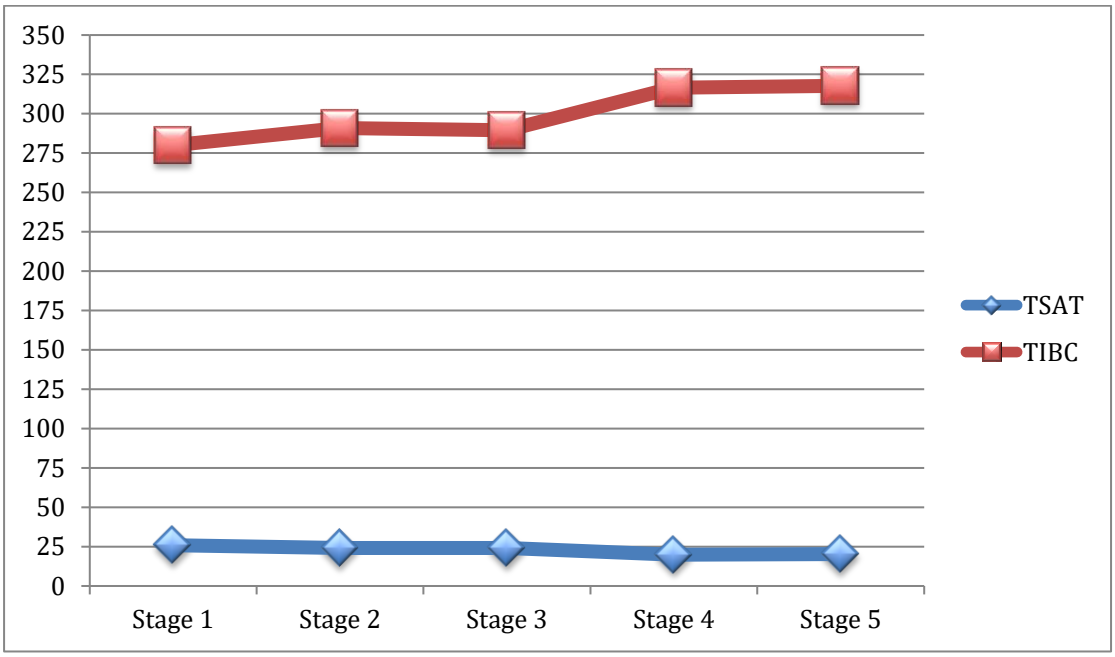
Table 17: Serum Transferrin saturation comparison with respect to Stage of CKD

		Transferrin saturation		P value
		Mean	SD	
Stage	Stage 1	25.89	3.16	0.525
	Stage 2	24.09	1.29	
	Stage 3	24.20	7.47	
	Stage 4	19.87	10.63	
	Stage 5	20.24	10.08	

In the study there was no significant difference in mean Transferrin saturation with respect to stage of CKD.

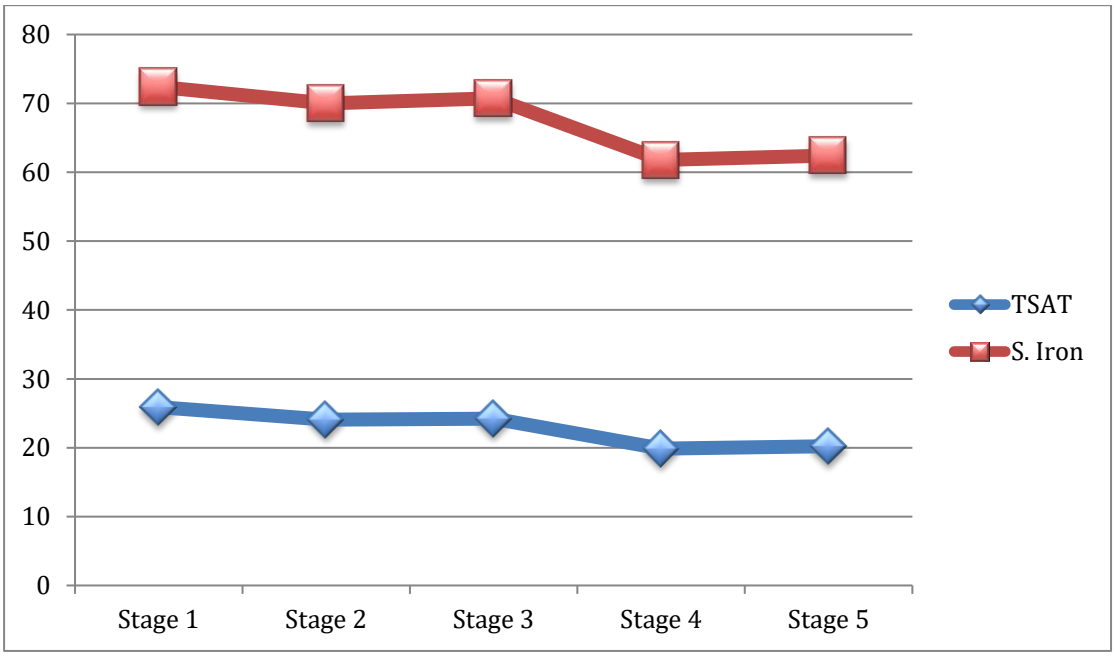


Graph 14: Bar diagram showing Serum Transferrin saturation comparison with respect to Stage of CKD



Graph 15: Line diagram showing correlation between TIBC and TSAT

In the study, there was a negative correlation between the TSAT and TIBC. As the TIBC increased, TSAT decreased



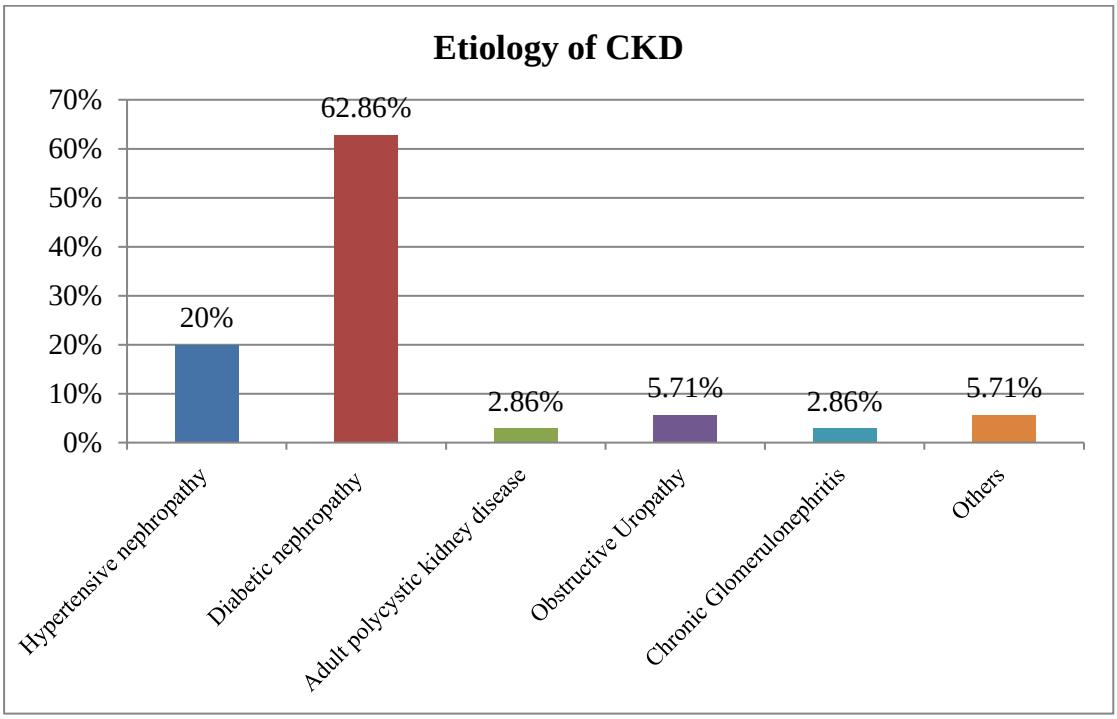
Graph 16: Line diagram showing correlation between Serum Iron and TSAT

There was a positive correlation between the TSAT and Serum Iron. Both Serum Iron and TSAT decreased in the study.

Table 18: Etiology of CKD

Etiological factors	Count	%
Hypertensive nephropathy	14	20%
Diabetic nephropathy	44	62.86%
Adult polycystic kidney disease	2	2.86%
Obstructive Uropathy	4	5.71%
Chronic Glomerulonephritis	2	2.86%
Others	4	5.71%

In the study 20% had Hypertensive nephropathy, 62.86% had Diabetic nephropathy, 2.86% had Adult polycystic kidney disease, 5.71% had Obstructive Uropathy, 2.86% had Chronic Glomerulonephritis and 5.71% had others.

