# PREVALENCE OF IRON OVERLOAD AMONG PATIENTS WITH TRANSFUSION DEPENDENT ANAEMIA ATTENDING MUHIMBILI NATIONAL HOSPITAL.

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**Muhimbili University of Health and Allied Sciences**

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**Department of Haematology and Blood Transfusion**

# PREVALENCE OF IRON OVERLOAD AMONG PATIENTS WITH TRANSFUSION DEPENDENT ANAEMIA ATTENDING MUHIMBILI NATIONAL HOSPITAL.

**By,**

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**Dissertation Submitted In (Partial) Fulfillment of the Requirement for the Degree of Master of Medicine (Haematology and Blood Transfusion) of**

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## CERTIFICATION

The undersigned certify they have read and hereby recommend for acceptance of dissertation entitled**"Prevalence of iron overload among patients with transfusion dependent anaemia attending Muhimbili National Hospital"** in partial fulfillment of the requirements for the degree of Master ofMedicine (Haematology and Blood Transfusion) of Muhimbili University of Health and AlliedSciences.

_____

**Dr. Magdalena Lyimo**
(Supervisor)

_____

Date

_____

**Dr. Mbonea Yonazi**
(Co-supervisor)

_____

Date

## DECLARATION AND COPYRIGHT

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

Signature _____ Date _____

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## ABSTRACT

**Background:**Blood transfusion provides a major rapid and effective treatment for transfusion dependent anaemia patients including those with thalassemia, sickle cell anaemia, myelodysplastic syndrome, aplastic anaemia and haemolytic anaemia. Unfortunately transfusional iron overload among transfusion dependent anaemia patients has been shown to be a potentially serious problem that is often overlooked because the symptoms are nonspecific and often develop gradually. A number of diagnostic tests are available, but their interpretation can be challenging. Once transfusional iron overload is diagnosed, the options for treatment are relatively straightforward in the majority of individuals. However, untreated individuals can develop life-threatening morbidity and mortality related to organ toxicity. Thus, it is important to identify iron overload before organ damage occurs.

**Objectives:**The study was performed to determine the prevalence of transfusional iron overload, to describe the clinical diagnosis,clinical presentation and iron status of individuals with transfusion dependent anaemia at MNH.

**Methodology:** This was a descriptive, cross sectional hospital based study involving patients from 2 years and abovewith transfusion dependent anemia who have received ≥10transfusions or ≥100mls/kg of red blood cells in their life time, attending inpatient and outpatient hematology services at Muhimbili National Hospital. A structured questionnaire wasused to obtain information on socio-demographic particulars, type of illnessand number of blood transfusions one has received in certain period of time. Blood samples were drawn for full blood count, serum ferritin, serum iron,serum transferrin and C-reactive protein.The collected data were analyzed by using SPSS version 20.0.

**Results:** Majority of patients were aged between 2 and 17 years 59 (52.7%), followed by 18-35 years 28 (25%). In a total of 112 study participants 60(53.6%) were males. Of these 112 study participants, majority were observed to have aplastic anaemia37(33%), followed by acute leukemia 27 (24.1%).Most of the patients were observed to have had received blood transfusion for more than 12 months.The clinical signs and symptoms that were observed in transfusion dependent anaemia patients were tachycardia, bradycardia, skin hyperpigmentation and hepatomegaly.Serum ferritin, iron, and transferrin saturation were observed to be markedly elevated and transferrin was low. Of the 112 patients, 74.1% had ferritin levels > 1000ng/ml and 71.4% had TSAT > 45%.

**Conclusion:**The prevalence of iron overload among transfusion dependent anaemia patients at Muhimbili National Hospital was 72.5%. Serum ferritin and transferrin saturation were observed to be significantly elevated in all clinical diagnoses observed in this study.

**Recommendation**: Having confirmed that iron overload is common among transfusion dependent anaemia patients, there is a need of screening for iron overload in a patient with a history of recurrent blood transfusion of ≥ 10 units or ≥ 100mls/kg in lifetime.

Ferritin level of > 1000ng/ml and TSAT of > 45% in a patient with the history of receiving blood transfusion of ≥ 10 units or ≥ 100mls/kg in life time, chelation therapy should be initiated since it is effective at reducing iron burden and preventing organ damage.

Serum ferritin and transferrin saturation are the easily available tests that can be used to diagnose transfusional iron overload in our setup.

## DEFINITION OF KEY TERMS.

**IRON OVERLOAD:**

It is the abnormal accumulation of iron in various body organs including the heart and liver as well as the endocrine system, leading to organ and system failure.

**TRANSFUSIONAL IRON OVERLOAD:**

Is the accumulation of iron in the liver and heart organs as well as in the endocrine system, in patients who receive or did receive frequent blood transfusions in lifetime**.**

**MULTIPLE BLOOD TRANSFUSIONS**

When a patient receives blood transfusions repeatedly as a treatment for transfusional dependent anaemia, it involves receiving 10 units or more of red blood cells in adults and ≥100mls/kg body weight in children in a lifetime**.**

**TRANSFUSION DEPENDENT ANAEMIA:**

Anaemia that is unresponsive to treatment but to blood transfusion, notably in conditions such as thalassemia major, sickle cell disease, myelodysplastic syndrome, aplastic anaemia and haemolytic anaemia.

