بسم الله الرحمن الرحيم

**Sudan University for Science and Technology**

**College of Graduate Studies**

# Study of Complete Blood Count and Iron Profile among Active Pulmonary Tuberculosis Patients, Khartoum State, 2017

# دراسة قياسات الدم الكامل وقياسات الحديد وسط المرضي المصابين بالسل الرئوى النشط ، ولاية الخرطوم ، 2017

*A dissertation submitted in partial fulfillment of the requirements of the M.Sc. Degree in Hematology and Immunohematology*

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# الاية

قال تعالي :

بسم الله الرحمن الرحيم

﴿ سَلَامٌ قَوْلًا مِن رَّبٍّ رَّحِيمٍ ﴾

صدق الله العظيم

سورة يس الاية (58)

# Dedications

I dedicate this research

To my parents.

To my sisters and my brothers.

To all of my teachers.

To my friends

To my faculty members in the hematology laboratory

To everyone who wish the best for me.

## Acknowledgements

Thanks at first and last for the light of our life our God Allah who gave us the strength and good health while doing this project and guided us through the way in this life and for Prophet Mohammed the prayer and peace from Allah to him.

Thanks extend my sincere: **Prof Babiker Ahmed Mohammed** who is credited with presenting this study in this way by his care and guidance.

Thanks to my friends and to any person who help my in this research study

# Abstract

The pulmonary tuberculosis is infectious and chronic disease; increase morbidity and mortality due to morbid condition develop during the stage of disease like anemia and Pancytopenia.

This was a case control study which aimed to study the CBC parameters and iron profile in active pulmonary tuberculosis patients to detect the effect on CBC and iron profile (S. iron, S. ferritin, TBIC, saturation %) and to detect frequency and the type of anemia in these patients.

One hundred twenty subjects were recruited for this study, 60 patients with active pulmonary tuberculosis patients and 60 healthy volunteers as a control group, blood samples were collected from all participants in EDTA and plain vacontainers tubes.

Patients' data were collected by structured interview questionnaire and analyzed by statistical package for social sciences (SPSS), version 11.5.

Complete blood count parameters were measured by automated hematology analyzer (Sysmex KX 21N) and iron profile was measured by spectrophotometer.

There was statistically significant decrease in hemoglobin concentration, MCV, MCH (P. value: *0.002, 0.009, 0.001*respectively) and no statistically significant change in PCV, RBCS count and MCHC. (P.value:*0.818, 0.409, 0.486* respectively) with statistically significant increased in RDW-SD, RDW-CV. (P. value: *0.001, 0.000* respectively) in active pulmonary tuberculosis patients when compared with healthy control.

There was statistically significant increased in WBCs count and absolute monocyte count (P. value: *0.009, 0.000* respectively) with no statistically significant difference in absolute neutrophil count, absolute lymphocyte count, absolute eosinophil count and absolute basophil count (P. value: *0.278, 0.177,0.160, 0.321*respectively) when compared with healthy control.

There was statistically significant increased platelet count (P. value: *0.009*) with statistically insignificant change in MPV, PDW, and P-LCR (P. value*: 0.068, 0.028, 0.055*respectively), when compared with healthy control.

There was statistically significant decrease in serum iron and TBIC (P. value*: 0.000, 0.000* respectively) and statistically significant increase in serum ferritin (P. value*: 0.000*) with statistically insignificant change in saturation% (P.value*:0.777*) when compared with healthy control.

There was statistically significant positive correlation between hemoglobin concentration and serum iron (P.value*:0.000*) and between hemoglobin concentration and saturation %,( P.value:0.002) with no correlation between hemoglobin concentration and serum ferritin(P.value*:0.212*)and between hemoglobin concentration and TBIC(P.value*:0.035*)in active pulmonary tuberculosis patients.

The frequency of anemia in active pulmonary tuberculosis is (48.3%) and the types of anemia are ACD (45%), IDA (3.3%) and the most types of anemia microcytic hypochromic anemia, secondly normocytic normochromic anemia.

# الخلاصة


يعتبرمرض السل الرئوى من الامراض المعدية والمزمنة التي تزيد من معدل الوفيات لاسباب عديدة منها فقر الدم المزمن و قلة الكريات الشامل.

هدفت دراسه الحالات والشواهد لقياس تعداد الدم الكامل وقياسات الحديد في مرضي السل الرئوي النشط.

شملت الدراسة 120 فرد 60 شخص مصابين بمرض السل الرئوى النشط و60 اصحاء كمجموعه ضابطة اخذت عينات الدم فى وعاء EDTA ووعاء plain.

بينات المريض اخذت عن طريق استبيان منظم وحللت عن طريق استخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية (11.5).

تم قياس تعداد الدم الكامل بواسطة جهاز(Sysmex KX 21N)وتم قياس قياسات الحديد بالمقياس الطيفي(Spectrophotometer).

اظهرت نتائج الدراسة نقصان ذو دلاله احصائية فى متوسط تركيزالهيموقلوبين (Hb) و حجم الكرية الوسطي(MCV) وهيموقلوبين الكرية الوسطي(MCH )وقيمة pالمطلقة:( *0.002* ، *0.009* ، *0.001* تباعا) ولا يوجد فرق ذو دلالة احصائية فى فى تعداد خلايا الدم الحمراء (RBCs)، حجم الكريات المكدوسة(PCV) والتركيزالوسطي لهيموقلوبين الكرية (MCHC) وقيمة P المطلقة: ( 0.409 ، 0.818 ،0.486 تباعا) مع زيادة ذات دلالة احصائية فى كل من معدل توزع خلايا الدم الحمراء .(RDW- CV) , (RDW-SD)وقيمة P المطلقة: (*0.001*،*0.000* تباعا) مقارنة مع المجموعة الضابطة.

اظهرت النتائج زيادة ذات دلالة احصائية في تعداد خلايا الدم البيضاء و التعداد المطلق لخلية الدم (Monocyte)وقيمة P المطلقة (*0.009*، *0.000 تباعا*) واظهرت النتائج لا فرق ذو دلالة احصائية في التعداد المطلق لكل من خلايا الدم Neutrophil Eosinophil, Lymphocyte ,Basophil, قيمهP المطلقة (*0.278*، *0.177*،*0.160*، *0.321* تباعا) مقارنة مع المجموعة الضابطة.

كما اظهرت النتائج زياده ذات دلالة احصائية في تعداد الصفائح الدموية (Platelet count)وقيمة P المطلقة (*0.009*) ولا فرق ذو دلاله احصائية فى كل من متوسط الصفيحة الدموية (MPV) ومتوسط

توزع الصفيحة الدموية (PDW) ونسبه الصفيحة الدموية الكبيرة بالنسبة لتعداد الصفائح الدموية ( P- LCR)وقيمة P المطلقة (*0.068*،*0.028*،*0.055* تباعا)،مقارنة مع المجموعة الضابطة.

اظهرت نتائج قياسات الحديد فى مصل الدم لاشخاص المصابين بالسل الرئوي النشط نقصان ذودلالة احصائية في كمية الحديد( S.iron) و سعة ارتباط الحديد الكلية (TIBC) وقيمة P المطلقة *0.000* ( ، *0.000*) كما اظهرت النتائج ذيادة ذو دلالة احصائية في مخزن الحديد(Ferritin) وقيمة P المطلقة (*0.000*) ولا يوجد فرق ذو دلالة احصائية في معدل تشبع الحديد (Saturation%) وقيمةP المطلقة (*0.777*). مقارنة مع المجموعة الضابطة.

اظهرت النتائج علاقة ذات دلالة احصائية موجبة بين تركيزالهيموقلوبين وكميه الحديد في مصل الدم و تركيز الهيموقلوبين ومعدل تشبع الحديد وقيمة P المطلقة( 0.000، *0.002* تباعا) ولا توجد علاقة ذات دلالة احصائية بين تركيز الهيموقلوبين ومخزن الحديد (Ferritin) و بين تركيز الهيموقلوبين و سعة ارتباط الحديد الكلية وقيمةP المطلقه(*0.212* ، *0.035* تباعا) مقارنة مع المجموعة الضابطة.

