Republic of Iraq

Ministry of Higher Education and

Scientific Research

University of Kerbala / College of Science

Department of Biology

Haematological indices and iron status in renal failure patients in Babylon Governorate

A Thesis

Submitted to the Council of College of Science at University of Kerbala in Partial Fulfillment of the Requirements for the Master Degree in Science / Biology

By

Hussein Hazim Madab AL-Ghanimi

Supervised By

Assist. Prof. Dr.

Wafaa S. Al-wazni

Assist. Prof. Dr.

Najlaa B. Al-Awadi

2017 A.B.

1438 A.H.

﴿ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍ ۚ وَٱللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾ ﴾

صدق الله العلي العظيم

المجادلة 11

Dedication

To the Great Prophet Mohammed peace be upon him

To the spirit of my mother

For her patience

To my family for their abundant support and understanding

Hussein H. AL-Ghanimi

Acknowledgements

First of all, I would like to praise Allah the Almighty, for helping me to complete this work and pray be to Prophet Mohammed and his progeny.

*I am extremely grateful to my supervisor "**Assist. Prof Dr. Wafaa S. Al-wazni** and, **Assist. Prof. Dr. Najlaa B. Al-awadi** " for giving me the opportunity to work under their supervision. I would like to thank them for the endless support, encouragement, and guidance. It was a pleasure working with them. Thanks to the University of Kerbala, College of Science and the Department of Biology for providing the necessary facilities during this study.*

*I want also to thank the staff of the Department of Biology-College of Science, particularly the Dean of the College Prof. Dr. **Nawras M. Al-Dahan** and the Head of the Department Assist.Prof.**Dr. Wafaa S. Al-wazni** for their kindness, support, care and cooperation during this work.*

My deepest thank also goes to the Laboratory Department – Marjan teaching hospital / Babylon Health Directorate for their kindness and support.

*I would also like to thank "**Dr. Hayder Mehdi** " in Marjan teaching Hospital in Hilla /Babil Governorate for sharing his knowledge, experience and the diagnosis of patients with renal failure.* **Thanks are due to the Renal failure Patients for their generosity and help during the course of the present work.**

Last but not least, I would like to thank all my respected friends and all members of my family for their enthusiasm and encouragements. I wouldn't have made it without you all.

I would like to extend my thanks to my dearest brothers (**Sultan Kreem , Heba Mahdi , Noor Hashim , Mohammed Jassim, Anwar Al-Bawi, Bahaa Kareem, Mahdi salih, and Haider Abbas**) for their help and encouragement.

Hussein H. AL-Ghanimi

Supervisors Certification

We certify that this thesis was prepared under our supervision at the College of Science, University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master of Science in Biology.

Member: Supervisor

Signature:

Name: Dr. Wafaa S. Al-wazni

Title: Assistant professor

Address: College of Science
University of Kerbala

Department of Biology

Member: Supervisor

Signature:

Name: Dr. Najlaa B. Al-awadi

Title: Assistant professor

Address: College of Medicine

University of Babylon

Department of pathology

Chair of the Biology Department.

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Head of Department

Signature:

Name: Assist. Prof. Dr. Wafaa S. Al-wazni

College of Science / University of Kerbala

Certification

We are the examining committee, certify that we have read this thesis and examined the student **(Hussein Hazim Madab AL-Ghanimi)** in its contents, and that according to our opinion, it is accepted as a thesis for the degree of **Master** of Science in Biology with (***Excellent***) estimation.

Chairman

Signature:
Name: Haider Kamil Zaidan
Title: Professor
Address: College of Science / University of Babylon.

Member

Signature:
Name: Haider Salih Jaffat
Title: Assistant professor
Address: College of Science /University of Kufa

Member

Signature:
Name: Ayyed H. Hassan
Title: Assistant professor
Address: College of Veterinary / Kerbala University

Member: Supervisor

Signature:
Name: Dr. Wafaa S. Al-wazni
Title: Assistant professor
Address: College of Science / Kerbala University
Department of Biology

Member: Supervisor

Signature:
Name: Dr. Najlaa B. Al-awadi
Title: Assistant professor
Address: College of Medicine /University of Babylon
Department of pathology

Dean of College of Science

Signature:
Name: Dr. Nawras M. Al-Dahan
Title: Assist. professor
Address: College of Science / University of Kerbala.
Date: / /2017

Table of Contents

Supervisors Certification.....I
Certification..... II
List of Table:..... VIII
List of Figure:..... X
List of Abbreviations XI
Summary: ..... A
Chapter one..... 1
1.1: Introduction 1
1.2: Aims of study:..... 2
Chapter Two 3
2: Kidney:..... 3
2.1: Anatomy and Structure:..... 3
2.2: Functions:..... 4
2.3: Renal Failure:..... 5
2.3.1: Major Causes of Renal Failure 5
1- High Blood Pressure (Hypertension):..... 5
2-Diabetes Mellitus:..... 6
3-Glomerulonephritis 6
4-Polycystic Kidney Disease 6
5-Kidney Infection or Pyelonephritis..... 6
6-Kidney Stone..... 7
7-Systemic Lupus Erythematous 7
2.3.2: Types of Renal Failure..... 7
2.3.3.: Acute kidney injury 7
2.3.3.1: Classification and major causes of Acute kidney injury 8
2.3.3.2: Complication of Acute kidney injury 8
2.3.4: Chronic Kidney Disease:..... 9
2.3.5: Clinical Signs and Symptoms:..... 9
2.3.6: Stages of Chronic Kidney disease: 10

2.3.7: Complication of Chronic Kidney disease: 11
1- Anemia 11
2- Uremia 12
3- Sexual Dysfunction 12
4- Altered Immune Function 12
2.3.8: Treatment and Prognosis of CKD: 12
2.3.8.1: Renal ReplacementTherapy: 13
2.3.8.2: Hemodialysis: 13
2.3.8.3: Peritoneal Dialysis: 14
2.3.8.4: Renal Transplantation: 14
2.4: Renal Function Tests: 14
2.4.1: Serum Urea: 14
2.4.2: Serum Creatinine: 15
2.5: Erythropoietin: 15
2.6: Haematological Parameters (RBC indices): 16
2.6.1: Red Blood Corpuscular: 16
2.6.2: Haemoglobin 16
2.6.3: Packed Cell Volume or Haematocrit: 17
2.6.4: White Blood Cells 18
2.6.5: Platelets 18
2.7: Iron Status 19
2.7.1: Iron: 19
2.7.2: Total-Iron Binding Capacity: 20
2.7.3. Transferrin: 20
2.7.4: Serum Ferritin: 21
2.8: Anemia in Patient with Renal Failure 22
2.8.1: Signs and symptoms of anemia with patients of AKI and CKD 25
2.8.2: Complications of anemia in patients with AKI and CKD 25
Chapter Three 26
3.1: Materials and Methods 26

3.1.1: Patients group: 26
3.1.2: Control Group 27
3.1.3: The design of the study 27
3.1.4: Sample collection: 28
3.1.5: Clinical Examination: 28
3.1.6: Instruments and tools used in this study: 29
3.1.7: Chemicals used in the study: 30
3.2: Methods: 30
3.2.1: Measurement of Serum Urea Concentration: 31
3.2.2: Measurement of Serum Creatinine Concentration: 32
3.2.3: Measurement of complete blood count: 34
3.2.3.1: Red Blood Corpuscular Morphology: 34
3.2.4: Iron Status 34
3.2.4.1: Measurement of serum iron (SI): 34
3.2.4.3: Measurement of Transferrin Saturation Percentage: 37
3.2.4.4: Measurement of Transferrin Concentration: 37
3.2.5: Measurement of serum Ferritin Concentration: 37
3.2.5.1: Estimated Total Iron Body Store (ETIBS) 39
3.2.6: Measurements of Erythropoietin 40
3.3: Statistical Analysis: 45
Chapter Four 46
4: The results 46
4.1: The patient group. 46
4.1.1: Social demographic characteristics of the study population 46
4.1.2: Distribution of renal failure patients and control groups by Gender. 47
4.2: Comparison Between Patients and control groups 48
4.2.1: Kidney function tests: 48
4.2.2: Iron status 49
4.2.3: Haematological parameter and Erythropoietin. 50
4.3: Comparison between Patients groups according to gender. 51

4.3.1: Kidney function tests Results according to gender difference: 51
4.3.2: Results of Iron status according to gender difference: 52
4.3.3: The Haematological parameter and Erythropoietin results according to gender difference:..... 53
4.1: Comparison among Patients of renal failure according to Duration of the disease. 54
4.4.1: Results of Kidney Function tests: 54
4.4.2: Results of Iron status tests according to Duration of the disease. 55
4.4.3: The Haematological parameter and Erythropoietin according to Duration of the disease..... 56
4.5: Comparison Between Patients and control groups according to age. 57
4.5.1: Results of Kidney function tests according to age group: 57
4.5.2: Results of Iron status according to age group :..... 58
4.5.3: Results of RBC, HB, PCV, RBC indices, PLT, WBC and EPO levels according to age group:..... 59
4.6.1: Distribution of anemia among AKI and CKD patients. 60
4.6.2: The association of blood indices with AKI and CKD patients. 60
4.6.3: The association of iron status with AKI and CKD patients. 61
4.6.4: The association of frequency of dialysis with AKI and CKD patients. 61
4.6.5: The association of Nutritional status with AKI and CKD patients. 62
4.6.6: Overall prevalence of anemia among AKI and CKD patients. 63
4.6.7: Prevalence of anemia among AKI and CKD patients by gender. 63
4.6.8: The associated diseases with AKI and CKD patients..... 64
4.6.9: Relationship of Erythropoietin Levels to Measured Parameters 64
4.7: Blood Smear..... 67
Chapter Five 70
5: Discussion 70
5.1: Social-demographic characteristics: 70
5.2: Kidney function test:..... 70
5.3: The status of Iron 71
5.4: Prevalence of anemia among renal failure patients 74

5.5: Morphological characteristics of renal failure anemia 79
Conclusions 80
Recommendation 81
References: 82

List of Table:

Table 4- 1 : Social demographic characteristics of the study population 46
Table 4- 2: Kidney function tests in patients with renal failure (AKI & CKD) and control group. 48
Table 4- 3 : Serum levels mean of Iron status components in patients and control groups. 49
Table 4- 4: RBC, Hb, PCV, RBC indices, PLT, WBC and S.EPO levels in patients groups comparison with control group 50
Table 4- 5: Serum creatinine and Blood urea rates in patients with renal failure groups according to gender difference. 51
Table 4- 6: Iron status in patients with renal failure groups according to gender difference 52
Table 4- 7: RBC, HB, PCV, RBC indices, PLT, WBC and S.EPO levels means in patients with renal failure groups according to gender difference. 53
Table 4-8: Kidney function tests among patients of renal failure according to Duration of the disease. 54
Table 4- 9: Iron status tests according to Duration of the disease. 55
Table 4- 10: The Haematological parameter and S.EPO level means according to Duration of the disease. 56
Table 4- 11: Serum creatinine and blood urea level means in the renal failure patients in comparison with control groups according to age groups. ... 57
Table 4-12: Iron status in the renal failure patients in comparison with control group according to age group. 58
Table 4- 13: RBC, HB, PCV, RBC indices, PLT, WBC and S.EPO levels in renal failure patients groups in comparison with control group according to age group. 59
Table 4- 14: The type of anemia among AKI and CKD patients 60
Table 4- 15: Blood indices among AKI and CKD patients. 60
Table 4- 16 : Association between renal failure patients with iron status. 61

Table 4-17: The frequency of dialysis (occasional or less than 8 time per month ≥ 12 times per month). 61
Table 4-18**:** The Nutritional status including: good oral intake vs. Poor oral intake (By history) 62
Table 4-19: The association Iron supplements with renal failure patients (By history) 62
Table 4- 20 : Prevalence of anemia among AKI and CKD patients. 63
Table 4- 21 : Prevalence of anemia among AKI and CKD patients by gender. . 63
Table 4- 22 : The associated diseases with AKI and CKD patients. 64
Table 4 -23*: Correlation between study Parameters in AKI patients (* No.*=13)*65
Table 4-24*: Correlation between study Parameters in CKD patients (* No.*=37)* 66

List of Figure:

Figure 2- 1: General organization of the kidneys and the urinary system(Hall, 2015) 3

Figure 2- 2 : Classification and major causes of AKI (Kasper *et al.*, 2005). 8

Figure 2- 3: The molecular structure of human erythropoietin, which is a 30.4 kDa 165-amino-acid glycoprotein with 2 disulphide bonds, 3 N-linked carbohydrate (oligosaccharide) chains and ≤14 sialic acid residues (Manolis *et al.*, 2005). 15

Figure 2- 4: Schematic representation of ferritin molecule. Ferritin is a spherical shell that consists of 24 subunits (or peptide chains) folded into ellipsoids, connected through noncovalent bounds (Kalantar-Zadeh *et al.*, 2006). 21

Figure 3- 1 : The study design..... 27

Figure 3- 3 Reagent of strip..... 38

Figure 3- 4: Standard curve of Erythropoietin (pg/ml) 45

Figure 4- 1: shows the distribution of renal failure patients and control groups by Gender. 47

Figure 4-3 : Shows Normochromic Normocytic anemia in patients with renal failure(40x)..... 67

Figure 4-4: Shows Normochromic Normocytic anemia in patients with renal failure (100x)..... 67

Figure4-5: Shows Hypochromic microcytic anemia in patients with renal failure characterizes smaller cells (<80 fL) (40 x). 68

Figure4-6: Shows Hypochromic microcytic anemia in patients with renal failure characterizes smaller cells (<80 fL) (100x). 68

Figure 4-7: Shows Macrocytic anemia in patients with renal failure characterizes larger cells (>100 fL) (100x)..... 69

List of Abbreviations

Abbreviations	Description
ACD	Anaemia of chronic disease
AKI	Acute kidney injury
ARF	Acute renal failure
CKD	Chronic kidney diseases
ELFA	Enzyme linked fluorescent assay
Epo	Erythropoietin
ESAs	Erythropoiesis-stimulating agents
ESRD	End-Stage Renal Disease
ETIBS	Estimated Total Iron Body Store
FER	Ferritin
GFR	Glomerular filtration rate
Hb	Hemoglobin
HD	Haemodialysis
HRP	Horseradish Peroxidase
HTN	Hypertension
IL-6	Interleukin -6
Kd	Kilo Dalton
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MTH	Marjan Teaching Hospital
PCV	Packed cell volume
PD	Peritoneal Dialysis
PLT	Platelet
RBC	Red blood corpuscular count
ROS	Reactive oxygen species
RRT	Renal ReplacementTherapy
S-ft	Serum ferritin
SI	Serum iron
SRP	Solid phase Receptacle
TIBC	Total Iron-Binding Capacity
Trf	Transferrin concentration
TSAT%	Transferrin saturation
UIBC	Unsaturated Iron binding capacity
WBCs	White blood cells count
WHO	The World Health Organization

Summary:

The patients with acute kidney injury (AKI) and chronic Kidney Disease (CKD) may develop multiple complications. Anemia is a common and important complication especially in chronic uremia. It can severe to necessitate blood transfusion despite of it's possible side effects to the uremic patients.

The aim of this study is to evaluation hematological indices (Red blood corpuscular count, Hemoglobin, Packed cell volume, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Platelet and White blood cells count), in addition to Erythropoietin and iron status (Serum iron, Total Iron-Binding Capacity, Unsaturated Iron binding capacity, Transferrin concentration, Transferrin saturation, Serum ferritin and Estimated Total Iron Body Store), in AKI and CKD patients in compares with control groups.

This study was conducted at Hemodialysis department / Marjan Teaching Hospital in Hilla/ Babylon governorate, during the period from November 2015 to May 2016. Measurements were performed at the laboratories of Marjan Teaching Hospital and Biology Department at the College of Sciences / University of Kerbala.

We have been randomly collecting samples from uremic patients group before dialysis. As well as from of normal control group. The classification ranged of age from patients and controls group between (15-77) years. The study included 88 people of both Gender and divided into three groups: the first group included 37 CKD patients, the second group included 13 AKI patients and the third group included 38 normal controls.

The result revealed a highly significant decrease ($p \leq 0.01$) in mean RBC, HB and PCV in patients with AKI and CKD when compared with the control group, It is found that there was a highly significant increase EPO level mean when compared with the control group ($p \leq 0.01$), while the results of the study showed

the presence of a significant decrease in mean of PLTs in patients with CKD when compared with the control group ($p \leq 0.05$).

In addition, the results showed a significant decrease ($p \leq 0.01$) in mean of MCV in patients with AKI when compared with control group. However, the results of the current study showed the presence of a significant decrease in the mean of MCH in patients with CKD compared with control group ($p \leq 0.05$). Meanwhile mean of MCHC was increased in patients with AKI and CKD compared with control group ($p \leq 0.01$).

The results showed a significant decrease ($p \leq 0.05$) in mean of the serum iron, TIBC, UIBC, Trf concentration, and TSAT% in patients with AKI and CKD when compared with control group. Moreover, a significant increase in Serum ferritin and ETIBS was found in CKD patients compared with control group ($p \leq 0.01$).

The results found a significant increase in mean TIBC in females compared with males in patients with renal failure. It was also noted a significant decrease ($P \leq 0.05$) in means of iron and TIBC in the age group, and a highly significant increase ($P \leq 0.01$) in mean of ferritin in the age group ≥46 years compared to other age groups.

The results of the current study indicated that there was negatively and non-significantly between serum level of EPO with RBC, HB, PCV, PLTs, Ferritin, Urea and Creatinine in patients with AKI. It was also observed a negatively and non- significantly between serum level of EPO with PCV, MCHC, PLTs and TSAT% with patients of CKD. It was also noted of positive correlation and significantly between EPO with RBC, HB, MCHC , PLTs and Urea and creatinine with patients of CKD.

This study concluded that Hematological parameters are commonly affected in AKI and CKD patients. Of all the parameters, red cell indices are the ones commonly affected. This study showed that anemia is prevalent among AKI and CKD patients by 96%.

Chapter one

1.1: Introduction

Renal failure (RF) is disorder in which the kidney loss its functions to remove unwanted substances and excess salt from the blood stream (Prasad *et al.*, 2012).

Kidney diseases represent a serious and prevalent health problem and manifestation includes changes in renal detoxification capacity, deregulation of salt and water balance and altered endocrine functions(Perco *et al.*, 2006). The pathophysiological spectrum of kidney diseases is broad and there are two general aspects, namely acute kidney injury (AKI) and chronic kidney diseases (CKD) (Khan *et al.*, 2005).

Acute kidney injury(AKI) which is defined as rapid decline of renal function which develops over days or weeks and is usually accompanied by a reduction in urine volume with an abnormally high serum creatinine, is common especially in hospitalized patients(Nakamura *et al.*, 2012; Kawakami *et al.*, 2013), AKI is associated with 40–80 % mortality rate and may also contribute to the anemia of critical illness (Lafrance and Miller, 2010).

Chronic kidney diseases (CKD) is considered a major global public health problem (McClellan and Powe, 2009) and it can be defined as a reduced excretory kidney function (Glomerular filtration rate(GFR)$\leq$ 60mL/min/1.73m^2) or evidence of kidney damage such as proteinuria for $\geq$ 3 months (Genovese *et al.*, 2010), CKD is considered the most widespread disease in the world. In US about 35,000 deaths recorded yearly due to the CKD. The proportion of renal disease death in the US could rise in the past sixteen years (Hooi *et al.*, 2013).

The renal failure is the five most common causes of death in patients over 5 years of age, in Iraq, the death rate in 2015 of about 6879 patients because of renal failure according to the statistics of the Republic of Iraqi-Ministry of Health/Environment. Male patients had slightly higher mortality rates (6.1/3716) than females (6.9/3163). These results did not include the figure in Kurdistan region, Al-Anbar, Salah Al-Deen and Nineveh governorates (The Republic of Iraqi Ministry of Health Environment, 2016).

Chronic kidney disease (CKD) is clinically silent in up to 90 % patients until it has reached an advanced stage (Chadban *et al.*, 2003; John *et al.*, 2004). Classified into five stages by National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/DOQI) based on degrees of reduction in GFR. The findings should be consistent for more than 3 months for stages 1–2 signs of kidney damage albuminuria, hematuria or sonographic abnormalities are also

required (Hallan and Orth, 2010). CKD presented with elevated blood urea and creatinine are found during routine examination (Walker and Colledge, 2013). Various symptoms and disorders including water electrolyte balance disorders, metabolic acidosis, anaemia, hypertension, hyperphosphataemia with bone disease(Zhang *et al.*, 2012) and hormonal disorders also occur frequently in patients (Niemczyk *et al.*, 2012).

Anemia is the major complication of CKD, partial loss of kidney function. Anemia might begin to develop in the early stages of CKD, when someone has 20-50% of normal kidney function. Anemia tends to worsen as CKD progresses. Most people who have total loss of kidney function, or kidney failure, have anemia (Brugnara and Eckardt, 2011)

Erythropoietin (EPO) is characterized as a hormone secreted from the peritubular cells in the adult kidney (Suresh *et al.*, 2012). The binding of erythropoietin to these progenitor cells saves them from apoptosis and therefore permits cell division and maturation into red cells (Van der Walt *et al.*, 2015).

1.2: Aims of study:

Anemia is a common complication in patients with renal failure, especially chronic type of it. This requires early diagnosis and the need for knowledge of its kind to be able to provide appropriate treatment for the patient especially if we know that a lot of drugs may have side effects. Also, ferritin ratio (Ferritin) in the blood may not represent the actual percentage of the storage of iron with patients of renal failure and could lead to difficulties in the description for the treatment of anemia. This study was aimed to done the following objective:

1- Study of hematological indices and Iron status in patients with AKI and CKD and to compare results with control group.

2- Determine the type of anemia in patients with AKI and CKD.

3- Determination the erythropoietin level and hemoglobin level among AKI and CKD patients.

4- Correlation between of these parameters.

Chapter Two

2: Kidney:

2.1: Anatomy and Structure:

The urinary system involves : kidneys, ureters, urinary bladder, and urethra (Mader, 2008).The kidneys are a pair of bean shaped, reddish brown organs about the size of one's fists, in adults 11-14 cm in length in adults, 5-6 cm in width and 3-4 cm in depth. Each kidney weights about 150g. The kidneys lie retroperitoneally on either side of the vertebral column at the level of Thorax12 to Lumber 3 (Kumar and Clark, 2002). There are three major regions of the kidney; renal cortex, renal medulla and the renal pelvis(Wu *et al.*, 2017). as show in figure (2-1)

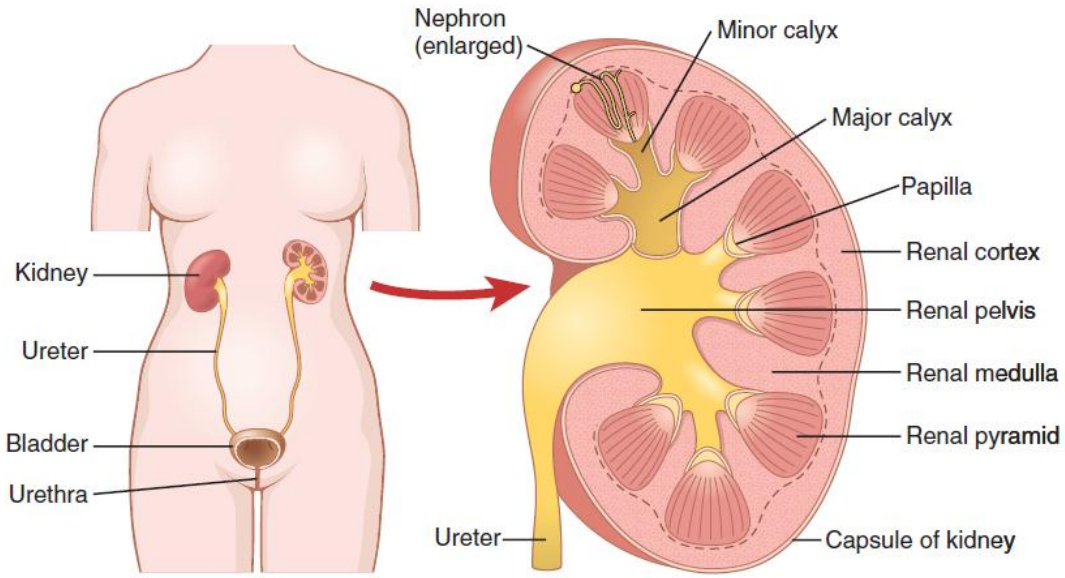


Figure 2- 1: **General organization of the kidneys and the urinary system(Hall, 2015)**

Each kidney involves over a million tiny structures known as nephrons. Nephron is the structural and functional units of the kidney(Elaine, 2012). Each renal corpuscle contains the enlarged end of a nephron, known as Bowman's capsule (glomerular capsule), and a network of capillaries, called the glomerulus (Rod *et al.*, 2004).

2.2: Functions:

There are several studies (Niemczyk *et al.*, 2012; Van der Meijden *et al.*, 2016) reported physiological function of the kidneys as summarized in the following paragraphs:

1. Excretion of Metabolic Waste Products, Foreign Chemicals, Drugs, and Hormone Metabolites: These products contain urea, creatinine, uric acid and products of Haemoglobin breakdown.

2. Regulation of Water and Electrolyte Balances

3. Glucose Synthesis: The kidneys synthesize glucose from amino acids and other precursors during prolonged fasting, a process referred to as gluconeogenesis.

4. Regulation of the pH of the blood.

5. Filtering of blood :- Proteins and blood cells are retained in the blood , while a large volume of filtrate is produced.

6. Regulation of blood pressure:- The kidneys regulate blood pressure in three ways, by adjusting the volume of blood in the body, adjusting the flow of blood both into, and out of, the kidneys, and via the action of the enzyme rennin.

7. Synthesis of Vitamin D:- Regulation of 1,25-dihydroxyvitamin D_3 Production . The kidneys produce the active form of vitamin D,1,25dihydroxyvitamin D3 (calcitriol), by hydroxylating this vitamin.

8.Secretion of Hormones: secrete hormone responsible for production of red blood cell in the bone marrow is called erythropoietin, also that kidney release a hormone called renin responsible for releasing aldosterone from cortex in adrenal gland , this hormone enhance reabsorption of sodium in kidney.

2.3: Renal Failure:

Renal failure occurs when an enough number of nephrons were damaged and therefore the kidneys incapable to perform their functions (Meyer and Hostetter, 2007).

The underlying causes may be due to many factors including diabetes, infections, auto immune diseases and other endocrine disorders, cancer, and toxic chemicals. Renal disease, systemic disease, or urinary tract disorder of non-renal origin(Mader, 2004; Fauci, 2008). This is characterized by uremia, the retention of nitrogen wastes in the blood and the reduction in the GFR (Meyer and Hostetter, 2007).