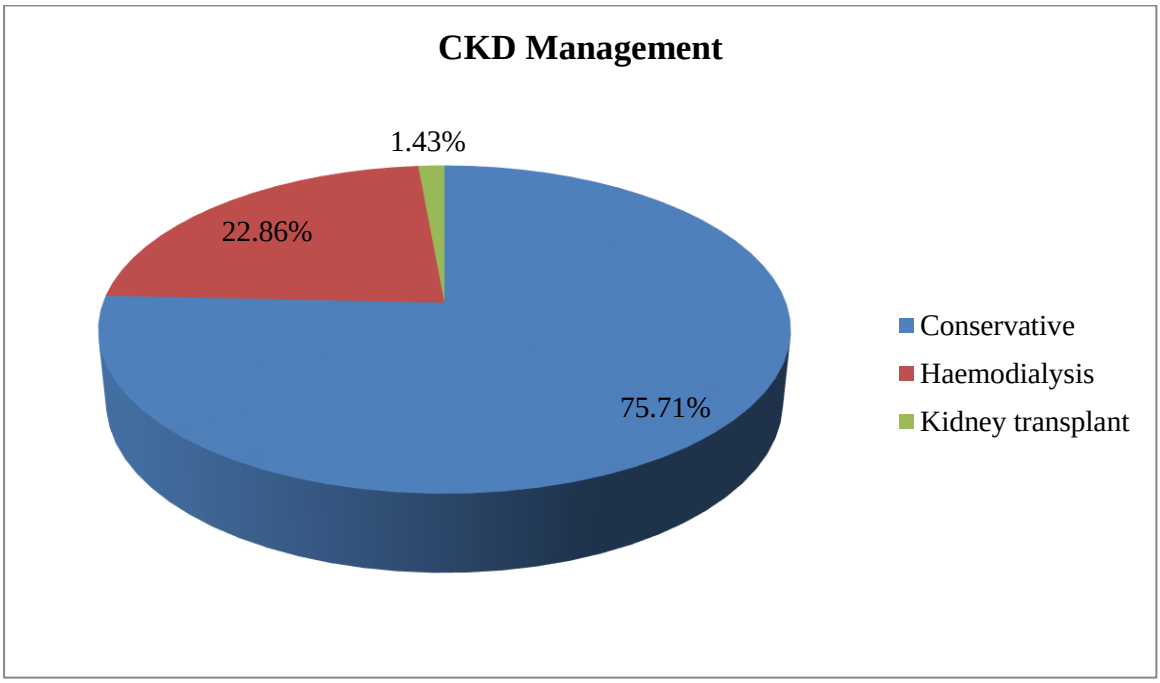


Graph 17: Bar diagram showing Etiology of CKD

Table 19: Management of CKD

Management	Count	%
Conservative	53	75.71%
Hemodialysis	16	22.86%
Kidney transplant	1	1.43%

In the study, 75.71% were managed conservatively, 22.86% by hemodialysis and 1.43% by the Kidney transplant.



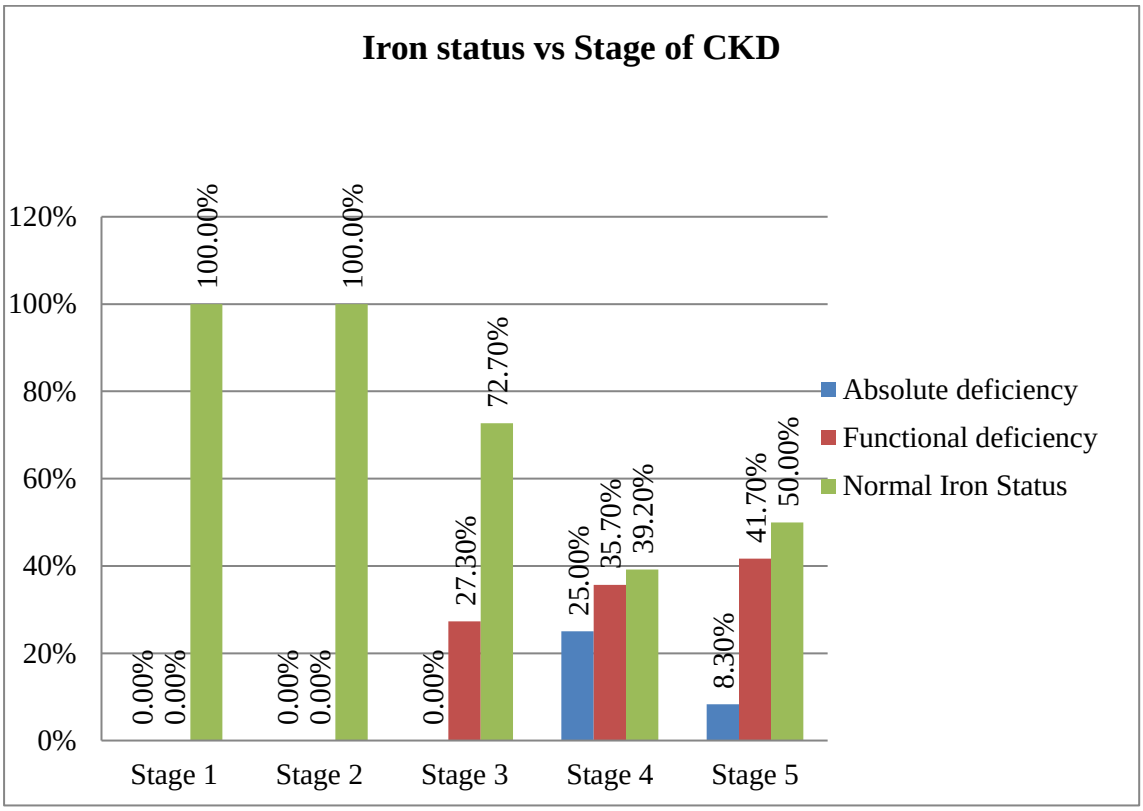
Graph 18: Pie diagram showing Management of CKD

Table 20: Association between Iron status and Stage of CKD

		Iron status					
		Absolute deficiency TSAT< 20%, Ferritin <100ng/ml		Functional deficiency Ferritin>100ng/ ml TSAT<20%		Normal Iron Status	
		Count	%	Count	%	Count	%
Stage	Stage 1	0	0.0%	0	0.0%	5	100.0%
	Stage 2	0	0.0%	0	0.0%	2	100.0%
	Stage 3	0	0.0%	3	27.3%	8	72.7%
	Stage 4	7	25%	10	35.7%	11	39.2%
	Stage 5	2	8.3%	10	41.7%	12	50.0%

$\chi 2 = 20.097$, df = 8, p = 0.01*

In the study in stage 1 and Stage 2 CKD, 100% had normal iron status, in stage 3, 27.3% had Functional deficiency and 72.7% had normal iron status, in stage 4, 25% had Absolute deficiency, 35.7% had Functional deficiency and 39.2% had normal iron status and in stage 5, 8.3% had absolute deficiency, 41.7% had Functional deficiency and 50% had normal iron status. There was a significant association between sage 1 and Iron status.



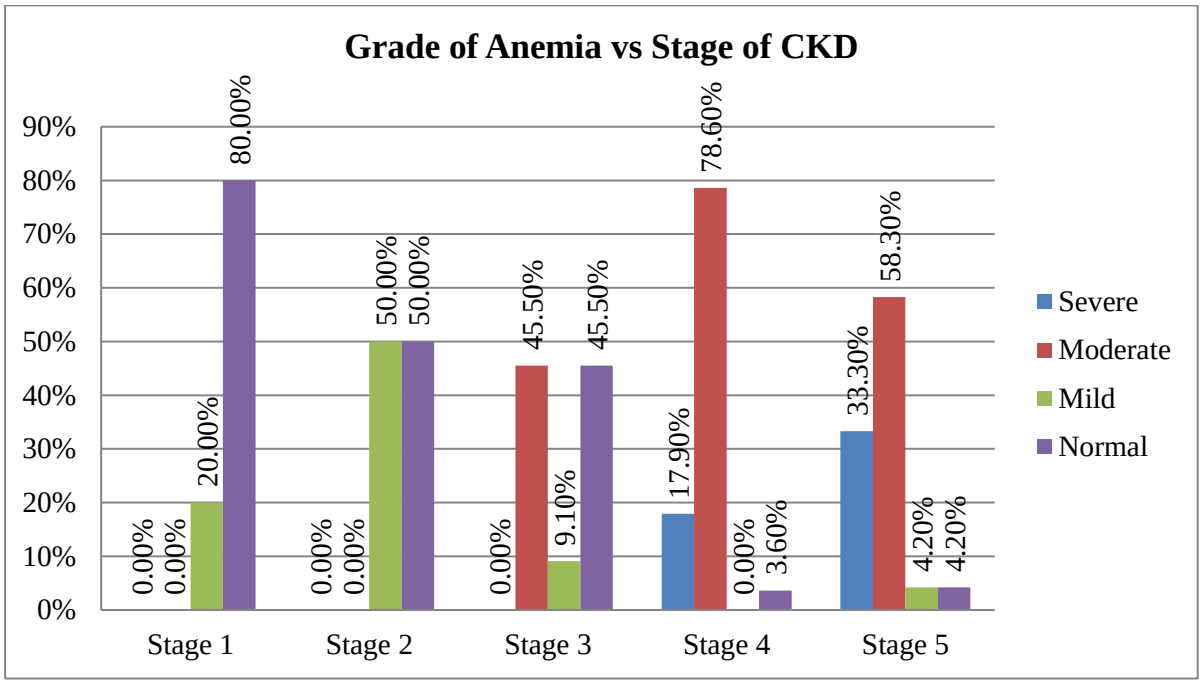
Graph 19: Bar diagram showing Association between Iron status and Stage of CKD