خلصت الدراسة الي ان انواع فقر الدم في الاشخاص المصابين بالسل الرئوي النشط : انيميا نقص الحديد(3.3%) وانيميا الامراض المزمنة (45%).

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# List of Abbreviation

| | |
|---|---|
| ACD | anemia of chronic disease |
| ALA | Amino laevulinic Acid |
| ANOVA | Analysis of Variance |
| AFB | Acid fast bacillary |
| BCG | BacilleCalmette-Guérin |
| CBC | Complete blood count |
| CFU-G | Colony formed unit granulocyte |
| CMML | Chronic myelomonocytic leukemia |
| DMT1 | divalentmetal transporter 1 |
| EEA1 | Early EndosomalAuto Antigen 1 |
| EDTA | Ethylene diamine tetra acetic acid |
| EPO | Erythropoietin |
| G-CSF | Granulocyte colony-stimulating factor |
| GM-CSF | Granulocyte/macrophage colony-stimulating factor |
| HSCs | Hematopoietic stem cells |
| HIV | Human immune deficiency virus |
| IDA | Iron deficiency anemia |
| IL-1 | Interlukin-1 |
| IL-6 | Interlukin-6 |
| INF-γ | Interferon - γ |
| MCH | mean cell hemoglobin |
| MCHC | Mean cell hemoglobin concentration |

| | |
|---|---|
| MCV | Mean cell volume |
| MPV | Mean platelet volume |
| PDW | Platelet distribution width |
| PTB | Pulmonary tuberculosis |
| PCV | Packed Cell Volume |
| PPD | Preservative protein derivates |
| RBCs | Red Blood Cell |
| RDW CV | Red cell distribution width coefficient variation |
| RDWSD | Red cell distribution width stander deviation |
| TB | Tuberculosis |
| TIBC | Total iron biding capacity |
| TNF-α | tumor necrosis factorα |
| WBCs | White blood cell |
| WHO | World Health Organization |
| XDR-TB | Extensively drug-resistant TB |

# Chapter one

## Introduction and Literature review

# Chapter one

# Introduction and Literature Review

## 1 Introduction

Anemia is still a major public health problem in many developing countries. The World Health Organization (WHO) Global Database on anemia for 1993-2005 showed that 25% or 1.62 billion people globally suffer from anemia. (WHO, 2008).

It is a condition characterized by lack of blood or in other word a reduction of total quantity of erythrocyte (red blood cells, RBC) or hemoglobin in the circulation which are necessary for normal function; this is caused by the inability of the bone marrow to replace the erythrocyte lost. (Haut, 2007; Blaser, 2001; Olde, 2005).

Anaemia is a common complication of pulmonary tuberculosis, the precise mechanism of anaemia in pulmonary tuberculosis is not clearly known but anaemia due to inflammation as well as of iron deficiency has been implicated, both are common in developing countries (Espinal *et al*., 2001).

Nutritional deficiency and mal absorption syndrome can deepen the severity of anaemia. However, the observation that patients with tuberculosis-associated anemia display an absence of bone marrow iron, suggests that iron deficiency as a possible cause of anaemia in patients with tuberculosis (Gladwion and Trattler, 2007).

The prevalence of anemia among TB patients ranges between 30 – 94%. (Gladwion and Trattler, 2007; Isanaka et al., 2012).

The increasing prevalence of anemia with age has been explained by increased chronic disease, poor nutritional status, decrease marrow cellularity, and low serum B12 level; therefore, old age could be considered as a risk factor for tuberculosis associated anemia. (Gladwion and Trattler, 2007).

On the other hand, a disturbance of iron homeostasis develops with increased Uptake and retention of iron within the reticuloendothelial system in chronic Infections such as tuberculosis because iron is an important growth factor of Mycobacterium tuberculosis. The iron retention in reticuloendothelial system is considered as one of the host defense mechanisms and many therapeutic trials are performed. Its affect of iron retention might be exaggeration, in women with tuberculosis because women are more likely than men to be iron deficient. This can explained female sex is a risk factor of anemia. (Gladwion and Trattler, 2007).

On the other hand iron deficiency can increase susceptibility to various infectious diseases, since macrophage require iron to function well (Schaible and Kaufmann, 2004).

Even mild iron deficiency cause a significant impaired in the immune status and reduces the capacity to control infections. (Dijkhcizen *et al.,* 2001).

**1.1 Hematopoiesis**

Is the process of blood cell production, differentiation, and development, the hematopoietic system consists of the bone marrow, liver, spleen, lymph nodes and thymus. **(**Mary and Turgeon, 2012).

**1.1.1 Stromal cells**

Growth and differentiation of hematopoietic cells in the bone marrow is regulated by the extracellular matrix and microenvironment provided by Stromal cells. These cells, including macrophages, fibroblasts in various stages of differentiation, endothelial cells, fat cells, and reticulum cells, mature hematopoietic stem cells and progenitor cells by producing growth factors like granulocyte/macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, or stem cell factor.( Munker *et al*., 2007).

### 1.1.2 Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotential stem cell that can give rise to the separate cell lineages; cell differentiation occurs from the stem cell via the committed haemopoietic progenitors' which are restricted in their developmental potential. (Hoffbrand *et al*., 2006).

### 1.1.3 Myelopoiesis

Under the influence of cytokines such as G-CSF, a myeloid progenitor cell, CFU-G, is formed. This cell then differentiates into the morphologically recognizable myeloid precursors: myeloblasts, promyelocytes, myelocytes, and metamyelocytes.

**1.1.3.1Myeloblasts:**

Are large cells (12–20 μm in diameter) and have a large nucleus with fine chromatin and several nucleoli, No cytoplasmic granules are present, up to 5% of myeloblasts present in normal marrow . (Munker *et al.*, 2007).

**1.1.3.2 Promyelocytes**

Are slightly larger neutrophilic precursors with granules in their cytoplasm.

**1.1.3.3 Myelocytes**

Which have smaller granules (secondary or specific granules), at this stage, a differentiation of the myelocytes into the neutrophil, eosinophil, and basophil series can be recognized. (Munker *et al.*, 2007).

**1.1.3.4 Metamyelocytes**

These cells can no longer divide and have a somewhat indented nucleus and numerous granules in their cytoplasm. (Munker *et al.*, 2007).

**1.1.3.5 Band form**

Between the mature neutrophil and the metamyelocyte, so called stab, or band forms are observed in which the nucleus is not yet fully segmented, occur normally in the peripheral blood (less than 8% of circulating neutrophils) and

are increased under hematopoietic stress, such as during infections. (Munker *et al.,* 2007).

**1.1.3.6 Leucocyte**

White blood cell, a term which includes all the mature peripheral blood cells of granulocyte, monocyte or lymphocyte lineage: neutrophils, eosinophils, basophils, lymphocytes and monocytes. (Kem, 2002).

**1.1.3.6.1Neutrophil**

Neutrophils are the most common type of WBCs in adults. Segmented neutrophils called polymorphonuclear neutrophil Leukocytes [PMNs or "polys"] have a nucleus divided into multiple distinct lobes connected by thin strands of chromatin, the cytoplasm has fine granules that stain lightly with the usual blood stains. Polys normally comprise ~50 to 70% of total WBCs, the primary function of neutrophils is phagocytosis, predominantly of bacteria; so they are the primary defense against bacterial infection, bacteria are killed by antimicrobial agents contained or generated within neutrophil granules, neutrophils circulates in the blood for ~10 hours and may live 1 to 4 days in the extravascular space. (Kem, 2002).

**1.1.3.6.2 Eosinophils**

They are about 12–17 μm in diameter. They usually have two nuclear lobes or segments, and the cytoplasm is packed with distinctive spherical gold/orange (eosinophilic) granules the underlying cytoplasm, which is usually is pale blue, prolonged steroid administration causes eosinopenia. Moderate eosinophilia occurs in allergic conditions; more severe eosinophilia (20–50× $10^9$ /l_) as well as parasitic infections and even greater numbers may be seen in other eosinophilic leukemia and the idiopathic hypereosinophilic syndrome. Reactive eosinophilia with very high counts may be seen in T-cell lymphoma, B-cell lymphoma and acute lymphoblastic leukemia. (Bain and Lewis *et al*., 2011).