In renal failure, there might be problems with increased fluid in the body (leading to swelling), raised acid levels, increased levels of potassium and phosphate, decreased levels of calcium and in later stages anemia (Liao *et al.*, 2012).

2.3.1: Major Causes of Renal Failure

There are several causes of CKD such as diabetes, hypertension, polycystic kidney disease, glomerulonephritis and, the most of people with polycystic kidney disease have a family history of the disease (Kes *et al.*, 2011).

Most kidney diseases attack the nephrons, causing them to lose their filtering capacity. Damage to the nephrons may occur quickly, because of injury or poisoning, but majority kidney diseases destroy the nephrons gradually and silently. Only after years or even decades will the damage become obvious. The majority of kidney diseases attack both kidneys at the same time(Chu and Sartorelli, 2007).There are many causes can lead to this state such as:

1- High Blood Pressure (Hypertension):

High blood pressure or Hypertension(HTN) makes the heart work harder and, over time, can harm blood vessels throughout the body, high blood pressure is known as the "silent killer" and can cause kidney disease (Kes *et al.*, 2011).

2-Diabetes Mellitus:

Diabetes Mellitus (DM) is a chronic disorder caused by inherited and/or acquired deficiency in manufacture of insulin by the pancreas, or by ineffectiveness of the insulin produced, it is projected by the year 2025, India alone has 57 million diabetics mainly of type2 diabetes constituting 90% of the diabetic population(Ramachandran *et al.*, 2002; Riaz *et al.*, 2009). Diabetes is the major cause of End Stage Renal Disease (ESRD) (Liao *et al.*, 2012).

3-Glomerulonephritis

Glomerulonephritis (GN) is a sort of kidney disease that incorporates the glomeruli. GN is the name given to a scope of conditions that can influence the glomeruli of the kidney. Salt and extra fluid can build up in the body if the kidneys are not working normally. This can result to complications such as HTN and, in certain cases, kidney failure can happen (Parmar, 2011).

4-Polycystic Kidney Disease

Polycystic Kidney Disease (PKD) is generally a genetic disease in which several grape-like, fluid filled cysts grow in the kidneys (Picken and Herrera, 2007). Most patients with PKD have a family history of PKD (Gupta *et al.*, 2011).

5-Kidney Infection or Pyelonephritis

Pyelonephritis (from Greek – pyelum, meaning "renal pelvis", – nephrons, meaning "kidney", and itis, meaning "inflammation") is an ascending urinary tract infection that has reached the pyelum or pelvis of the kidney. Severe cases of pyelonephritis can lead to pyonephrosis (pus accumulation around the kidney), urosepsis (a systemic inflammatory response of the body to infection), kidney failure and even death (Ramakrishnan and Scheid, 2005). Pyelonephritis is most often caused by a bacterium (*Escherichia coli*) or virus infecting the kidneys(Hadjifrangiskou *et al.*, 2011). Frequent infections of this type can reason damage to the kidneys leading to kidney failure (Bostrom and Freedman, 2010).

6-Kidney Stone

A kidney stone is a hard mass that develops when calcium oxalate or different chemicals in the urine form crystals that stick together. These crystals may grow into stones ranging in size from a grain of sand to a golf ball. Kidney stones can form in the ureters (the tubes that carry urine from the kidneys to the bladder) and block the flow of urine(Stamatelou *et al.*, 2003; Vupputuri *et al.*, 2004).

7-Systemic Lupus Erythematous

Systemic Lupus Erythematous (SLE) is an autoimmune disease, which means the body's immune system mistakenly attacks healthy tissue. This results in long-term (chronic) inflammation (Hari *et al.,* 2009; Su *et al.,* 2007). It can influence the skin, kidneys, joints, lungs, nervous system and other organs of the body, most patients feel fatigue and have rashes, arthritis and fever (Hahn *et al.*, 2008).If the kidneys become involved, they lose the capability to filter wastes out of the blood and other normal functions of kidneys (Ruiz-Irastorza *et al.*, 2010).

2.3.2: Types of Renal Failure

Renal failure can broadly be classify into two main forms acute and chronic (Nitescu, 2007). Acute kidney injury (AKI), which is often reversible with adequate treatment, and Chronic Kidney Disease (CKD), which is frequently not reversible. In both cases, there is usually an underlying cause (Mishra *et al.*, 2016).

2.3.3.: Acute kidney injury

Acute kidney injury (AKI), previously known as acute renal failure (ARF) Kinsey *et al.* (2008); Mishra *et al.* (2016), is defined as an abrupt deterioration of renal function enough to result in the failure of the urinary elimination of nitrogenous waste products (Rees and Shaw, 2007; Srisawat *et al.*, 2010) AKI is

potentially reversible if the precipitating factors can be corrected before permanent renal damage occurs (Cerveró *et al.*, 2008).

2.3.3.1: Classification and major causes of Acute kidney injury

Acute kidney injury (AKI) can result from a variety of causes, generally divided into three categories: prerenal, postrenal and intrinsic (Cerveró *et al.*, 2008), the causes of AKI as show in the figure (2 -2).

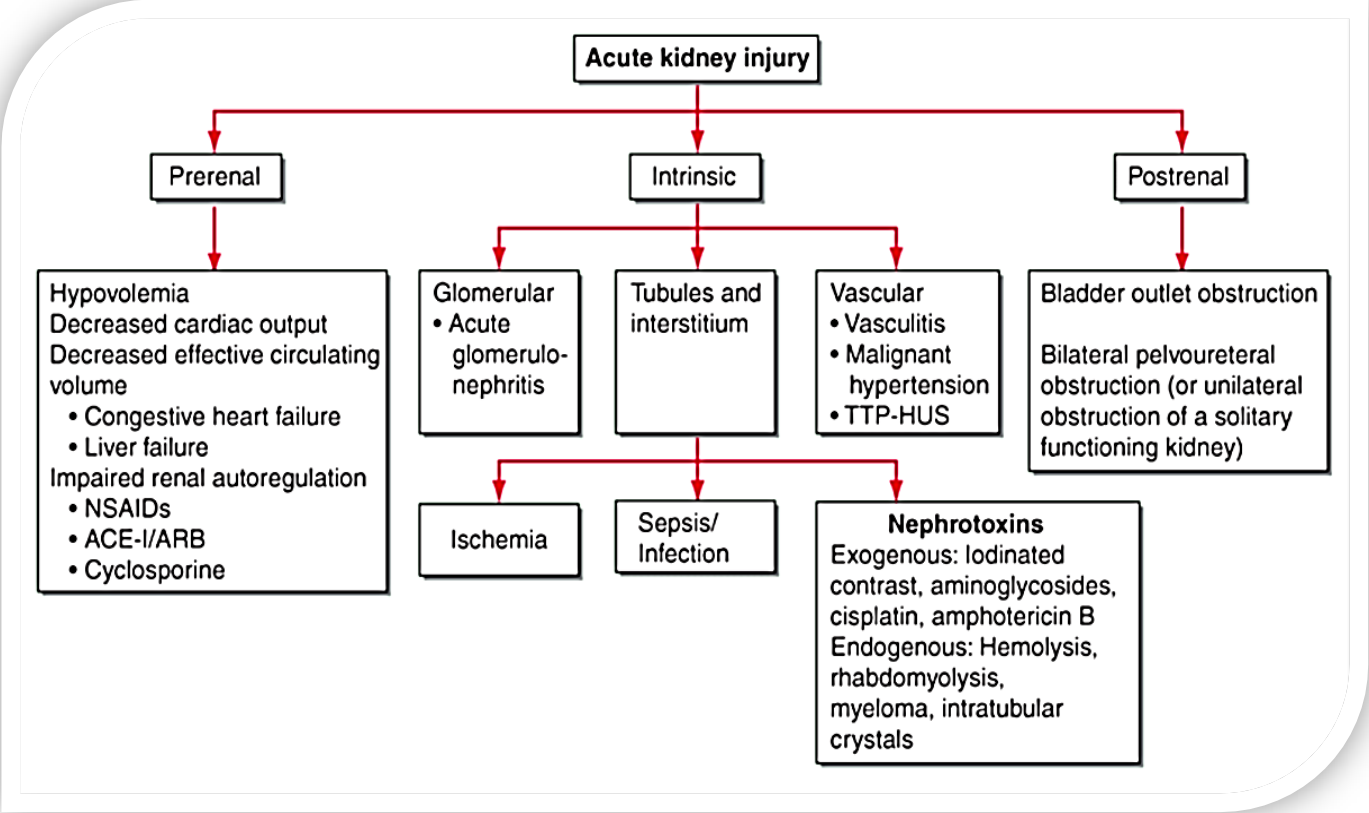


Figure 2- 2 : **Classification and major causes of AKI (Kasper *et al.*, 2005).**

2.3.3.2: Complication of Acute kidney injury

The incidence of acute kidney injury (AKI), has grown by more than fourfold in the United States since 1988 and is estimated to have a yearly incidence of 500 per 100,000 population, higher than the yearly incidence of stroke. AKI is associated with a markedly increased risk of death in hospitalized individuals, particularly in those admitted to the ICU where in-hospital mortality rates may exceed 50%. AKI occurs suddenly and is usually initiated by underlying causes, for example dehydration, infection, serious injury to the kidney or the chronic use of over the counter pain medications like Tylenol

(acetaminophen) or Advil (ibuprofen) (Liano and Pascual, 1996; Libório *et al.*, 2011).

2.3.4: Chronic Kidney Disease:

Chronic Kidney Disease (CKD) frequently result in end stage renal disease (ESRD)which without renal replacement therapy would lead to death (Mehdi, 2011); It is defined as either renal damage or decreased renal function for ≥ 3 months (Gooneratne *et al.*, 2008).

Chronic Kidney Disease (CKD) was generally defined according to the presence of kidney damage or GFR (GFR ≤60 mL/min per 1.73 m^2) for ≥3 months (Levin and Rocco, 2006; Peter, 2007; Raptis *et al.*, 2013)

Also called chronic renal failure, is slow loss of kidney function over time. It may be so slow that symptoms do not occur until kidney function is less than one-tenth of normal. The final stage of chronic kidney disease is called end-stage renal disease (ESRD). The kidneys no longer function and the patient needs dialysis or a kidney transplant (Ahmed, 2015).

Chronic Kidney Disease (CKD) patients associated with calcium-phosphorus load, hyperparathyroidism, malnutrition, anemia, hemodynamic overload, inflammation and hyperhomocysteinemia (Hage *et al.*, 2009). CKD is considered by an exceptionally high mortality rate (Peter, 2007)

2.3.5: Clinical Signs and Symptoms:

The signs and symptoms of renal failure occur gradually and do not become evident until the disease is far advanced (Bostrom and Freedman, 2010). and waste accumulates in the body and the blood, This condition known as azotemia .Very low levels of azotemia might produce few, if any, symptoms (Colledge *et al.*, 2010; Walker and Colledge, 2013) .

Signs and symptoms of renal failure can vary from person to person which may include, nausea, vomiting, loss of appetite, fatigue and weakness,

sleep problems, changes in urine output, reduced mental sharpness, muscle twitches and cramps, hiccups, swelling of the feet and ankles, persistent itching, chest pain, if fluid accumulates around the lining of the heart, shortness of breath due to extra fluid on the lungs (may also be caused by anemia), EPO synthesis is decreased leading to anemia and the patient has puffiness around the eyes, especially in the morning. Complications in renal failure include hypertensive crisis, acidosis, hyperkalemia, pulmonary Odem, and infection (Walker and Colledge, 2013).

2.3.6: Stages of Chronic Kidney disease:

Recent professional guidelines classify the severity of Chronic Kidney Disease (CKD) in five stages, with stage1 being the mildest and usually causing few symptoms and stage 5 being a severe illness with poor life expectancy if untreated (Table 2-1). CKD requiring dialysis or transplantation is known as End-Stage Renal Disease (ESRD) (Peter, 2007). In young adults, normal GFR is approximately120-130ml/min/1.73m^2 and decline with age (Lindeman *et al.*, 1985).

Table 2- 1: Classification of the Stages of Chronic Kidney Disease According To Guidelines by the Kidney Disease Outcomes Quality Initiative of the United States National Kidney Foundation (Walker and Colledge, 2013).

Stage	Definition	Description	Clinical presentation
1	Kidney damage with normal or high GFR >90.	mild CKD	Asymptomatic
2	Kidney damage and GFR 60-89.		
3A	GFR 45-59	moderate CKD	Usually asymptomatic
3B	GFR 40-44		Most are non-progressive or progress very slowly.
4	GFR 15-39	severe CKD	First symptoms often at GFR<20.electrolytes problems likely as GFR falls.
5	GFR < 15 or on dialysis	kidney failure	Significant symptoms and complications are usually present. Dialysis initiation varies but usually at GFR < 10

2.3.7: Complication of Chronic Kidney disease:

1- Anemia

It's one of the common diseases associated with CKD , especially in the ESRD, where anemia is described as severe and chronic (Furuland *et al.*, 2003)

Anemia is the major complication of CKD, it occurs in all stages but becomes more distinct at the latter stages of kidney failure(KDOQI, 2007). A significant increase in the prevalence of anemia develops as the creatinine clearances (GFR) falls to ≤ 70 ml/min in males and to ≤ 50 ml/min in females. Generally more than one half of patients with CKD are estimated to have an anemic state (Bolignano *et al.*, 2015).

2- Uremia

Uremia was the term for the contamination of the blood with urine (Li *et al.*, 2000).This included reduced urine output, which was thought to be caused by the urine mixing with the blood instead of being voided through the urethra, the term uremia is now used for the illness accompanying kidney failure (Meyer and Hostetter, 2007).

3- Sexual Dysfunction

Alterations in physiologic sexual responses, reproductive ability, and libido are common. The cause probably is multifactorial and may result from high levels of uremic toxins, neuropathy, altered endocrine function, psychological factors, and medications (e.g., antihypertensive drugs). Impotence occurs in as many as 56% of male patients receiving dialysis (Finkelstein *et al.*, 2007; Anantharaman and Schmidt, 2007)

4- Altered Immune Function

Infection is a common complication and cause of hospitalization and death of patients with CKD. Immunologic abnormalities decrease the efficiency of the immune response to infection(Kato *et al.*, 2008). All aspects of inflammation and immune function may be affected adversely by the high levels of urea and metabolic wastes, including a decrease in granulocyte count, impaired humoral and cell mediated immunity, and defective phagocyte function (Haroun *et al.*, 2003). Although persons with ESRD have normal humoral responses to vaccines, a more aggressive immunization program may be needed. Skin and mucosal barriers to infection also may be defective (Eleftheriadis *et al.*, 2007).

2.3.8: Treatment and Prognosis of CKD:

In CKD reduced intake of salt and potassium, vitamin supplements, avoidance of injury or infection, weight administration, electrolytes monitoring, and monitoring of vital signs, monitoring of cardiac status, and mental status are

important. Peritoneal or haemodialysis is the treatment of choice when other measures fail (Kellum and Decker, 2001; Snyder and Pendergraph, 2005).

Drug therapy includes Anti-hypertensive for hypertension, such as non-dihydropyridinecalcium channel blocker and an angiotensin converting enzyme inhibitor to achieve a lower than usual target blood pressure of less than130/80 mm Hg, diuretics for edema and hypertension , phosphate binders for hyper-phosphatemia (Michael and Gabreil, 2004), antibiotics, anticonvulsants for seizures, anti-emetics (drugs that prevent vomiting) for nausea, laxatives for constipation, calcium, recombinant human Epo for anemia (Nowrousian, 2007).

2.3.8.1: Renal ReplacementTherapy:

Since 1960s, Renal ReplacementTherapy (RRT) has provided life- saving and life sustaining treatment for patients affected with ESRD (Levey *et al.*, 2003) . This generally occurs when GFR falls below 15 ml/minute /1.37 m^2. RRT consists of hemodialysis, peritoneal dialysis , continues RRT. Renal transplantation increases the survival of patients with ESRD when compared to other therapeutic options (Groothoff, 2005).There are two different types of dialysis can be done hemodialysis and peritoneal dialysis (Colledge *et al.*, 2010).

2.3.8.2: Hemodialysis:

Hemodialysis (HD) starts when the patient has symptomatic progressive renal failure but before the development of serious complications, frequently with a plasma creatinine of 600-800 mmol/l (6.8-9.0 mg/dl) (Walker and Colledge, 2013). HD is a treatment that removes wastes and excess fluid from the blood when the kidneys cannot do so adequately. It can be done in a dialysis center or at the home (Chan *et al.*, 2002).

Complications of HD differ among mild complications such as headache, nausea, and vomiting and severe complications such as hemolysis and seizures (Rosner, 2005).

2.3.8.3: Peritoneal Dialysis:

Peritoneal Dialysis (PD) is a home-based treatment that may be done anywhere (at work, home, or when sleeping),it must be done daily, patient require a minor operation to place a catheter in abdomen (Williams *et al.*, 2004).During treatment a cleansing solution, known as dialysis, flows into abdomen through a soft tube known as a PD catheter. Wastes and excess fluid pass from blood into the cleansing solution. Removing the used solution and adding fresh solutions takes about a half hour and is known as an "exchange." (Walker and Colledge, 2013).

2.3.8.4: Renal Transplantation:

Renal transplantation is the renal replacement treatment of choice because it improves survival rates, quality of life and cost as compared to dialysis is recommended for patients with ESRD who can tolerate transplanting surgery (Knoll *et al.*, 2005)

Kidneys for transplantation may come from either a living or deceased donor. A healthy kidney may be transplanted into a patients with End stage of renal failure. It is essential to monitor its function closely and to inhibit immune system by drugs like prednisone, tacrolimus , cyclosporine A (Sandimmune®) and lymphocyte proliferation inhibitors (azathioprine and mycophenolate mofetil) (Cerveró *et al.*, 2008) In transplant patients, graft failure may be attributed to complications associated with morbid obesity, such as heart disease, HTN, and DM (Rama and Grinyó, 2010).

2.4: Renal Function Tests:

2.4.1: Serum Urea:

Urea is a major disposal form of amino groups generated from amino acids and accounts approximately 90% of the nitrogen containing components of urine. Urea is synthesis by the liver, and then it is transported to the kidneys where it is filtered and released in the urine (Harvey and Ferrier, 2011). Estimation of serum urea and creatinine is generally regarded as the first line

investigation of renal functions, increased urea level is associated with nephritis, renal ischemia, urinary tract obstruction (Beckett *et al.*, 2010).

2.4.2: Serum Creatinine:

Creatinine is produced from creatine and phosophocreatine, both of which are released from muscles (Ceriotti *et al.*, 2008).The level of creatinine in the blood stream is fairly constant; however creatinine level may be changed by muscle mass changes (Mendelssohn *et al.*, 1999). Normal serum creatinine (55-120) µmol/L (Beckett *et al.*, 2010). High creatinine blood level may indicate serious damage or disease of the kidney is present, also can mean heart failure, dehydration, excessive blood loss that causes shock, gout and heavy protein meal intake (Chhetri *et al.*, 2008). In addition, medications (e.g. cimetidine or trimethoprim) may increase serum creatinine level by inhibition of creatinine excretion (Mendelssohn *et al.*, 1999).

2.5: Erythropoietin:

Human Erythropoietin (EPO) is an endogenous 30.4 kDa glycoprotein hormone containing a 165-amino acid residue backbone and two di-sulphide bonds (Fig. 1-3) (Egrie and Browne, 2001). In response to hypoxia and stimulates the proliferation and differentiation of erythrocytic progenitors in the bone marrow (Macdougall, 2002).

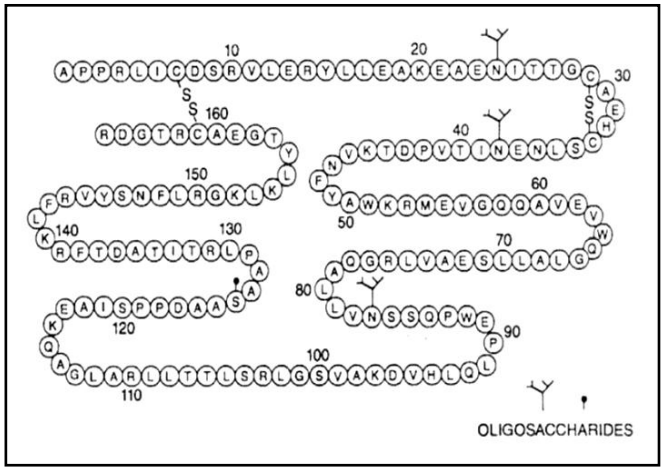


Figure 2- 3: The molecular structure of human erythropoietin, which is a 30.4 kDa 165-amino-acid glycoprotein with 2 disulphide bonds, 3 N-linked carbohydrate (oligosaccharide) chains and ≤14 sialic acid residues (Manolis *et al.*, 2005).

The use of EPO Stimulating Agents as it is prepared as recombinant human erythropoietin (EPO), may result in formation of anti-erythropoietin antibodies thereby leading to pure red cell aplasia and erythropoietin resistance hence the hemoglobin level suddenly starts to decline, despite continued therapy with EPO at the same or even increased doses (McGonigle *et al.*, 1984).

In CKD, the EPO levels may be normal, but inadequate for the degree of anemia. The mechanisms impairing EPO production in diseased kidneys remain poorly understood; Dialysis may improve renal anemia and the efficiency of EPO-stimulating agents. The anemia of chronic disease are mediated by inflammatory cytokines through the Inhibitions of erythropoietin manufacture and efficiency and reduced iron availability (Van der Walt *et al.*, 2015).

There are several types of Epoetin manufacture: Epoetin or Neorecorman , Darbepoetin Epoetin delta (Dynepo) Pdpoetin (Drüeke *et al.*, 2006).

The side effects of Erythropoietin Manufacturer: such as Hypertension, Headache, Joint pain, topical clotting of blood at the injection site , Skin rash, Muscle pain and other symptoms , but there are rare : Allergic reaction, Seizures and some cardiovascular diseases (Macdougall, 2001)**.**

2.6: Haematological Parameters (RBC indices):

2.6.1: Red Blood Corpuscular:

The Red Blood Corpuscular (RBC) also known as Erythrocytes are cells that carry oxygen to all parts of the body. In humans, mature RBC are flexible biconcave disks that lack a cell nucleus and most organelles (Zhang *et al.*, 2008). The production can be stimulated by the hormone EPO, synthesized by the kidney (Nemeth and Bodine, 2007).

2.6.2: Haemoglobin

Hemoglobin (Hb) is the protein molecule in RBC that carries CO_2 from the lungs to the body's tissues and returns CO_2 from the tissues to the lungs. It is the main protein component of RBC (92% of dry weight), the protein is a tetramer

with a molecular weight of ~ 64 kDa (Ashton, 2013).The normal values of Hb within (13.5 - 17.5) g/dL for males and (12–16) g/dL for females (Benjamin *et al.*, 2015).

If the untreated anemia, hemoglobin concentrations typically fall below 10 g/dl, and frequently to half or less of the normal value. With blood oxygen carrying capacity thus markedly diminished, cardiac output must increase in order to maintain normal tissue oxygen delivery, and even in the absence of pulmonary disease, patients are vulnerable to tissue hypoxia during exertion and at times of acute illness (Cody *et al.*, 2005).

2.6.3: Packed Cell Volume or Haematocrit:

Packed Cell Volume (PCV) is the percentage of the total blood volume occupied by RBC. PCV can be used as an indicator for anemia and to indicate the degree of fluid loss in dehydration (Albu *et al.*, 2004). The PCV is important, along with Hb and RBC, to determine anemia or polycythemia. The normal values of PCV within 39–49% for male and 35–45% for females (Benjamin *et al.*, 2015).

PCV level was decreased in CKD patients undergoing HD due to the supine position of the HD patients and consequent haemodilution caused by redistribution of water from the extra- to intra- vascular space (Inagaki *et al.*, 2001).

In addition to a higher incidence of atherosclerotic disease and CV comorbidities, several small studies have emphasized the existence of platelet dysfunction in CKD patients. Many pathways of platelet activation have been hypothesized to play a causative role in this increased thrombotic and bleeding risk. Alterations in primary haemostasis as a result of intrinsic platelet abnormalities and impaired platelet–vessel wall interactions have been described for a long time in CKD patients and speculatively associated with an enhanced bleeding risk. After vascular injury, shear-induced platelet aggregation, involving von Willebrand factor (vWF) and platelet glycoproteins (GP) Ia and IIb-IIIa, is a determinant process in primary haemostasis. In uraemic patients,

part of the platelet dysfunction could be explained by decreased GP IIb-IIIa availability due to receptor occupancy by fibrinogen and vWF fragments or uraemic toxins. An impairment of cytoskeletal organization leading to decreased cell spreading was described at baseline in platelets from uraemic patients, an abnormality which was more evident after thrombin stimulation(Morel *et al.*, 2013).

2.6.4: White Blood Cells

White Blood Cells (WBCs), also known as Leukocytes, are cells of the immune system. The role of WBCs is to defend against invading pathogens by distinguish foreign material and either engulf cells or release membrane disrupting chemicals that can destroy the organism (Ashton, 2013).

There are five types of leukocytes exist, classified into granulocytes (neutrophils, basophils and eosinophils) and a granulocytes (monocytes and lymphocytes) (Ashton, 2013)

An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. Excessive numbers of WBCs are most often due to the response of normal B.M to infection or inflammation. In some instances, leukocytosis is a sign of more serious primary B.M disease (leukemias or myeloproliferative disorders) (Abramson and Melton, 2000)

2.6.5: Platelets

Platelets (PLTs), also called thrombocytes, are specialized disk shaped cells in the blood that are contributed in the formation of blood clots. PLTs can identify a disruption in the lining of a blood vessel and react to form a wall to stop bleeding (Gregg and Goldschmidt-Clermont, 2003). They are small, irregularly-shaped nuclear cells, approximately (0.5-3) mm in diameter; their level in plasma is controlled indirectly by the level of thrombopoietin.

The average half-life of PLTs is approximately 10 days. PLTs play an important role in hemostasis, Decrease in the number of PLTs lead to a condition known as thrombocytopenia and may lead to increased bleeding, which this may lead to nose bleeding, gum bleeds, and increased bruising (Gregg and Goldschmidt-Clermont, 2003).

2.7: Iron Status

2.7.1: Iron:

Iron is an essential element for life. The control of this essential but actually toxic substance is an important part of several aspects of human health and disease. The majority of the human body's iron is contained in RBC (Fleming and Bacon, 2005).

Humans consume iron in two forms, heme iron (ferrous) that is present in meat, liver, blood …etc. and Non-heme iron is generally present in plants and the majority of it is ferric state (Huang, 2003).