Table 21: Association between Grade of Anemia and Stage of CKD

		Anemia									
		Severe		Moderate		Mild		Normal		Total	
		Count	%	Count	%	Count	%	Count	%	Count	%
Stage	Stage 1	0	0.0%	0	0.0%	1	20.0%	4	80.0%	5	7.1%
	Stage 2	0	0.0%	0	0.0%	1	50.0%	1	50.0%	2	2.9%
	Stage 3	0	0.0%	5	45.5%	1	9.1%	5	45.5%	11	15.7%
	Stage 4	5	17.9%	22	78.6%	0	0.0%	1	3.6%	28	40.0%
	Stage 5	8	33.3%	14	58.3%	1	4.2%	1	4.2%	24	34.3%

$\chi 2 = 46.36$, df = 12, p <0.001*

In the study in stage 1 CKD, 20% had mild anemia, in Stage 2 CKD, 50% had mild anemia, in Stage 3 45.5% had moderate, 9.1% had mild anemia, in Stage 4, 17.9% had severe, 78.6% had moderate anemia and in stage 5, 33.3% had severe, 58.3% had moderate and 4.2% had mild anemia. There was a significant association between the grade of anemia and stage of CKD.



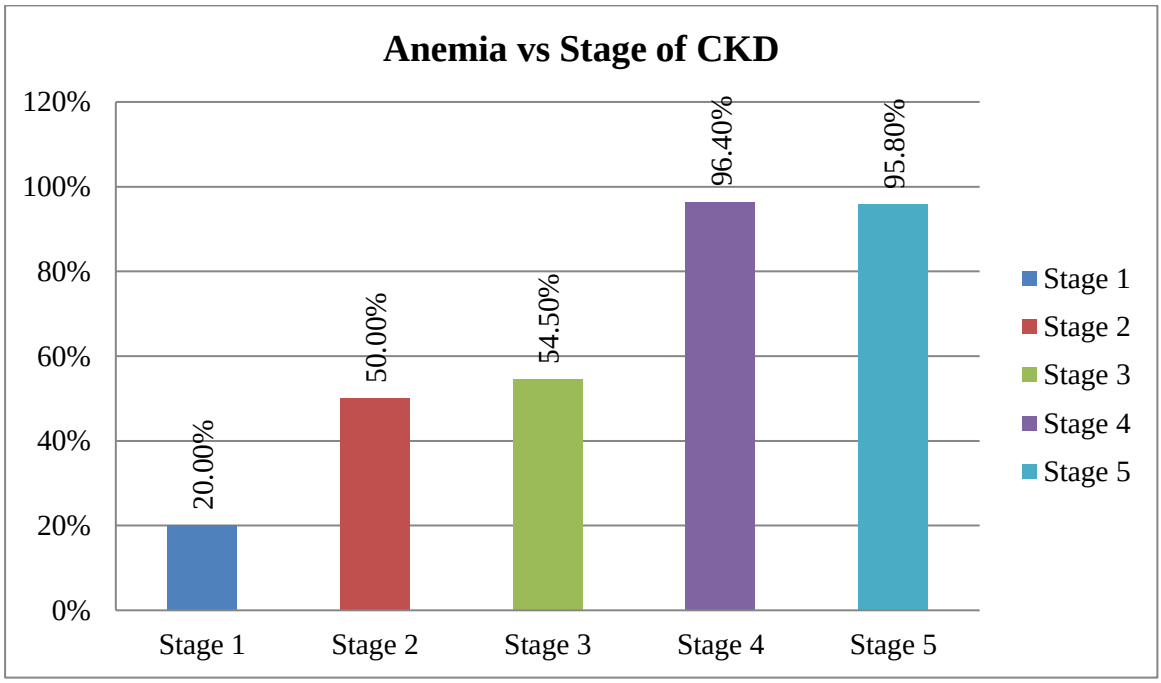
Graph 20: Bar diagram showing Association between Grade of Anemia and Stage of CKD

Table 22: Association between Anemia and Stage of CKD

		Anemia			
		Anemia		No Anemia	
		Count	%	Count	%
Stage	Stage 1	1	20.0%	4	80.0%
	Stage 2	1	50.0%	1	50.0%
	Stage 3	6	54.5%	5	45.5%
	Stage 4	27	96.4%	1	3.6%
	Stage 5	23	95.8%	1	4.2%

$\chi 2 = 28.11$, df = 4, p <0.001*

In Stage 1, 20% had anemia, in Stage 2 - 50% had anemia, in stage 3 – 54.5% had anemia, in Stage 4 – 96.4% had anemia and in Stage 5 – 95.8% had anemia. There was a significant association between stage of CKD and Anemia.



Graph 21: Bar diagram showing Association between Anemia and Stage of CKD

6. DISCUSSION

In this study, we evaluated iron status among CKD patients and estimated the percentage of patients having functional versus absolute iron deficiency.

A male (77.1%) predominance over females (22.9%) with CKD was seen in our study, which is similar to the work of Weeratunga P et al. [62]

This explains the role of sex hormones in the pathogenesis of kidney injury. Testosterone induces podocyte apoptosis leading to glomerulosclerosis) and TGF-β1 expression (which causes tissue fibrosis), while estradiol inhibits these processes. [63]

The average age of patients in this study was 50 years with most them between 55-65 years, which is similar to the age published in the center for disease control and prevention 2016. [64]

CKD diagnosis was made according to KDIGO guidelines. Duration of CKD in our study, patients with CKD for <1 year were 30%, 62.9% had CKD for 1 to 5 years and 7.1% had CKD for >5 years. Structural damage was assessed using ultrasound KUB to see corticomedullary distortion and kidney size and functional damage were assessed by proteinuria.

In our study 20% had no protein in urine, 28.6% had 30-100 mg/dL Proteinuria, 35.7% had 100-300 mg/dL of Proteinuria, 14.3% had 300-1000 mg/dL of Proteinuria and 1.4% had > 1000mg/dL of Proteinuria.

The mean systolic (146.14 +/- 22.08) and diastolic (88.57 +/- 13.32) blood pressures were on the higher side. This is not unexpected as hypertension is a common cause of CKD and CKD also causes secondary hypertension. The presence of hypertension could accelerate CKD progression. Hypertension leads to high intra-glomerular pressure, which impairs the glomerular filtration and thus damages glomeruli leading

to an increase in protein filtration. Microalbuminuria often is the first sign of CKD. (65)

In the study 15.7% were underweight, 64.3% had Normal BMI and 20% were overweight. Dry weight for patients on ESRD was taken to measure BMI. Uremic state in ESRD and malnutrition impedes weight gain.

Obese and overweight were at a higher risk of developing advanced CKD than lean weight adults. (66)

The mean S.Cr and estimated creatinine clearance (using MDRD) for estimation of GFR among CKD patients in our study was in accordance with study done by B Kumar et al, which showed Creatinine-based GFR estimation is more exact estimation of 24 hour creatinine clearance and kidney function than measuring serum creatinine alone. (67)

In our study high mean creatinine levels may reflect the late presentation of the majority of CKD patients. Percentage of patients who presented in CKD stage 4 and 5 were 74.2%. Because of many challenges in access to care, more than 50% of patients with advanced CKD are first seen when the eGFR is <15 ml/min per 1.73 m2.

The correlation between the stages of CKD and serum creatinine was statistically significant in all the stages of CKD. As CKD progresses so does serum creatinine.

In our study, 62.86% had Diabetic nephropathy which was most common (68) followed by Hypertensive nephropathy(20%) due to growing prevalence of T2DM and hypertension, Adult polycystic kidney disease, Obstructive Uropathy, and Chronic Glomerulonephritis were among the other causes.

Many patients with diabetic nephropathy in this study were managed at local clinics and referred only when they developed overt renal impairment. Therefore the low

frequency of diabetic nephropathy may merely reflect the tip of an iceberg of the true prevalence of diabetic nephropathy in this population.

In our study, 75.71% were managed conservatively, 22.86% by hemodialysis and 1.43% by a Kidney transplant.