**1.1.3.6.3 Basophils**

The earliest stage at which they can be identified is the promyelocytes stage, at which large, black–violet stained granules are visible, close relations of basophilic, granulocytes are tissue basophils or tissue mast cells, tissue basophils have a round nucleus underneath large basophilic granules. Basophils play role in anaphylactic reactions, elevated basophil counts are seen above all in hypersensitivity reactions of various kinds. Basophils are also increased in chronic myeloproliferative bone marrow diseases, especially chronic myeloid leukemia (Theml *et al*., 2004).

**1.1.3.6.4 Monocytes**

The monocyte is an exceptionally pleomorphic blood cell ranging from 12 - 20 um in size, its cytoplasm often has irregular borders and stains a characteristic grayish blue. The nucleus is seldom rounded; usually it is deeply indented and lobulated its loose, delicate chromatin pattern is unique among the blood cells, nucleoli are rarely present. (Heilmeyer and Begemann, 2004).

Monocytes are increased in the following conditons: Chronic infection (e.g., tuberculosis), Recovery from severe neutropenia in neoplastic or a plastic disorders Benign neutropenia (Hays and Jamieson, 2008).

Monocytosis always present at the end of acute infections; chronic especially in Endocarditis lenta, listeriosis, Brucellosis, Tuberculosis ,monocytosis in/as *neoplasia*, e.g., Para neoplastic in cases of Disseminating tumors, Bronchial carcinoma, breast carcinoma, Hodgkin disease, Myelodysplasias especially CMML, and Acute monocytic leukemia. (Theml, *et al*., 2004).

**1.1.3.6.6 Lymphocytes**

Lymphocytes are small, round- to ovoid shaped cells that range in size from 7 μm to 15 μm with round to oval nuclei. Some normal lymphocytes are medium-sized

due to an increase in the amount of cytoplasm. The nucleus appears dense or coarse and clumped with ridges of chromatin and parachromatin. Nucleoli, if

present, are small and inconspicuous. The majority of lymphocytes have a scant amount of pale blue to basophilic a granular cytoplasm. (Hays and Jamieson, 2008).

Lymphocytes, Large Granular (Atypical Lymphocytes): These are large with abundant cytoplasm-containing areas having azurophilic granules, the nucleus has clumped chromatin and no visible nucleoli, these cells are suppressor/cytotoxic T

lymphocytes or natural killer cells, theses are commonly found with viral infections.

Reactive Lymphocytes**:** They tend to be large with abundant cytoplasm, they are.

Indented by surrounding red cells, and they may have blue skirting around the

Cytoplasm these lymphocytes have deeply basophilic cytoplasm and resemble plasma cells. Reactive lymphocytes are frequently seen in children with viral diseases, but the condition where reactive lymphocytes demonstrating all the Downey type cells are seen is usually infectious mononucleosis. (Hays and Jamieson, 2008).

### 1.1.4 Thrombopoiesis

Platelets are formed in the bone marrow by megakaryocytes, and are subsequently released in to the vascular compartment where they play an essential role in haemostasis. (Firkin *et al*., 2011).

#### 1.1.4.1The megakaryocytic series

Megakaryoblast, these cells amount to less than 8% of the total megakaryocyte population, the next stage in the sequence of maturation Promegakaryocyte make up to 25% of megakaryocyte have deeply basophilic cytoplasm containing some bascvpl1ilic granules. The nucleus may be lobulated, this larger than its precursor because it has undergone endoreduplication (nuclear replication without cell

division) leads to formation of very large cells, containing up to 32 times the normal diploid content of DNA, the final stage of maturation is Mature megakaryocytes about 30-90 μm in diameter, and contain 4 to 16 nuclear lobes with coarsely clumped chromatin, light blue stain cytoplasm and contains red-purple granules. Platelets formed by protrusion into the bone marrow sinusoids of pseudopods of megakaryocyte cytoplasm (Firkin *et al*., 2011).

**1.1.4.2 Platelets**

Are the small, a nucleate, terminal stage of development of the megakaryocytic series, they are discoid and have a diameter of 1-4 μm. The cytoplasm stains light- blue and contains small red-purple granules which are centrally located in platelets in blood films. (Firkin *et al*., 2011).

Thromboietin the major regulator of platelet production and is constitutively

Produced by the liver and kidneys and Thrombopoietin increases the number and

Rate of maturation of megakaryocytes via c-Mpl receptor.

The normal platelet count is approximately 250 x$\times 10^9$ /l (range 150-400 $\times 10^9$ /l) and the normal platelet lifespan is 7-10 days. Up to one-third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to 90% in cases of massive splenomegaly. (Hoffbrand et *al*., 2006).

Platelets play a key role in hemostasis and thrombosis. Their surface membrane is exquisitely designed for interaction with specific components of the vascular endothelial cell matrix. During hemostasis, circulating platelets rapidly adhere to the exposed vascular sub endothelium of damaged blood vessels. Adhesion induces a variety of intracellular biochemical events that lead to platelet aggregation, the secretion of granule contents, and the expression of procoagulant activity to support fluid phase coagulation. (Narayanan and Ellinor, 2001).

### 1.1.5 Erythropoiesis

The process of erythropoiesis includes all steps of Haemopoiesis, starting with the initial specification of haemopoietic stem cells (HSCs) from mesoderm during embryogenesis. HSCs either undergo self - renewal or, through the process of lineage specification, differentiate and proliferate to form committed erythroid.

Progenitors. Finally, they undergo terminal differentiation through a series of erythroblastic maturation stages to develop into red blood cells (Hoffbrand, *et al*., 2011).

#### 1.1.5.1 Immature red cell precursors

##### 1.1.5.1.1 Proerythroblasts

Proerythroblasts are 20 μm diameters, and have a very dense nuclear structure with a narrow layer of cytoplasm, homogeneous in appearance, with a lighter zone at the center; they stain deep blue after Romanowsky staining. (Theml, *et al*., 2004).

##### 1.1.5.1.2 Basophilic erythroblasts (macroblasts)

Their nuclei are smaller and the chromatin is more coarsely structured.

The maturation of cells in the erythrocyte series is closely linked to the activity of

Macrophages (transformed monocytes), which phagocyte nuclei expelled from Normoblasts and iron from senescent erythrocytes, and pass these cell components on to developing erythrocytes. (Theml, *et al*., 2004).

#### 1.1.5.2 Mature red blood precursor cells

##### 1.1.5.2.1 Polychromatic erythroblasts

The immature cells in which the cytoplasm displays a grayish blue hue, which are still able to divided.

##### 1.1.5.2.2 Orthochromatic erythroblasts

These cells the cytoplasm is already taking on a pink hue, which contains a lot of hemoglobin and is no longer able to divide. (Theml, *et al*., 2004).

**1.1.5.2.3 Reticulocytes**

The nuclei of the latter gradually condense into small black spheres without structural definition that eventually are expelled from the cells. The now enucleated young erythrocytes contain copious ribosomes that precipitate into reticular ("net-like") structures after special staining, (Theml, et al., 2004).

**1.1.5.3 Erythrocytes**

Are highly differentiated cells that have no nuclei or cytoplasmic organelles. erythrocytes are circular biconcave discs with a mean diameter of 7.2 μm. (Range 6.7–7.7 μm) in dried fixed smears and about 7.5 μm in the living state, they are eosinophilic and consequently appear red with a central area of pallor in Romanowsky stained smear the normal level of RBC for the male is $5.4\times10^9$ cell/μl and for female is $4.8\times10^9$ cell/μl. (Porwit *et al*., 2011).

**1.1.6 Hemoglobin**

Hemoglobin molecules are composed of four globin chains to which an iron-Containing porphyrin, heme, is attached, the hemoglobin is protein that transports oxygen in red blood cells from the lungs to the tissues. (Longo, 2010).

**1.1.6.1Heme**

The prosthetic group for hemoglobin, but which is essential for the function of all.

Aerobic cells. Approximately 85% of heme is synthesized in the erythropoietic marrow, the remainder being produced mainly by the liver. Heme is composed of an iron atom coordinated to four pyrrole rings of porphyrin through the nitrogen atom on each pyrrole ring, the rate of heme synthesis in the liver is regulated largely by the enzyme ALA synthase, the formation of porphobilinogen, uroporphyrin and coproporphyrin takes place in the cytoplasm, and the final assembly of the protoporphyrin ring occurs in the mitochondria. The final step is made with a mitochondrial enzyme

ferrochelatase (heme synthetase). Iron is then incorporated to form heme. (Shinton, 2008).