Most well- nourished population in industrialized countries has 3- 4 grams of iron. About, 2.5 g is contained in the hemoglobin required to carry oxygen through the blood. Additional 400 mg is devoted to cellular proteins that use iron for essential cellular processes like storing oxygen myoglobin, or performing energy-producing redox reaction (cytochromes). Three to four mg, attached to transferrin in the plasma (Fleming and Bacon, 2005).

Large amounts of free iron in the circulation, will lead to damage the critical cells in the liver, the heart, and other organs. Iron toxicity is frequently the results of iron overload such as syndromes associated with genetic disease, repeated transfusion and other causes (Ganz, 2007)

Intestinal iron absorption is limited to the duodenum and is ensured by the mature enterocytes present at the top of the villi. In humans, only 1-2 mg of a normal daily diet will be absorbed (Beaumont, 2004; Andrews, 2008).

Another control is a decrease in iron export to plasma in response to inflammation in which inflammatory cytokines regulate the synthesis of hepcidin (Loreal *et al.*, 2005)

2.7.2: Total-Iron Binding Capacity:

Total-Iron Binding Capacity (TIBC) is a medical laboratory test that measures the blood's capacity to bind iron with transferrin. It is achieved by drawing blood and assessing the maximum amount of iron that it can carry, which indirectly measures Trf, Since Trf is the most dynamic carrier, TIBC is less expensive than a direct measurement of Trf (Yamanishi *et al.*, 2003). The TIBC most not be confused with the unsaturated iron binding capacity (UIBC). The UIBC is calculated by subtracting the serum iron from the TIBC (Gambino *et al.*, 1997) .

2.7.3. Transferrin:

The ferric iron binds to Apotransferrin (the iron-free form of transferrin) in the plasma to form Fe^{+3}-transferrin complex, which is the major type of iron present in blood. Transferrin (Trf) is typically 80 kDa a soluble glycoprotein and a bilobal molecule i.e. it contains an N-terminal (amino acids 1-336) and a C-terminal (amino acids 337-679) globular domain. The Trf is produced in liver, retina, testis and brain (Thevis *et al.*, 2003; Andrews and Schmidt, 2007).

Essentially all circulating plasma iron normally is bound to Trf. This chelation serves three purposes: it renders iron soluble under physiologic conditions, it prevents iron- mediated free radical toxicity, and it facilitates transport into cells (Ponka *et al.*, 1998; Andrews and Schmidt, 2007).

Serum iron, TIBC, and calculated transferrin saturation (TSAT%) tests are used to screen for and monitor conditions of iron deficiency and iron overload, although the usefulness of these tests for diagnosing iron deficiency may be debated (Guyatt *et al.*, 1992)

2.7.4: Serum Ferritin:

Ferritin is the principal, iron storage proteins in most living organisms throughout development from Human through invertebrates, plants and microorganisms (Crichton, 2016), it's found in blood, liver, spleen, bone marrow, intestine (mucosal cells), synovial fluids and milk (Chatterjea and Shinde, 2012)

Ferritin molecule takes the shape of a hollow sphere with an external diameter of 12 to 13 nm (Figure 2-4). The outer shell is composed of 19 kDa light (L) and heavy (H) is 21 kDa polypeptide chains, the so-called ferritin subunits, folded into four-helix bundles (Theil, 1987; Ganz, 2007). The H subunit has ferroxidase activities that are necessary for the oxidation of incoming ferrous ions, while as the L subunit has a nucleation site which is involved in iron-core formation (De Domenico *et al.*, 2008; Harrison and Arosio, 1996; Torti and Torti, 2002).

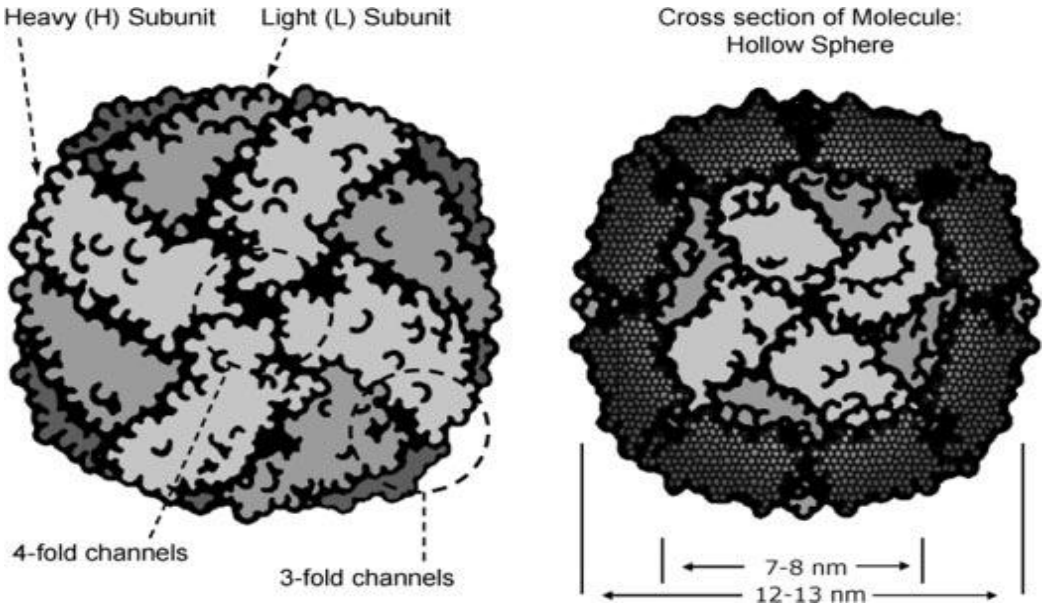


Figure 2- 4: Schematic representation of ferritin molecule. Ferritin is a spherical shell that consists of 24 subunits (or peptide chains) folded into ellipsoids, connected through noncovalent bounds (Kalantar-Zadeh *et al.*, 2006)**.**

The serum ferritin level relates with total body iron stores thus, the serum ferritin is considered the best convenient laboratory test to assess iron stores (Jackson *et al.*, 2007).

Ferritin levels drop early in the development of iron deficiency, before serum iron and transferrin saturation become abnormally low. A rise in serum ferritin may be the primary indication of iron overload, long before the signs and symptoms of hemochromatosis appear. However, the secretion of ferritin from harm tissues in hepatitis, acute inflammatory conditions, and a variety of tumors as well dramatically increases the serum ferritin level. In these situations, normal ferritin values can mask the occurrence of iron deficiency (Kaplan and Kazmierczak, 2003)

Thus low ferritin level is a reliable indicator of iron deficiency, while a normal to moderately high serum ferritin does not rule out iron deficiency or indicate sufficient (Kalantar-Zadeh *et al.*, 2006).

Serum ferritin is also an acute phase reactant and able to be increased in inflammation (Kalender *et al.*, 2002). Thus, It's possible that high levels of serum ferritin are engendered by inflammation independently of iron stores (Kalantar-Zadeh *et al.*, 2006).

2.8: Anemia in Patient with Renal Failure

In 1836 Richard Bright, for the first time, described the association of CKD and anemia, when he observed pallor in the development of Brigit's disease (Remuzzi and Minetti, 2000).

Anemia is a state in which the number of circulating RBC, the concentration of Hb or PCV is lower than normal for age and sex (Smith, 2010).The WHO considers men with a Hb concentration ≤13.0 g/dL or PCV ≤39 % anemic and women with Hb ≤12.0 g/dL or PCV ≤36 % to be anemic (Hahn, 2007)

The causes of anemia are multifactorial ranging from erythropoietin deficiency to nutritional anemia due to iron deficiency, vitamin deficiencies (B12, folate), hemolysis, bleeding and adverse effects of cytotoxic or immunosuppressive drugs and Angiotensin converting enzyme inhibitors.

However, erythropoietin deficiency is the most significant cause of anemia in CKD (Van der Walt *et al.*, 2015).

Anemia associated with the normocytic and normochromic RBC are hypoproliferative anemia. This includes early iron deficiency (before hypochromic microcytic red cells develop), acute and chronic inflammation (including many malignancies), and kidney disease, hypometabolic states as protein malnutrition and anemia from being marrow damage(Kasper *et al.*, 2008).

Iron deficiency is also found in patients with CKD which may be unconditional; often due to poor nutritional iron intake, some-times as a result of blood loss, even when there is an imbalance between demand and supply of iron to erythroid marrow. Iron deficiency leads to reduction in formation of red blood cell hemoglobin causing hypochromic microcytic anemia. In addition, presence of uremic inhibitors like parathyroid, cytokines , shorten half-life of matured blood cells and either vitamin B12 or folate deficiencies result (Srinivasan *et al.*, 2016)

Iron deficiency has also been considered as important cause of anemia in CKD patients and these patients manifest iron deficiency as ‘‘absolute’’ or ‘‘functional’’ iron deficiency(Nairz *et al.*, 2016).

In CKD patients iron stores (absolute) are depleted as a result of decreased intake due to malnutrition, Blood loss for laboratory tests, aggravated by hospitalizations, decreased appetite associated with uremia and increased loss through chronic GIT bleeding due to blood vessel fragility associated with uremia , platelet dysfuctionatial related to uremia, chronic blood retention in the dialysis circuit(Macdougall *et al.*, 2016).

Functional iron deficiency occurs when there is a need for a greater amount of iron to support hemoglobin synthesis than can be released from iron

store. In CKD there is an impaired release of stored iron from macrophages and hepatocytes to transferrin(Nairz *et al.*, 2016)

The diagnosis of the absolute Iron deficiency anemia rests on the presence of microcytic, hypochromic anemia with, concentration of serum ferritin level ≤ 100 ng/ml, TSAT% ≤20%, low MCV percentage of hypochromic red cells ≥6%.The diagnosis of functional iron deficiency anemia rests on the presence of hypochromic, microcytic anemia with, concentration of serum Ferritin level ≥ 100ng/ml, TSAT% ≤20%, The normal of MCV and MCHC and percentage of Hypochromic red cells (HRC) ≥6% (Worwood, 2006; Pasricha *et al.*, 2010).

Angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) can contribute to anemia in CKD patients. A circulating natural inhibitor of bone marrow, N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) has been implicated in the pathogenesis of ACEI-induced anemia. The ACE enzyme is responsible for the degradation of AcSDKP, thus blockade of ACE with ACEI leads to increased circulating levels of AcSDKP and therefore bone marrow inhibition (McGonigle *et al.*, 1984).

Presence of inflammatory mediators (cytokines) such as tumor necrosis factor alpha, interleukin-1 and 6 are elevated in CKD and are associated with rheumatoid disease (arthritis, lupus), chronic infections and dysmetabolic state seen in late in CKD disease. These mediators interfere maturation of RBC precursors (Tsirpanlis *et al.*, 2004; Macdougall and Cooper, 2002).

A normocytic normochromic anemia is also common in patients with a variety of inflammatory disorders and there are many contributing factors(Ida, 2015). In inflammation, from whatever cause, IL-6 stimulate the liver to produce hepcidin. Hepcidin decreases iron absorption from the bowel and blocks iron utilization in the bone marrow. Again, some chemotherapeutic agents induce anemia by impairing hematopoiesis (Wilson *et al.*, 2007). In addition, nephrotoxic effects of particular cytotoxic agents, such as platinum salts, can also lead to the persistence of anemia through reduced EPO production by the kidney (Stasi *et al.*, 2002).

2.8.1: Signs and symptoms of anemia with patients of AKI and CKD

Weakness , fatigue or feeling tired , headaches , problems with concentration , paleness , dizziness , difficulty breathing or shortness of breath and chest pain (Somvanshi *et al.*, 2012).

2.8.2: Complications of anemia in patients with AKI and CKD

An unusually fast heartbeat is there, mainly when exercising, The harmful enlargement of muscles in the heart and Heart failure, which does not mean the heart suddenly stops working. Instead, heart failure is a long-lasting condition in which the heart can't pump enough blood to meet the body's needs (Somvanshi et al., 2012).

The spread of anemia (with or without CKD) rises with age , which means that, as the US population ages, the number of people influenced by anemia in CKD will also increase(Bowling *et al.*, 2011; Stauffer and Fan, 2014).

Successful treatment of anemia in patients with renal disease may decrease the decline of kidney function. The target Hb levels are approximately (10 – 12) g/dL when treating anemia in CKD. The treatment of anemia is costly and therefore rational consideration is mandatory. The evaluation of anemia in patients with CKD should include a CBC with red cell indices (MCV and MCHC). Anemia CKD is usually normochromic and normocytic. Vit-B12 and folate deficiency may cause macrocytosis, whereas iron deficiency or inherited disorders of Hb may lead to microcytosis. Iron studies should be performed to evaluate the level of iron in tissue stores or the sufficiency of iron supply for erythropoiesis (Van der Walt *et al.*, 2015).

Chapter Three

3.1: Materials and Methods

3.1.1: Patients group:

This study was conducted at Hemodialysis department / Marjan Teaching Hospital in Hilla /Babylon Governorates, during the period from November 2015 to May 2016. The practical part of the study was performed at the laboratories of Marjan Teaching Hospital and Biology Department at the College of Sciences / University of Karbala.

Fifty patients were divided into study groups: AKI patients group included 13 (26%) subjects aged 28-70 years and CKD patients group included 37(74%) subjects range aged (15- 77) years.

The renal failure patients were classified into two groups according to the duration of renal failure disease as follows:

1. Group I (GI): the group of patients with disease length of (less than 3 months) was considered the groups with acute kidney injure duration depend on of diagnosis of doctor.
2. Group II (GII): the group of patients with disease more than 3 months was considered the group with chronic kidney disease duration Genovese *et al.* (2010).

The patients were divided into three age ranges: the 1st age range (15-30) years include (8) patients; the 2nd age range (31-45) years include (8) patients and the 3rd age range (≥46) years include (34) patients. The patients were diagnosed as renal failure based on medical history, clinical examination and investigations.

Exclusion Criteria: Patients who suffered from the following conditions were excluded from the current study; liver diseases, including positive HBsAg and anti-HCV Ab, chronic inflammation including inflammatory bowel diseases, malignancy and thyroid diseases.

3.1.2: Control Group

The control group consisted of 38 subjects. They included normal individuals who had negative past medical history and were free from signs and symptoms of kidney diseases, anemia, diabetes mellitus, hypertension, dyslipidemia, thyroid disease and obesity. The age range was from (15-69) years, average (41.53 ±14.88) years.

3.1.3: The design of the study was show in figure 3-1

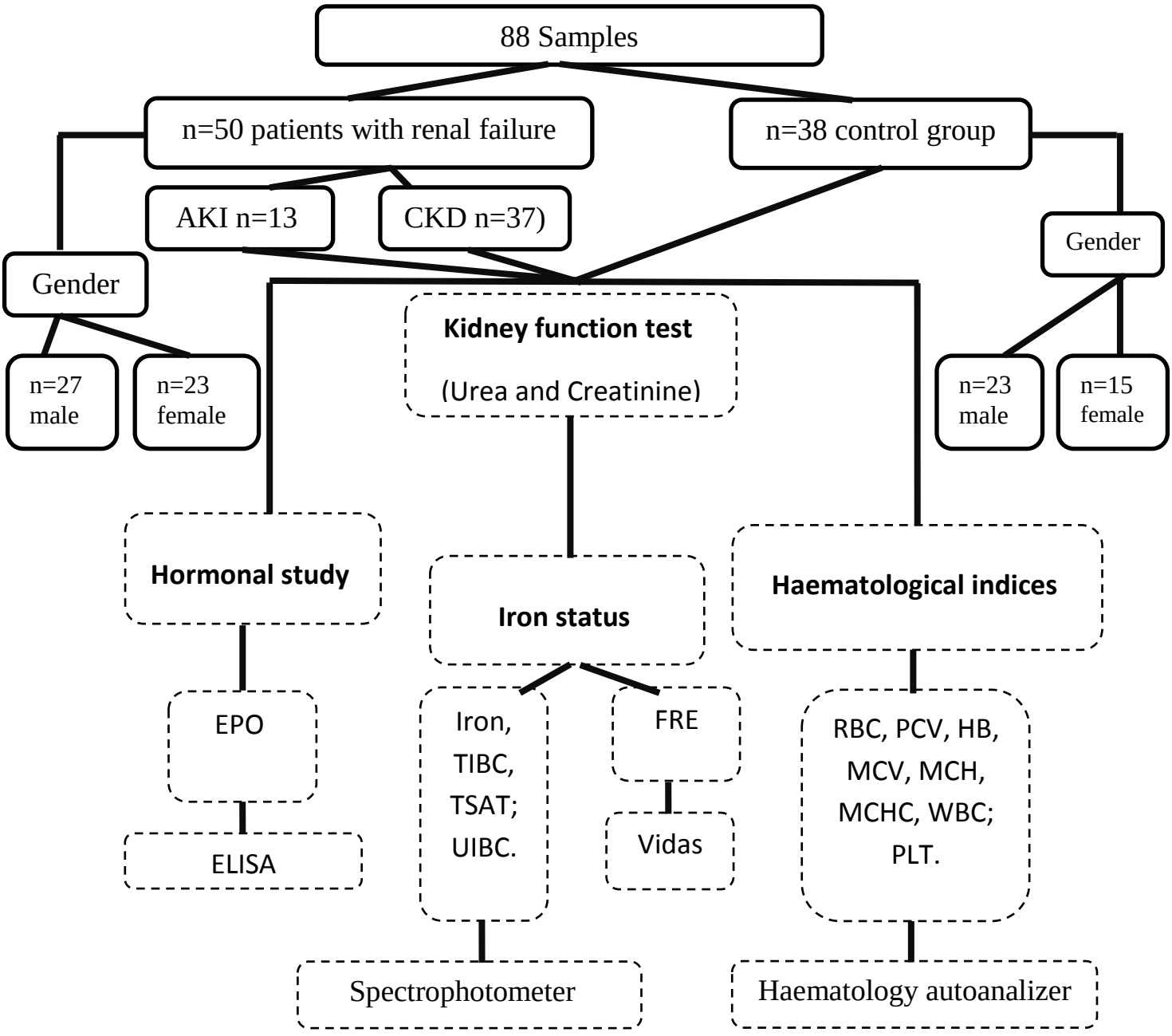


Figure 3- 1 : The study design

3.1.4: Sample collection:

The patients were diagnosed by physician based on history, clinical Examination.

Blood samples were collected from each patient after an overnight fasting by venipuncture using Ten milliliters disposable syringes. Blood was divided into 2 parts:

- First part (3ml) was put in a plain tube and left to clot for (30 min) in room temperature and then separated by centrifugation at (3000 Xg) for (10 min). The obtained serum was divided into two parts; the first part (1 ml) was kept in the eppendrof tube which is used to analyze of kidney functional tests (serum creatinine and urea). The second part of serum (2 ml) also kept in the eppendrof tube which transferred immediately in the freezer at (-20 c) for subsequent analysis (iron, TIBC, TSAT%, Transferrin conc., UIBC, Ferritin and EPO).

- Second part (2ml) was put in an EDTA tube, mixed gently and put on shaker for RBC, PCV, Hb, MCV, MCH, MCHC, WBC and PLT measurements.

3.1.5: Clinical Examination:

Every patient included in this study, was asked about his illness, age, work, housing, the date of diagnosis of the disease, the history of dialysis, duration of dialysis. All patients were also asked about history of anemia, route of administration and duration of treatment of anemia and the type of treatment, chronic diarrhea or vomiting, peptic ulcer disease or surgery, significant weight changes, hereditary type of anemia, blood loss, malabsorption, smoking, personal diet habits.

3.1.6: Instruments and tools used in this study:

The main instruments used in this work and their sources are listed in table (3-1).

Table 3 -1 : The main instruments and Tools used in the study and their sources.

No	*The instrument*	*The source*
1.	Disposal syringe	Changzhou Hangfulai, china
2.	Plain tube	AFma–Dispo, Jordan.
3.	EDTA tube	AFma–Dispo, Jordan.
4.	Water bath	Memmert, Germany.
5.	Centrifuge eppendorf	Universal ,Germany.
6.	Spectrophotometer	CECIL CE-1011,England.
7.	Haematology autoanalizer	Swelab Alfa ,Sweden.
8.	Incubator	Fisher Scientific (USA)
9.	Micro – pipette 50 µl	Human, Germany.
10.	Micro – pipette 100 -1000	Human, Germany.
11.	Centrifuge	Beckman, Germany.
12.	Refrigerator	Concord, Lebanon.
13.	Microscope slide	China.
14.	Light Microscope	Olympus, Japan.
15.	Vidas	BioMerieux, Frances.
16.	ELISA – reader	ELX50 Biotech, USA.
17.	ELISA – washer	ELX50 Biotech, USA.
18.	Eppendrof tube	China
19.	Sensitive balance (2000g)	Denver, USA.

3.1.7: Chemicals used in the study:

The chemicals and Kits are used in this work and their sources are listed in table (3-2).

Table 3- 2: Chemicals and Kits used in the present study

No	*The material*	*The source*
1.	Urea kit	Biolab, France.
2.	Creatinine Kit	Cromatest, Spain.
3.	Iron Kit	Human, Germany.
4.	TIBC Kit	Human, Germany.
5.	Ferritin Kit	BioMérieux, France.
6.	Epo ELISA kit	Elabscience, china.
7.	Methanol	china
8.	Leishman stain	china

3.2: Methods:

The following laboratory tests were done for all cases including:

Parameters of the study

- Renal function test (urea and creatinine).
- Complete blood picture indices.
- Iron status (Iron, TIBC, FER, UIBC and TSAT %).
- Serum EPO.

3.2.1: Measurement of Serum Urea Concentration:

Serum urea concentration was measured by enzymatic colorimetric method using a kit supplied by (Biolab), France.

Principle

Serum urea concentration was determined enzymatically according to the following reaction: (Chaney and Marbach, 1962; Searcy *et al.*, 1967)

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with the salicylate and Alkaline hypochlorite in presence of sodium Nitroprusside as coupling agent to yiled a green cromophore forming indophenol (2,2 dicarboxyl indophenol)

$$NH_4^+ + \text{Salicylate} + \text{Hypochlorite} \xrightarrow[OH^-]{\text{Nitroprusside}} \text{indophenol (green color)}$$

The intensity of the green color of the sample was measured using the spectrophotometer. The intensity of the color is proportional to the urea concentration in the sample.

R1 Enzyme reagent	Urease	≥ 500 U/ml
R2 Buffer chromogen (color reagent)	Phosphate buffer pH 6.9 EDTA Sodium salicylate Sodium nitroprusside	20 mmol/L 2 mmol/L 60 mmol/L 3.4 mmol/L
R3 Alkaline hypochlorite	Sodium hydroxide (NaOH) Sodium hypochlorite (NaClO)	10 mmol/L 150 mmol/L
CAL Urea Standard	Urea	(8.3) mmol/L

Preparation of working solution was done by dispensing one vial of R1 into one vial of R2 and mixing by turning upside down several times. Reagent stability: for 4 weeks at 2-8 ˚C and for 7 days 15-25 ˚C.

Procedure

In clean test tubes, the following were added:

Tubes	Blank	Serum sample	CAL. Standard
Working solution	1.0 ml	1.0 ml	1.0 ml
Sample	-	10 µl	-
CAL. Standard	-	-	10 µl
The contents of the above tubes were mixed by shaking gently and then Incubated for 5 minutes at 37 or for 10 minutes at 16-25°C Pipette			
R3	1.0 ml	1.0 ml	1.0 ml
The contents of the above tubes were mixed by shaking gently and then incubated for 5 minutes at 37 or for 10 minutes at 16-25°C			

The absorbance (A) of the standard and samples were measured against the blank at wavelength of 600 nm.

Calculation of Serum Urea*:*

$$\text{Serum Urea} = \frac{\text{A sample}}{\text{A standard}} \quad \text{X standard conc. (n)} = \text{mg/dL}$$

Conversion factor:

mg/dL = mmol/l X 6

3.2.2: Measurement of Serum Creatinine Concentration:

Serum urea concentration was measured by colorimetric method using a kit supplied by (Cromatest), Spain.

Principle:

The procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine reacts with alkaline picrate solution forms an orange color (creatinine picrate complex) .The intensity of the orange color creatinine picrate is proportional to creatinine concentration in the sample (Fabiny and Ertingshausen, 1971; Labbe *et al.*, 1996)

Creatinine + Picric acid ⟶ Creatinine picrate complex

Colorimetric reaction of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pretreatment step. This reaction

has been improved (specificity, speed and adaptability) by the development of an initial-rate method.

Reagents:

Reagent®	Contents	Initial Concentration of Solution
Vial R1 (Base)	Disodium phosphate Sodium hydroxide	6.4 mmol/L 150 mmol/L
Vial R2 (Dye)	Sodium dodecyl sulfate Picric acid pH= 4.0	0.75 mmol/L 4 mmol/L
Vial R3	Creatinine Standard	177 µmol/L (2 mg/dL)

Reagent Preparation:

Vial R_1 and vial R_2 content (1 volume/1 volume) were mixed. A graduated test-tube was used. All the assays were performed at room temperature.

Procedure

Solution	Blank(optional)	Standard	Assay
Working reagent (R1+R2)	1 mL	1 mL	1mL
D.W	100 µL	-	-
Standard	-	100 µL	-
Specimen	-	-	100 µL

The above materials were mixed well. After 30 seconds, recorded absorbance A_1 at 490 nm (490-510) against reagent blank or distilled water. Exactly two minutes after the first reading, record absorbance A_2.

Calculation:

$$\textbf{Sample concentration} = \frac{\textbf{A sample}}{\textbf{A standard}} \times \textbf{n}$$

n= Concentration of standard (177) µmol/L (2 mg/dL).

3.2.3: Measurement of complete blood count:

The blood samples were collected in EDTA tubes and complete blood count (CBC) indices were directly measured by automated hematological equipment.

Principle

Methodology: Electrical resistance for counting, hemiglobincyanide method and STF method for hemoglobin.

- Test parameters: total white blood cells count (WBCs), red blood corpuscular count (RBC), platelet count (PLTs), hemoglobin (Hb), packed cell volume (PCV), erythrocyte indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured by coulter counter (Swelab Alfa),Sweden.

3.2.3.1: Red Blood Corpuscular Morphology:

Freshly prepared blood films were stained using Leishmann's stain. By using oil immersion, 500 erythrocytes counted (Evatt *et al.*, 1992).

3.2.4: Iron Status

3.2.4.1: Measurement of serum iron (SI):

The kit was supplied by Human Company-Germany.

Principle:

Iron (III) reacts with chromazurol B (CAB) and cetytrimethylammonium bromide (CTMA) to form a coloured ternary complex with an absorbance maximam at 623 nm (Garčic, 1979).

The intensity of the color produced is directly proportional to the concentration of iron in the sample.

The test can also be used in combination with the TIBC kit (REF 10670) to determine the total iron binding capacity.