Many patients with advanced CKD were managed conservatively because they were unaffordable for renal replacement therapy (RRT). In our center, only 1 patient had a kidney transplant.

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.

In our study, an overall prevalence of anemia was 82.8% with increased prevalence in stage 4 and 5. mild anemia was seen in CKD stage 1-5, moderate in Stage 3-5, and severe in stage 4-5.

This correlates to findings of a study, which stated that the prevalence of anemia increases with stage of CKD. [(69)]

Anemia in CKD is usually evident when a patient's creatinine clearance is <30 ml/min/1.73 m3 or serum creatinine (S Cr) is more than 3 mg/dl. (66,67,68)

Mild anemia in advanced disease can be explained by ongoing treatment with ESA, transfusions and iron therapy (according to KDIGO guidelines which states that irrespective of ESA treatment IV/oral therapy should be given).

Iron estimation and regular monitoring are important in the management of anemia of CKD patients.

In our study mean Hb was 9.36 ± 2.53 gm%. Hemoglobin is an important marker of iron status, and it also assesses how the tissue iron is mobilized to target cells and utilized for heme synthesis. [70]

Serum iron, TIBC, and ferritin are regularly estimated as it comes in iron profile done in this hospital, though estimation of tissue ferritin and transferrin is rare.

The range of serum iron levels in all the CKD patients in our study was 12-150 µg/dL.

This may also be because CKD patients were on the iron supplement as part of their treatment protocol as recommended by KDOQI guidelines.

As CKD stage advanced serum iron and TSAT values decreased, which was similar to the work of Gangadhar et al, (2010) in India on the predictive value of iron store markers in anemia of CKD in 207 patients. [71] Except for TIBC that was higher in our study and it increased with the stage of CKD.

This above findings suggests that as the CKD advances the incidence of iron deficiency increases (decreased iron & TSAT with increasing TIBC)

TSAT is a value of serum iron divided by the total iron-binding capacity.

A positive correlation between iron and TSAT was observed. TSAT is an indicator of circulating iron and the positive correlation with serum iron reflects that once the circulating iron levels increases there is also increase in the binding of iron to transferrin proportionately .

There was, however, a negative correlation between TIBC and TSAT. With decreasing TSAT, TIBC increases.

Ferritin is an acute phase reactant, its level is elevated in acute and chronic inflammatory states, independent of iron status. Therefore any clinical evidence of infection and liver disease was excluded in the study.

High ferritin level implicates iron overload, leading to oxidative stress. High risk of Cerebro-/cardio-vascular diseases, infection, and mortality in hemodialysis patients is seen with elevated ferritin levels. [72,73]

The mean ferritin levels among all CKD stages were relatively adequate expect for stage 2 which may be due to low sample size in the group.

This indicates that majority had adequate iron stores but there was insufficient mobilization from the stores suggesting higher prevalence of FID rather than AID.

Estimation of hemoglobin and ferritin alone does not give a complete picture of the iron status, which supports many of previous studies.

International treatment guidelines generally recommend that IV iron is to be discontinued when serum ferritin is greater than 500-1000 ng/ml to avoid iron overload. [74]

Patients were diagnosed to have a functional deficiency if the Ferritin value >100 and transferrin saturation (TSAT) value<20.

And absolute iron deficiency will be diagnosed based on the Ferritin <100 and TSAT <20.

Amongst the 82.8% of anemic CKD patients in our study, 47.1% had iron deficiency.

The patients who had absolute iron deficiency were only 12.8% indicating more prevalence of Functional Iron Deficiency (32.85%), who have high serum ferritin levels. Kopelman et al showed that FID associated with elevated ferritin levels in dialysis patients, and there was a significant improvement in FID status with IV iron supplementation. Positive association with serum iron, serum ferritin, and tissue iron reflects that the availability of sufficient iron bound to transferrin. [75]

7. SUMMARY

The findings in this study include the following

- As CKD advances, incidence of anemia increased, amongst the early stage 11.4%, stage 4 96.4% and stage 5 95.8%.
- Prevalence of anemia among CKD patients is 82.8% and showed a high prevalence of iron deficiency anemia (47.1% had iron deficiency).
- Among 47.1% iron deficient patients, 12.8% had absolute iron deficiency and 32.85 %of patients had functional iron deficiency.
- An increased prevalence of Functional iron deficiency anemia was noted than absolute IDA with normal or high ferritin levels.
- As stage advanced Serum iron and TSAT decreased with increasing TIBC suggesting increasing prevalence of iron deficiency anemia as CKD progresses.
- Mean ferritin values were relatively adequate among various stages suggesting that anemia was due to inadequate mobilization from the iron stores.
- A positive correlation seen between TSAT and serum iron, On the other hand a negative correlation is observed between TSAT and TIBC.
- The leading causes of CKD were diabetic nephropathy followed by hypertensive nephropathy and others.
- The mean age of CKD patients was found to be 50 years in Kolar, India.
- A male preponderance was observed in the CKD population.
- Stage 4 and 5 CKD patients were 74.2%.
- Many patients with CKD had high blood pressure levels and body mass index suggesting poor control.

8. CONCLUSIONS

1. In the vast majority of patients, this study implies anemia due to inadequate endogenous EPO production(normal iron status) and defective iron supply for erythropoiesis(functional iron deficiency).

2. 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.

3. An early management of anemia is needed in CKD patients as anemia leads to CKD progression and CVD in these patients.

4. ESAs are the main treatment of anemia of CKD and for this adequate iron stores are necessary to permit an optimal response. A reduction of 70% in ESA dosage has been noted in patients receiving IV iron.

5. An Iron study is important to know the types of iron deficiency, as FID will need IV iron supplement compared to the AID, which needs oral iron.

6. Target blood pressure of <130/80 should be achieved as recommended by KDOQI, ISN guidelines in all CKD patients.

7. At last the study supports current KDOQI recommendation of routine assessment of iron status in all the patients of CKD with anemia. This not only avoids the in advert use of ESA which has its own complications as outlined above but also to avoid iron overload in patients with adequate iron stores.

9. LIMITATIONS

The study had certain limitations.

- Relatively small sample size
- We were not able to investigate the other etiologies of anemia in this observed population due to the high cost of investigations (The study could have learned more by investigating vitamin B12, folate, erythropoietin levels)
- The use of novel iron biomarkers like reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR), the percentage of hypochromic red cells (PHRC), serum hepcidin and NGAL were not used to estimate iron indices.
- The study population was in advanced CKD stages, which might have overestimated the prevalence of anemia among the CKD study population.

10. REFERENCES

1. [Internet]. Kdigo.org. 2018 [cited 4 November 2018]. Available from: http://kdigo.org/wp-content/uploads/2017/02/2017-KDIGO-CKD-MBD-GL-Update.pdf
2. National Kidney Foundation (India) [Internet]. Nkfi.in. 2018 [cited 25 November 2018]. Available from: http://nkfi.in/.
3. Gansevoort R, Correa-Rotter R, Hemmelgarn B et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. The Lancet. 2013;382(9889):339-352.
4. UpToDate [Internet]. Uptodate.com. 2018 [cited 4 November 2018]. Available from: https://www.uptodate.com/contents/epidemiology-of-chronic-kidney-disease
5. Thomas R, Kanso A, Sedor J. Chronic Kidney Disease and Its Complications. Primary Care: Clinics in Office Practice. 2008;35(2):329-344.
6. Agarwal R. Iron deficiency anemia in chronic kidney disease: Uncertainties and cautions. Hemodialysis International. 2017;21: S78-S82.
7. Getahun W, Belachew T, Wolide A. Burden and associated factors of anemia among pregnant women attending antenatal care in southern Ethiopia: cross sectional study. BMC Research Notes. 2017;10(1).
8. Subbiah A, Chhabra Y, Mahajan S. Cardiovascular disease in patients with chronic kidney disease: a neglected subgroup. Heart Asia. 2016;8(2):56-61.
9. Weiss G. Iron metabolism in the anemia of chronic disease. Biochimica et Biophysica Acta (BBA) - General Subjects. 2009;1790(7):682-693.