### 1.1.6.2 The Role of iron in hemoglobin synthesis

Iron is the most abundant transition metal in the body; Iron uptake is precisely controlled to maintain iron balance. In the duodenum dietary free iron is reduced to ferrous iron and taken up from the intestinal lumen into the enterocytes by the iron transport protein divalent metal transporter 1 (DMT1). DMT1 is instrumental in the uptake of iron by erythropoietic cells as well, once absorbed, iron may be stored as ferritin in the enterocytes or exported into the circulation by another iron transport protein, ferroportin 1 (fpn1). (Marry and Turgeon, 2012).

Iron is a transitional metal and micronutrient which is essential for several Physiological functions in the body. Iron is also a potent pro-oxidant known to catalyze the formation of reactive oxygen species (Rajpathak et al., 2009).

## 1.2. Anaemia

Is a condition in which the number of red blood cells (and consequently their Oxygen-carrying capacity) is insufficient to meet the body's physiologic needs (WHO, 2007).

anaemia a reduction in the haemoglobin concentration in the blood, in comparison with what would be found in a normal individual of the same age and gender *(*Bain and Gupta ,2003).

### 1.2.1Iron deficiency anemia (IDA)

Anaemia caused by a lack of adequate supplies of iron, iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributable to iron deficiency and accounts for ~841,000 deaths annually worldwide. Africa and parts of Asia bear 71% of the global mortality burden; North America represents only 1.4% of the total morbidity and mortality associated with iron deficiency. *(*Bain and Gupta, 2003).

### 1.2.2 Anemia of chronic disease (ACD)

This is the type of anemia mostly associated with infection, inflammation and cancer. It is characterized by hypoferremia, hyperferitinemia, reduction in transferrin concentration and increases in iron stores. These are also the main factors or parameters to distinguish anemia of chronic diseases from that of iron deficiency anemia which showed the opposite characteristics. Most of the studies

Showed that 10%-40% of cancer patients also suffered from ACD.Cancer disease itself is considered as one of the leading causes of ACD and is related with the progression of cancer disease.

There are several mechanisms by which ACD are caused such as bone marrow metastasis leading to myelosuppression or cytokines production like interlukine-1,Interlukine-6, tumor necrosis factor (TNF-α) and interferon (INF-γ) which will

either inhibit erythropoietin (EPO) synthesis by the kidney (i.e., inhibit erythropoiesis) or inhibit action on erythroid precursors leading to retention of iron in the reticuloendothelial system, gastrointestinal tract and hepatocytes or cytokine mediated failure of erythropoiesis. Several studies had proven that there are several cytokines in case of solid cancer diseases interfering with EPO synthesis. Other mechanisms includes bleeding, hemodilution, hypersplenism, hemophagocytosis and autoimmune, furthermore chemotherapy and radiotherapy both play a major role in the onset and severity of anemia since both of these two

factors lead to bone marrow suppression and reduce EPO produced from the kidneys. However, anemia seems to be more common among patients with hematological cancer such as in multiple myeloma (Haut, 2007; Brown and Olde, 2005; Weiss *et al*., 2005).

### 1.2.2.1Causes of Anemia of Chronic Diseases (ACD)

This is mainly caused by cytokine including interlukine-1, interlukine-6, interferon-γ and tumor necrosis factor-α These cytokines caused impairment of erythropoietin (EPO) synthesis, reduce erythrocytes life span and anemia of chronic disease which encompasses inflammation, infection, tissue injury, and conditions (such as cancer) associated with the release of pro inflammatory cytokines is one of the most common forms of anemia seen clinically and probably the most important in the differential diagnosis of iron deficiency because many of the features of the anemia are brought about by inadequate iron delivery to the marrow, despite the presence of normal or increased iron stores.

This is reflected by a low serum iron, increased red cell protoporphyrin, a hypo proliferative marrow, transferrin saturation in the range of 15–20%, and a normal or increased serum ferritin. The serum ferritin values are often the most distinguishing feature between true iron deficiency anemia and the iron deficient erythropoiesis associated with inflammation. Typically, serum ferritin values increase three fold over basal levels in the face of inflammation. All of these changes are due to the effects of inflammatory cytokines and hepcidin, the key iron regulatory hormone, acting at several levels of erythropoiesis, Interleukin 1 (IL-1) directly decreases EPO production in response to anemia. IL-1, acting through accessory cell release of interferon γ (IFN- γ), suppresses the response of the erythroid marrow to EPO an effect that can be overcome by EPO administration in vitro and in vivo. In addition, tumor necrosis factor acting through the release of IFN-γ by marrow stromal cells, also suppresses the response to EPO. Hepcidin, made by the liver, is increased in inflammation and acts to suppress iron absorption and iron release from storage sites, the overall result is a chronic hypo proliferative anemia with classic changes in iron metabolism. The anemia is further compounded by a mild to moderate shortening in red cell survival, with chronic inflammation, bone marrow

replacement which is associated with inhibition of the body ability for the production of RBC, this condition of bone marrow suppression is associated with specific types of cancer like breast, prostate, lymphoma and acute leukemia, also bone marrow suppression is mainly caused by chemotherapy and radiotherapy which are the main treatment for cancer. (Haut, 2007).

### 1.2.3 Other hypoproliferative anemias:

The hypoproliferative anemias can be divided into four categories:

1- Chronic inflammation,

2- Renal disease

3- Endocrine and nutritional deficiencies (hypo metabolic states),

4- Marrow damage with chronic inflammation, renal disease, or hypo metabolism. (Longo *et al*., 2010).

## 2. Tuberculosis (TB)

is the world's second most common cause of death from infectious diseases , In 2011 a total of 8.7 million new active TB cases and 1.4 million TB related deaths were estimated worldwide ; 70% of these deaths were among HIV uninfected people (WHO,2012) .

Smear positivity is the most important predictor of TB infectiousness when smear positive TB patients initiate TB therapy containing rifampicin and isoniazid, there is a rapid multifold reduction in bacillary load expelled in sputum which minimizes the risk for transmission (Jindani *et al*., 2003).

About 90% of patients are Likely to become smear and/or culture negative (smear and/or Culture conversion) after two to three months of TB chemotherapy (Fitzwater, 2010; Parikh *et al*., 2012).

However, approximately 10% of TB patients are still culture positive 60 days following initiation of anti TB therapy (Fitzwater, 2010).

Implying a potential for persistent infectiousness. Furthermore longer smear conversion times have been associated with subsequent poor TB treatment outcomes and relapse within two years of follow up (Chien *et al.,* 2013; Tiwari *et al*., 2012).

Some risk factors have been identified for delayed time to smear conversion. These include cavitatory lesions, high initial sputum smear acid fast bacillary counts, multi drug resistant TB, old age, diabetes and duration of symptoms before treatment (Fitzwater, .2010; Guoler, 2007; Senkoro *et al*., 2010).

### 2.1 Pulmonary tuberculosis (PTB)

Refers to a case of TB involving the lung parenchyma, Miliary tuberculosis is classified as pulmonary TB because there are lesions in the lungs, Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs. (WHO, 2008).

### 2.1.1 Mycobacterium tuberculosis

Is the most important causal agent of tuberculosis, other species, such as *M. bovis, M. kansasii, M. gordonae, M. avium, M. fortuitum,* and *M. phlei*, considered pathogenic but mostly in immune compromised subjects (Murray *et al*., 2009)

### 2.1.2 Tuberculosis patient

Any person who presents with symptoms or signs suggestive of TB. The most common symptom of pulmonary TB is a productive cough for more than 2 weeks, which may be accompanied by other respiratory symptoms (shortness of breath, chest pains, haemoptysis) and/or constitutional symptoms (loss of appetite, weight loss, fever, night sweats, and fatigue).( WHO,2009).

### 2.1.3 Pathophysiology:

TB is spread by aerosolized droplets from patients with active pulmonary TB, the mycobacteria proliferate in alveolar macrophages and are transported to hilar lymph nodes and are subsequently spread to almost any other part of the body, especially the upper lobes of the lung, the pleura, lymph nodes, bones, and genitourinary and central nervous systems. (Kirmani *et al*, 2013).