Contents of kit:

RGT 2 X 100 ml CAB Reagent

RGT CAB (Ready for use)	0.18 mmol/l
CTMA	2.2 mmol/l
Guanidinium chloride	2.6 mmol/l
Sodium acetate buffer (pH)	45 mmol/l
STD: 5 ml Standard : Ready for use Iron (ionised)	100 µg/dl or 17.9 µmol/l

Reagent Stability:

Reagent (RGT) is stable even after opening up to the stated expiry data when stored at 2…25˚C. Contamination of the reagents was absolutely avoided.

Procedure:

1.50µl of sample, distilled water and STD, each were put into plan tubes.

2. 1000µl of RGT was added to the tubes.

3. The tubes were mixed well, and incubated for15 minutes at 20-25˚C .

4. The absorbance of the sample and standard was measured against the reagent blank within 60 minutes.

Calculation of serum iron:

$$C = \frac{\Delta A_{sample}}{\Delta A_{STD}} \times 100 \quad (\mu g/dl)$$

$$C = \frac{\Delta A_{sample}}{\Delta A_{STD}} \times 17.9 \ (\mu mol/L)$$

2.2.4.2: Measurement of serum Total Iron-Binding Capacity:

The kit was supplied by Human Company-Germany.

Principle:

The iron-binding protein transferrin in serum is saturated upon treatment with an excess of Fe^{+3} ions. Unbound (excess) iron is adsorbed onto aluminium oxide and precipitated. The transferrin-bound iron (TIBC) in the supernatant is then determined (Starr, 1980).

Kit Contents:

1. FE: 1×100 ml Iron Solution.

Iron-(III)-chloride. 0.09 mmol/l

2. ALOX: 2×25g Aluminium Oxide.

Measuring spoon for aluminium oxide.

Reagent Stability:

The reagents are stable up to the stated expiry date when stored at 15…25 ˚C

Assay Procedure:

Before carrying out the assay procedure, the kit was left at room temperature for 30 minutes to equilibrate, as suggested by the manufacture. After that, the assay was carried out following the instructions in the kit's leaflet, which are summarized in the following steps:

The reaction was performed into test tubes:

FE	1.0 ml
Sample(serum)	0.5 ml

The tubes were mixed well. After 3-5 minutes, add one level measuring spoonful of aluminum oxide ALOX (approximately 0.25-0.35 g) . Cap and place on a rotator or roller mixer for 10 min .Remove tubes and allow to stand for 3 min upright or centrifuge for 1 min at 5,000 rpm .

Take off the cap prior to centrifugation.

The clear supernatant was used as “sample” for the respective iron determination.

Calculation of total iron binding capacity (TIBC):

To calculate TIBC, the multiply the result of the iron is determined in the supernatant by the diluent factor 3.

TIBC = Iron conc. in supernatant (µmol/L) x 3 (Dilution Factor).

Calculation Unsaturated Iron binding capacity (UIBC)

When the serum iron (SI) determination is performed concurrently with the TIBC and the result subtracted from the TIBC value, the difference yields the unsaturated iron-binding capacity (UIBC), or serum transferrin not bound to iron (Tietz, 1995).

UIBC = TIBC - Serum iron concentration.

3.2.4.3: Measurement of Transferrin Saturation Percentage:

Transferrin saturation percentage (TSAT%) is calculated from the following equation: (McLaren *et al.*, 2001)

TSAT% =Iron×100/TIBC

3.2.4.4: Measurement of Transferrin Concentration:

The percentage of transferrin saturation was calculated from the serum iron and transferrin concentrations by using the formula:

Transferrin (Trf) g/L = Serum iron (µmol/L) / (3.98 X TSAT %)

The formula is based on the maximal binding of 2 mol Fe^{3+}/mol of transferrin and a molecular weight of 79,570 for transferrin(Morgan, 2002).

3.2.5: Measurement of serum Ferritin Concentration:

Serum ferritin (S-Ft) was measured by VIDAS using kits supplied by BioMérieux, Frances.

Principle:

Serum concentration of ferritin was Measures by automated equipment (Vidas), the assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).The solid phase Receptacle (SRP) serves as the solid phase as well as the pipetting device for the assay .Dispensed in the sealed reagent strips. All assay steps are performed

automatically by the instrument .The reaction medium is cycled in and out of the SRP several times. The sample is taken and transferred into the well containing the antigen labeled with alkaline phosphate (conjugate).competition occurs between the antigen present in the sample and the labeled antigen for specific anti-ferritin antibody coated on the interior of the SRP. During the final detection step, the substrate (4-Methyle-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugated enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyle-umbelliferyl) the fluorescence of which is measured at 450 nm (Kouegnigan *et al.*, 2015). As show in figure (3-3).

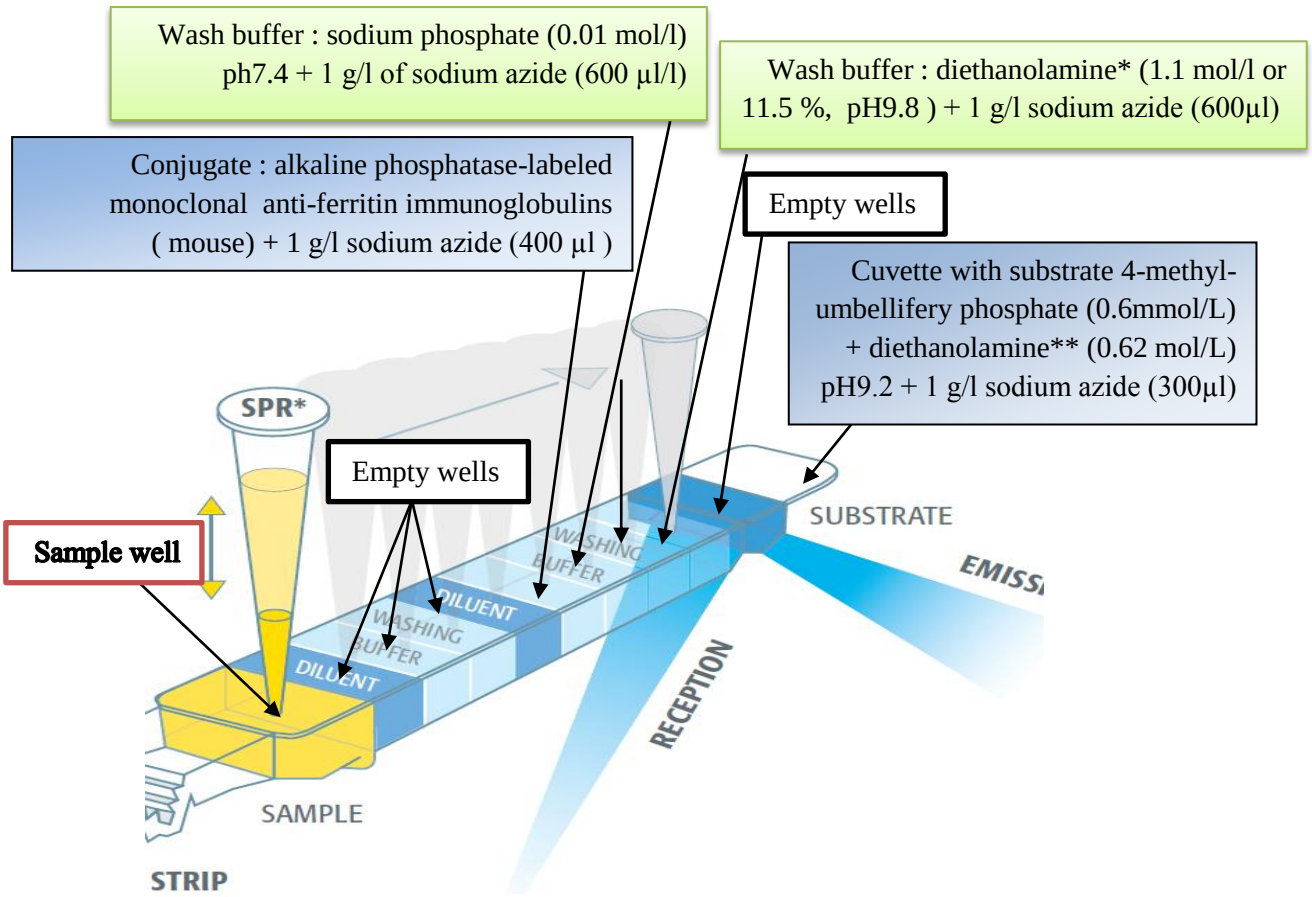


Figure 3- 2 Reagent of strip

Reagents:

FRE strip are ready to use (Sample well, Empty wells, Conjugate : alkaline phosphatase-labeled monoclonal anti-ferritin immunoglobulins (mouse) + sodium azide, Wash buffer, Cuvette with substrate 4-methyl-umbellifery phosphate + diethanolamine + sodium azide).

- FRE SRP is ready to use coated with monoclonal anti-ferritin Ig (mouse).
- FRE control 1 x 2 ml (liquid) C1.
- Calibrator 1 x 2 ml (liquid) S1.
- FER dilution buffer 1 x 25 ml (liquid) R1
- Master lot Entry

Procedure

9. The required reagents from the refrigerator was removed and allow them to come to room temperature for at least 30 minutes.
10. Use on "FER" strip and one "FER" SPR for each sample. Control or calibrator to be tested .Make sure the storage pouch has been carefully released after the required SRPs have been removed.
11. The test is identified by the "FER"code on the instrument. The calibrator must be identified by "S1" and testes in duplicate. If the control needs to be tested .it should be identified by "C1".
12. Mix the calibrator, control and sample using vortex type mixer (for serum).
13. For this test, the calibrator, control, and sample test portion is 100 µl
14. Insert the FER" SPR and "FER" strip into instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent strips match.
15. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
16. Restopper the vial and return them to 2-8C after pipetting.
17. The assay will be completed within approximately 30 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
18. Dispose of the used SPRs and reagent strips into an appropriate recipient.

3.2.5.1: Estimated Total Iron Body Store (ETIBS)

ETIBS µmol/L = serum ferritin in µg/l × 143 (Burtis *et al.*, 2012)

3.2.6: Measurements of Erythropoietin

This ELISA Kit for quantitative determination of Erythropoietin (EPO) concentration in human serum was supplied by Elabscience, china.

Principle of the test

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to EPO. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for EPO and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain EPO, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of EPO. You can calculate the concentration of EPO in the samples by comparing the OD of the samples to the standard curve.

Kit Components：

Item	Specifications	Storage
Micro ELISA Plate	8 wells ×12 strips	4℃/-20℃#
Reference Standard	2 vials	4℃/-20℃#
Reference Standard & Sample Diluent	1vial 20mL	4℃
Concentrated Biotinylated Detection Ab	1vial 120μL	4℃/-20℃#
Biotinylated Detection Ab Diluent	1vial 10mL	4℃
Concentrated HRP Conjugate	1vial 120μL	4℃(shading light)
HRP Conjugate Diluent	1vial 10mL	4℃
Concentrated Wash Buffer (25×)	1vial 30mL	4℃
Substrate Reagent	1vial 10mL	4℃(shading light)
Stop Solution	1vial 10mL	4℃
Plate Sealer	5pieces	
Manual	1 copy	
Certificate of Analysis	1 copy	

#: keep the kit at 4℃ if it's used within 30 days, keep at -20℃ for longer storage.

Reagent preparation

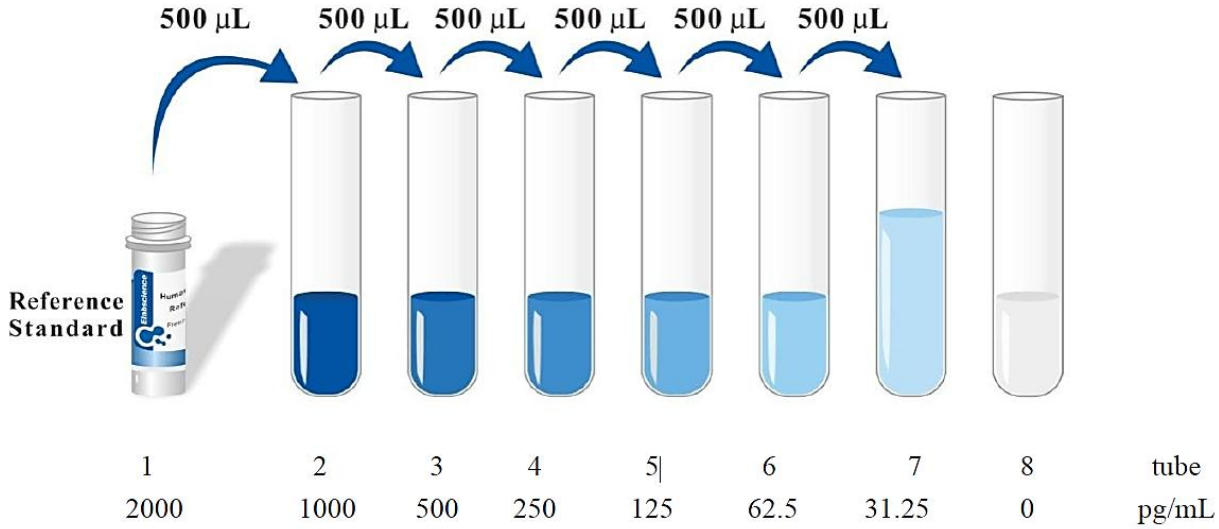
Bring all reagents to room temperature (18-25℃) before use.

Wash Buffer - Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard – Prepare standard within 15 minutes before use. Centrifuge at 10,000×g for 1 minute, and reconstitute the Standard with **1.0mL** of Reference Standard &Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 2000pg/mL.

Then make serial dilutions as needed (making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows:**2000、1000、500、250、125、62.5、31.25、0 pg/mL**. If you want to make standard solution at the concentration of 1000pg/mL, you should take0.5mL standard at 2000pg/mL, add it to an EP tube with 0.5mL Reference Standard &Sample Diluent, and mix it. Procedures to prepare the remained concentrations are all the same. The undiluted standard serves as the highest standard (2000pg/mL). The Reference Standard &Sample Diluent serves as the zero (0 pg/mL).

(Standards can also be diluted according to the actual amount, such as 200µL/tube).



Biotinylated Detection Ab – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Centrifuge the stock tube before use, dilute the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Dilute the Concentrated HRP Conjugate to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent: As it is sensitive to light and contaminants, so you shouldn't open the vial until you need it! The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent shouldn't be dumped back into the vial again.

Note: Please don't prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.

Washing Procedure:

1. **Automated Washer:** Add 350µL wash buffer into each well, the interval between injection and suction should be set about 60s.

2. **Manual wash:** Add 350μLWash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. **All the reagents were mixed thoroughly by gentle swirling before pipetting. Avoid foaming.** It's recommended that all samples and standards be assayed in duplicate.

1. **Add Sample:** Add 100μL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃.

2. **Biotinylated Detection Ab:** Remove the liquid of each well, don't wash. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.

3. **Wash:** Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

4. **HRP Conjugate:** Add 100μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.

5. **Wash:** Repeat the wash process for five times as conducted in step 3.

6. **Substrate:** Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color

change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.

7. **Stop**: Add 50μLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.

8. **OD Measurement**: Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm. User should open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

9. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should retest it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Typical data

As the Optical Density values of the standard curve may vary according to the condition of actual assay performance (e.g. operator, pipetting, technique, washing technique or temperature effects), the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only. As show in table (3-3)

Table 3- 3: Typical standard curve of EPO

concentration (pg/mL)	OD	OD – OD $_{blank}$
0	0.083	0
31.25	0.134	0.051
62.5	0.183	0.1
125	0.25	0.167
250	0.508	0.425
500	0.901	0.818
1000	1.634	1.551
2000	2.459	2.376

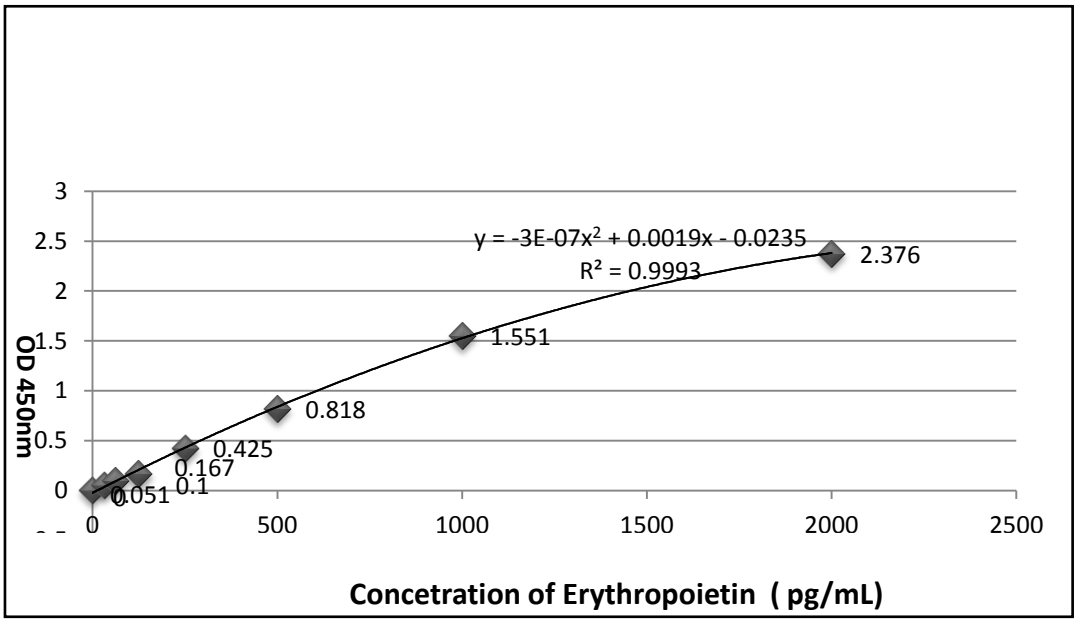


Figure 3- 3: Standard curve of Erythropoietin (pg/ml)

3.3: Statistical Analysis:

Statistical analysis was done using the software statistical package for social sciences (SPSS version 18), the results were expressed as mean ± standard deviations (Mean ± S.D). Statistical analysis for the significance of differences of the quantitative data was done by using Student's t-test for two independent means and ANOVA test for more than two independent means while the Pearson's correlation coefficient was used for the determination of the correlation between two quantitative data in different groups. Pearson's chi square (X^2) test and fisher exact test used to find the association between the categorical variable. P-values ≤ 0.05 were considered statistically significant (Ren *et al.*, 2016).

Chapter Four

4: The results

4.1: The patient group.

4.1.1: Social demographic characteristics of the study population

In table (4-1), shows the association of renal failure patient with Social demographic. There was non-significant association between renal failure patient with gender, social class and area of living.

Table 4- 1 **: Social demographic characteristics of the study population**

Group / Variable	Renal failure patients		**P values**
	Acute No. (%)	**Chronic No.(%)**	
Gender			
Male	8(61.5)	19(51.4)	0.2
Female	5(38.5)	18(48.6)	
Social class			
Rich	1(7.7)	8(21.6)	0.631
Medium	6(46.2)	13(35.1)	
Poor	6(46.2)	16(43.2)	
Area			
Rural	4(30.8)	17(45.9)	0.268
Urban	9(69.2)	20(54.1)	

P values ≤ 0.05 is a significant

Fisher's Exact Test

4.1.2: Distribution of renal failure patients and control groups by Gender.

Distribution of renal failure patients and control groups by Gender, Majority (21.6) and (9.10) of the male had AKI and CKD patients, respectively, as show in Figure (4-1).

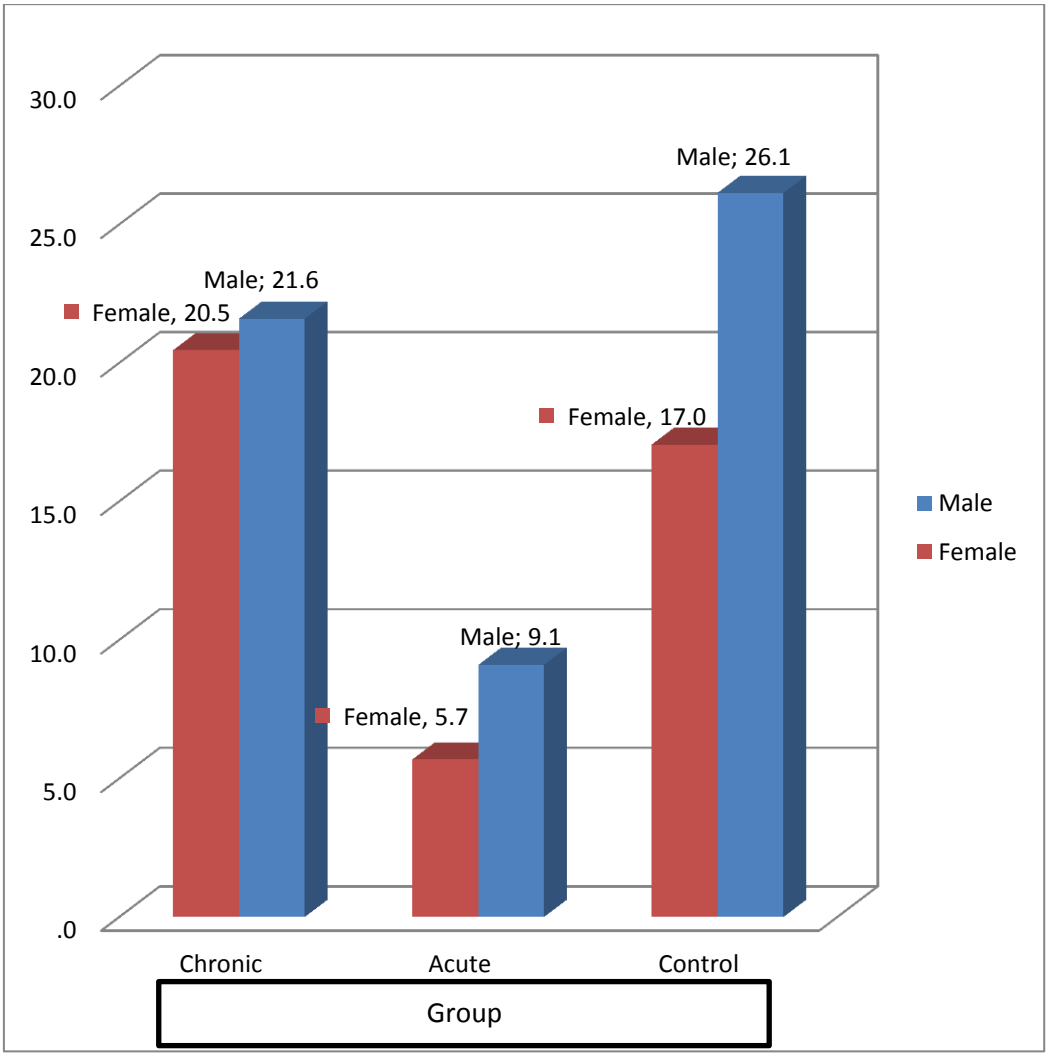


Figure 4- 1: shows the distribution of renal failure patients and control groups by Gender.

4.2: Comparison Between Patients and control groups

4.2.1: Kidney function tests:

The Serum levels of Creatinine and Blood urea in the renal failure patients (AKI & CKD) and control group are presented in table (4-2).

The table (4-2) shows highly significant increase in serum levels of creatinine and Blood urea levels among patients of AKI and CKD as compared with control groups (P≤0.01), when AKI and CKD patients were compared, no significant were found in serum urea and creatinine.

Table 4- 2: Kidney function tests in patients with renal failure (AKI & CKD) and control group.

Group / Parameter	Means± S.D		
	Normal control n=38	Acute kidney injure n=13	Chronic kidney disease n=37
S.Creatinine μmol/L	81.63±12.87	712.08±198.49**	607.16±202.36**
S.Urea mmol	4.86±1.10	24.15±7.45**	25.10±6.27**

*** P values ≤ 0.01 : highly significant different in comparing with control group.*

(●): significant different in comparing AKI and CKD groups.

4.2.2: Iron status

The serum levels of iron status parameters in the renal failure patients (AKI & CKD) and control group.

The table (4-3) shows highly significant decrease in iron, total iron binding capacity (TIBC) and transferrin levels (P≤0.001) in patients with AKI and CKD as compared with control groups, on the other hand, this table shows a significant decrease in TSAT% in AKI patients as compared with their related control groups (P≤0.05). Moreover, highly significant decrease in UIBC levels in patients of CKD as compared with control groups (P≤0.01). In addition, a significant increase in Ferritin and ETIBS levels in AKI and CKD of patients as compared with control groups (P≤0.01).

Table 4- 3 : Serum levels mean of Iron status components in patients and control groups.

Groups / Parameters	Means± S.D		
	Normal control n=38	**AKI n=13**	**CKD n=37**
Iron µmol/L	22.69±8.56	10.15±5.61**●	17.23±3.64**
TIBC µmol/L	62.14±10.64	45.48±17.37**	40.67±12.73**
U I B C µmol/L	49.46±12.72	35.33±10.86	25.23±12.59**
TSAT (%)	38.15±12.42	22.14±9.73*●	47.52±19.48
Trf (g/L)	0.16±0.03	0.11±0.03**	0.10±0.05**
Ferritin ng/ml	63.46±17.97 (22.00-241)	84.76±13.66● (1.00-977.00)	158.59±28.33** (1.00-1142.85)
ETIBS µmol/L	8.29±8.89	10.06±8.89●	21.01±20.07**

( ) P values ≤ 0.05 : significantly different in comparing with control group.*

*(**) P values ≤ 0.01 : highly significant different in comparing with control group.*

(●): significant different in comparing AKI and CKD groups.

4.2.3: Haematological parameter and Erythropoietin.

The Haematological parameter and Erythropoietin (EPO) in the renal failure patients (AKI & CKD) and control groups.

The statistical analysis showed highly significant decrease (P≤0.01) in mean of RBC, Hb, PCV and MCH in patients of AKI and CKD compared to the healthy control group. Moreover, the table refers to a significant decrease in MCV in patients of AKI as compared with control group. On the other hand, this table shows a significant increase (P≤0.01) in MCHC levels among patients of AKI and CKD as compared with their associated control group. In addition, significant decreases in PLT in CKD patients as compared with control groups (P≤0.01), furthermore significant increase in EPO level in patients with AKI and CKD as compared with their associated control groups, are presented in the table (4-4).

Table 4- 4: RBC, Hb, PCV, RBC indices, PLT, WBC and S.EPO levels in patients groups comparison with control group.