10. Guidi G, Lechi Santonastaso C. Advancements in anemias related to chronic conditions. Clinical Chemistry and Laboratory Medicine. 2010;48(9).
11. Lankhorst C, Wish J. Anemia in renal disease: Diagnosis and management. Blood Reviews. 2010;24(1):39-47.
12. Zager RA, Johnson AC, Hanson SY et al. Parenteral iron formulations: a comparative toxicologic analysis and mechanisms of cell injury. Am. J. Kidney dis, 40(1), 90-103.
13. Macdougall I, Cavill I, Hulme B, Bain B, McGregor E, McKay P et al. Detection of functional iron deficiency during erythropoietin treatment: a new approach. BMJ. 1992;304(6821):225-226.
14. Besarab A. Evaluating iron sufficiency: A clearer view. Kidney International. 2001;60(6):2412-2414.
15. Fishbane S, Kowalski EA, Imbriano LJ et al. The Evaluation of Iron Status in Hemodialysis Patients. J Am Soc Nephrol. 1996 Dec;7(12):2654-7.
16. Chung M, Chan J, Moorthy D, Hadar N, Ratichek S, Concannon T et al. Biomarkers for Assessing and Managing Iron Deficiency Anemia in Late-Stage Chronic Kidney Disease: Future Research Needs [Internet]. Ncbi.nlm.nih.gov. 2018 [cited 20 November 2018]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK143610/
17. Guyton A, Hall J. Guyton & Hall, Tratado de fisiología médica. Barcelona: Elsevier España; 2016.
18. Ross and Wilson Human Anatomy and Physiology PDF 12th Edition | Homeostasis | Tissue (Biology) [Internet]. Scribd. 2018 [cited 4 November 2018]. Available from: https://www.scribd.com/document/341658298/Ross-and-Wilson-Human-Anatomy-and-Physiology-PDF-12th-Edition

19. Koury M, Haase V. Anaemia in kidney disease: harnessing hypoxia responses for therapy. Nature Reviews Nephrology. 2015;11(7):394-410.
20. Agarwal S, Dash S, Irshad M, Raju S, Singh R, Pandey R. Prevalence of chronic renal failure in adults in Delhi, India. Nephrology Dialysis Transplantation. 2005;20(8):1638-1642.
21. Agarwal S, Srivastava R. Chronic Kidney Disease in India: Challenges and Solutions. Nephron Clinical Practice. 2009;111(3):c197-c203.
22. Varughese S, John GT, Alexander S, et al. Pre-tertiary hospital care of patients with chronic kidney disease in India. Indian J Med Res 126: 28–33, 2007
23. Ene-Iordache B, Perico N, Bikbov B et al. Chronic kidney disease and cardiovascular risk in six regions of the world (ISN-KDDC): A cross-sectional study. Lancet Glob Health 4: e307–e319, 2016
24. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular diagnostics. Chapter 24, Kidney Function Tests.4th edition. Elsevier; 2006:1920-1925.
25. Traynor J, Mactier R, Geddes C, Fox J. How to measure renal function in clinical practice. BMJ. 2006;333(7571):733-737.
26. National Kidney Foundation [Internet]. National Kidney Foundation. 2018 [cited 4 November 2018]. Available from: https://www.kidney.org/
27. Mula-Abed W, Al Rasadi K, Al Riyami D. Estimated Glomerular Filtration Rate (eGFR): A Serum Creatinine-Based Test for the Detection of Chronic Kidney Disease and its Impact on Clinical Practice. Oman Medical Journal. 2012;27(2):108-113.
28. Levey A, Stevens L, Schmid C et al. A New Equation to Estimate Glomerular Filtration Rate. Annals of Internal Medicine. 2009;150(9):604.

29. Glomerular filtration rate (GFR) kidney test information | myVMC [Internet]. myVMC. 2018 [cited 4 November 2018]. Available from: https://www.myvmc.com/investigations/glomerular-filtration-rate-gfr/
30. Pradeep A, Vecihi B,(2012) Chronic Kidney Disease updated Mar 28,2012. retrieved 24 may 2012
31. Parmar M. Chronic renal disease. BMJ. 2002;325(7355):85-90.
32. Schmidt R, Dalton C. Treating anemia of chronic kidney disease in the primary care setting: cardiovascular outcomes and management recommendations. Osteopathic Medicine and Primary Care. 2007;1(1):14.
33. Isah H S (2007) Renal function in; Essential clinical biochemistry (1st ed) Zaria tamaza publication, pp 231
34. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2012;1823(9):1434-1443.
35. Linder M. Mobilization of Stored Iron in Mammals: A Review. Nutrients. 2013;5(10):4022-4050.
36. Abbas A, Aster J, Kumar V, Perkins J. Robbins basic pathology.
37. Adamson J. Normal Iron Physiology. 2018.
38. Jameson J, Fauci A, Kasper D, Hauser S, Longo D, Loscalzo J et al. Harrison's principles of internal medicine. [Miejsce nieznane]: The McGraw-Hill Companies; 2018.
39. Girelli D, Nemeth E, Swinkels D. Hepcidin in the diagnosis of iron disorders. Blood. 2016;127(23):2809-2813.
40. Stauffer M, Fan T. Prevalence of Anemia in Chronic Kidney Disease in the United States. PLoS ONE. 2014;9(1):e84943.

41. Babitt J, Lin H. Mechanisms of Anemia in CKD. Journal of the American Society of Nephrology. 2012;23(10):1631-1634.
42. Jelkmann W. Regulation of erythropoietin production. The Journal of Physiology. 2011;589(6):1251-1258.
43. Wish J. Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation. Clinical Journal of the American Society of Nephrology. 2006;1(Supplement 1): S4-S8.
44. Fukumoto S. Phosphate metabolism and vitamin D. BoneKEy Reports. 2014;3.
45. Rossert J. Anti-Erythropoietin Antibodies and Pure Red Cell Aplasia. Journal of the American Society of Nephrology. 2004;15(2):398-406.
46. Pratt M, Lewis-Barned N, Walker R, Bailey R, Shand B, Livesey J. Effect of angiotensin converting enzyme inhibitors on erythropoietin concentrations in healthy volunteers. British Journal of Clinical Pharmacology. 1992;34(4):363-365.
47. WHO | Assessing the iron status of populations [Internet]. Who.int. 2018 [cited 6 November 2018]. Available from: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.
48. Beard J, Tobin B. Iron status and exercise. 2018.
49. Bothwell TH (1979) Iron metabolism in man. London, Blackwell Scientific Publications. [Internet]. Sciepub.com. 2018 [cited 20 November 2018]. Available from: http://www.sciepub.com/reference/12145

50. Kalantar-Zadeh K. The Fascinating but Deceptive Ferritin: To Measure It or Not to Measure It in Chronic Kidney Disease?. Clinical Journal of the American Society of Nephrology. 2006;1(Supplement 1): S9-S18.
51. Zaritsky J, Young B, Wang HJ et al. Hepcidin—a potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol*2009;4:1051-1056.
52. Larson DS, Coyne DW. Understanding and exploiting hepcidin as an indicator of anemia due to chronic kidney disease. *Kidney Res Clin Pract*2013;32:11-15.
53. Kim I, Kim J, Lee D, Lee S, Rhee H, Song S et al. Plasma neutrophil gelatinase-associated lipocalin is associated with iron status in anemic patients with pre-dialysis chronic kidney disease. Clinical and Experimental Nephrology. 2017;22(1):28-34.
54. Coyne D. A Comprehensive Vision for Intravenous Iron Therapy. American Journal of Kidney Diseases. 2008;52(6): S14-S20.
55. Coyne D. It's Time to Compare Anemia Management Strategies in Hemodialysis. Clinical Journal of the American Society of Nephrology. 2010;5(4):740-742.
56. Horl W. Clinical Aspects of Iron Use in the Anemia of Kidney Disease. Journal of the American Society of Nephrology. 2007;18(2):382-393.
57. Besarab A, Coyne D. Iron supplementation to treat anemia in patients with chronic kidney disease. Nature Reviews Nephrology. 2010;6(12):699-710.
58. Gaddis, ML, Gaddis, GM. Introduction to biostatistics: Part 4, Statistical inference techniques in hypothesis testing. *Ann Emerg Med*. 1990;19:820–825.

59. Patra P. Sample size in clinical research, the number we need. Int J Med Sci Public Health. 2012;1:5–9.
60. Sunder R, Richard J: An Introduction to Biostatistics, A manual for students in health sciences, New Delhi: Prentice Hall of India. 4th edition, 86-160. (2006)
61. Elenbaas RM, Elenbaas JK, Cuddy PG. Evaluating the medical literature, part II: Statistical analysis. *Ann Emerg Med*. 1983;12:610–620.
62. Weeratunga P, Selvarajah M, Sivayoganthan S et al. Clinicopathological correlates of chronic kidney disease of unknown etiology in Sri Lanka. Indian J. Nephrol. 2016;26(5):357.
63. Sandberg K. Mechanisms underlying sex differences in progressive renal disease. Gend Med. 2008;5(1):10-23.
64. Crude Prevalence of CKD Stages 1–4 by Age 2015–2016 [Internet]. Nccd.cdc.gov. 2018 [cited 29 October 2018]. Available from: https://nccd.cdc.gov/ckd/detail.aspx?QNum=Q9&Strat=Age
65. Keane WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper for the National Kidney Foundation. *Am J Kidney Dis*. 1999;33:1004-1010.
66. Herrington W, Smith M, Bankhead C et al. Body-mass index and risk of advanced chronic kidney disease: Prospective analyses from a primary care cohort of 1.4 million adults in England. PLOS ONE. 2017;12(3):e0173515.
67. Kumar B. Retrospective Comparison of Estimated GFR using 2006 MDRD, 2009 CKD-EPI and Cockcroft-Gault with 24 Hour Urine Creatinine Clearance. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2017;.