### 2.1.4 Immunity and Pathogenesis*:*

M. tuberculosis is an intracellular pathogen that is able to establish Lifelong infection, at the time of exposure, M. tuberculosis enters the respiratory airways and minute infectious particles penetrate to the alveoli, where they are phagocytized by alveolar macrophages. In contrast with most phagocytized bacteria, M. tuberculosis prevents fusion of the phagosome with lysosomes by blocking the specific bridging molecule, early endosomal auto antigen 1(EEA1) at the same time, the phagosome is able to fuse with other intracellular vesicles, permitting access to nutrients and facilitating intravacuole replication, By inactivating the oxidants that are formed, phagocytized bacteria are also able to evade macrophage killing mediated by reactive nitrogen intermediates formed between nitric oxide and superoxide anions. (Murray *et al*., 2009).

### 2.1.5 Diagnosis

#### 2.1.5.1 Clinical presentation

In active pulmonary TB, patients may present with nonproductive cough and (3 weeks or more in duration), fevers, chills, night sweats, and weight loss. Hemoptysis may occur in advanced disease, latent TB patients are asymptomatic.

#### 2.1.5.2 Diagnostic criteria

The diagnosis of active pulmonary TB is made with laboratory findings of acid fast organisms on sputum with a positive nucleic acid amplification test for Mycobacterium tuberculosis complex or culture growing M. tuberculosis, Culture-negative pulmonary TB is diagnosed with active TB symptoms, no alternative diagnosis, and improvement on TB therapy. TB skin testing (PPD) cannot be used to rule out TB in active infection, Latent TB is diagnosed with a positive PPD or interferon-γ release Assay (preferred in patients who have had bacillus Calmette-Guérin vaccine). (Kirmani *et al*., 2013).

#### 2.1.5.3 Differential Diagnosis

The differential diagnosis includes non-tuberculous mycobacterial infections, fungal infections, malignancies, lung abscess, septic emboli, and anti neutrophil cytoplasmic antibody–associated vasculitis, which can all cause cavitary pulmonary lesions and symptoms suggestive of TB (Kirmani *et al*, 2013).

### 2.2 Multi drug resistance tuberculosis (MDR-TB)

Is defined as tuberculosis caused by mycobacterium tuberculosis showing invitro resistance to isoniazid and rifampicin with or without resistant to any other drugs. (WHO, 2010).

MDR-TB results from either infection with organisms which are already drug-resistant or may develop in the course of a patient's treatment. (WHO, 2013).

## 2.3Extensively drug-resistant TB (XDR-TB)

is a form of TB caused by organisms that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second–line anti-TB injectable drugs (amikacin, kanamycin or capreomycin).(WHO,2013).

## 2.4Essential anti tuberculosis drugs:

1. **Isoniazid**

Isoniazid, the hydrazide of isonicotinic acid, is highly bactericidal against replicating tubercle bacilli**;** Isoniazid is normally taken orally but may be administered intramuscularly or intravenously to critically ill patients (American Thoracic Society, 2003).

2. **Rifampicin**

A semi synthetic derivative of rifamycin, rifampicin is a complex macro cyclic antibiotic that inhibits ribonucleic acid synthesis in a broad range of microbial pathogens. It has bactericidal action and a potent sterilizing effect against tubercle bacilli in both cellular and extracellular locations; rifampicin is lipid-soluble **(**American Thoracic Society, 2003).

3. **Streptomycin**

Is an aminoglycoside antibiotic derived from *Streptomyces griseus* that is used in the treatment of TB and sensitive Gram-negative infections. Streptomycin must be administered by deep intramuscular injection (American Thoracic Society, 2003).

4. **Ethambutol**

A synthetic congener of 1, 2-ethanediamine, ethambutol is active against *M. tuberculosis, M. bovis* and some nonspecific mycobacteria. It is used in combination with other anti-TB drugs to prevent or delay the emergence of resistant strains. Ethambutol is administered orally. **(**American Thoracic Society, 2003).

5. **Pyrazinamide**

Pyrazinamide is a synthetic analogue of nicotinamide that is only weakly bactericidal against *M. tuberculosis* but has potent sterilizing activity, Pyrazinamide is administered orally. Adults (usually for the first 2 or 3 months of TB treatment **(**American Thoracic Society, 2003).

**Table1.1: recommended dose of TB treatment (WHO Model Formulary, *2008*).**

| | **Recommended dose** | | | |
|---|---|---|---|---|
| **Drug** | **daily** | | **3times per week** | |
| | **Dose and range** (mg/kg body weight | **Maximum** (mg) | **Dose and range** (mg/kg body weight) | **Maximum** (mg) |
| Isoniazid | 5(4-6) | 300 | 10(8-12) | 900 |
| Rifampicin | 10(8-12) | 600 | 10(8-12) | 600 |
| Pyrazinamide | 25(20-30) | _ | 35( 30-35) | _ |
| Ethamputol | 15(15-20) | _ | 30(25-35) | _ |
| Streptomycin | 15(15-18) | _ | 15(12-18) | 1000 |

**2.5 Vaccination:**

The introduction of Bacille Calmette-Guérin (BCG) and chemotherapy in the past century marks an important advance in the history of tuberculosis (TB), which accounted for optimism to fight the disease especially in endemic area. BCG remains as the most widely used vaccine worldwide and has been given to more than 4 billion individuals with astonishing safety records. (McShane, 2011; Ottenhoff and Kaufmann, 2012).

### 1.3 Previous studies:

A prospective study is conducted by Bashir et al, in period from June 2006 to December 2008 at Port Sudan tuberculosis diagnostic center involved100 newly discovered Ziehl Neelsen stain positive selected along with 50 apparently healthy adult selected to determine Hemoglobin concentration (Hb), serum iron, total iron binding capacity (TIBC) and transferrin saturation% , Hemoglobin concentration, serum iron, TIBC, and transferrin saturation were lower in the patient group than in the control group ($P<0.02$); anemia was observed in 44(44%) of pulmonary tuberculosis patients, of which 15 (34%) of cases were anemia of chronic disease, 7 (16%) of cases were iron deficiency anemia, 2 (5%) of cases were macrocytic anemia and 8 (18%) of cases were normocytic normochromic anemia ( Bashir et al,2015).

A study conducted in Korea by Yim, (2006) among 880 patients, 472 patients (53.6%) were male and 408 (46.4%) were female. Prevalence and characteristics of anemia was identified in 281 patients (31.9%) at the time of diagnosis of TB. 133 (28.2%) of men and 148 (36.3%) of women with TB had anemia. In 45 patients, the hemoglobin concentration was less than 10 g/dL. No male patient had a hemoglobin concentration less than 8.0 g/dL and no female patient had a hemoglobin concentration less than 7.0 g/dL ,Normocytic normochromic anemia was most common, and was identified in 202 (71.9%) patients; and microcytic hypochromic anemia was next common (26 patients,9.1%) (Yim, 2006).

A study was conducted by Bala, et al in period March 2013 to march 2014, on hematological parameters in pulmonary tuberculosis patients Total numbers of 80 subjects were selected of Z-N positive. Hemoglobin (Hb) concentration, red blood cell (RBC), packed cell volume (PCV), mean cell volume (MCV) and mean cell hemoglobin (MCH) lower in tuberculosis patients than control group, while increased value observed in total leukocyte count (TLC), mean cell hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), and

Anemia noticed in 59% patients, including mild, moderate and severe. In peripheral blood film examination, microcytic hypochromic blood picture was most common. (Bala, 2015).

The study conducted by Saeed, Khartoum state, during the Period between Aprils to August 2013, 40 patients with pulmonary tuberculosis (25 males and 15 females) and 20 healthy controls (14 males and 6 females) CBC were carried out. Significant lower values in Hemoglobin (Hb) concentration, Red Cell Count and PCV (P= 0.00) with normal MCV, MCH and MCHC in T.B patients compared with controls group. Significant increase in TWBCs count, absolute monocyte count and absolute neutrophil count in T.B patients compared with control. The most type of anemia was normocytic normochromic anemia, and significant increase in platelet count in T.B patients compared with controls group. (Saeed, 2016).