**DELAYED PUBERTY:**

The absence of testicular development by age 14 years in boys and the absence of breast development by the age of 13 years and no menstrual periods by age 16 years in girls.

**SEVERITY OF ANAEMIA (WHO):**

Mild anaemia- Hb of 11-11.9g/dl in a non-pregnant woman and 11-12.9g/dl in a man.
Moderate anaemia- Hb of 8-10.9g/dl in both women and men
Severe anaemia- Hb below 8g/dl in both women and men

## LIST OF ABREVIATIONS

| | |
|---|---|
| **CBC** | Complete Blood Count |
| **CRP** | C Reactive Protein |
| **DCytb** | Duodenal Cytochrome b |
| **DFO** | Deferoxamine |
| **DMT1** | Divalent Metal Transport 1 |
| **DNA** | Deoxyribonucleic acid |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **HEPC1** | Hepcidin gene 1 |
| **HFE** | Human factors engineering. |
| **HH** | Hereditary Haemochromatosis |
| **HHC** | Hereditary Hemochromatosis |
| **HIV** | Human Immunodeficiency Virus |
| **LIC** | Liver iron concentration |
| **LMIC** | Low Medium Income Countries |
| **MCH** | Mean Corpuscular Haemoglobin |
| **MCHC** | Mean Corpuscular Haemoglobin Concentration |
| **MNH** | Muhimbili National Hospital |
| **MRI** | Magnetic resonance imaging |
| **NTBI** | Non-transferrin bound Iron |
| **PCV** | Packed Cell Volume |
| **PI** | Principal Investigator |
| **RBCs** | Red Blood Cells |
| **TDA** | Transfusional dependent anaemia |
| **TIBC** | Total Iron Binding Capacity |
| **TSAT** | Transferrin Saturation |
| **UK** | United Kingdom |
| **USA** | United States of America |
| **WHO** | World Health Organization |

# CHAPTER ONE

## 1.0 INTRODUCTION

Iron overload is a condition resulting from increased total body iron stores, with or without organ dysfunction and it is divided into hereditary and acquired.

Hereditary iron overload is mainly due to HFE gene mutation, while acquired causes are due to increased intake, parenteral iron administration and red bloodcell transfusion.(1)

A number of conditions associated with chronic anemia may result in the need for multiple blood transfusions and a consequent risk for transfusional iron overload. These conditions may **syndrome,** include leukemia, aplastic anaemia, myelodysplastic sickle cell disease, thalassemia major and other types of anaemiasuch as post bone marrow transplant with poor red cell line engraftment and post chemotherapy anaemia.(1)

Transfusional iron overload remains to be a major serious health related disease condition among the patients with refractory anemia, it has been reported that patients receiving multiple blood transfusions are commonly in need of receiving transfusions of more than or equal to 10 transfusions or 20 units of red blood cells.(2)

The human body has no active mechanism for the excretion iron. Iron overload occurs when the body's iron transport and storage capacities have been exceeded. Accumulation of free iron in tissues occurs within few years in transfusion dependent patients. Iron toxicity occurs when free iron in tissues causes organ damage and dysfunction .(3)

In transfusional iron overload, free iron accumulates in various organs after iron storage proteins become saturated. This free iron is transported to and deposited primarily in parenchymal cells of the liver, heart, pancreas and endocrine tissues(4).

The mechanism of iron toxicity in parenchymal cells in involved organs is basically related to formation of hydroxyl radicals from superoxide or hydrogen peroxide, after the reduction of ferric to ferrous iron and these reactive oxygen species have a greater capacity for chronic cell toxicity and DNA damage.(5)

Study done by Taher A et al shows that patients with thalassemia intermedia having refractory anaemia had features of ineffective erythropoiesis which resulted to inappropriate suppression of the iron regulating peptide hepcidin which contributesto iron overload.(6)(7)

Hepcidin is a 25-amino acid peptide produced by hepatocytes, it is a key iron regulatoryhormone. It acts by inhibiting intestinal iron absorption and iron release from hepatic stores and from macrophages recycling senescent RBCs. Increased hepcidin levels

have been associated with anemia of inflammation, and hepcidin deficiency may be the common pathogenic feature of hereditary hemochromatosis .(8)

Serum ferritin has been extensively used as an easily accessible serum marker for transfusional iron overload. The ferritin level that has been used as a cutoff point for iron toxicity varies in studies from 1000ng/ml to 3000ng/ml. The relationship between serum ferritin and total body iron stores has been clearly established by strong correlations with hepatic iron concentration and amount of iron removed by venesection(9).