68. McClellan WM, Flanders WD. Risk factors for progressive chronic kidney disease. J Am Soc Nephrol. 2003;14: S65–S70.
69. Robinson B, Artz A, Culleton B, Critchlow C, Sciarra A, Audhya P. Prevalence of Anemia in the Nursing Home: Contribution of Chronic Kidney Disease. Journal of the American Geriatrics Society. 2007;55(10):1566-1570.
70. G. Chinnapu Reddy P. Iron Indices in Patients with Functional Anemia in Chronic Kidney Disease [Internet]. PubMed Central (PMC). 2018 [cited 27 November 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975187/
71. Gangadhar, T., Srikanth, P., & Suneetha, Y. Predictive Value of Iron Store Markers in Anemia of Chronic Kidney Disease.Journal of Chemical and Pharmaceutical Research, (2010). 2(3), 400-410.
72. Hasuike Y, Nonoguchi H, Tokuyama M et al. Serum ferritin predicts prognosis in hemodialysis patients: the Nishinomiya study. Clinical and Experimental Nephrology. 2010;14(4):349-355.
73. Kuragano T, Matsumura O, Matsuda A et al. Association between hemoglobin variability, serum ferritin levels, and adverse events/mortality in maintenance hemodialysis patients. Kidney International. 2014;86(4):845-854.
74. Fishbane S, Kalantar-Zadeh K, Nissenson A. Serum Ferritin in Chronic Kidney Disease: Reconsidering the Upper Limit for Iron Treatment. Seminars in Dialysis. 2004;17(5):336-341.
75. Kopelman RC, Smith L, Peoples L et al. Functional iron deficiency in hemodialysis patients with high ferritin. Hemodial Int 2007; 11(2):238–246.
76. United States Renal Data System Annual Data Report 2013 | GHDx [Internet]. Ghdx.healthdata.org. 2018 [cited 20 November 2018]. Available from:

http://ghdx.healthdata.org/record/united-states-renal-data-system-annual-data-report-2013

77. WHO | WHO Global Database on Anaemia [Internet]. Who.int. 2018 [cited 20 November 2018]. Available from: http://www.who.int/vmnis/anaemia/en/

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

79. McClellan WM, Flanders WD, Langston RD. Anemia and renal insufficiency 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.

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

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

82. Remuzzi G, Rossi E. Hematologic consequences of renal failure. In: Brenner BM, Ed. The Kidney. 5th ed. Philadelphia:WB Saunders Co;1995:2170-85.

83. Lee GR. The anemias associated with renal disease, liver disease, endocrine disease, and pregnancy. In: Lee GR, Foester J, Lekuns J, Paraskevas F, Greer JPRodgers GM, eds. Wintrobe clinical hematology. 10th ed. Baltimore: Williams and Wilkins;1999:1497-1517.

84. Monograph. Signs and symptoms of uremia. In: Block RM, Alfred HJ, Fan PY, Stoff JS, eds. Rose and Block's clinical problems in nephrology. 1st ed. Boston: Little, Brown and Company;1996:497-523.

85. Brenner B. Brenner & Rector's The Kidney. Philadelphia: Saunders; 2011.

INFORMED CONSENT FORM

SERUM IRON INDICES IN PATIENTS WITH CHRONIC KIDNEY DISEASE.

STUDY NUMBER:

SUBJECT'S NAME: **HOSPITAL NUMBER:**

If you agree to participate in this study we shall collect information (as per proforma) from you or a person responsible for you or both. We will collect the treatment and relevant details from your hospital record. The information collected from you will be used for dissertation and publication only. The institutional ethical committee has reviewed this study. The care you get will not change if you don't agree to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

You can withdraw from the study any time and this won't change your future care.

I the patient/patient's attender have read or have been read to us and understood the purpose of the study, the risk and benefits associated with my involvement in the study and the information which will be collected and disclosed during the study. I have had the opportunity to ask questions regarding this study and my questions were answered to my satisfaction. I, the undersigned agree to participate in this study and

authorize the collection and disclosure of my personal information for dissertation and publication only.

Signature/thumb impression of the patient: Date:

Name and signature of the witness: Date:

Name and signature of the person obtaining consen Date:

PROFORMA FOR DATA COLLECTION

NAME: IP NO:

AGE:

SEX:

ADDRESS:

OCCUPATION:

DETAILED HISTORY:

ANTHROPOMETRIC MEASUREMENT:

HEIGHT:

WEIGHT:

BODY MASS INDEX:

HIP WAIST RATIO:

GENERAL PHYSICAL EXAMINATION:

PULSE:

BLOOD PRESSURE:

SYSTEMIC EXAMINATION:

CARDIOVASCULAR EXAMINATION:

RESPIRATORY EXAMINATION:

PER ABDOMINAL EXAMINATION:

CENTRAL NERVOUS SYSTEM EXAMINATION:

LABORATORY DATA:

1.CBC

2.RFT

3.SERUM IRON INDICES .(Serum iron,Serum Ferritin, transferrin saturation(TSAT)

4.eGFR calculation by using modification of diet in renal disease(MDRD) formula

eGFR (mL/min/1.73m2)= 175 x [SerumCreatinine(umol/L) x 0.0113]-1.154 x Age(years)-0.203 (x 0.742 if female)