## 1.4 Rationale:

Pulmonary tuberculosis is an infectious and chronic disease; anemia in patient with active pulmonary tuberculosis develops during stage of disease increase morbidity and mortality due to morbid conditions like anemia with cardiac failure, Pancytopenia.

This a study was conducted to evaluate effect of pulmonary tuberculosis in the complete blood count and iron profile and to determine types of anemia among this patients with tuberculosis, and this may have a positive impact on monitoring and treatment of these patients .

## 1.5 Objectives:

### 1.5.1 General objective:

To study complete blood count and iron profile among Sudanese patients with active pulmonary tuberculosis.

### 1.5.2 Specific objective:

1- To measure complete blood count parameters and iron profile in active pulmonary tuberculosis patients using sysmex KX 21N and spectrophotometer.

2- To compare the complete blood count parameters and iron profile in active pulmonary tuberculosis patients and apparently healthy controls.

3- To determine frequency types of anemia in active pulmonary tuberculosis patient using hemoglobin, PCV, red blood cell count and indices, , iron profile and peripheral blood picture .

4-To correlate between hemoglobin and iron profile in active pulmonary tuberculosis patients.

# Chapter Two

# Materials and Methods

# Chapter Two

# Materials And Methods

## 2.1 Materials

### 2.1.1Study design, area and duration

This is a case control study, conducted in Khartoum state in the period from January 2017 to October 2017.

### 2.1.2Study population

One hundred and twenty individual were participate in this study and classified into two groups: 60 active pulmonary tuberculosis patients and 60 apparently healthy volunteers as control group.

### 2.1.3 Inclusion criteria

Case group were active pulmonary tuberculosis patients, control group were healthy individual.

### 2.1.4Exclusion criteria

Patients with any chronic disease such as hypertension and/ or diabetes mellitus were excluded from study.

### 2.1.5Ethical considerations

The specimens and information that collected from the participating were under privacy and confidentially. The aim of the research was explain for the subjects under the study in simple language and they understood the research idea and agree to participate in the study.

## 2.2 Methods

### 2.2.1 Blood collection

### 2. 2.1.1 Procedure

The person was sitting on table then the arm was positioned on the armrest so that the vein identified and after that the skin was cleaned with 70% alcohol and allowed to dry. Tourniquet was applied to the arm, sufficiently tightly to distend the vein, but not so tightly that discomfort may arise, the personal details was checked up on the forearms and blood vials, 2.5ml of blood sample was

collected by syringe. Then blood sample was collected in EDTA.K3 (1.2mg/ml blood) anticoagulant. EDTA blood sample was analyzed by sysmex KX-21N (semi-automated hematological analyzer). (mitchel2006). and 2.5ml of blood sample was collected by syringe in plan vacoteriner.

**2.2.2Sysmex KX-21N**

**2.2.2.1Principle of KX-21N**

This instrument perform blood count to measure WBC and differentional counts RBCSs, HCT, MCV, MCH, MCHC, Hb and platelet count. Blood sample is aspirated, measured to predetermine, and then diluted at specified ratio. Then fed into each transducer, Transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes, between which flow direct current blood cells suspended in diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As a direct current resistance change the blood cell count is calculated by counting the pulses, and histogram of blood cell sizes is blotted by various analysis data. Hemoglobin is measured by non cyanide hemoglobin analysis methods which rapidly converts blood hemoglobin as oxy hemoglobin and contain no poisonous substance making it suitable for automated methods. (Diamond, 1999).

**2.2.2.2Procedure** (whole blood mode)

Blood is aspirated from the sample probe into the sample rotor valve:

4microliter of blood measured by the sample rotor valve is diluted into 1:500 with 1.996microliter of diluents and brought to the mixing chamber as diluted sample (first step dilution)

Out of the 1:500 dilution sample 40 micro liters is measured by the sample rotor valve, diluted into 1:25000 with 1.96 micro liters of diluents then transferred to the RBC/plt transducter chamber (second step dilution). 250 micro liter of sample in RBC/platelet transducer chamber is aspirated through the aperture. At

this time RBCs and platelet are counted by the DC detection method. (Sysmex corporation1998)

### 2.2.3 Blood film

To comment of the red blood cells, white blood cells with differentional count and platelets

### 2.2.4Iron profile

Sample are collected in plan vacoteriner and centrifuged to obtained serum.

### 2.2.4.1Serum iron

#### 2.2.4.1.1Principle of the method

Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by means of ascorbic acid. Ferrous ions react with ferrozine forming a colored complex that can be measured by spectrophotometry.

#### 2.2.4.1.2Procedure

Bring the reagent into room temperature pipette in to labeled test tube

| | **Reag.blank** | **Sample blank** | **sample** | **standard** |
|---|---|---|---|---|
| **Distilled water** | 200 | - | - | - |
| **Sample** | - | 200 | 200 | - |
| **Iron stander** | - | - | - | 200 |
| **Reagent A** | - | 1.0ml | - | - |
| **Working reagent** | 1.0ml | - | 1.0ml | 1.0ml |

Mix thoroughly and let stand the tubes for 5 minute at room temperature, read the absorbance A of the sample blanks at 560nm against DW, read absorbance A of the sample s and stander at 560nm against the reagent blank, calculate

A sample –A sample blank/A standard× C standard=C sample **.**

**Reference values:**

Men: 65-175μg/dl =11.6-31.3μmol/L. Women: 50-170μg/ dl =9.0-30.4μmol/L. Burtis et al, 2005; Young, 2000; Friedman and Young, 2001).*(*

**2.2.4.2Total iron binding capacity**

**2.2.4.2.1Principle of the method:**

Excess of $Fe^3$ is add to the sample to saturate serum transferrin, uncomplexed $Fe^3$+ is precipitated with magnesium carbonate and the iron bonded to protein in the supernatant is then spectrophotometrically.

**2.2.4.2.2procedure**

Pipette in to labeled tubes

| | |
|---|---|
| Sample | 0.5 |
| Reagent A | 1.0ml |

mix thoroughly and let stand 5-30 RT, add to each tubes one spoonful of reagent A mix thoroughly the tubes at RT for30-60 during this time mix thoroughly several time, centrifuge t minimum of 3000 rpm for 10 minutes, carefully collected supernatant and measure the iron concentration using the kit Iron-ferrozine.

**Calculations**

TIBC=iron concentration× 3(dilution)

**Reference values:**

Infant: 100-400μg/dl=18-72μmol/l.

Adult: 250-425μg/dl=45-76μmol/l.

**2.2.4.3 Iron saturation (%)**

Iron saturation: 100×serum iron concentration/TBIC =iron saturation (%).

**Reference values:**

Men: 20-50% , Women: 15-50%.

(Burtis et al, 2005; Young, 2000; Friedman and Young, 2001).

### 2.2.4.4Serum ferritin

#### 2.2.4.4.1Principle of the method

Ferritin-turbilatex is a quantitative turbidimetric test for the measurement of ferritin in human serum or plasma. Latex particles coated with specific anti human ferritin are agglutinated when mixed with sample s containing ferritin. The agglutinated cases and absorbance change, dependent upon the ferritin contents of the sample that can be quantified by comparison from a calibrator of known ferritin concentration.

#### 2.2.4.4.2Procedure

Bring the reagent and photometer at room temperature adjust instrument to zero with distilled water pipette into cuvette

| | |
|---|---|
| **Diluent R1** | 800 |
| **Latex R** | 200 |
| **Calibrator or sample** | 90 |

Mix and read the absorbance immediately A1and after 5 minutes A2 of sample addition, calculation ferritin concentration in the sample is calculated d by interpolation of its (A2-A1) in calibration curve.

**Reference values**

Men: 30-220μg/l Women: 20-110 μg/l. (Knovich et al., 2009).

### 2.3Data collection and analysis

Data was collected using structured interview questionnaire, and then analyzed using statistical package for social sciences (SPSS), version (11.5). Qualitative data was represented as frequency and percentage. Quantitative data was presented as mean and SD. Independent T-test used to compare means of quantitative variable in two groups. Person correlation test used to correlate between two quantitative variables. ANOVA was used to compare between quantitative variable and qualitative (more than two branches).Chi-square test was used to investigate the association between two qualitative variables.