Groups / Parameters	Means± S.D		
	Normal control n=38	**AKI n=13**	**CKD n=37**
RBC(10^{12}/l)	5.06±0.55	2.94±0.65**	2.67±0.74**
Hb(g/dl)	14.00±1.24	8.10±1.99**	7.67±1.93**
PCV(%)	43.70±3.78	23.94±6.26**	22.55±5.95**
MCV(fl)	88.16±3.63	80.88±8.57*●	85.35±7.99
MCH(pg)	27.84±2.52	27.65±3.10	29.26±3.06*
MCHC(pg)	32.11±1.43	34.18±1.32**	34.25±1.31**
PLT(10^9/l)	231.74±79.45	222.38±90.03	179.59±72.65**
WBC(10^9/l)	6.89±1.50	7.15±3.13	6.08±2.88
S.EPO(pg/ml)	103.85±33.48	280.32±51.93**●	205.33±73.40**

( ) P values ≤ 0.05 : significantly different in comparing with control group.*

*(**) P values ≤ 0.01 : highly significant different in comparing with control group.*

(●):P values ≤ 0.05 : significant different in comparing between AKI and CKD groups.

4.3: Comparison between Patients groups according to gender.

4.3.1: Kidney function tests Results according to gender difference:

The Serum levels of Creatinine and Blood urea in the comparison between male and female of renal failure patients are presented in the table (4-5).

The table (4-5) shows highly significant increase in Serum levels means of Blood urea levels means in males compared females (P≤0.01)

Table 4- 5: Serum creatinine and Blood urea rates in patients with renal failure groups according to gender difference.

Group / Parameter	Means± S.D		P Values
	Renal failure patients		
	Males Groups	Females Groups	
S.Creatinine (μmol/L)	568.05±160.22	531.83±149.90	0.21
S.Urea (mmol)	26.29±6.64	23.4±5.90**	**0.01**

**P values ≤ 0.01 is a significant ** P values ≤ 0.01 is a highly significant.

4.3.2: Results of Iron status according to gender difference:

The iron status parameters in the comparison between male and female of renal failure patients are presented in the table (4-6).

According to the table (4-6) indicates significant decrease in total iron binding capacity (TIBC) levels means in males compared females (P≤0.05)

Table 4- 6: Iron status in patients with renal failure groups according to gender difference.

Group / Parameter	Means± S.D		P Values
	Renal failure patients		
	Males Groups	Females Groups	
Iron (µmol/L)	17.85±10.76	16.57±8.57	0.61
TIBC (µmol/L)	38.31±14.92	43.11±20.42**	**0.03**
U I B C (µmol/L)	23.73±14.74	26.82±12.68	0.69
TSAT (%)	49.37±13.55	44.74±18.36	0.43
Trf (g/L)	0.10±0.05	0.11±0.05	0.65
Ferritin (ng/ml)	221.57±50.48	213.56±90.62	0.64
ETIBS (µmol/L)	29.94±6.46	29.15±5.92	0.87

** P values ≤ 0.01 is a highly significant

4.3.3: The Haematological parameter and Erythropoietin results according to gender difference:

The Haematological parameter and Erythropoietin (EPO) in the comparison between male and female of renal failure patients.

According to the table (4-7) indicates highly significant decrease in MCHC mean in males compared females (P≤0.05)

Table 4- 7: RBC, HB, PCV, RBC indices, PLT, WBC and S.EPO levels means in patients with renal failure groups according to gender difference.

Group / Parameter	Means± S.D		P Values
	Renal failure patients		
	Males Groups	Females Groups	
RBC(10^{12}/l)	2.81±0.74	2.51±0.73	0.22
HB(g/dl)	8.15±2.09	7.17±1.65	0.12
PCV(%)	24.08±6.44	20.92±5.08	0.11
MCV(fl)	85.80±7.20	84.88±8.94	0.73
MCH(pg)	29.23±2.12	29.30±3.89	0.94
MCHC(pg)	34.07±1.26	34.45±1.37**	**0.01**
PLT(10^9/l)	180.00±50.69	179.17±58.32	0.97
WBC(10^9/l)	6.19±3.27	5.96±2.49	0.81
S.EPO(pg/m)	205.78±86.58	206.46±73.56	0.99

** P values ≤ 0.01 is a highly significant

4.1: Comparison among Patients of renal failure according to Duration of the disease.

4.4.1: Results of Kidney Function tests:

The serum level of creatinine and Blood urea in the renal failure patients (AKI & CKD) according to Duration of the disease.

Results in the table (4-8) showed non-significant differences ($p \geq 0.05$) in the level of mean urea and creatinine among patients of renal failure according to duration of the disease groups.

Table 4-8: Kidney function tests among patients of renal failure according to Duration of the disease.

parameter	**Duration of the disease**	**Means± S.D**	**P values**
S.Urea (mmol)	≤3 months	24.08±7.41	0.297
	3months- 1 year	27.58±7.53	
	≥ 1 year	24.24±5.45	
S.Creatinine (μmol/L)	≤3 months	712.08±198.49	0.297
	3months- 1 year	655.67±179.47	
	≥ 1 year	583.88±211.95	

P values ≤ 0.05 is a significant.

4.4.2: Results of Iron status tests according to Duration of the disease.

Results in the table (4-9) showed significant ($p \leq 0.05$) in the level of mean iron, TSAT%, Ferritin and ETIBS among patients of renal failure according to duration of the disease groups.

Table 4- 9: **Iron status tests according to Duration of the disease.**

parameter	Duration of the disease	Means± S.D	P values
Iron (µg/dl)	≤3 months	10.15±5.61	**0.02***
	3months- 1 year	20.00±3.01	
	≥ 1 year	15.89±3.82	
TIBC(µmol/L)	≤3 months	45.48±12.37	0.157
	3months- 1 year	47.30±12.26	
	≥ 1 year	37.44±11.54	
U I B C (µmol/L)	≤3 months	35.33±1.86	0.277
	3months- 1 year	27.87±2.00	
	≥ 1 year	23.97±2.76	
TSAT(%)	≤3 months	22.14±9.73	**0.02***
	3months- 1 year	50.00±15.81	
	≥ 1 year	49.29±9.72	
Trf (g/L)	≤3 months	0.11±0.03	0.427
	3months- 1 year	0.12±0.06	
	≥ 1 year	0.10±0.05	
Ferritin(ng/ml)	≤3 months	63.47±15.97	**0.008****
	3months- 1 year	99.66±31.71	
	≥ 1 year	189.74±57.51	
ETIBS (µmol/L)	≤3 months	9.08±2.29	**0.008****
	3months- 1 year	15.59±1.59	
	≥ 1 year	25.53±2.66	

**P values ≤ 0.05 is a significant*

***P values ≤ 0.01 is highly a significant*

4.4.3: The Haematological parameter and Erythropoietin according to Duration of the disease.

Results in the table (4-10) showed a highly significant ($p \leq 0.01$) in the level of mean S.EPO in patients of renal failure according to duration of the disease groups.

Table 4- 10: The Haematological parameter and S.EPO level means according to Duration of the disease.

Parameter	Duration of the disease	Means± S.D	P Values
RBC(10^{12}/l)	≤3 months	2.94±0.65	0.503
	3months- 1 year	2.70±0.77	
	≥ 1 year	2.65±0.75	
HB(g/dl)	≤3 months	8.10±1.99	0. 765
	3months- 1 year	7.80±2.28	
	≥ 1 year	7.61±1.79	
PCV (%)	≤3 months	23.94±6.26	0.764
	3months- 1 year	22.83±6.89	
	≥ 1 year	22.41±5.60	
MCV(fl)	≤3 months	80.88±8.57	0.210
	3months- 1 year	84.17±3.80	
	≥ 1 year	85.92±9.39	
MCH(pg)	≤3 months	27.65±3.10	0.260
	3months- 1 year	28.95±1.91	
	≥ 1 year	29.41±3.51	
MCHC(pg)	≤3 months	32.39±0.95	0.774
	3months- 1 year	32.78±1.94	
	≥ 1 year	32.38±1.75	
PLT(10^9/l)	≤3 months	257.00±91.12	0.174
	3months- 1 year	194.33±59.40	
	≥ 1 year	206.48±44.94	
WBC(10^9/l)	≤3 months	6.95±1.57	0.542
	3months- 1 year	7.50±1.55	
	≥ 1 year	6.26±2.04	
S.EPO(pg/ml)	≤3 months	281.42±52.43	**0.0001****
	3months- 1 year	266.62±54.72	
	≥ 1 year	177.07±31.30	

***P values ≤ 0.01: is highly a significant.*

4.5: Comparison Between Patients and control groups according to age.

4.5.1: Results of Kidney function tests according to age group:

Kidney function tests comparison between patients and control groups according to age group are given below.

Results in the table (4-11) showed a significant increase ($p \leq 0.01$) in the level of means urea and creatinine among age groups in the patients with renal failure.

Table 4- 11: Serum creatinine and blood urea level means in the renal failure patients in comparison with control groups according to age groups.

Groups / Parameters	Age group	Control groups		Renal failure groups		
		n	Means±S.D	n	Means± S.D	P values
S.Creatinine (μmol/L)	15-30	11	84.33±14.51	8	616.50±115.37	**0.001****
	31-46	12	83.75±9.95	8	572.62±154.62	**0.001****
	≥46	15	76.87±13.70	34	571.81±180.25	**0.001****
S.Urea (mmol)	15-30	11	4.57±1.91	8	24.14±6.00	**0.001****
	31-46	12	4.70±1.91	8	24.05±8.45	**0.001****
	≥46	15	5.20±1.37	34	25.57±6.03	**0.001****

** P values ≤ 0.01 is a highly significant

4.5.2: Results of Iron status according to age group :

In the table (4-12), there was showed highly significant decrease (P≤0.01) in level of means iron, TIBC, UIBC, Trf concentration and ETIBS among the age group in patients with renal failure compared to the healthy control group.

Table 4-12: Iron status in the renal failure patients in comparison with control group according to age group.

Groups / Parameters	Age groups	Means ± S.D				
		n	Control groups	n	Renal failure groups	P values
Iron (µmol/L)	15-30	10	22.95±7.52	8	17.38±8.62	0.12
	31-46	13	21.75±7.38	9	**9.86±2.88**	**0.001***
	≥46	15	24.63±9.49	33	**16.40±1.79**	**0.009***
TIBC (µmol/L)	15-30	10	64.50±13.89	8	**41.85±11.79**	**0.023***
	31-46	13	58.84±10.36	9	**41.90±12.19**	**0.006***
	≥46	15	45.35±7.47	33	**41.14±13.20**	**0.001****
U I B C (µmol/L)	15-30	10	39.73±19.08	8	26.00±11.79	0.17
	31-46	13	39.83±14.45	9	36.91±9.19	0.381
	≥46	15	39.80±11.04	33	**26.04±5.20**	**0.006***
TSAT(%)	15-30	10	40.22±20.88	8	41.53±9.30	0.94
	31-46	13	36.89±14.87	9	28.78±3.90	0.147
	≥46	15	38.39±13.79	33	43.63±4.60	0.420
Trf (g/**L**)	15-30	10	0.15±0.04	8	0.10±0.05	**0.019***
	31-46	13	0.15±0.03	9	0.11±0.07	0.103
	≥46	15	0.16±0.02	33	0.10±0.03	**0.001****
Ferritin (ng/ml)	15-30	10	86.21±22.69	8	168.12±32.14	0.11
	31-46	13	74.11±20.37	9	46.80±8.82	0.19
	≥46	15	93.33±35.41	33	148.18±47.41	0.07
ETIBS (µmol/L)	15-30	10	17.16±7.58	8	21.69±5.79	0.12
	31-46	13	11.83±1.81	9	5.14±1.56	0.37
	≥46	15	6.12±2.82	33	**20.35±4.06**	**0.02***

*P values ≤ 0.05 is a significant ** P values ≤ 0.01 is a highly significant

4.5.3: Results of RBC, HB, PCV, RBC indices, PLT, WBC and EPO levels according to age group:

The statistical analysis showed highly significant (P≤0.01) in level of means RBC, Hb, PCV, MCHC, PLT and S.EPO levels means among the age group in patients with renal failure compared to the healthy control group, presented in the table(4-13).

Table 4- 13: RBC, HB, PCV, RBC indices, PLT, WBC and S.EPO levels in renal failure patients groups in comparison with control group according to age group.

Groups / Parameters	Age group	Control group		Renal failure group		
		n	Means± S.D	n	Means± S.D	P values
RBC(10^{12}/l)	15-30	11	4.84±0.47	8	3.02±0.65	**0.001****
	31-46	12	5.03±0.48	8	2.64±0.82	**0.001****
	≥46	15	5.25±0.62	34	2.69±0.72	**0.001****
HB(g/dl)	15-30	11	13.65±1.33	8	8.98±1.80	**0.001****
	31-46	12	14.04±1.35	8	7.34±2.39	**0.001****
	≥46	15	14.23±1.09	34	7.61±1.80	**0.001****
PCV (%)	15-30	11	42.53±3.62	8	26.54±5.76	**0.001****
	31-46	12	43.19±4.33	8	21.81±7.41	**0.001****
	≥46	15	44.97±3.28	34	22.31±5.57	**0.001****
MCV(fl)	15-30	11	87.93±3.80	8	87.93±5.58	0.11
	31-46	12	85.78±4.09	8	83.06±11.69	0.544
	≥46	15	86.17±6.88	34	83.58±7.90	0.3
MCH(pg)	15-30	11	29.36±1.76	8	29.86±1.52	0.53
	31-46	12	28.62±1.17	8	28.24±4.39	0.817
	≥46	15	28.99±1.22	34	28.75±3.09	0.7
MCHC(pg)	15-30	11	32.13±1.46	8	33.95±1.05	**0.008****
	31-46	12	32.59±0.74	8	33.93±0.91	**0.002****
	≥46	15	31.71±1.76	34	34.37±1.43	**0.001****
PLT(10^9/l)	15-30	11	230.18±32.69	8	159.25±64.55	**0.006****
	31-46	12	253.42±128.14	8	223.13±25.45	0.608
	≥46	15	215.53±48.30	34	190.50±76.80	0.2
WBC(10^9/l)	15-30	11	7.14±1.90	8	5.54±2.71	0.15
	31-46	12	7.07±1.30	8	7.09±1.88	0.988
	≥46	15	6.57±1.17	34	6.38±2.81	0.7
S.EPO(pg/ml)	15-30	11	52.00±20.75	8	246.58±27.90	**0.001****
	31-46	12	173.52±153.05	8	240.42±38.13	**0.001****
	≥46	15	39.88±21.39	34	293.07±26.89	**0.001****

*P values ≤ 0.05 is a significant ** P values ≤ 0.01 is a highly significant

4.6.1: Distribution of anemia among AKI and CKD patients.

In the table (4-14), shows the association of renal failure patient with type of anemia. There was significant association between renal failure patient and normocytic normochromic (P≤0.05).

Table 4- 14: The type of anemia among AKI and CKD patients

Group / Variable	Renal failure patients		P values
	AKI No. (%)	**CKD No. (%)**	
Microcytic hypochromic	6(46.15)	9(24.32)	0.39
Normocytic normochromic	7(53.84)	27(72.97)	**0.03***
Macrocytic hypochromic	0(0.0)	1(2.70)	0.707

* P values ≤ 0.05 is a significant.

- Fisher exacta test.

4.6.2: The association of blood indices with AKI and CKD patients.

In the table (4-15), shows the association of renal failure patient with blood indices. There was significant association (P≤ 0.05) between renal failure patient and MCV.

Table 4- 15: Blood indices among AKI and CKD patients.

Group / Variable	Renal failure patients		P values
	AKI No. (%)	**CKD No. (%)**	
MCV			
Low	6(40.0)	9(60.0)	**0.039***
Normal	7(20.6)	27(79.4)	
High	0(0.0)	1(100.0)	
MCH			
Low	6(37.5)	10(62.5)	0.301
Normal	7(20.6)	27(79.4)	
MCHC			
Low	5(33.3)	10(66.7)	0.707
Normal	8(22.9)	27 (77.1)	

* P values ≤ 0.05 is a significant

- Fisher exacta test

4.6.3: The association of iron status with AKI and CKD patients.

In the table (4-16), shows the association of renal failure patient with Iron indices. There was significant association between renal failure patient and iron level.

Table 4- 16 : Association between renal failure patients with iron status.

Variable	Renal failure patients		P values
	Acute No. (%)	Chronic No. (%)	
Iron: Low Normal	 8(61.53) 5(38.47)	 11(42.30) 26 (57.7)	**0.05***
TSAT%: less than 20% less than 20% more than 50%	6(46.2) 5(38.5) 2 (15.4)	9(24.3) 26(70.3) 2(5.4)	0.25
Ferritin: less than 100 ng/ml more than 100 ng/ml more than 800 ng/ml	6(46.2) 5(38.5) 2 (15.4)	9(24.3) 26(70.3) 2(5.4)	0.25

* P values ≤ 0.05 is a significant

4.6.4: The association of frequency of dialysis with AKI and CKD patients.

In the table (4-17), shows the association of renal failure patient with frequency of dialysis. There was no significant association between renal failure patient and frequency of dialysis.

Table 4-17: The frequency of dialysis (occasional or less than 8 time per month ≥ 12 times per month).

Group	Frequency of dialysis		P values
	less than 8 time per month	≥ 12 times per month	
Acute n=13	3(23.1%)	10(76.9%)	0.508
Chronic n=37	13(35.1%)	24(64.9%)	

P values ≤ 0.05 is a significant

- Fisher's Exact Test

4.6.5: The association of Nutritional status with AKI and CKD patients.

In the table (4-18), shows the association of renal failure patient with nutritional status. There was no significant association between renal failure patient and nutritional status.

Table 4-18: The Nutritional status including: good oral intake vs. Poor oral intake (By history)

Group / Nutritional status	Renal failure patients		P values
	AKI No. (%)	CKD No. (%)	
Good	3(23.07)	4(10.18)	0.357
Poor	10(76.92)	33(89.19)	

P values ≤ 0.05 is a significant

- Fisher's Exact Test

In the table (4-19), shows the association of renal failure patient with Iron supplement. There was significant association (P≤ 0.01) between renal failure patient and Iron supplement.

Table 4-19: The association Iron supplements with renal failure patients (By history)

Group / Iron supplement	Renal failure patients (n=50)		P values
	AKI No. (%)	CKD No. (%)	
Yes	4(30.8)	34(91.9)	**0.001***
No	9(69.2)	3 (8.1)	

*P values ≤ 0.05 is a significant

- Fisher's Exact Test

4.6.6: Overall prevalence of anemia among AKI and CKD patients.

In the table (4-20), shows the association of renal failure patient with prevalence of anemia. There was no significant association between renal failure patient and prevalence of anemia.

Table 4- 20 : Prevalence of anemia among AKI and CKD patients.

Group / Degree of anemia	Renal failure patients (n=50)		P values
	AKI n (%)	CKD n (%)	
Severe	3(23.08)	12(32.34)	0.835
Moderate	8(61.54)	18(48.64)	
Mild	2 (15.38)	7(18.92)	

P values ≤ 0.05 is a significant

- Fisher's Exact Test

4.6.7: Prevalence of anemia among AKI and CKD patients by gender.

In the table (4-21), shows the association of renal failure patient with prevalence of anemia by gender. There was no significant association between renal failure patient and prevalence of anemia.

Table 4- 21 : Prevalence of anemia among AKI and CKD patients by gender.

Group / Degree of anemia	Renal failure patients (n=50)		P Values
	Male No. (%)	Female No. (%)	
Severe	8 (29.63)	7 (30.43)	0.284
Moderate	12(44.44)	14 (60.87)	
Mild	7 (25.93)	2 (8.61)	

P values ≤ 0.05 is a significant

- Fisher's Exact Test

4.6.8: The associated diseases with AKI and CKD patients.

In the table (4-22), shows the association of renal failure patient with prevalence of anemia. There was significant between renal failure patient and association diseases.

Table 4- 22 : The associated diseases with AKI and CKD patients.

Group / Associated diseases	Renal failure patients			P values
	Control No. (%)	AKI No. (%)	CKD No. (%)	
HTN	5(83.33)	3(23.08)	12(57.14)	**0.01****
DM,HTN	1(16.66)	10(76.92)	9(42.86)	

* * P values ≤ 0.01 is a highly significant

- Fisher's Exact Test

4.6.9: Relationship of Erythropoietin Levels to Measured Parameters

The results of the current study indicated that there was negative and non-significant between serum level of EPO with RBC, Hb, PCV, PLTs, Ferritin, Urea and Creatinine in patients with AKI, are present in table (4-23).

The results of the present study also observed a negatively and non-significant between serum level of EPO with PCV, MCV, MCHC, PLTs and TSAT% with patients of CKD. It was also noted of positive correlation and no significantly between EPO with RBC, Hb, MCH, PLTs, urea and creatinine of CKD patients, as shown present in table (4-24).

Table 4 -23*: Correlation between study Parameters in AKI patients (No.=13)*

Parameters		Urea	Iron	U I B C	TSAT%	Ferritin	RBC	HB	PCV	MCV	MCH	MCHC	PLT	WBC	EPO(pg/ml)
Creatinine(μmol/L)	*r value*	0.39	0.03	0.11	0.00	-0.38	0.38	0.21	0.28	-0.13	-0.31	-0.55	0.35	0.35	-0.42
	p value	0.19	0.93	0.72	0.99	0.20	0.19	0.49	0.35	0.67	0.30	0.05	0.24	0.24	0.15
Urea(mmol)	*r value*		0.16	-0.36	0.34	0.24	0.36	0.13	0.20	-0.32	-0.47	-0.52	0.46	0.07	-0.45
	p value		0.61	0.23	0.26	0.44	0.23	0.66	0.51	0.29	0.10	0.07	0.11	0.83	0.12
Iron (µmol/L)	*r value*			0.03	0.88**	-0.29	0.33	0.09	0.11	-0.32	-0.30	-0.06	0.71**	0.54*	0.41
	p value			0.93	0.00	0.34	0.27	0.77	0.72	0.29	0.32	0.84	0.01	0.05	0.17
U I B C (µmol/L)	*r value*				-0.37	-0.33	0.08	0.23	0.22	0.46	0.41	-0.08	0.08	0.56*	0.36
	p value				0.21	0.28	0.80	0.46	0.46	0.11	0.17	0.81	0.78	0.05	0.22
TSAT(%)	*r value*					-0.19	0.42	0.19	0.21	-0.32	-0.31	-0.08	0.60*	0.22	0.22
	p value					0.54	0.16	0.53	0.50	0.29	0.30	0.80	0.03	0.48	0.47
Ferritin (ng/ml)	*r value*						-0.34	-0.45	-0.40	-0.33	-0.38	-0.22	-0.12	-0.15	-0.19
	p value						0.25	0.12	0.18	0.27	0.19	0.47	0.70	0.62	0.53
RBC (10^{12}/l)	*r value*							0.88**	0.91**	0.17	-0.02	-0.54*	0.54	0.27	-0.32
	p value							0.00	0.00	0.57	0.94	0.05	0.06	0.37	0.28
HB (g/dl)	*r value*								0.99**	0.61*	0.45	-0.32	0.20	0.12	-0.13
	p value								0.00	0.03	0.12	0.29	0.51	0.71	0.66
PCV (%)	*r value*									0.55*	0.35	-0.46	0.29	0.16	-0.20
	p value									0.05	0.23	0.12	0.34	0.60	0.51
MCV (fl)	*r value*										0.94**	0.05	-0.35	-0.07	0.24
	p value										0.00	0.87	0.24	0.81	0.43
MCH (pg)	*r value*											0.38	-0.51	-0.18	0.41
	p value											0.19	0.08	0.56	0.16
MCHC (pg)	*r value*												-0.57*	-0.37	0.53
	p value												0.04	0.22	0.06
PLT (10^9/l)	*r value*													0.69**	-0.18
	p value													0.01	0.56
WBC (10^9/l)	*r value*														0.24
	p value														0.42

(*): Correlation is significant at 0.05 level. (**): Correlation is a highly significant at 0.01 level. (r value) : correlation coefficient value

Table 4-24: *Correlation between study Parameters in CKD patients (* No.*=37)*

Parameters		Urea	Iron	U I B C	TSAT%	Ferritin	RBC	HB	PCV	MCV	MCH	MCHC	PLT	WBC	EPO
Creatinine (μmol/L)	*r value*	0.44**	-0.11	-0.02	-0.11	-0.06	-0.01	0.06	0.02	-0.06	0.05	0.27	0.24	0.04	0.02
	p value	0.01	0.50	0.92	0.52	0.72	0.98	0.72	0.90	0.74	0.79	0.11	0.15	0.82	0.89
Urea (mmol)	*r value*		-0.15	-0.04	-0.13	0.21	0.17	0.18	0.16	-0.09	-0.03	0.15	0.21	0.18	0.55**
	p value		0.38	0.83	0.45	0.20	0.32	0.28	0.35	0.59	0.86	0.39	0.21	0.28	0.00
Iron (µmol/L)	*r value*			-0.44**	0.82**	0.35*	0.18	0.19	0.24	0.14	-0.05	-0.43**	0.09	-0.06	0.06
	p value			0.01	0.00	0.03	0.29	0.27	0.15	0.43	0.77	0.01	0.62	0.72	0.73
U I B C (µmol/L)	*r value*				-.713**	-0.22	0.03	-0.08	-0.11	-0.21	-0.13	0.14	-0.20	-0.23	0.21
	p value				0.00	0.20	0.87	0.62	0.52	0.21	0.44	0.40	0.24	0.18	0.22
TSAT(%)	*r value*					0.36*	-0.05	0.19	0.24	0.33*	0.16	-0.35*	0.04	-0.02	-0.13
	p value					0.03	0.77	0.25	0.16	0.05	0.36	0.03	0.80	0.92	0.43
Ferritin (ng/ml)	*r value*						0.11	0.05	0.03	0.07	0.09	0.09	0.05	-0.09	0.14
	p value						0.52	0.76	0.85	0.69	0.59	0.60	0.77	0.58	0.42
RBC (10^{12}/l)	*r value*								0.99**	0.07	-0.05	-0.32	-0.06	0.00	0.05
	p value								0.00	0.68	0.76	0.06	0.73	0.99	0.77
HB (g/dl)	*r value*									0.05	-0.12	-0.44**	-0.06	-0.02	0.03
	p value									0.77	0.50	0.01	0.74	0.92	0.85
PCV (%)	*r value*										.93**	0.14	-0.38*	-0.40**	-0.26
	p value										0.00	0.40	0.02	0.01	0.12
MCV (fl)	*r value*											0.41**	-.34*	-0.31	-0.20
	p value											0.00	0.04	0.06	0.23
MCH (pg)	*r value*												0.02	0.14	0.09
	p value												0.90	0.43	0.61
MCHC (pg)	*r value*													.50**0	-0.04
	p value													0.00	0.83
PLT (10^9/l)	*r value*														0.25
	p value														0.13
WBC (10^9/l)	*r value*														
	p value														

(*): Correlation is significant at 0.05 level. (**): Correlation is a highly significant at 0.01 level. (r value) : correlation coefficient value

4.7: Blood Smear

Note the presence of sparsely distributed RBC, various sizes of RBC (Microcytes, Macrocytes, Target cells), as show in below figures

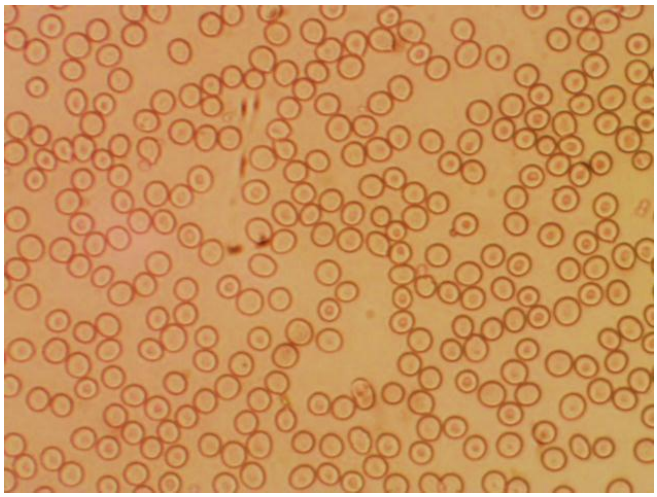


Figure 4-2 : Shows Normochromic Normocytic anemia in patients with renal failure(40x)

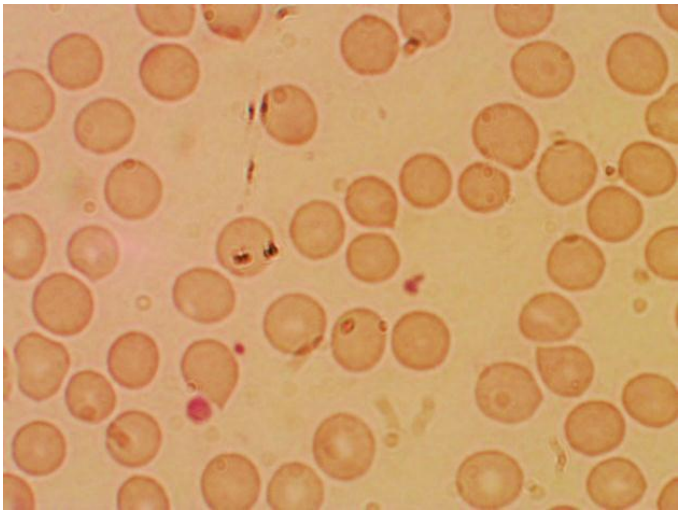


Figure 4-3: Shows Normochromic Normocytic anemia in patients with renal failure (100x)

A normocytic anemia is defined as an anemia with a mean corpuscular volume (MCV) of (80-100fL) which is the normal range. However, the hematocrit and hemoglobin is decreased.