5.USG KUB

6.Urine routine

KEY TO MASTER CHART

A/G ratio – albumin globulin ratio

AID – absolute iron deficiency

Alb – albumin

ALP – alkaline phosphatase

ALT – alanine transaminase

AST – aspartate transaminase

BP – blood pressure

BMI - body mass index

DB – direct bilirubin

eGFR – estimated GFR

ESRD – end stage renal disease

F - Female

FID – functional iron deficiency

GGT – gamma glutamyl transferase

Glo – globulin

H.No - Hospital Number

Hb – hemoglobin

M - Male

MRD – medico renal disease

NIS – normal iron stores

Sl.No - Serial Number

T2DM – type 2 diabetes mellitus

TB- total bilirubin

TIBC – transferrin iron binding capacity

TP – total protein

USG - Ultrasonography

sl no.	H.no.	Age	Sex	CKD in years	T2DM	Hypertension	height (m)	weight	BMI	waist hip ratio	Pulse	BP	Hb	Blood Urea	Serum Creatinine	S. iron	TIBC	S. ferritin	Transferrin saturation	iron status	usg KUB	URINE Protein	eGFR	Stage	TB	DB	AST	ALT	ALP	TP	ALB	GLO	AG	GGT
1	403747	61	M	5	Yes	no	1.62	65	24.76756592	0.6	86	160/100	8.6	78	3	63	300	155	21	NIS	Grade 3 MRD	2+	22.76108551	4	0.1	0.1	24	34	79	7.1	3.7	3.4	1.088	57
2	185294	45	M	3	Yes	yes	1.58	70	28.04037814	1.1	76	180/110	6.5	56	5	36	325	243	11.07692308	FID	Grade 3 MRD	3+	13.42762888	ESRD	0.3	0.1	56	44	88	7.2	4.2	3	1.400	85
3	592472	48	M	2	Yes	no	1.77	62	19.78997095	0.5	78	140/90	10.4	96	1.9	70	265	168	26.41509434	NIS	Grade 3 MRD	nil	40.4798811	3	0.5	0.1	34	37	98	6.8	3.8	3	1.267	29
4	530846	63	M	7	Yes	yes	1.76	66	21.30681818	0.95	92	190/90	7.9	76	2.4	38.5437	289	157	13.33692042	FID	Grade 3 MRD	1+	29.25384463	4	0.7	0	23	54	100	6.4	4.3	2.1	2.048	26
5	535080	45	M	2	no	yes	1.82	58	17.50996256	0.5	99	170/110	9.8	68	3.2	55	320	234	17.1875	FID	Grade 3 MRD	1+	22.4733409	4	0.2	0	34	26	111	6.5	4.4	2.1	2.095	65
6	509091	60	M	2	Yes	no	1.76	49	15.81869835	0.4	89	220/130	8.4	69	2.9	44	288	354	15.27777778	FID	Grade 3 MRD	2+	23.74875578	4	0.6	0.1	14	34	43	6.6	3.6	3	1.200	50
7	602429	50	M	2	Yes	yes	1.67	48	17.21108681	0.56	76	140/90	4.6	90	3.6	26	401	56	6.483790524	AID	Grade 3 MRD	1+	19.20210579	4	0.8	0.1	24	23	46	7.6	3.7	3.9	0.949	88
8	580713	40	M	1	Yes	yes	1.65	55	20.2020202	0.67	75	120/19	5.9	113	4	44	305	44	14.42622951	AID	Grade 3 MRD	2+	17.79169999	4	0.3	0.2	33	45	53	7.7	3.9	3.8	1.026	68
9	587468	55	M	2	Yes	yes	1.69	59	20.65754	0.66	89	160/100	7.9	103	3.6	46	299	176	15.38461538	FID	Grade 3 MRD	nil	18.83415512	4	0.9	0.2	32	76	64	6.8	4.1	2.7	1.519	69
10	314926	35	M	3	Yes	no	1.79	78	24.34380949	0.9	74	160/90	13.9	97	5	100	261	245	38.31417625	NIS	Grade 3 MRD	nil	14.13043908	ESRD	1	0.1	32	76	76	6.1	4.3	1.8	2.389	56
11	592494	30	M	1	no	yes	1.56	56	23.01117686	0.98	80	170/80	8.9	142	4.2	33	270	141	12.22222222	FID	Grade 3 MRD	3+	17.82902357	4	1	0.1	24	65	66	6.2	4.4	1.8	2.444	67
12	572273	65	M	2	Yes	no	1.73	63	21.0498179	0.7	65	160/90	13	100	2	37	287	300	12.89198606	FID	Grade 3 MRD	2+	35.87590196	3	0.2	0.1	23	49	121	6.4	3.5	2.9	1.207	68
13	492971	60	M	5	Yes	yes	1.74	65	21.46915048	0.8	68	140/90	9.7	95	1.8	27	276	178	9.782608696	FID	Grade 1 MRD	2+	41.17785123	3	0.3	0.2	43	48	128	7	4	3	1.333	75
14	594095	31	M	4	Yes	yes	1.67	71	25.4580659	0.9	76	160/90	9.9	86	5	45	306	13	14.70588235	AID	Grade 2 MRD	1+	14.48288379	ESRD	0.9	0	38	39	79	7.3	4.1	3.2	1.281	54
15	512755	60	M	7	Yes	no	1.78	58	18.30576947	0.65	98	120/90	5.6	45	4.4	15	288	156	5.208333333	FID	Grade 3 MRD	1+	14.67924647	5	0.7	0.1	27	39	48	6.8	4.2	2.6	1.615	67
16	555314	50	M	2	Yes	yes	1.66	55	19.95935549	0.65	68	130/90	6.2	102	4.9	87	297	143	29.29292929	NIS	Grade 3 MRD	1+	13.45351191	5	0.6	0.2	29	47	47	6.9	3.8	3.1	1.226	58
17	98151	70	M	8	Yes	no	1.81	53	16.17777235	0.5	78	140/90	7.6	76	4.5	90	276	254	32.60869565	NIS	Grade 3 MRD	1+	13.86279215	ESRD	0.5	0.1	25	45	56	6.6	4.3	2.3	1.870	67
18	624819	30	M	1	no	yes	1.82	62	18.71754619	0.5	93	140/100	14.5	68	4.5	78	281	164	27.75800712	NIS	Grade 3 MRD	2+	16.46455508	4	0.1	0.1	32	56	68	6.9	3.9	3	1.300	85
19	625731	70	M	3	Yes	no	1.7	67	23.183391	0.76	83	160/90	8.9	88	3.9	59	362	216	16.29834254	FID	Grade 3 MRD	1+	16.35194419	4	0.8	0	37	55	88	6.7	3.5	3.2	1.094	65
20	620737	60	M	6 months	Yes	yes	1.72	64	21.63331531	0.67	63	170/80	10.4	120	2.8	112	270	176	41.48148148	NIS	Grade 3 MRD	nil	24.73020878	4	1.2	0	20	34	98	7.1	3.6	3.5	1.029	85
21	622859	52	M	2	no	yes	1.83	69	20.60378035	0.66	93	150/90	11	96	2.3	86	277	184	31.04693141	NIS	Grade 2 MRD	nil	31.94701262	3	0.3	0	31	37	122	6.8	3.8	3	1.267	85
22	615960	40	M	6 months	Yes	no	1.75	73	23.83673469	0.76	99	130/80	13	56	2	90	304	98	29.60526316	NIS	Grade 1 MRD	2+	39.59187427	3	0.6	0.2	33	23	53	7.6	4.4	3.2	1.375	57
23	626911	78	M	10	Yes	yes	1.69	64	22.40817899	0.8	84	150/100	8.8	83	3	150	302	89	49.66887417	NIS	Grade 2 MRD	2+	21.6530821	4	0.8	0.1	34	33	39	6.7	4.5	2.2	2.045	85
24	570505	46	M	1.5	Yes	no	1.66	52	18.87066338	0.7	76	140/70	7.6	45	3.6	50	401	152	12.46882793	FID	Grade 3 MRD	3+	19.52989594	4	1	0.1	24	45	64	7.7	4.1	3.6	1.139	29
25	362647	47	M	5	Yes	yes	1.6	48	18.75	0.75	94	100/60	11.5	95	1.9	121	356	77	33.98876404	NIS	Grade 1 MRD	2+	40.65325594	3	0.3	0.1	14	64	68	6.7	3.7	3	1.233	26
26	261253	57	M	3	Yes	no	1.76	72	23.24380165	0.7	69	120/60	10.9	135	2.4	70	356	32	19.66292135	AID	Grade 3 MRD	1+	29.85427206	4	1	0	3	65	121	6.2	3.9	2.3	1.696	50
27	286460	50	M	2 months	Yes	no	1.67	67	24.02380867	0.87	87	120/80	5.5	38	5	23	384	345	5.989583333	FID	Grade 3 MRD	2+	13.14348576	ESRD	1	0	32	44	128	6.3	4.4	1.9	2.316	88
28	470120	57	M	2 months	Yes	yes	1.65	64	23.50780533	0.85	78	110/80	7.8	94	2.9	33	276	155	11.95652174	FID	Grade 2 MRD	3+	23.99733253	4	0.2	0.1	32	48	79	7	3.6	3.4	1.059	68
29	411294	64	M	3	no	yes	1.69	56	19.60715661	0.7	97	130/80	6.4	66	3.6	37	254	76	14.56692913	AID	Grade 3 MRD	1+	18.26355153	4	0.4	0.1	11	39	48	7.3	3.8	3.5	1.086	69
30	450721	21	M	3 months	no	no	1.79	65	20.28650791	0.78	79	120/60	8.6	65	6	86	311	111	27.65273312	NIS	Grade 2 MRD	2+	12.70035309	ESRD	0.9	0.1	23	29	47	6.7	3.9	2.8	1.393	86
31	526283	46	M	4.5	no	yes	1.56	75	30.81854043	0.98	96	140/70	8.8	84	3.6	75	266	132	28.19548872	NIS	Grade 3 MRD	2+	19.52989594	4	0.8	0.1	43	39	56	6.8	4	2.8	1.429	56
32	558181	64	M	1.5	Yes	no	1.73	49	16.37208059	0.68	69	160/90	11	56	1.8	56	288	178	19.44444444	FID	Grade 1 MRD	1+	40.64188562	3	0.7	0	23	47	68	6.9	4.1	2.8	1.464	67