# Chapter Three

# Results

# Chapter Three

## Results

one hundred twenty (120) venous blood samples were collected in EDTA and plain vacoteriner tubes from participants, active pulmonary tuberculosis (60 case, 47 male and 13 female) and (60) health individual (47 male and 13 female) their age ranged between17-60 years(mean 34.2 ± SD:10.8)

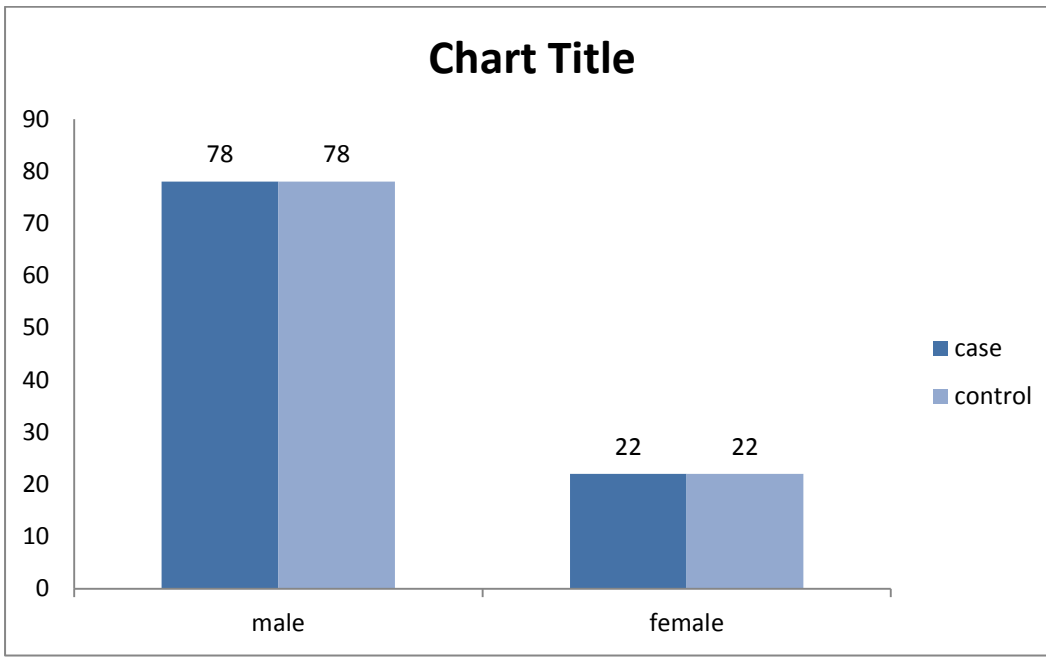


**Figure 1.1 Gender distributions among study groups**

There were statistically significant decrease in hemoglobin concentration, MCV, MCH in active pulmonary tuberculosis than control and no statistically significant changes in PCV, RBCS count and MCHC between case and control groups with statistically significant increase in RDW SD and RDW CV in active pulmonary tuberculosis than health individual.

**Table 2.1** comparisons of mean HB, PCV and red blood cells count and indices between case and control groups:

| **parameter** | **Active pulmonary tuberculosis mean± SD** | **Control group mean± SD** | **P-value** |
|---|---|---|---|
| HB g/dL | 12.2±2.46 | 13.4±1.43 | 0.002 |
| PCV g/dL | 38.7±9.00 | 39.0±6.2 | 0.818 |
| RBCS× $10^6$/μL | 4.6±0.63 | 4.7 ±0.44 | 0.409 |
| MCV fl | 79.9±10.9 | 83.9±3.71 | 0.009 |
| MCH pg | 26.6±3.20 | 28.3±1.9 | 0.001 |
| MCHC mg/dl | 36.8±3.49 | 33.7±1.3 | 0.486 |
| RDW SD fl | 44.6±5.48 | 41.7±3.5 | 0.001 |
| RDW CV% | 14.8±2.51 | 13.4±1.2 | 0.000 |

There was statistically significant increased white blood cells count between case and control group (7.4 ± 2.4 vs. 6.4± 1.24×$10^3$ /μl respectively, P. value *=0.009*) with statistically significant increase in absolute monocyte count in patients with active pulmonary tuberculosis than control groups.

There were no statistically significant changes in absolute neutrophil count, absolute eosinophil count, absolute lymphocyte count, and absolute basophil count between case and controls group showed that in table (2.1).

**Table 2.2:** comparison of the white blood cell count and absolute count between case and control groups.

| **parameter** | **Active pulmonary tuberculosis mean± SD** | **Control group mean± SD** | **P- value** |
|---|---|---|---|
| Mean of WBCS Count (×$10^3$/μl) | 7.4± 2.49 | 6.4± 1.2 | 0.009 |
| Absolute neutrophil Count (×$10^3$/μl) | 4.51±5.7 | 3.69± 1.0 | 0.278 |
| Absolute monocyte Count(×$10^3$ μl) | 1.19 ±1.03 | 0.49 ± 0.25 | 0.000 |
| Absolute lymphocyte count(×$10^3$ /μl) | 2.55±2.48 | 2.11±0.58 | 0.177 |
| Absolute Eosinophil Count(×$10^3$ /μl) | 0.225±0.30 | 0.124±0.90 | 0.160 |
| Absolute Basophil Count(×$10^3$ /μl) | 0.015±0.12 | 0.00±0.00 | 0.321 |

There was statistically significant increased platelet count in active pulmonary tuberculosis than control group (293.7±108.79vs 251.8 ± 54.77 respectively, P. value =0.009), with no statistically significant change in PDW, MPV, P-LCR between case and control groups

**Table2.3:** Comparison of platelet count and indices in case and control group:

| **parameter** | **Active pulmonary tuberculosis mean± SD** | **Control group mean± SD** | **P- value** |
|---|---|---|---|
| platelet count | 293.7±108.79 | 251.7± 54.77 | 0.009 |
| Mean PDW | 12.6 ±2.106 | 13.3± 2.19 | 0.068 |
| Mean MPV | 10.1±1.29 | 10.5± 0.96 | 0.028 |
| Mean P-LCR | 26.2±7.64 | 29.0±8.67 | 0.055 |

There were statistically significant decrease in serum iron and total binding capacity in active pulmonary tuberculosis than control groups, there were no statistically significant changes in saturation % between case and control with statistically significant increase in serum ferritin in active pulmonary tuberculosis than healthy controls.

**Table 2.4**: comparison of iron profile between case and control group

| **parameter** | **Active pulmonary tuberculosis** | **Control group** | **P value** |
|---|---|---|---|
| serum iron mg/dl | 75.4±43.3 | 124.8±29.3 | 0.000 |
| TIBC | 215.3±106.51 | 312.4±63.94 | 0.000 |
| Saturation % | 39.6±23.600 | 40.5±8.92 | 0.777 |
| Serum ferritin | 241.8±80.39 | 112± 32.32 | 0.000 |

There was statistically significant positive correlation between HB and S.iron and between HB and saturation%, the was statistically no correlation between HB and S. ferritin, and between HB and TIBC

**Table 2.5**: correlation between HB and iron profile in case and control

| **parameter** | **Person correlation ( r)** | **P .value** |
|---|---|---|
| HB/S.iron | 0.598 | 0.000 |
| HB/S. ferritin | -0.164 | 0.212 |
| HB/TIBC | 0.273 | 0.035 |
| HB/saturation% | 0.338 | 0.002 |

The result showed that the frequency of anemia in active pulmonary tuberculosis is (48.3%) and types of anemia according to HB, PCV, MCV, MCH, iron profile and peripheral blood picture among active pulmonary tuberculosis ACD (45%), IDA (3.3%), The result showed that the microcytic anemia is more common type of anemia in active pulmonary tuberculosis (31.7%), and normocytic anemia are second type of anemia in case (28.3%).