Normochromic is a form of anemia in which the concentration of hemoglobin in the red blood corpuscular (RBC) is within the standard range. However, there are insufficient numbers of RBC. This includes: aplastic, posthemorrhagic, and hemolytic anemias and anemia of chronic disease.

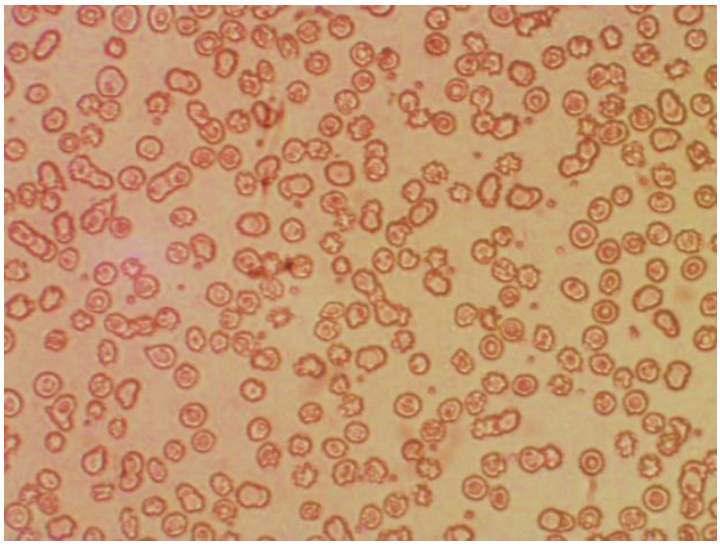


Figure4-4: Shows Hypochromic microcytic anemia in patients with renal failure characterizes smaller cells (<80 fL) (40 x).

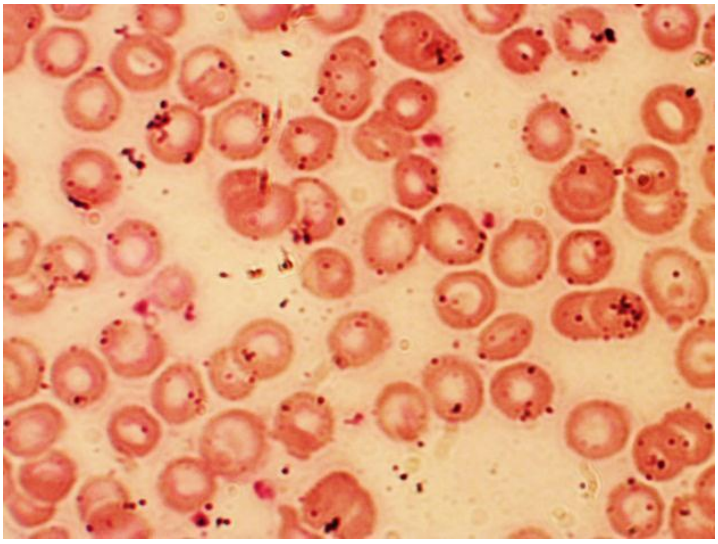


Figure4-5: Shows Hypochromic microcytic anemia in patients with renal failure characterizes smaller cells (<80 fL) (100x).

Microcytic anaemia is any of several types of anemia characterized by small red blood corpuscular (RBC) (called Microcytes). The normal mean corpuscular volume (abbreviated to MCV on full blood count results) is 80-100 fL, with smaller cells (<80 fL) described as microcytic and larger cells (>100 fL) as macrocytic (the latter occur in macrocytic anemia).

In microcytic anemia, the red blood corpuscular (RBC) are usually also hypochromic, meaning that the RBC appear paler than usual. This is reflected by a lower-than normal mean corpuscular hemoglobin

concentration (MCHC), a measure representing the amount of hemoglobin per unit volume of fluid inside the cell; normally about 32-36 g/dL. Typically, therefore, anemia of this category is described as microcytic, hypochromic anaemia.

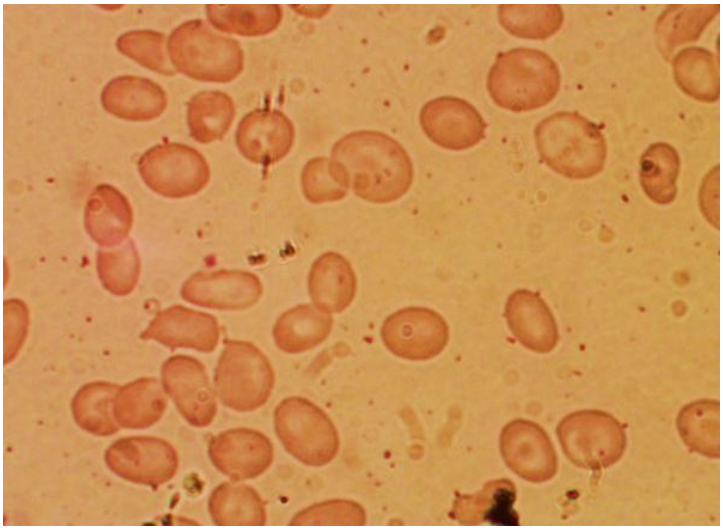


Figure 4-6: Shows Macrocytic anemia in patients with renal failure characterizes larger cells (>100 fL) (100x)

A macrocytic class of anemia (defined as blood with an insufficient concentration of hemoglobin) in which the red blood corpuscular (RBC) are larger than their normal volume. The normal erythrocyte volume in humans is about 80 to 100 femtoliters (fL= 10^{-15} L). In metric terms the size is given in equivalent cubic micrometers (1 μm^3 = 1 fL). The condition of having erythrocytes which (on average) are too large, is called macrocytosis. In contrast, in microcytic anemia, the erythrocytes are smaller than normal.

In a macrocytic anemia, the larger RBC is always associated with insufficient numbers of cells and often also insufficient hemoglobin content per cell. Both of these factors work to the opposite effect of larger cell size, to finally result in a total blood hemoglobin concentration that is less than normal (i.e., anemia).

Chapter Five

5: Discussion

5.1: Social-demographic characteristics:

This study was applied on acute kidney injure and chronic kidney disease patients among other patients with different kidney disease seen at Hemodialysis department / Marjan Teaching Hospital in Hilla/Babil Governorate.

The majority of patients were males 61.5% - 51.4% with acute kidney injure and chronic kidney disease, respectively, like other studies ,this study approved that (Akinsola *et al.*, 2000; Ijoma *et al.*, 2010). Men suffer renal failure more than females because in developing countries men tend to seek medical attention more than females, show in table (4-1).

The means of ages in this study were 53. 23±12.78 and 51.19±17.95 years which coordinate to 47.0±2.0 and 45.0+15.0 years in the Indonesia and Nigerian study, respectively (Suega *et al.*, 2005; Ijoma *et al.*, 2010).The increasing age is a traditional risk factor for renal failure patients as age increases, the GFR decreases. Consequently, renal failure patient is common in adult as compared to young age. Hypertension (HTN) and Diabetes mellitus (DM) were identified as the main underlying disease for renal failure patients in this study. Therefore aggressive screening programme of patients, especially those at risk such as HTN and DM is in place, thereby decreasing progression of CKD.

5.2: Kidney function test:

The results of the current study showed that there are a highly significant differences increases ($P \leq 0.01$) in the means values of serum urea and creatinine in AKI and CKD patients compared to the control groups. When AKI and CKD patients were compared, no significant difference were found in serum urea and creatinine, as shown in table (4-2), these results are in agreement with the previous studies (Mavromatidis *et al.*, 1998; Ahssan *et al.*, 2010; Schaalan and Mohamed, 2016).

These studies concluded that urea and creatinine are quantitatively the most important solute excreted by the kidney and it was the first organic solute detected in the blood of patients with AKI and CKD. This elevation in urea and creatinine concentration occurs because, in AKI and CKD patients the kidney lose its ability to eliminate nitrogenous wastes from the blood results in accumulation of these substances in the blood (Mohamed, 2016).

Some researches pointed out that serum urea and serum creatinine were the main indicators for renal failure at both types (acute and chronic), but others indicated that at all stages of renal insufficiency, the serum creatinine was a much more reliable indicator of renal function than blood urea because blood urea is far more likely to be affected by dietary and physiological conditions not related to renal function. Moreover, the differences in the results of urea and creatinine among both genders in all age groups were highly significant ($P \leq 0.01$).These results were similar to those obtained by some researchers who pointed out that the mean values of urea and creatinine in patients with renal failure was not significant if the results of both indicators were distributed among patients ages and gender (Chhetri *et al.*, 2008).

5.3: The status of Iron

The results of the present study showed that the levels of iron, TIBC, Trf and TSAT% were significantly decreased in patients with AKI and CKD, while the level of ferritin was significantly elevated in patients with CKD compared to control group ($P \leq 0.01$), as shown in tables (4-3), these results agreed with the studies (Andrews, 1999; Besarab *et al.*, 2000; Fishbane, 2006; Lenga *et al.*, 2007; Mavromatidis *et al.*, 1998).

The results of this study showed an increase in the serum ferritin level in AKI patients compared to control group, that increase of Serum ferritin level in AKI patients may be due to Inflammation, as show in table (4-3). These results agreed with the study (Góes *et al.*, 2013)

The results of the current study showed an increase in the TSAT% in patients with CKD, These results agreed with the studies (Jeffrey *et al.*, 2012; Deori and Bhuyan, 2017). This result may be due to Intravenous iron injection and increased treatment with hemodialysis.

However, serum iron, TIBC, TSAT%, or serum ferritin were used as objective markers for body iron stores. among these biomarkers or indices, the serum ferritin is considered the best single indicator of total body iron (Baer *et al.*, 1994). Serum ferritin is an acute phase reactant and elevated level of serum ferritin in AKI and CKD patients is mainly due to the inflammation (Kalender *et al.*, 2002; Kennedy *et al.*, 2004; De Zoysa and Lee, 2007)

Ferritin is the principal iron storage protein participating in iron metabolism. Serum level of ferritin usually reflects the amount of iron storage in the body, physicians have measured serum ferritin in order to evaluate iron deficiency or overload. Although a rise in serum ferritin concentration occurs in iron overload; without it, hyperferritinemia (elevation serum levels of ferritin) has been reported in some inflammatory diseases, malignancies and renal failure. Some cytokines have been reported to be responsible for the elevation of ferritin production. However, the hyperferritinemia is not a result, but profoundly participates in the disease process (Ganz, 2003; Andrews, 2004; Kennedy *et al.*, 2004)

Iron deficiency status, in this study, absolute iron deficiency was defined with serum ferritin of less than 100 ng/ml and TSAT% of less than 20%. Functional iron deficiency was defined with serum ferritin of above 100 ng/ml and TSAT% below 20%. Adequate iron store was defined with serum ferritin of 100-800 ng/ml and TSAT% of 20-50% and Iron overload was considered when patient had serum ferritin of more than 800 ng/ml TSAT% of more than 50% (National, 2006; Pasricha *et al.*, 2010).

In the current study, 6 (46.2%) and 9 (24.3%) of AKI and CKD patients were iron deficient, respectively, as indicated by serum ferritin ≤100 ng/ml and

TSAT% ≤ 20% where by 5 (38.5%) and 26 (70.3%) had functional iron deficiency as indicated by serum ferritin ≥ 100 ng/ml and TSAT% ≤ 20% and all were anemic, as shown in table (4-16).

Our opinion hemodialysis itself may be contributed to the anemia. Iron deficiency can result from unavoidable dialyzer blood loss, clotted dialysis membranes and frequent blood sampling.

In another study conducted by Malyszko *et al.* (2006) reported that the prevalence of functional iron deficiency was 21% of 200 studied hemodialysis patients as indicated by ferritin. This was also found to be associated with high hepcidin levels and inflammatory markers.

The findings of our study agreed with Post *et al.* (2006), who, while studying iron deficiency anemia and the role of intravenous iron among renal failure patients, found out that 28.4% of 102 anemic CKD patients had iron deficiency (serum ferritin less than 100 ng/ml and TSAT% less than 20%) and 41% had functional iron deficiency.

However, in contrast to the findings of the current study, Hsu *et al.* (2002) reported that 62.6% of CKD patients with anemia were iron deficient, as indicated by serum ferritin which was less than 100 ng/mL and TSAT% less than 20%. While 25.8% of patients had functional iron deficiency anemia as indicated by serum ferritin which was more than 100ng/ml and less than 20% TSAT%**.** This upward trend of high proportional of CKD patients with iron deficiency done elsewhere could be attributed to small sample size (n=50) investigated in the current study compared to (15,837) in the previous study**.**

The present study is dissimilar with the study of Łukaszyk *et al.* (2015) who, while studying iron status and inflammation in early stages of CKD, found out that 17% (n= sixty nine anemic) CKD patients had absolute iron deficiency and 12% had functional iron deficiency.

5.4: Prevalence of anemia among renal failure patients

This study demonstrated the type and the high prevalence of anemia in AKI and CKD patients at Marjan Teaching Hospital with overall prevalence of forty eight (96%). A similar finding to this study was displayed in the study conducted in northern Tanzania were the prevalence was 92.4 % among fifty two CKD patients seen Kilimanjaro Christian Medical Centre (Kilonzo, 2010).

Suega *et al.* (2005) in Indonesia and Afshar *et al.* (2010) in Iran, reported the prevalence rate of anemia in pre-dialysis patients 73.1% and 75.0% respectively, and in Africa a Nigerian studies documented 77.5% and 87% prevalence of anemia in CKD patients (Akinsola *et al.*, 2000; Ijoma *et al.*, 2010).

The results of this study revealed that some of red blood cell indices including RBC count and Hb, PCV and MCV levels were significantly decreased in patients group, compared with the control group ($P \leq 0.05$) and anemia was considerable in patients with AKI as show in table (4-4).

Our study also showed that AKI does not cause significant difference in platelet count in these patients were slightly decreased in comparison to the control group ($P \leq 0.05$).

The study of Hales *et al.* (1994) reported the presence of anemia in 90% of patients with AKI as a result of increase in serum urea and presence of oliguria. In their study 53 of the 56 patients had a mild anemia (PCV≤ 35%) during their hospital stay. This finding is similar with our findings that our studded patients 3(23.07%) with AKI had a PCV below 35%. In the study of Michele Hales, Forty-three of the patients had a PCV below 30%, but this finding was observed in our study 10 patients (76.92%) of all patients had PCV lower than 30%. The possible cause of this difference is the serum urea level.

About patients with CKD, they were anemic and most RBC indices including RBC count, Hb level and PCV level were significantly lower in comparison with the control group ($P \leq 0.05$) as show in table (4-4).

The findings of this study were in line with a similar study that was done by McClellan *et al.* (2004), which revealed the presence of anemia in 47.7% of 5222 patients with CKD before dialysis and It also showed the increased prevalence of anemia as kidney function decreased.

It was reported that CKD patients with anemia had lower hematological indices and the degree of changes depended on the severity of renal failure (Khanam *et al.*, 2007). This study agrees with the previous studies as significant decreases observed in the PCV, Hb and platelet count in patients with CKD. Normochromic normocytic anemia observed as the most common type of anemia in CKD patients. Further, it was observed that hypertension systemic and diabetes mellitus are more common causes for CKD.

Wastiet *et al.* (2013) reported that mean of RBC, Hb and PCV were significantly lowered in CKD patients and similarly MCH and MCHC indices also decreased significantly.

It was reported that normochromic normocytic anemia was the most common hematological abnormality in patients with CKD. Anemia can be correlated with severity of renal failure. The higher the blood urea lead to severe anemia (Sunita *et al.*, 2014).

Asif *et al.* (2015) reported that among hematological parameter, Hb is the most commonly affected.

The most frequent morphologic feature of anemia was normochromic normocytic followed by hypochromic microcytic anemia, which has different causes, but the most common is iron deficiency particularly due to decrease in iron intake or iron loss (Fernández-Rodríguez *et al.*, 1999). With early

recognition and treatment of anemia, prevalence of iron deficiency anemia among CKD patients, decreased (Suega *et al.*, 2005).

The platelet count in patients of this study was highly significant as it is lower than control groups ($P \leq 0.01$). Although the mean platelet count (179.59×10^9/l) does not show that, the patients are not in a potential bleeding risk but thrombocytopenia was an important risk factor for bleeding among a minority of our patients of CKD.

Another similar study was done Mohamed (2010) revealed that the patients with renal failure are at high risk of bleeding because of thrombocytopenia and platelet dysfunction. This study also found a mild thrombocytopenia among patients but a minority of patients (13.51%) had a platelet count that could put patients at risk of bleeding.

The study of Suresh *et al.* (2012) was done on hematological changes in Fifty patients suffering from CKD. This study showed abnormal haematological parameters due to impaired production of EPO and other factors like the increase hemolysis, suppression of bone marrow erythropoiesis and gastrointestinal blood loss.

The results also showed that was significant differences in the mean of MCH in patient with CKD compared to control group as show in table (4-4), this study agrees with two studies of (Malyszko *et al.*, 2006; Pereira *et al.*, 2010).

The results of this study revealed an increase in MCH, MCHC and a decrease in MCV that may be related to protein loss from erythrocyte membrane during the Hemodialysis procedure, that previously described by this study (Costa *et al.*, 2008).

The results in table (4-4) demonstrates non-significant increase ($p \leq 0.05$) in WBCs count in patients with CKD groups as compared with control group. This results are matched with the results of study of Pereira *et al.* (2010) found that total WBCs count increased non-significantly compared with control group.

The present study shows a highly significant differences(P≤0.001)increase in serum level of EPO in AKI patients with anemia compared to the control group, this study agrees with the study (Góes *et al.*, 2013).As show in tables(4-4).

The results of the current study showed a significant increase in serum level of EPO in AKI patients with anemia compared to the control group (P≤0.001), this study agrees with the study (Yamashita *et al.*, 2016). As show in tables (4-4), suggesting that they are unable to mount an appropriate EPO response and the action of EPO may be inhibited by proinflammatory cytokines.

The results of this study showed high serum levels of EPO in patients with AKI was observed, may be due to the decreased renal hypoperfusion, which leads to compensatory increase of EPO production along with activation of the renin angiotensin and sympathetic nervous systems. Angiotensin II activity may also up regulate EPO serum levels (Gossmann *et al.*, 2001). The results of this study was different from a study conducted in Cairo, Egypt by Schaalan and Mohamed (2016) whom reported a decrease in serum level of EPO in AKI patients compared to control group.

Several researchers suggested a relation between EPO levels and activity of the renin-angiotensin system. It was demonstrated that angiotensin II increase EPO levels by stimulating the AT1-receptor (Gossmann *et al.*, 2001; Kato *et al.*, 2005), The use of both Angiotensin Converting Enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) have been related to reduction of serum levels of EPO (Robles *et al.*, 2009).

The present study shows that the mean value of EPO increased in CKD patients in compared to the control groups, these results are different from the study (Jones *et al.*, 2005) .These findings suggest that anemia appeared first when the GFR falls below 40 ml/minute, and its present in most patients with ESRD because in renal failure, erythropoietin production usually insufficient to stimulate adequate red blood cells production by bone marrow. Also Levin (2002) showed that it is well recognized that patients with CKD have low Hb

and the etiology of the anemia was eventually determined to be primarily due to the decrease in EPO production and activity. As show in table (4-4).

This study showed that serum levels of EPO in anemic-renal failure of the fifty patients compared to Thirty-eight normal control group. In this study, it was noticed that the level of EPO was significantly higher in both AKI and CKD patients, as compared to the normal control groups. In AKI level of EPO was 280.32±51.93 Pg/ml significantly higher $p \leq 0.01$ and In CKD patients 205.33±80.40 Pg/ml significantly higher than normal 103.85±84.48 Pg/ml $p \leq 0.01$, increase in serum EPO level may be because resistance of erythropoietin receptors to the recombinant human erythropoietin (rHuEPO). As show in tables (4-4).

In non-dialysis patients with declining renal function, progressive anemia is noticed despite of no decrease in serum EPO level, may be that erythropoietin tissues may be less sensitive to EPO in CKD (Artunc and Risler, 2007; Chandra *et al.*, 1988) and many studies have shown that patients placed on hemodialysis, manifested with improvement in PCV, without significant changes in plasma EPO levels(Radtke *et al.*, 1980), suggestion that an inhibitor was removed by dialysis.

A number of patients with AKI and CKD have remained anemic despite of the presence increase EPO level on bioassay, I think that the sensitivity of bone marrow has decreased to circulating EPO in these patients.

Fukushima *et al.* (1986) in Japan studied the serum EPO and inhibitors of erythropoiesis in anemic CKD patients fifty-four compared to twenty-six normal control group. In this study it was noted that the level of EPO was significantly higher in both pre and dialysis patient of thirty five as compared to control groups. The level of EPO in dialysis patients was 141.2+ 109 mU/ml and in seven pre-dialysis patients was 99.9+ 45.0mU/ml, which is significantly higher than control (42.0±25.8 mU/ml) and it was not correlated to concentration of Hb and serum creatinine.

5.5: Morphological characteristics of renal failure anemia

It is well identified that the anemia of renal failure is normocytic normochromic type as the loss of renal mass could be the principle mechanism (Suega *et al.*, 2005) . In this study, the morphology of fifty anemic of thirteen AKI and Thirty-seven CKD patients were normocytic normochromic anemia in seven (53.84%) and twenty-seven (72.97%) of patients, microcytic hypochromic anemia in six (46.15%) and nine (24.32) of patients and macrocytic hypochromic anemia in 0(0.0) and one (2.70%) of patients in respectively, as show in table (4-14).

In another study was agreement from a study conducted by Suega *et al.* (2005) in Indonesia, the morphology of Thirty-three anemic CKD patients was reported to be normochromic normocytic in Twenty-six (78.8%) of patient, macrocytic in Seven (21.2%) of patient, and no hypochromic anemia was found.

The results of this study was similar from a study conducted George (2015) in Indian, the morphology of fifty anemic CKD patients was reported to be normochromic normocytic in 62% of patients, hypochromic microcytic anemia 30% of patients, macrocytic anemia in 8% of patients.

The results of this study was similar from a study conducted Afshar *et al.* (2010) in Iran, the morphology of one hundred anemic CKD patients was reported to be normochromic normocytic in 80% of patients, hypochromic anemia 15% of patients and macrocytic anemia in 5% of patients, these result were different from another study conducted in New Delhi India by Talwar and Gupta (2002) whom reported 30% of the patients had normocytic normochromic anemia, 5% of patient had macrocytic anemia while 60% of patient had microcytic hypochromic anemia, the researcher concluded that majority of patients 65% had microcytic hypochromic anemia due to iron deficiency.

Conclusions

1. Hematological parameters are commonly affected in AKI and CKD. Of all the parameters, red cell indices are the ones commonly affected.
2. Anemia in AKI and CKD is a known risk factor and untreated anemia leads to increased morbidity and mortality.
3. This study shows that anemia is prevalent among AKI and CKD patients by forty eight (96%) where moderate degree of anemia is most frequent finding in both gender and the degree of anemia is severe in male as compared to female.
4. In the vast majority of patients, this study gives an evidence of inadequate endogenous EPO production and defective iron supply for anemia in CKD patients.
5. Both oral and parenteral iron are shown to decrease the proportion of people who require blood transfusion and increase Hb levels, without any benefit to mortality. Further attempts at a low risk of bias, powered to measure clinically significant endpoints, are still required.