33	312525	52	M	1	Yes	yes	1.74	50	16.51473114	0.76	79	160/100	10.8	20	5	67	298	154	22.48322148	NIS	Grade 3 MRD	2+	13.03925538	ESRD	0.5	0	28	56	88	6.9	4.3	2.6	1.654	68
34	545578	75	M	7	Yes	yes	1.67	61	21.87242282	0.85	98	120/90	12	40	0.9	70	302	48	23.17880795	NIS	normal	2+	87.57488235	2	0.4	0	27	44	98	6	4.4	1.6	2.750	75
35	599333	67	M	5	Yes	no	1.78	60	18.9370029	0.75	85	150/100	12	60	1.8	68	300	123	22.66666667	NIS	Grade 3 MRD	3+	40.26569495	3	1.2	0.1	25	34	122	7.1	3.5	3.6	0.972	54
36	5502257	60	M	3 months	Yes	yes	1.66	76	27.58020032	0.99	84	140/100	13	70	5	88	266	145	33.08270677	NIS	Grade 3 MRD	nil	12.66592074	ESRD	0.3	0.2	28	37	127	6.8	4	2.8	1.429	67
37	461116	51	M	6 months	no	yes	1.81	65	19.8406642	0.7	93	170/80	11	74	4.4	70	344	43	20.34883721	AID	Grade 3 MRD	1+	15.17161176	4	0.5	0.2	37	54	43	6.4	4.5	1.9	2.368	58
38	582746	60	M	7	Yes	no	1.82	66	19.92512982	0.69	82	190/90	7	119	4.9	110	365	176	30.1369863	NIS	Grade 3 MRD	1+	12.96468218	ESRD	0.7	0.2	20	24	46	7.4	4	3.4	1.176	67
39	10208	37	F	3 months	Yes	yes	1.56	58	23.8330046	0.9	91	150/90	6	112	4.1	12	377	170	3.183023873	FID	Grade 3 MRD	2+	13.03524624	5	0.9	0.1	35	26	59	6.5	3.9	2.6	1.500	85
40	374730	60	F	2	Yes	yes	1.47	55	25.45235781	0.87	70	180/110	9	89	3.3	76	261	111	29.11877395	NIS	Grade 3 MRD	1+	15.18053123	4	0.4	0.1	23	34	39	6.6	5.1	1.5	3.400	65
41	228740	60	F	3	Yes	no	1.83	53	15.82609215	0.66	79	130/90	6.7	90	3.9	44	311	200	14.14790997	FID	Grade 4 MRD	2+	12.51882302	ESRD	0.6	0	24	23	64	7.6	3.3	4.3	0.767	85
42	475094	60	F	6	Yes	yes	1.65	62	22.77318641	0.78	70	120/60	7.7	94	2.8	66	278	132	23.74100719	NIS	Grade 3 MRD	nil	18.34981492	4	1	0.2	34	45	76	7.7	3.1	4.6	0.674	85
43	533870	40	F	2 months	Yes	no	1.69	67	23.45856238	0.86	80	140/70	12	73	0.6	69	288	176	23.95833333	NIS	normal	3+	117.8723763	1	0.3	0	23	64	66	6.7	3.5	3.2	1.094	68
44	512102	40	F	3	no	yes	1.66	64	23.22543185	0.88	88	160/100	9	57	2	65	267	165	24.34456929	NIS	Grade 3 MRD	3+	29.37717071	4	0.6	0	33	76	103	6.8	4	2.8	1.429	44
45	399613	60	F	7	Yes	no	1.6	69	26.953125	0.94	89	180/110	8.8	29	3.4	56	301	189	18.60465116	FID	Grade 3 MRD	2+	14.66646284	5	1	0.1	24	44	79	6.3	4.2	2.1	2.000	57
46	428506	40	F	6	Yes	yes	1.49	69	31.0796811	0.99	97	120/80	8.9	30	3.1	34	312	56	10.8974359	AID	Grade 3 MRD	3+	17.71606183	4	0.1	0.1	43	49	98	6.4	3.8	2.6	1.462	85
47	606237	43	F	2	Yes	no	1.52	64	27.70083102	0.87	77	150/90	7.6	90	2	45	333	125	13.51351351	FID	Grade 3 MRD	4+	28.94903216	4	0.2	0.1	33	48	48	7	4.3	2.7	1.593	29
48	623585	64	F	7	no	yes	1.55	52	21.64412071	0.68	76	150/100	4.8	89	2.8	12	321	58	3.738317757	AID	Grade 3 MRD	1+	18.11097609	4	0.3	0	3	37	47	7.2	3.9	3.3	1.182	26
49	513897	60	F	5	Yes	no	1.59	48	18.98659072	0.66	66	120/80	13	65	1.5	78	288	154	27.08333333	NIS	Grade 1 MRD	1+	37.70880271	3	0.4	0	32	39	56	7.3	4.4	2.9	1.517	58
50	526283	30	F	2 months	Yes	yes	1.6	58	22.65625	0.72	87	160/90	10.1	47	5	48	333	211	14.41441441	FID	normal	nil	10.81806933	ESRD	1.2	0.1	24	47	88	6.9	3.7	3.2	1.156	50
51	416554	24	F	2	no	no	1.6	67	26.171875	0.92	78	170/80	7	106	5.6	87	276	143	31.52173913	NIS	Grade 4 MRD	2+	9.931729782	5	0.1	0.1	11	45	98	7	3.8	3.2	1.188	88
52	11416	26	F	6 months	Yes	yes	1.59	64	25.31545429	0.89	98	140/70	7.8	198	7.9	90	311	121	28.93890675	NIS	Grade 4 MRD	1+	6.569245726	5	0.5	0.2	43	44	122	6.6	4	2.6	1.538	68
53	612346	46	F	6 months	Yes	no	1.62	56	21.33821064	0.84	89	150/100	6.9	108	4.5	78	312	121	25	NIS	Grade 4 MRD	nil	11.20133225	5	0.9	0.2	38	34	127	7	4.2	2.8	1.500	69
54	511633	30	M	2	Yes	yes	1.63	59	22.20633069	0.88	67	130/90	8	65	5	59	276	88	21.37681159	NIS	Grade 3 MRD	nil	14.57960826	ESRD	0.4	0.1	27	37	46	7	4.4	2.6	1.692	56
55	618421	65	M	5	no	yes	1.55	66	27.47138398	0.98	87	120/80	9.7	75	4	112	421	79	26.60332542	NIS	Grade 3 MRD	2+	16.1218254	ESRD	0.6	0.1	29	54	59	7	4.5	2.5	1.800	67
56	576832	40	M	6	Yes	yes	1.63	49	18.44254582	0.7	78	110/80	8.9	84	2.6	86	412	121	20.87378641	NIS	Grade 3 MRD	2+	29.24929705	4	1	0.1	32	26	39	7	4	3	1.333	68
57	538195	43	M	6 months	Yes	no	1.59	50	19.77769867	0.7	69	160/100	9.9	93	4.5	90	299	68	30.10033445	NIS	Grade 2 MRD	3+	15.30423119	4	0.3	0.2	28	44	64	6	3.5	2.5	1.400	75
58	628637	25	F	6 months	no	no	1.52	61	26.40235457	0.9	80	140/70	9.8	39	3.5	150	414	95	36.23188406	NIS	Grade 3 MRD	2+	16.94261395	4	0.9	0.1	35	23	76	7.4	4	3.4	1.176	54
59	603881	40	M	2	Yes	yes	1.56	60	24.65483235	0.98	92	120/80	8.8	48	3.8	50	261	155	19.15708812	FID	Grade 3 MRD	1+	18.87662752	ESRD	0.1	0	20	45	48	6.6	3.5	3.1	1.129	44
60	610201	63	M	1	Yes	no	1.49	76	34.23269222	1.12	90	140/90	7.8	57	5	45	386	321	11.65803109	FID	Grade 3 MRD	2+	12.54109155	ESRD	0.4	0	35	56	47	6.9	4	2.9	1.379	23
61	583077	65	M	5	Yes	yes	1.67	65	23.30668005	0.87	91	160/100	13	66	1.6	70	267	82	26.21722846	NIS	Grade 3 MRD	3+	46.41271601	3	0.8	0.1	31	44	56	6	3.6	2.4	1.500	41
62	610676	51	M	2	Yes	no	1.66	66	23.95122659	0.67	99	140/90	6.7	90	3.9	23	381	105	6.036745407	FID	Grade 2 MRD	1+	17.43763379	4	1.2	0.2	23	55	68	6.7	4.1	2.6	1.577	46
63	594547	78	M	7	Yes	yes	1.72	51	17.23904813	0.56	87	140/90	6	184	6.6	33	351	76	9.401709402	AID	Grade 4 MRD	2+	8.716937837	5	0.9	0	33	24	122	7.4	3.9	3.5	1.114	58
64	611590	38	M	3	no	yes	1.65	57	20.93663912	0.66	71	140/70	7.8	90	4	37	333	167	11.11111111	FID	Grade 3 MRD	2+	17.97792461	ESRD	0.4	0	24	44	127	7.5	3.5	4	0.875	59
65	614678	26	M	2 months	no	no	1.56	49	20.13477975	0.86	72	130/80	13	20	1.4	70	280	45	25	NIS	Grade 3 MRD	1+	65.21410105	2	1	0.1	43	33	59	6.7	3.8	2.9	1.310	75
66	615314	53	M	3	Yes	yes	1.64	54	20.07733492	0.8	79	180/110	10.6	23	1.5	75	277	121	27.07581227	NIS	Grade 3 MRD	2+	52.11653581	3	0.6	0.2	3	64	39	6.7	4	2.7	1.481	58
67	617939	32	M	2 months	no	yes	1.78	67	21.14631991	0.81	88	140/100	13.7	24	0.8	69	256	100	26.953125	NIS	normal	nil	119.2618562	1	0.5	0.1	44	35	68	6.9	4.2	2.7	1.556	86
68	293406	50	M	5	yes	yes	1.66	66	23.95122659	0.85	98	150/90	13.9	16	0.9	67	267	102	25.09363296	NIS	normal	nil	95.08808215	1	0.1	0	11	44	103	6.3	4.5	1.8	2.500	58
69	620300	70	M	5	yes	no	1.81	57	17.3987363	0.7	67	140/90	12.5	44	0.8	89	289	95	30.79584775	NIS	normal	nil	101.7400877	1	0.4	0	23	37	56	7.2	3.6	3.6	1.000	85
70	620354	50	M	2	yes	no	1.82	90	27.17063157	0.98	87	140/100	14.2	32	0.6	68	300	99	22.66666667	NIS	normal	nil	151.8222422	1	0.8	0	28	29	98	6.7	3.7	3	1.233	56
																		64.65917973																