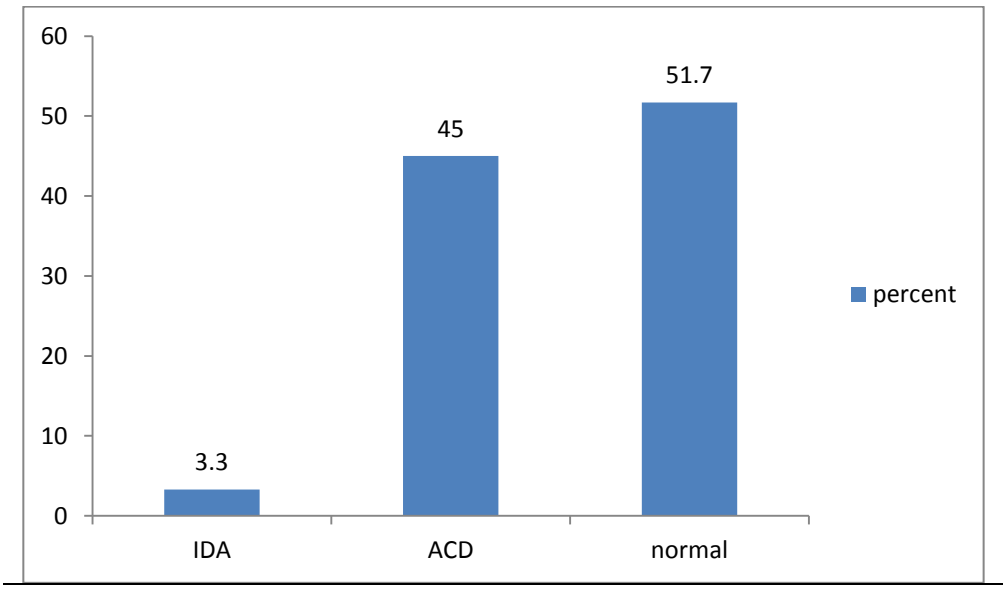


Figure 1.2 Frequency and type of anemia in active pulmonary tuberculosis patients Gender distributions among study groups

**Table 2.6 duration and anemia**

| **Duration** (month) | **anemia** | | | **total** |
|---|---|---|---|---|
| | IDA | ACD | normal | |
| Group1(1-8) | 1 | 23 | 24 | 48 |
| Group2(9-17) | 0 | 3 | 4 | 8 |
| Group3(18-26) | 1 | 0 | 1 | 1 |
| Group4(27-60) | 0 | 1 | 2 | 3 |

# Chapter Four

## Discussion, Conclusion, Recommendation

## 4.1 Discussion

This study was carried out to measure the complete blood count parameters and iron profile and to determine frequency and the type of anemia among active pulmonary tuberculosis patients in Khartoum state.

The result showed that the mean of hemoglobin concentration, MCV, and MCH in active pulmonary tuberculosis patients were statistically significantly decrease with statistically significant increased in RDW-SD and RDW-CV and no statistically significant difference in RBCs count, PCV, and MCHC than healthy control, this result agree with Bala statistically significant decrease in HB, MCV, MCH, and disagree with Bala in PCV, and RBCs statistically decrease and statistically increased MCHC. The difference may be due to study area and sample size (Bala, 2015).

The result also agrees with Saeed in mean of hemoglobin concentration, MCV and MCH statistically significant decrease and disagrees with Saeed in RBC sand PCV statistically significant decreased and statistically significant increased MCHC (Saeed, 2016).

The result showed that statistically significant increased in TWBCs count, and absolute monocyte count with no statistically difference in absolute neutrophil count, absolute lymphocyte count, absolute eosinophil count, and absolute basophil count, in compared with healthy controls, this result was agree with Saeed in TWBCs count and absolute monocyte count statistically significant increased and disagree in absolute neutrophil count statistically significant increased. (Saeed, 2016).

The result showed that significant increased in platelet count and no significant difference in PDW, MPV, P-LCR, when compared with healthy controls .this result agree with Bala statistically increased in platelet count, (Bala 2015),

And agree with Saeed statistically significant increased in platelet count (Saeed, 2016).

The results of iron profile showed that statistically significant decrease in S.iron, TBIC, and statistically significant increase in S. ferritin, with no difference in saturation% when compared with healthy controls. These results agree with Bashir in mean of hemoglobin concentration, serum iron, TIBC, saturation%. (Bashir et al., 2015).

The result showed that statistically significant positive correlation between hemoglobin concentration and S.iron, and between hemoglobin concentration and saturation%, with no statistically correlation between hemoglobin concentration and S. ferritin, and between hemoglobin concentration and TIBC.

The result showed that The frequency of anemia in active pulmonary tuberculosis patients is(48.3%) and type of anemias are ACD (45%), IDA (3.3%) this result agree with the result of Bashir of which anemia was observed in (44%) of tuberculosis patients, and disagree with Bashir in types of anemia in active pulmonary tuberculosis patients of which (34%) of cases were anemia of chronic disease, (16%) of cases were iron deficiency anemia, (5%) of cases were macrocytic anemia and (18%) of cases were normocytic normochromic anemia ( Bashir et al,2015).

the result of the frequency of anemia are agree with Oliveira in prevalence of iron deficiency anemia (2.4%) , and disagree with Oliveira in prevalence of anemia of chronic disease (75.9% ) .( Oliveira, et al. 2014 ).

## 4.2 Conclusion

- I concluded that in active pulmonary tuberculosis there was statistically significant decreased in HB concentration, MCV, MCH ,S.iron, and no significant difference in RBCS count, PCV, MCHC ,TIBC ,and saturation % MPV ,PDW,P.LCR with statistically significant increased in WBCs count, absolute monocyte count, RDW-SD , RDW-CV , S. ferritin and platelet count with statistically significant positive correlation between HB and S.iron and saturation % .
- The frequency of anemia in active pulmonary tuberculosis patients is (48.3%); ACD (45%), IDA (3.3%).

## 4.3 Recommendations:

- Further study with increased sample size should conduct in the future.
- The patients with active pulmonary tuberculosis should have their CBC and iron profile checked at diagnosis and monitor after that to categorize them according to type of anemia for proper monitoring and treatment.
- Further study in iron status and its role in host defense mechanism in infections.
- Further hematology and immunological study in cytokines and pro inflammatory mediators and hepcidin and its role in infection should be conducted in these patients.

# Chapter Five

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# Appendixes

# Sudan University for Science and Technology

## College of Graduate Studies

Study of Complete Blood Count and Iron Profile Among Active Pulmonary Tuberculosis Patients

Questionnaire

Serial No (  )

**Demographical data:**

Age……

Gender:  female (  )  male (  )

Diagnosis……

Duration of disease…….

Chronic disease……..

**Laboratory Results:**

CBC: twbcs……$\times 10^3$ /µl. RBCs……($10^6$ /µl). Hb ……. (mg/dl) HCT ……(mg/dl ) MCV……..(fl). MCH……. (Pg) MCHC…….(mg/dl) . RDW SD……..(Fl). CV……..(%)

Absolute count ($\times 10^3$ /µl): neutrophil…… lymphocyte…… monocyte ….. eosinophil… basophil…

Platelets count……..($\times 10^3$ /µl). PDW … ……..(fL).MPV……. (fL) P-LCR……..(%).

Iron profile:

Serum iron… ……. µg/dl

Serum ferritin……. µg/l.

Saturation.....%

TBIC…….. µg/dl.

Method of blood sample collection: Requirements for blood collection:

- Ethylene diamine tetra acetic acid (EDTA.K3) vacontainers

-Plan vacoteriner

- Cotton.

- Alcohol (70%). - Syringe and tourniquet.

**Sysmex KX-21N: Reagents and materials**

Commercial close system reagents were provided by sysmex KX-21N operators and consist of: cell pack and stromatolyser; diluents and lysing reagent for use sysmex. Detergent and cell cleaner: use for cleaning solution to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of sysmex automated analyzer (Dimond, 1999)

**Serum iron reagent**

Reagent: Guanidinium chloride 1.0mol/Lactate buffer 0.4mol/L PH 4.0.

Reagent: ferrozine 8mmol/L ascorbic acid 200mmol/L.

Iron standard: iron 200μg/dl (35.8 umol/L) aqueous primary standard.

**Serum ferritin Reagent**

R1 Diluent: tris buffer 20mmol/L PH 8.2 preservative.

R2: anti human ferritin anti body coated latex particles PH 8.2 preservative.

FERR- CAL.