Recommendation

1. Control of blood sugar and blood pressure is essential in uremic patients to reduce future complications.
2. Frequent assessment of iron and ferritin level in uremic patients to prevent iron poisoning.
3. There is a need for early diagnosis and treatment of anemia in AKI and CKD patients as anemia leads to CKD progression and cardiovascular disease.
4. The main treatment for anemia in AKI and CKD is ESAs and adequate iron store are necessary to permit an optimal response, therefore it is highly recommended to do iron studies for all patients to establish types of iron deficiency as absolute iron deficiency needs oral iron therapy; while functional iron deficiency needs intravenous iron supplement.
5. This was a hospital based study, it is therefore recommended to do similar study using large AKI and CKD sample size at a community level to include all stages of CKD and more causative factors related to anemia than in the present study .
6. It is suggested to study the level of thyroid hormones and thrombopoietin in patients with AKI and CKD as they may be factors participating in pathogenesis of anemia.
7. Additional work is advised to study renin effect on blood pressure in patients with AKI and CKD.
8. Further studies are recommended to discover new anti-inflammatory drugs to overcome problem of EPO resistance to the recombinant human erythropoietin (rHuEPO).
9. Avoid smoking and drug abuse with proper diet and exercise are important lifestyle modifications.

References:

Abramson, N. and Melton, B. (2000). Leukocytosis: basics of clinical assessment. *American family physician 62*(9): 2053-2066.

Afshar, R.; Suzan, S.; Salimi, J. and Ahmadzadeh, M. (2010). Hematological profile of chronic kidney disease (CKD) patients in Iran, in pre-dialysis stages and after initiation of hemodialysis. *Saudi Journal of Kidney Diseases and Transplantation 21*(2): 368-371.

Ahmed, R. A. K. (2015). Changes of Some Haematological Parameters in Chronic Renal Failure patients after. M.Sc. Hematology and Immunohematology, Sudan University Of Science And Technology.

Ahssan, A. L.; Salih, M. H. and Sahib, A. A.-A. (2010). Biochemical Studies for Some Enzymes in Sera of Patients With renal Failure. *Journal of Kerbala University 8*(2): 77-166

Akinsola, A.; Durosinmi, M. and Akinola, N. (2000). The haematological profile of Nigerians with chronic renal failure. *African journal of medicine and medical sciences 29*(1): 13-16.

Albu, S.; Joyce, E.; Paniwnyk, L.; Lorimer, J. and Mason, T. (2004). Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinus officinalis for the food and pharmaceutical industry. *Ultrasonics Sonochemistry 11*(3): 261-265.

Anantharaman, P. and Schmidt, R. J. (2007). Sexual function in chronic kidney disease. *Advances in chronic kidney disease 14*(2): 119-125.

Andrews, N. C. (1999). Disorders of iron metabolism. *New England Journal of Medicine 341*(26): 1986-1995.

Andrews, N. C. (2004). Anemia of inflammation: the cytokine-hepcidin link. *The Journal of clinical investigation 113*(9): 1251-1253.

Andrews, N. C. (2008). Forging a field: the golden age of iron biology. *blood 112*(2): 219-230.

References

Andrews, N. C. and Schmidt, P. J. (2007). Iron homeostasis. *Annu. Rev. Physiol. 69*(1): 69-85.

Artunc, F. and Risler, T. (2007). Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. *Nephrol Dial Transplant 22*(10): 2900-2908.

Ashton, N. (2013). Physiology of red and white blood cells. *Anaesthesia & Intensive Care Medicine 14*(6): 261-266.

Asif, N.; Hasan, S. and Hassan, K. (2015). Hematological Changes in Patients of Chronic Renal Disease and Their Response to Treatment with Erythropoietin. *Int. j. pathol 13*(1): 14-19.

Baer, D. M.; Tekawa, I. S. and Hurley, L. B. (1994). Iron stores are not associated with acute myocardial infarction. *Circulation 89*(6): 2915-2918.

Beaumont, C. (2004). Molecular mechanisms of iron homeostasis. *Medecine sciences 20*(1): 68-72.

Beckett, G.; Walker, S. W.; Rae, P. and Ashby, P. (2010). Lecture Notes: Clinical Biochemistry The Atrium, Sothern Gate, Chichester, West Sussex PO19 8SQ UK, *John Wiley & Sons,*: 56-63.

Benjamin, I.; Griggs, R. C.; Wing, E. J. and Fitz, J. G. (2015). Andreoli and Carpenter's Cecil essentials of medicine. *Elsevier Health Sciences*: 392-399.

Besarab, A.; Amin, N.; Ahsan, M.; Vogel, S. E.; Zazuwa, G.; Frinak, S.; Zazra, J. J.; Anandan, J. and Gupta, A. (2000). Optimization of epoetin therapy with intravenous iron therapy in hemodialysis patients. *Journal of the American Society of Nephrology 11*(3): 530-538.

Bolignano, D.; D'Arrigo, G.; Pisano, A. and Coppolino, G. (2015). Pentoxifylline for anemia in chronic kidney disease: A systematic review and meta-analysis. *PLoS ONE 10*(8): 1-16.

Bostrom, M. A. and Freedman, B. I. (2010). The spectrum of MYH9-associated nephropathy. *Clinical Journal of the American Society of Nephrology 5*(6): 1107-1113.

Bowling, C. B.; Inker, L. A.; Gutiérrez, O. M.; Allman, R. M.; Warnock, D. G.; McClellan, W. and Muntner, P. (2011). Age-specific associations of reduced estimated glomerular filtration rate with concurrent chronic kidney disease complications. *Clinical journal of the American Society of Nephrology 6*(12): 2822-2838.

Brugnara, C. and Eckardt, K. (2011). Hematologic aspects of kidney disease. Brenner and Rector's The Kidney, *Saunders, Philadelphia , p. 2081-2120*.

Burtis, C. A.; Ashwood, E. R. and Bruns, D. E. (2012). Tietz textbook of clinical chemistry and molecular diagnostics. United States of America, *Elsevier Health Sciences*: 703-1699.

Ceriotti, F.; Boyd, J. C.; Klein, G.; Henny, J.; Queralto, J.; Kairisto, V. and Panteghini, M. (2008). Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clinical chemistry 54*(3): 559-566.

Cerveró, A. J.; Bagán, J. V.; Soriano, Y. J. and Roda, R. P. (2008). Dental management in renal failure: patients on dialysis. *Med Oral Patol Oral Cir Bucal 13*(7): 419-426.

Chadban, S. J.; Briganti, E. M.; Kerr, P. G.; Dunstan, D. W.; Welborn, T. A.; Zimmet, P. Z. and Atkins, R. C. (2003). Prevalence of kidney damage in Australian adults: The AusDiab kidney study. *Journal of the American Society of Nephrology 14*(2): 131-138.

Chan, C.; Floras, J. S.; Miller, J. A. and Pierratos, A. (2002). Improvement in ejection fraction by nocturnal haemodialysis in end‐stage renal failure patients with coexisting heart failure. *Nephrology Dialysis Transplantation 17*(8): 1518-1521.

Chandra, M.; Clemons, G. K. and McVicar, M. I. (1988). Relation of serum erythropoietin levels to renal excretory function: evidence for lowered set point for erythropoietin production in chronic renal failure. *The Journal of pediatrics 113*(6): 1015-1021.

Chaney, A. L. and Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical chemistry 8*(2): 130-132.

Chatterjea, M. N. and Shinde, R. (2012). Textbook of medical biochemistry. New Delhi, *Jaypee Bros. Medical Publishers*: 578-580.

Chhetri, P.; Manandhar, D.; Pahari, L.; Chhetri, S. and Chaudhary, S. (2008). Acute renal failure in Nepal Medical College Teaching Hospital. *Nepal Medical College journal 10*(2): 132-135.

Chu, E. and Sartorelli, A. C. (2007). Basic and clinical pharmacology. *Cancer chemotherapy. 10th ed. Boston: McGraw-Hill*: 878-907.

Cody, J. D.; Daly, C.; Campbell, M. K.; Khan, I.; Rabindranath, K. S.; Vale, L.; Wallace, S. A.; MacLeod, A. M.; Grant, A. M. and Pennington, S. (2005). Recombinant human erythropoietin for chronic renal failure anaemia in pre‐dialysis patients. *The Cochrane Library 22*(6): 474−478.

Colledge, N. R.; Walker, B. R. and Ralston, S. H. (2010). Davidson's Principles and Practice of Medicine. illust. Robert Britton . Edinburgh: Churchill Livingstone. *Elsevier*: 478-518.

Costa, E.; Rocha, S.; Rocha-Pereira, P.; Castro, E.; Miranda, V.; Faria, M. d. S.; Loureiro, A.; Quintanilha, A.; Belo, L. and Santos-Silva, A. (2008). Changes in red blood cells membrane protein composition during hemodialysis procedure. *Renal failure 30*(10): 971-975.

Crichton, R. (2016). Iron metabolism: from molecular mechanisms to clinical consequences Siuthern,Chichester,West,PO18SQ,United Kingdom, *John Wiley & Sons*: 183-222.

De Domenico, I.; Ward, D. M. and Kaplan, J. (2008). Regulation of iron acquisition and storage: consequences for iron-linked disorders. *Nature Reviews Molecular Cell Biology 9*(1): 72-81.

De Zoysa, M. and Lee, J. (2007). Two ferritin subunits from disk abalone (Haliotis discus discus): cloning, characterization and expression analysis. *Fish & shellfish immunology 23*(3): 624-635.

Deori, R. and Bhuyan, B. (2017). Iron status in chronic kidney disease patients. *International Journal of Research in Medical Sciences 4*(8): 3229-3234.

Drüeke, T. B.; Locatelli, F.; Clyne, N.; Eckardt, K.-U.; Macdougall, I. C.; Tsakiris, D.; Burger, H.-U. and Scherhag, A. (2006). Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *New England Journal of Medicine 355*(20): 2071-2084.

Egrie, J. C. and Browne, J. K. (2001). Development and characterization of novel erythropoiesis stimulating protein (NESP). *Nephrology Dialysis Transplantation 16*(suppl 3): 3-13.

Elaine, N. (2012). Essentials on Human Anatomy and Physiology, 7th ed. USA: San Francisco. . 479-501.

Eleftheriadis, T.; Antoniadi, G.; Liakopoulos, V.; Kartsios, C. and Stefanidis, I. (2007). Basic science and dialysis: disturbances of acquired immunity in hemodialysis patients. *Seminars in dialysis 20*(5): 440-451.

Evatt, B. L.; McArthur, J. R.; Lewis, S. M.; Gibbs, W. N.; Organization, W. H. and Control, C. f. D. (1992). Fundamental diagnostic hematology: Anemia. Ch 5 Geneva, *World Health Organization*: 74-79.

Fabiny, D. L. and Ertingshausen, G. (1971). Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. *Clinical chemistry 17*(8): 696-700.

Fauci, A. S. (2008). Harrison's principles of internal medicine. New York, USA *McGraw-Hill, Medical Publishing Division*: 1549-1553.

Fernández-Rodríguez, A. M.; Guindeo-Casasús, M. C.; Molero-Labarta, T.; Domínguez-Cabrera, C.; Hortal-Cascón, L.; Pérez-Borges, P.; Vega-Díaz, N.; Saavedra-Santana, P. and Palop-Cubillo, L. (1999). Diagnosis of iron deficiency in chronic renal failure. *American Journal of Kidney Diseases 34*(3): 508-513.

Finkelstein, F. O.; Shirani, S.; Wuerth, D. and Finkelstein, S. H. (2007). Therapy Insight: sexual dysfunction in patients with chronic kidney disease. *Nature Clinical Practice Nephrology 3*(4): 200-207.

Fishbane, S. (2006). Iron supplementation in renal anemia. *Semin Nephrol 26*(4): 319-324.

Fleming, R. E. and Bacon, B. R. (2005). Orchestration of iron homeostasis. *The New England Journal of Medicine 352*(17): 1741-1744.

Fukushima, Y.; Fukuda, M.; Yoshida, K.; Yamaguchi, A.; Nakamoto, Y.; Miura, A. B.; Harada, T. and Tsuchida, S. (1986). Serum Erythropoietin levels and inhibitors of erythropoiesis in patients with chronic renal failure. *Tohoku J Exp Med 150*(1): 1-15.

Furuland, H.; Linde, T.; Ahlmén, J.; Christensson, A.; Strömbom, U. and Danielson, B. G. (2003). A randomized controlled trial of haemoglobin normalization with epoetin alfa in pre‐dialysis and dialysis patients. *Nephrology Dialysis Transplantation 18*(2): 353-361.

Gambino, R.; Desvarieux, E.; Orth, M.; Matan, H.; Ackattupathil, T.; Lijoi, E.; Wimmer, C.; Bower, J. and Gunter, E. (1997). The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. *Clinical chemistry 43*(12): 2408-2412.

Ganz, T. (2003). Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood 102*(3): 783-788.

Ganz, T. (2007). Molecular control of iron transport. *Journal of the American Society of Nephrology 18*(2): 394-400.

Garčic, A. (1979). A highly sensitive, simple determination of serum iron using chromazurol B. *Clinica Chimica Acta 94*(2): 115-119.

Genovese, G.; Friedman, D. J.; Ross, M. D.; Lecordier, L.; Uzureau, P.; Freedman, B. I.; Bowden, D. W.; Langefeld, C. D.; Oleksyk, T. K. and Knob, A. L. U. (2010). Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science 329*(5993): 841-845.

George, S. V. (2015). A study to assess changes in the hematological profile in chronic kidney disease. *Hypertension 4*(6): 1-3.

Góes, M. A.; Iizuka, I. J.; Quinto, B. M.; Dalboni, M. A.; Monte, J. C.; Santos, B. C.; dos Santos, O. F. P.; Pereira, V. G.; Durão, M. d. S.; Batista, M. C. and Cendoroglo, M. (2013). Serum Soluble-Fas, Inflammation, and Anemia in Acute Kidney Injury. *Artificial Organs 10.1111*(12019): n/a-n/a.

Gooneratne, I.; Ranaweera, A.; Liyanarachchi, N.; Gunawardane, N. and Lanerolle, R. (2008). Epidemiology of chronic kidney disease in a Sri Lankan population. *International journal of diabetes in developing countries 28*(2): 60.

Gossmann, J.; Burkhardt, R.; Harder, S.; Lenz, T.; Sedlmeyer, A.; Klinkhardt, U.; Geiger, H. and Scheuermann, E.-H. (2001). Angiotensin II infusion increases plasma erythropoietin levels via an angiotensin II type 1 receptor-dependent pathway. *Kidney international 60*(1): 83-86.

Gregg, D. and Goldschmidt-Clermont, P. J. (2003). Platelets and cardiovascular disease. *Circulation 108*(13): e88-e90.

Groothoff, J. (2005). Long-term outcomes of children with end-stage renal disease. *Pediatric Nephrology 20*(7): 849-853.

Gupta, K.; Hooton, T. M.; Naber, K. G.; Wullt, B.; Colgan, R.; Miller, L. G.; Moran, G. J.; Nicolle, L. E.; Raz, R. and Schaeffer, A. J. (2011). International clinical practice guidelines for the treatment of acute

uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical infectious diseases 52*(5): e103-e120.

Guyatt, G. H.; Oxman, A. D.; Ali, M.; Willan, A.; McIlroy, W. and Patterson, C. (1992). Laboratory diagnosis of iron-deficiency anemia. *Journal of general internal medicine 7*(2): 145-153.

Hadjifrangiskou, M.; Kostakioti, M.; Chen, S. L.; Henderson, J. P.; Greene, S. E. and Hultgren, S. J. (2011). A central metabolic circuit controlled by QseC in pathogenic Escherichia coli. *Molecular microbiology 80*(6): 1516-1529.

Hage, F. G.; Venkataraman, R.; Zoghbi, G. J.; Perry, G. J.; DeMattos, A. M. and Iskandrian, A. E. (2009). The scope of coronary heart disease in patients with chronic kidney disease. *Journal of the American College of Cardiology 53*(23): 2129-2140.

Hahn, B. H.; Grossman, J.; Ansell, B. J.; Skaggs, B. J. and McMahon, M. (2008). Altered lipoprotein metabolism in chronic inflammatory states: proinflammatory high-density lipoprotein and accelerated atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis research & therapy 10*(4): 1.

Hahn, D. (2007). CBE-Morphological classification of anaemia. *IMVS Newsletter 65*: 8-10.

Hales, M.; Solez, K. and Kjellstrand, C. (1994). The anemia of acute renal failure: association with oliguria and elevated blood urea. *Renal failure 16*(1): 125-131.

Hall, J. E. (2015). Guyton and Hall textbook of medical physiology. Philadelphia,USA, *Elsevier Health Sciences*: 419-420.

Hallan, S. I. and Orth, S. R. (2010). The conundrum of chronic kidney disease classification and end-stage renal risk prediction in the elderly–what is the right approach? *Nephron Clinical Practice 116*(4): 307-316.

Hari, P.; Bagga, A.; Mahajan, P. and Dinda, A. (2009). Outcome of lupus nephritis in Indian children. *Lupus 18*(4): 348-354.

Haroun, M. K.; Jaar, B. G.; Hoffman, S. C.; Comstock, G. W.; Klag, M. J. and Coresh, J. (2003). Risk factors for chronic kidney disease: a prospective study of 23,534 men and women in Washington County, Maryland. *Journal of the American Society of Nephrology 14*(11): 2934-2941.

Harrison, P. M. and Arosio, P. (1996). The ferritins: molecular properties, iron storage function and cellular regulation. *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1275*(3): 161-203.

Harvey, R. A. and Ferrier, D. R. (2011). Biochemistry. Philadelphia, Baltimore, New York, London, *Wolters Kluwer Health/Lippincott Williams & Wilkins*: 253 – 255.

Hooi, L. S.; Ong, L. M.; Ahmad, G.; Bavanandan, S.; Ahmad, N. A.; Naidu, B. M.; Mohamud, W. N. W. and Yusoff, M. F. M. (2013). A population-based study measuring the prevalence of chronic kidney disease among adults in West Malaysia. *Kidney international 84*(5): 1034-1040.

Hsu, C. Y.; McCulloch, C. E. and Curhan, G. C. (2002). Iron status and hemoglobin level in chronic renal insufficiency. *J Am Soc Nephrol 13*(11): 2783-2786.

Huang, X. (2003). Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 533*(1): 153-171.

Ida, H. (2015). [Pathogenesis and Clinical Examination of Autoinflammatory Syndrome]. *Rinsho byori. The Japanese journal of clinical pathology 63*(10): 1207-1212.

Ijoma, C.; Ulasi, I.; Ijoma, U. and Ifebunandu, N. (2010). High prevalence of anemia in predialysis patients in Enugu, Nigeria. *Nephrology Reviews 2*(14): 14.

Inagaki, H.; Kuroda, M.; Watanabe, S. and Hamazaki, T. (2001). Changes in major blood components after adopting the supine position during haemodialysis. *Nephrology Dialysis Transplantation 16*(4): 798-802.

Jackson, D. J.; Wörheide, G. and Degnan, B. M. (2007). Dynamic expression of ancient and novel molluscan shell genes during ecological transitions. *BMC evolutionary biology 7*(1): 160.

Jeffrey, S. B.; Thomas A Golper and Alice M Sheridan (2012). Use of iron preparations in hemodialysis patients. *Neph Dialysis Trans 12* (334).

John, R.; Webb, M.; Young, A. and Stevens, P. E. (2004). Unreferred chronic kidney disease: a longitudinal study. *American Journal of Kidney Diseases 43*(5): 825-835.

Jones, K. B.; Anderson, D. W. and Longmore, G. D. (2005). Effects of recombinant hematopoietins on blood-loss anemia in mice. *The Iowa orthopaedic journal 25*(2005): 129.

Kalantar-Zadeh, K.; Kalantar-Zadeh, K. and Lee, G. H. (2006). 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 1*(Supplement 1): 9-18.

Kalender, B.; Mutlu, B.; Ersöz, M.; Kalkan, A. and Yilmaz, A. (2002). The effects of acute phase proteins on serum albumin, transferrin and haemoglobin in haemodialysis patients. *International journal of clinical practice 56*(7): 505-508.

Kaplan, L. and Kazmierczak, E. (2003). Clinical chemistry: theory, analysis, correlation. London, *Mosby*: 668-669.

Kasper, D. L.; Braunwald, E.; Fauci, A. S.; Hauser, S. L.; Jameson, J. L. and Longo, D. L. (2005). Harrisons' Principles of Internal Medicine. USA, *McGraw-Hill, Medical Publishing Division.* : 1644-1645.

Kasper, S.; Wald B; Fauci F; Longo H; J., J. and Harisons (2008). Principles of internal Medicine. . New York, USA, *MC Graw Hill Company*: 1549-1553.

Kato, H.; Ishida, J.; Imagawa, S.; Saito, T.; Suzuki, N.; Matsuoka, T.; Sugaya, T.; Tanimoto, K.; Yokoo, T. and Ohneda, O. (2005). Enhanced erythropoiesis mediated by activation of the renin-angiotensin system via angiotensin II type 1a receptor. *The FASEB journal 19*(14): 2023-2025.

Kato, S.; Chmielewski, M.; Honda, H.; Pecoits-Filho, R.; Matsuo, S.; Yuzawa, Y.; Tranaeus, A.; Stenvinkel, P. and Lindholm, B. (2008). Aspects of immune dysfunction in end-stage renal disease. *Clinical Journal of the American Society of Nephrology 3*(5): 1526-1533.

Kawakami, T.; Ren, S. and Duffield, J. S. (2013). Wnt signalling in kidney diseases: dual roles in renal injury and repair. *The Journal of pathology 229*(2): 221-231.

KDOQI, N. K. F. N. K. D. O. Q. I. (2007). KDOQI Clinical Practice Guideline and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease: 2007 Update of Hemoglobin Target. *American Journal of Kidney Diseases 50*(3): 471-530

Kellum, J. A. and Decker, J. M. (2001). Use of dopamine in acute renal failure: a meta-analysis. *Critical care medicine 29*(8): 1526-1531.

Kennedy, A.; Kohn, M.; Lammi, A. and Clarke, S. (2004). Iron status and haematological changes in adolescent female inpatients with anorexia nervosa. *Journal of paediatrics and child health 40*(8): 430-432.

Kes, P.; Basić-Jukić, N.; Ljutić, D. and Brunetta-Gavranić, B. (2011). The role of arterial hypertension in the development of chronic renal failure. *Acta Medica Croatica 65*(3): 78–84.

Khan, R. N.; Vohra, E. A. and Suleman, W. (2005). Factors determining outcome of acute renal failure patients. *Journal-Pakistan Medical Association 55*(12): 526.

Khanam, S.; Begum, N.; Begum, S. and Hoque, A. E. (2007). Changes in hematological indices in different stages of chronic renal failure. *Journal of Bangladesh Society of Physiologist 2*: 38-41.

Kilonzo, K. (2010). Chronic Renal Failure and Associated Risk Factors among Medical Admission at KCMC. M.Sc., Tumaini University.

Kinsey, G. R.; Li, L. and Okusa, M. D. (2008). Inflammation in acute kidney injury. *Nephron Experimental Nephrology 109*(4): e102-e107.

Knoll, G.; Cockfield, S.; Blydt-Hansen, T.; Baran, D. and Kiberd, B. (2005). Kidney Transplant Working Group of the Canadian Society of Transplantation. Canadian Society of Transplantation: consensus guidelines on eligibility for kidney transplantation. *CMAJ 173*(10): S1-25.

Kouegnigan, R. L.; Essola, R. L.; Mbourou, E. A.; Mouguiama, R. M.; Issanga, P. B.; Biyoghe, A. S.; Batchilili, B.; Akone, A. S. and Djoba, S. J. F. (2015). Blood Transfusion, Serum Ferritin, and Iron in Hemodialysis Patients in Africa. *Journal of blood transfusion 2015*(2015): 5.

Kumar, P. and Clark, M. (2002). Textbook of clinical medicine. *Pub: Saunders (London) 8*(54): 299-308.

Labbe, D.; Vassault, A.; Cherruau, B.; Baltassat, P.; Bonète, R.; Costantini, A.; Guerin, S.; Houot, O.; Lacour, B. and Nicolas, A. (1996). SFBC selected method for plasmatic creatinine measurement: validation and transferability. *Annales de Biologie Clinique 8*(54): 299-308.

Lafrance, J. P. and Miller, D. R. (2010). Acute kidney injury associates with increased long-term mortality. *J Am Soc Nephrol 21*(2): 345-352.

Lenga, I.; Lok, C.; Marticorena, R.; Hunter, J.; Dacouris, N. and Goldstein, M. (2007). Role of oral iron in the management of long-term hemodialysis patients. *Clinical Journal of the American Society of Nephrology 2*(4): 688-693.

Levey, A. S.; Coresh, J.; Balk, E.; Kausz, A. T.; Levin, A.; Steffes, M. W.; Hogg, R. J.; Perrone, R. D.; Lau, J. and Eknoyan, G. (2003). National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Annals of internal medicine 139*(2): 137-147.

Levin, A. (2002). The role of anaemia in the genesis of cardiac abnormalities in patients with chronic kidney disease. *Nephrology Dialysis Transplantation 17*(2): 207-210.

Levin, A. and Rocco, M. (2006). KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease-Foreword. *AMERICAN JOURNAL OF KIDNEY DISEASES 47*(5): 9-145.

Li, Z.; Lew, N. L.; Lazarus, J. M. and Lowrie, E. G. (2000). Comparing the urea reduction ratio and the urea product as outcome-based measures of hemodialysis dose. *American journal of kidney diseases 35*(4): 598-605.

Liano, F. and Pascual, J. (1996). the Madrid Acute Renal Failure Study Group: Epidemiology of acute renal failure: A prospective, multicenter, community-based study. *Kidney Int 50*(3): 811-818.

Liao, M.-T.; Sung, C.-C.; Hung, K.-C.; Wu, C.-C.; Lo, L. and Lu, K.-C. (2012). Insulin resistance in patients with chronic kidney disease. *BioMed Research International 2012*: 1-12.

Libório, A. B.; Abreu, K. L. S.; Silva Jr, G. B.; Lima, R. S.; Barreto, A. G.; Barbosa, O. A. and Daher, E. F. (2011). Predicting hospital mortality

in critically ill cancer patients according to acute kidney injury severity. *Oncology 80*(34): 160-166.

Lindeman, R. D.; Tobin, J. and Shock, N. W. (1985). Longitudinal studies on the rate of decline in renal function with age. *Journal of the American Geriatrics Society 33*(4): 278-285.

Loreal, O.; Haziza-Pigeon, C.; Troadec, M.-B.; Detivaud, L.; Turlin, B.; Courselaud, B.; Ilyin, G. and Brissot, P. (2005). Hepcidin in iron metabolism. *Current Protein and Peptide Science 6*(3): 279-291.

Łukaszyk, E.; Łukaszyk, M.; Koc-Żórawska, E.; Tobolczyk, J.; Bodzenta-Łukaszyk, A. and Małyszko, J. (2015). Iron status and inflammation in early stages of chronic kidney disease. *Kidney and Blood Pressure Research 40*(4): 366-373.

Macdougall, I. C. (2001). An overview of the efficacy and safety of novel erythropoiesis stimulating protein (NESP). *Nephrology Dialysis Transplantation 16*(suppl 3): 14-21.

Macdougall, I. C. (2002). Optimizing the use of erythropoietic agents–pharmacokinetic and pharmacodynamic considerations. *Nephrology Dialysis Transplantation 17*(suppl 5): 66-70.

Macdougall, I. C.; Bircher, A. J.; Eckardt, K.-U.; Obrador, G. T.; Pollock, C. A.; Stenvinkel, P.; Swinkels, D. W.; Wanner, C.; Weiss, G. and Chertow, G. M. (2016). Iron management in chronic kidney disease: conclusions from a "Kidney Disease: Improving Global Outcomes"(KDIGO) Controversies Conference. *Kidney international 89*(1): 28-39.

Macdougall, I. C. and Cooper, A. C. (2002). Erythropoietin resistance: the role of inflammation and pro‐inflammatory cytokines. *Nephrology Dialysis Transplantation 17*(suppl 11): 39-43.

Mader, S. (2004). Understanding human anatomy and physiology 5th ed. *McGraw-Hill Higher Education*: 227.

Mader, S. S. (2008). Human Biology,10th Ed.McGraw Hill, New York**:** 188.

Malyszko, J.; Malyszko, J. S.; Pawlak, K. and Mysliwiec, M. (2006). Hepcidin, iron status, and renal function in chronic renal failure, kidney transplantation, and hemodialysis. *American journal of hematology 81*(11): 832-837.

Manolis, A. S.; Tzeis, S.; Triantafyllou, K.; Michaelidis, J.; Pyrros, I.; Sakellaris, N.; Kranidis, A. and Melita, H. (2005). Erythropoietin in heart failure and other cardiovascular diseases: hematopoietic and pleiotropic effects. *Current Drug Targets-Cardiovascular & Hematological Disorders 5*(5): 355-375.

Mavromatidis, K.; Fytil, C.; Kynigopoulou, P.; Fragia, T. and Sombolos, K. (1998). Serum ferritin levels are increased in patients with acute renal failure. *Clin Nephrol 49*(5): 296-298.

McClellan, W.; Aronoff, S. L.; Bolton, W. K.; Hood, S.; Lorber, D. L.; Tang, K. L.; Tse, T. F.; Wasserman, B. and Leiserowitz, M. (2004). The prevalence of anemia in patients with chronic kidney disease. *Curr Med Res Opin 20*(9): 1501-1510.

McClellan, W. M. and Powe, N. R. (2009). Introduction to the Proceedings of a Centers for Disease Control and Prevention Expert Panel Workshop: Developing a comprehensive public health strategy for preventing the development, progression, and complications of CKD. *American Journal of Kidney Diseases 53*(3): S1-S3.

McGonigle, R. J.; Wallin, J. D.; Shadduck, R. K. and Fisher, J. W. (1984). Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney international 25*(2): 437-444.

McLaren, C.; Li, K.-T.; Gordeuk, V. R.; Hasselblad, V. and McLaren, G. D. (2001). Relationship between transferrin saturation and iron stores in the African American and US Caucasian populations: analysis of data

from the third National Health and Nutrition Examination Survey. *Blood 98*(8): 2345-2351.

Mehdi, W. A. (2011). Determination of resistin and several antioxidants in sera of patients with chronic renal failure. *Australian Journal of Basic and Applied Sciences 5*(8): 196-203.

Mendelssohn, D. C.; Barrett, B. J.; Brownscombe, L. M.; Ethier, J.; Greenberg, D. E.; Kanani, S. D.; Levin, A. and Toffelmire, E. B. (1999). Elevated levels of serum creatinine recommendations for management and referral. *Canadian Medical Association Journal 161*(4): 413-417.

Meyer, T. W. and Hostetter, T. H. (2007). Uremia. *New England Journal of Medicine 357*(13): 1316-1325.

Michael, I. O. and Gabreil, O. E. (2004). Chronic renal failure in children of benin, Nigeria. *Saudi Journal of Kidney Diseases and Transplantation 15*(1): 79.

Mishra, N. R.; Meher, A. K. and Behera, J. R. (2016). Spectrum of Acute Kidney Injury Under 14 Years of Age a Tertiary Care Experience. *Indian Journal of Research 5*(6).

Mohamed (2010). Evaluation of hemostatic mechanism in Sudanese renal failure patients attending Khartoum teaching hospital. M.Sc., SUST Repository.

Mohamed, R. J. (2016). The Effect of Acute Renal Failure on the Levels of Some Parameters. *Journal of Kerbala University 14*(1): 77-84.

Morel, O.; Muller, C.; Jesel, L.; Moulin, B. and Hannedouche, T. (2013). Impaired platelet P2Y12 inhibition by thienopyridines in chronic kidney disease: mechanisms, clinical relevance and pharmacological options. *Nephrology Dialysis Transplantation 28*(8): 1994-2002.

Morgan, E. H. (2002). Transferrin. Haeberli, A. eds. Human protein data 1992 VCH Weinheim Cited by: Lenivon Bonsdorff, Enni Lindeberg, Leila

Sahlstedt, Jari Lehto &Jaakko Parkkinen. Bleomycin detectable Iron Assay for Non-Transferrin bound Iron in Hematologic Malignancies. *Clinical Chemistry 48*(2): 307-314.

Nairz, M.; Theurl, I.; Wolf, D. and Weiss, G. (2016). Iron deficiency or anemia of inflammation? *Wiener Medizinische Wochenschrift 166*(13): 411-423.

Nakamura, M.; Seki, G.; Iwadoh, K.; Nakajima, I.; Fuchinoue, S.; Fujita, T. and Teraoka, S. (2012). Acute kidney injury as defined by the RIFLE criteria is a risk factor for kidney transplant graft failure. *Clinical transplantation 26*(4): 520-528.

National, C. C. f. C. C. (2006). Anaemia management in chronic kidney disease: national clinical guideline for management in adults and children:Royal College of Physicians, *Royal College of Physicians: 51-61*.

Nemeth, M. J. and Bodine, D. M. (2007). Regulation of hematopoiesis and the hematopoietic stem cell niche by Wnt signaling pathways. *Cell research 17*(9): 746-758.

Niemczyk, S.; Niemczyk, L. and Romejko-Ciepielewska, K. (2012). Basic endocrinological disorders in chronic renal failure. *Endokrynol Pol 63*(3): 250-257.

Nitescu, N. (2007). Studies on pathophysiological mechanisms in experimental models of acute renal failure. *Inst of Clincial Sciences. Dept of Anesthesiology & Intensive Care Medicine*: 1240-1247.

Nowrousian, M. R. (2007). Recombinant Human Erythropoietin (rhEPO) in clinical oncology: Scientific and clinical aspects of anemia in cancer. *Springer Science & Business Media*: 502.

Parmar, M. S. (2011). Acute Glomerulonephritis. *Medscape, Jun*: 1-6.

Pasricha, S.-R.; Flecknoe-Brown, S. C.; Allen, K. J.; Gibson, P. R.; McMahon, L. P.; Olynyk, J. K.; Roger, S. D.; Savoia, H. F.; Tampi,

R. and Thomson, A. R. (2010). Diagnosis and management of iron deficiency anaemia: a clinical update. *Med J Aust 193*(9): 525-532.

Perco, P.; Pleban, C.; Kainz, A.; Lukas, A.; Mayer, G.; Mayer, B. and Oberbauer, R. (2006). Protein biomarkers associated with acute renal failure and chronic kidney disease. *European journal of clinical investigation 36*(11): 753-763.

Pereira, R.; Costa, E.; Gonçalves, M.; Miranda, V.; Sameiro Faria, M. d.; Quintanilha, A.; Belo, L.; Lima, M. and Santos Silva, A. (2010). Neutrophil and monocyte activation in chronic kidney disease patients under hemodialysis and its relationship with resistance to recombinant human erythropoietin and to the hemodialysis procedure. *Hemodialysis International 14*(3): 295-301.

Peter, W. S. (2007). Introduction: chronic kidney disease: a burgeoning health epidemic. *Journal of Managed Care Pharmacy 13*(9 Supp D): 2-5.

Picken, M. M. and Herrera, G. A. (2007). The burden of "sticky" amyloid: typing challenges. *Archives of pathology & laboratory medicine 131*(6): 850-851.

Ponka, P.; Beaumont, C. and Richardson, D. R. (1998). Function and regulation of transferrin and ferritin;. Seminars in hematology; 35(1) p. 35-54. *35***:** 35-54.

Post, J. B.; Wilkes, B. M. and Michelis, M. F. (2006). Iron deficiency in patients with chronic kidney disease: potential role for intravenous iron therapy independent of erythropoietin. *Int Urol Nephrol 38*(3-4): 719-723.

Prasad, N.; Barai, S.; Gambhir, S.; Parasar, D.; Ora, M.; Gupta, A. and Sharma, R. (2012). Comparison of glomerular filtration rate estimated by plasma clearance method with modification of diet in renal disease prediction equation and Gates method. *Indian journal of nephrology 22*(2): 103.

Radtke, H. W.; Frei, U.; Erbes, P. M.; Schoeppe, W. and Koch, K. M. (1980). Improving anemia by hemodialysis: Effect on serum erythropoietin. *Kidney international 17*(3): 382-387.

Rama, I. and Grinyó, J. M. (2010). Malignancy after renal transplantation: the role of immunosuppression. *Nature Reviews Nephrology 6*(9): 511-519.

Ramachandran, A.; Snehalatha, C. and Viswanathan, V. (2002). Burden of type 2 diabetes and its complications-the Indian scenario. *Current Science Bangalore 83*(12): 1471-1476.

Ramakrishnan, K. and Scheid, D. C. (2005). Diagnosis and management of acute pyelonephritis in adults. *Am Fam Physician 71*(5): 933-942.

Raptis, V.; Kapoulas, S. and Grekas, D. (2013). Role of asymmetrical dimethylarginine in the progression of renal disease. *Nephrology 18*(1): 11-21.

Rees, L. and Shaw, V. (2007). Nutrition in children with CRF and on dialysis. *Pediatric Nephrology 22*(10): 1689-1702.

Remuzzi, G. and Minetti, L. (2000). Hematologic consequences of renal failure. *Brenner and Rector's The Kidney 2*: 2079-2102.

Ren, X.; Meng, Z.; Liu, M.; Zhu, M.; He, Q.; Zhang, Q.; Liu, L.; Song, K.; Jia, Q. and Jia, Q. (2016). No associations exist between mean platelet volume or platelet distribution width and thyroid function in Chinese. *Medicine 95*(40): 1-38.

Riaz, S.; Alam, S. S.; Raza, M.; Hasnain, S. and Akhtar, M. W. (2009). Obesity as risk factor and study of obesity related proteins in diabetes mellitus. *African Journal of Biotechnology 8*(5): 737-744.

Robles, N. R.; Angulo, E.; Grois, J. and Barquero, A. (2009). Comparative effects of fosinopril and irbesartan on hematopoiesis in essential hypertensives. *Renal failure 26*(4): 399-404.

Rod , R. S.; Trent , D. S.; Philip, T. and Philip, t. (2004). Anatomy and physiology. New York *Hill compopanies*: 947-948.

Rosner, M. H. (2005). Hemodialysis for the non-nephrologist. *Southern medical journal 98*(8): 785-792.

Ruiz-Irastorza, G.; Ramos-Casals, M.; Brito-Zeron, P. and Khamashta, M. A. (2010). Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Annals of the rheumatic diseases 69*(1): 8-20.

Schaalan, M. F. and Mohamed, W. A. (2016). Determinants of hepcidin levels in sepsis-associated acute kidney injury: Impact on pAKT/PTEN pathways. *Journal of immunotoxicology 13*(5): 751-757.

Searcy, R.; Reardon, J. and Foreman, J. (1967). A new photometric method for serum urea nitrogen determination. *The American journal of medical technology 33*(1): 15.

Smith, R. E. J. (2010). The Clinical and Economic Burden of Anemia. *The American Journal of Managed Care 16*(3): 59-66.

Snyder, S. and Pendergraph, B. (2005). Detection and evaluation of chronic kidney disease. *Interventions 100*(1): 24-25.

Somvanshi, S.; Khan, N. Z. and Ahmad, M. (2012). Anemia in chronic kidney disease patients. *Clinical Queries: Nephrology 1*(3): 198-204.

Srinivasan, R.; Fredy, I. C.; Chandrashekar, S.; Saravanan, J.; Mohanta, G. P. and Manna, P. K. (2016). Assessment of erythropoietin for treatment of anemia in chronic kidney failure- ESRD patients. *Biomedicine & Pharmacotherapy 82*: 44-48.

Srisawat, N.; Hoste, E. E. and Kellum, J. A. (2010). Modern classification of acute kidney injury. *Blood purification 29*(3): 300-307.

Stamatelou, K. K.; Francis, M. E.; Jones, C. A.; Nyberg, L. M. and Curhan, G. C. (2003). Time trends in reported prevalence of kidney stones in the United States: 1976–19941. *Kidney international 63*(5): 1817-1823.

Starr, R. (1980). Use of an alumina column in estimating total iron-binding capacity. *Clinical chemistry 26*(1): 156-158.

Stasi, R.; Abriani, L.; Beccaglia, P.; Terzoli, E. and Amadori, S. (2002). Anemia induced by solid tumor chemotherapy. *Recenti progressi in medicina 93*(11): 585-601.

Stauffer, M. E. and Fan, T. (2014). Prevalence of anemia in chronic kidney disease in the United States. *PLoS One 9*(1): 2-5.

Su, B. Y.-J.; Su, C.-Y.; Yu, S.-F. and Chen, C.-J. (2007). Incidental discovery of high systemic lupus erythematosus disease activity associated with cytomegalovirus viral activity. *Medical microbiology and immunology 196*(3): 165-170.

Suega, K.; Bakta, M.; Dharmayudha, T. G.; Lukman, J. S. and Suwitra, K. (2005). Profile of anemia in chronic renal failure patients: comparison between predialyzed and dialyzed patients at the Division of Nephrology, Department of Internal Medicine, Sanglah Hospital, Denpasar, Bali, Indonesia. *Acta Med Indones-Indones J Intern Med 37*(4): 190-193.

Sunita, G.; Arvind, A. K. and Pravin, S. P. A. (2014). Study of Haematological Changes in Chronic Renal Failure. *Sch. J. App. Med. Sci. 2(4A)*(2): 1232-1234.

Suresh, M.; Mallikarjuna, N.; M, S. B. S.; Bandi, H. K. and Shravya, G. (2012). Hematological Changes in Chronic Renal Failure. *International Journal of Scientific and Research Publications 2*(9): 1-4.

Talwar, V. and Gupta, H. (2002). Clinicohaematological profile in chronic renal failure. *The Journal of the Association of Physicians of India 50*: 228-233.

The Republic of Iraqi Ministry of Health Environment (2016). Annual Statistical Report Nouri , T.; Shaker, K.; Hussein, A.; Abdul Karim, R. and Safitan, A. Library and Archives Baghdad, Baghdad, Iraq**:** 1-280.

Theil, E. C. (1987). Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. *Annual review of biochemistry 56*(1): 289-315.

Thevis, M.; Loo, R. R. O. and Loo, J. A. (2003). Mass spectrometric characterization of transferrins and their fragments derived by reduction of disulfide bonds. *Journal of the American Society for Mass Spectrometry 14*(6): 635-647.

Tietz, N. W. (1995). Clinical guide to laboratory tests. *WB Saunders Co*.

Torti, F. M. and Torti, S. V. (2002). Regulation of ferritin genes and protein. *Blood 99*(10): 3505-3516.

Tsirpanlis, G.; Bagos, P.; Ioannou, D.; Bleta, A.; Marinou, I.; Lagouranis, A.; Chatzipanagiotou, S. and Nicolaou, C. (2004). Exploring inflammation in hemodialysis patients: persistent and superimposed inflammation. *Kidney and Blood Pressure Research 27*(2): 63-70.

Van der Meijden, K.; Bakker, A.; van Essen, H.; Heijboer, A.; Schulten, E.; Lips, P. and Bravenboer, N. (2016). Mechanical loading and the synthesis of 1, 25 (OH) 2 D in primary human osteoblasts. *The Journal of steroid biochemistry and molecular biology 156*: 32-39.

Van der Walt, I.; Swanepoel, C.; Mahala, B. and Meyers, A. (2015). Important complications of chronic kidney disease. *SAMJ: South African Medical Journal 105*(4): 321-321.

Vupputuri, S.; Soucie, J. M.; McClellan, W. and Sandler, D. P. (2004). History of kidney stones as a possible risk factor for chronic kidney disease. *Annals of epidemiology 14*(3): 222-228.

Walker, B. R. and Colledge, N. R. (2013). Davidson's principles and practice of medicine. New York Oxford Philadelphia , London New, *Elsevier Health Sciences*: 467.

Wastiet , A.; Sumaira Iqbal; Fatima, N. and Haider, a. S. (2013). Hematological disturbances associated with chronic Kidney Disease and

kidney transplant patients *International Journal of Advanced Research 1*(10): 48-54.

Williams, J.; Topley, N.; Craig, K.; Mackenzie, R.; Pischetsrieder, M.; Lage, C. and Passlick-Deetjen, J. (2004). The Euro-Balance Trial: the effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. *Kidney Int 66*: 408– 418.

Wilson, J.; Yao, G.; Raftery, J.; Bohlius, J.; Brunskill, S.; Sandercock, J.; Bayliss, S.; Moss, P.; Stanworth, S. and Hyde, C. (2007). A systematic review and economic evaluation of epoetin alfa, epoetin beta and darbepoetin alfa in anaemia associated with cancer, especially that attributable to cancer treatment. *NIHR Journals Library 11*(13): 1-4.

Worwood, M. (2006). Chapter 7 - Iron deficiency anaemia and iron overload A2 - Lewis, S. Mitchell. Dacie and Lewis Practical Haematology (Tenth Edition). Bain, B. J. and Bates, I. Philadelphia, *Churchill Livingstone***:** 131-160.

Wu, C. J.; Bao, M. L.; Wang, Q.; Wang, X. N.; Liu, X. S.; Shi, H. B. and Zhang, Y. D. (2017). Acute kidney damage induced by low and iso osmolar contrast media in rats: Comparison study with physiologic MRI and histologic gene examination. *Journal of Magnetic Resonance Imaging 45*(1): 291-302.

Yamanishi, H.; Iyama, S.; Yamaguchi, Y.; Kanakura, Y. and Iwatani, Y. (2003). Total iron-binding capacity calculated from serum transferrin concentration or serum iron concentration and unsaturated iron-binding capacity. *Clinical chemistry 49*(1): 175-178.

Yamashita, T.; Noiri, E.; Hamasaki, Y.; Matsubara, T.; Ishii, T.; Yahagi, N.; Nangaku, M. and Doi, K. (2016). Erythropoietin concentration in acute kidney injury is associated with insulin-like growth factor-binding protein-1. *Nephrology (Carlton) 21*(8): 693-699.

Zhang, H.; Ho, Y. F.; Che, C. T.; Lin, Z. X.; Leung, C. and Chan, L. S. (2012). Topical herbal application as an adjuvant treatment for chronic kidney disease–a systematic review of randomized controlled clinical trials. *Journal of advanced nursing 68*(8): 1679-1691.

Zhang, J.; Johnson, P. C. and Popel, A. S. (2008). Red blood cell aggregation and dissociation in shear flows simulated by lattice Boltzmann method. *Journal of biomechanics 41*(1): 47-55.

الخلاصة

يعاني المرضى المصابون بالعجز الكلوي بنوعيه الحاد AKI والمزمن CKD من عدد كبير من المضاعفات ومن أهم هذه المضاعفات فقر الدم الذي قد يكون شديدا ، و يتطلب عملية نقل دم للمريض تعرضه للعديد من المخاطر بما تحمله هذه العملية من مشاكل كثيرة.

لذا هدفت الدراسة الحالية الى تقيم مؤشرات الدم لدى مرضى الفشل الكلوي مثل كريات الدم الحمر (Red Blood corpuscular) ، مستوى خضاب الدم (Hemoglobin) ، حجم مكداس الدم (Packed Cell Volumes)، الصفيحات الدموية(Platelets) والعدد الكلي لخلايا الدم البيض (White blood cells). بالاضافة الى هرمون الارثروبويتين (Erythropoietin). كما تم قياس كل دلائل حالة الحديد وتضمنت الحديد الكلي في المصل(Serum Iron)، سعة ارتباط الحديد الكلي (Total Iron Binding Capacity) ، سعة ارتباط الحديد غير المشبَع (Unsaturation of Iron Binding Capacity)، مستوى الترانسفرين Transferring concentration، نسبة تشبع الترانسفرين (Transferring Saturation%) ، و بروتين الفرتين (Ferritin) وخزين الحديد الكلي المقدر . (Estimation of Total Iron Body Store) ومن ثم المقارنة بين نوعي الفشل الكلوي مع مجموعة السيطرة.

وقد أجريت هذه الدراسة على المرضى المراجعين لقسم الكلية الاصطناعية (الديلزة الدموية) في مستشفى مرجان التعليمي/ مديرية صحة بابل – العراق، للمدة من تشرين الثاني 2015 الى أيار 2016. تم اجراء الجزء العملي في مختبرات مستشفى مرجان التعليمي وفي قسم علوم الحياة– كلية العلوم/ جامعة كربلاء.

تم جمع العينات بصورة عشوائية من المرضى قبل الديلزه . اضافة الى جمع عينات السيطرة و تراوحت اعمار المجموعتين من المرضى والاصحاء بين (15 –77) وضمَت الدراسة 88 شخصا من كلا الجنسين ، قسموا إلى ثلاث مجاميع : المجموعة الأولى : ضمَت 37 مريضاً بالعجز الكلوي المزمن و المجموعة الثانية : شملت 13 مريضاً بالعجز الكلوي الحاد ، فضلا عن مجموعة السيطرة التي ضمَت 38 شخصا اصحاء . وقورنت المجاميع بشكل عام وكذلك حسب الجنس والعمر ومدة المرض.

كشفت نتائج التحليل الإحصائي وجود انخفاض معنوي ($p\leq0.01$) في معدل تعداد كريات الدم الحمر RBC ، معدل مستويات خضاب الدمHb و معدل حجم مكداس الدم PCV لدى مرضى الفشل الكلوي الحاد والمزمن عند مقارنتها مع مجموعة السيطرة ، وجد أن هناك ارتفاع معنويا ($p\leq0.01$) عند تقدير معدل مستويات هرمون الارثروبويتين EPO عند مقارنتها مع مجموعة السيطرة . في حين أظهرت النتائج الى وجود انخفاض معنوي في معدل تعداد الصفيحات الدموية PLTs لدى مرضى الفشل الكلوي المزمن عند مقارنتها مع مجموعة السيطرة ($p\leq0.05$) .

كذلك بينت النتائج حصول انخفاض معنوي($p\leq0.05$) في دلائل كريات الدم الحمر المتمثلة بمعدل حجم الخلية(MCV) لدى مرضى الفشل الكلوي الحاد مقارنة بالسيطرة. في حين أظهرت نتائج الدراسة الحالية إلى وجود ارتفاع معنوي ($p\leq0.05$) في معدل وزن خضاب الدم (MCH) لدى مرضى الفشل الكلوي المزمن مقارنة بالسيطرة . بينما ارتفع معدل مستويات خضاب الدم داخل الكرية (MCHC) لدى مرضى الفشل الكلوي الحاد والمزمن عند مقارنتها مع مجموعة السيطرة .

وقد أظهرت نتائج الفحوص حالة الحديد لعينات الدم إلى وجود انخفاض معنوي ($p\leq0.01$) في معدل مستويات الحديد Iron، معدل سعة ارتباط الحديد الكلي TIBC، معدل سعة ارتباط الحديد غير المشبَع UIBC و معدل مستويات بروتين الترانسفرين Trf في مصل مرضى العجز الكلوي الحاد والمزمن عند مقارنتها مع مجموعة السيطرة . بينما انخفض معدل مستويات النسبة المئوية للترانسفرين TSAT% لدى مرضى العجز الكلوي الحاد عند مقارنتها بالسيطرة.في حين أظهرت النتائج وجود ارتفاع معنوي ($p\leq0.05$) في معدل مستويات الفرتين (ferritin) وخزين الحديد الكلي المقدر ETIB لدى مرضى العجز الكلوي المزمن عند مقارنتها بالسيطرة.

كما بينت النتائج الحالية إن معدل سعة ارتباط الحديد(TIBC) ارتفاعاً معنويا ($P\leq0.01$) في مصل الإناث بالمقارنة مع الذكور لدى مرضى الفشل الكلوي، وقد لوحظ أيضا وجود زيادة معنوية ($P\leq0.05$) في معدل سعة ارتباط الحديد(TIBC) في الفئة العمرية ، في حين سجلت أعلى زيادة في معدل مستويات الفرتين (ferritin)في الفئة العمرية 46 سنة فما فوق مقارنة بالفئات العمرية الأخرى ($P\leq0.01$).

أشارت نتائج الدراسة الحالية إلى عدم وجود علاقة ارتباطيه بين مستويات هرمون الارثروبويتين EPO وتعداد كريات الدم الحمر RBC ، ومستويات خضاب الدمHb ، حجم مكداس الدمPCV ، تعداد الصفيحات الدموية PLTs ،الفرتين Ferritin ، مستويات اليوريا Urea والكرياتينين Creatnine لدى مرضى الفشل الكلوي الحاد AKI. بينما لوحظ وجود علاقة ارتباطيه بين هرمون الارثروبويتينEPO وتعداد خلايا الدم الحمر RBC، خضاب الدم Hb، تعداد الصفيحات الدموية PLTs، الحديد Iron والفرتين Ferritin لدى مرضى الفشل الكلوي المزمن CKD. ولوحظ أيضا عدم وجود علاقة ارتباطيه بين هرمون الارثروبويتين EPO واحجام مكداس الدم PCV ، الصفيحات الدموية PLTs ونسب تشبع للترانسفرين TSAT% لدى مرضى الفشل الكلوي المزمن CKD .

نستنتج من هذه الدراسة إلى أن المعايير الدموية تتأثر لدى مرضى الفشل الكلوي الحادAKI والمزمنCKD، ومؤشرات الدم هي الاكثر تاثراً. وأظهرت هذه الدراسة أن فقر الدم منتشر بين مرضى الفشل الكلوي الحاد والمزمن بنسبة96%.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية العلوم

قسم علوم الحياة

مؤشرات الدم و حالة الحديد في مرضى الفشل الكلوي في محافظة بابل

رسالة مقدمة الى

مجلس كلية العلوم / جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في العلوم / علوم الحياة.

من

حسين حازم مدب الغانمي

بأشراف

ا.م.د.وفاء صادق الوزني

ا.م.د.نجلاء بدر العوادي

1438هجـ

2017م