Liver biopsy has been used as the gold standard diagnostic test for transfusion iron overload and allowing assessing the degree of transfusion iron overload and estimating prognosis of the chronic effect of iron overload.(10)(11)

MRI is the noninvasive means of imaging choice and can detect iron deposition in the liver, heart, joints, and pituitary. However, in assessment of myocardial iron loading with the use of gradient echo T2* has been reliable confirmatory test.(12)

CT scanning has a limited sensitivity for the assessment of hepatic iron overload. However, an elevated hepatic CT density associated with an elevated serum ferritin indicates iron overload but normal hepatic CT density does not exclude iron overload.(13)

Transfusional iron overload may be prevented and treated by an efficient Iron Chelators. Iron chelators are agents that promote negative iron balance by binding and allowing excretion of iron in a nontoxic form.(14)

Iron chelators which are often used includeDeferoxamine, Deferiprone and Deferasirox (15).

Study has shown phlebotomy has great benefit among patients of SCD who have presented with stroke.(16)however options for the treatment of iron overload following other haematological causes of overload have not yet revealed its greatest feasibility to use but many studies have documented its efficacy in early and late post-transplant setting.(17)

## 1.2 LITERATURE REVIEW

### 1.2.1 Transfusional iron overload overview

Clinically diagnosed thalassemia major and intermedia were collected from different hospitals in India for their serum ferritin estimation and it was observed that 10 to 12 units of red blood cells are enough to raise serum ferritin to 1000ng/ml.(18)

Chong Gao from China analyzed 13 patients who were transfusion dependent for more than one year so as to assess the clinical outcomes of transfusion-associated iron overload in patients with refractory anaemia, he observed that most people would develop iron overload transfusion after 10-20 units of red blood cells.(2)

Patients with high risk of developing transfusional iron overload were those with beta thalassemia and 57.4% had higher levels of serum ferritin after being multiply transfused.(19)

Comparison of iron overload among subjects with thalassemia major and sickle cell disease done in USA, Canada and UK, showed that subjects of thalassemia major had more evidence of diabetes, hypogonadism, hypothyroidism and growth failure and by multivariate analysis indicated that endocrinopathies was more likely in thalassemia major than sickle cell disease .(20)

African iron overload is a disorder characterized by abnormally elevated levels of iron in the body. It originated from Sub Saharan Africa, whereby individuals were affected after being exposed from drinking a traditional, homemade beer that contained high amounts of iron. Apart from the high dietary iron content, a genetic defect also contributes to iron overload in Africa. (21)

Mutation in the ferroportin 1 gene is associated with mild anaemia and tendency to iron loading, this was observed from a study done in USA among African American general population. (22)

Researchers believe that African iron overload often goes unrecognized and is under diagnosed, making it difficult to determine its true frequency in the general population. Symptoms of the following chronic blood transfusional iron overload can be similar to those of African iron overload and the comparisons may be useful for a differential diagnosis. (23)

### 1.2.2 The prevalence and outcome of transfusional iron overload in patients with transfusional dependent anaemia.

The prevalence of transfusional iron overload in patients with transfusion dependent anaemia has not been well documented in LMIC.

A comparative studies done in United States, Canada and United Kingdome involved 31 subjects concerning organ dysfunction in adolescents and adults with transfusional iron overload and thalassemia and sickle cell disease and a control group of sickle cell disease without iron overload. It showed that, transfused sickle cell disease and thalassemia patients were hospitalized more frequently with longer duration of stay in the hospitals, also number of deaths were more in transfused subjects.(24)

Twenty five aplastic anaemia patients who were diagnosed to have transfusional iron overload were studied in Korea and it was found that endocrinopathies are common in transfusional iron overload, although transfusional iron overload has been considered to be less harmful than hereditary hemochromatosis. (25)

Adult patients with sickle cell disease are at jeopardy to develop serious complications caused by iron overload not only that, but also iron overload is a prognostic indicator of morbidity and mortality in patients with sickle cell disease. The reasons of Iron overload to cause complications was due to its effect of accelerate the immuno modulator of blood transfusion and the immunosuppression caused by the disease itself. (26)

Among transfused sickle cell disease and thalassemia major patients, both transfused patients had elevated levels of iron storage and subjects with thalassemia major had a greater risk for endocrinopathies than those with sickle cell disease. This suggested that, endocrine organ failure is often noted to be a complication of chronic red blood cell transfusion related iron overload.(20)

The factors that determine iron loading into the heart and endocrine organs in TM remain unclear. A key candidate that may be critical in determining the distribution of iron to various organs is plasma non transferrin bound iron (NTBI). However there have been no studies that examine the relationship between the progression of iron overload and NTBI levels in thalassemia or sickle cell disorders. (27)

In sickle cell anaemia, the relationship between iron overload and distribution to organs such as the heart is even less well defined than in Thalassemia major. In sickle cell anaemia ineffective erythropoiesis does not play a major role and, in the absence of blood transfusion, iron overload is not seen. However, the management of sickle cell anaemia in recent years has increasingly included the use of blood transfusions to prevent and treat complications such as stroke, chest syndrome and venous ulcers. (28)

This in turn has resulted in patients developing significant iron overload, necessitating treatment of some patients with iron chelating agents. End organ damage from iron overload is less well described in sickle cell anaemia than in thalassemia major, even in the context of a high transfusional iron load and high hepatic iron loading. (29)

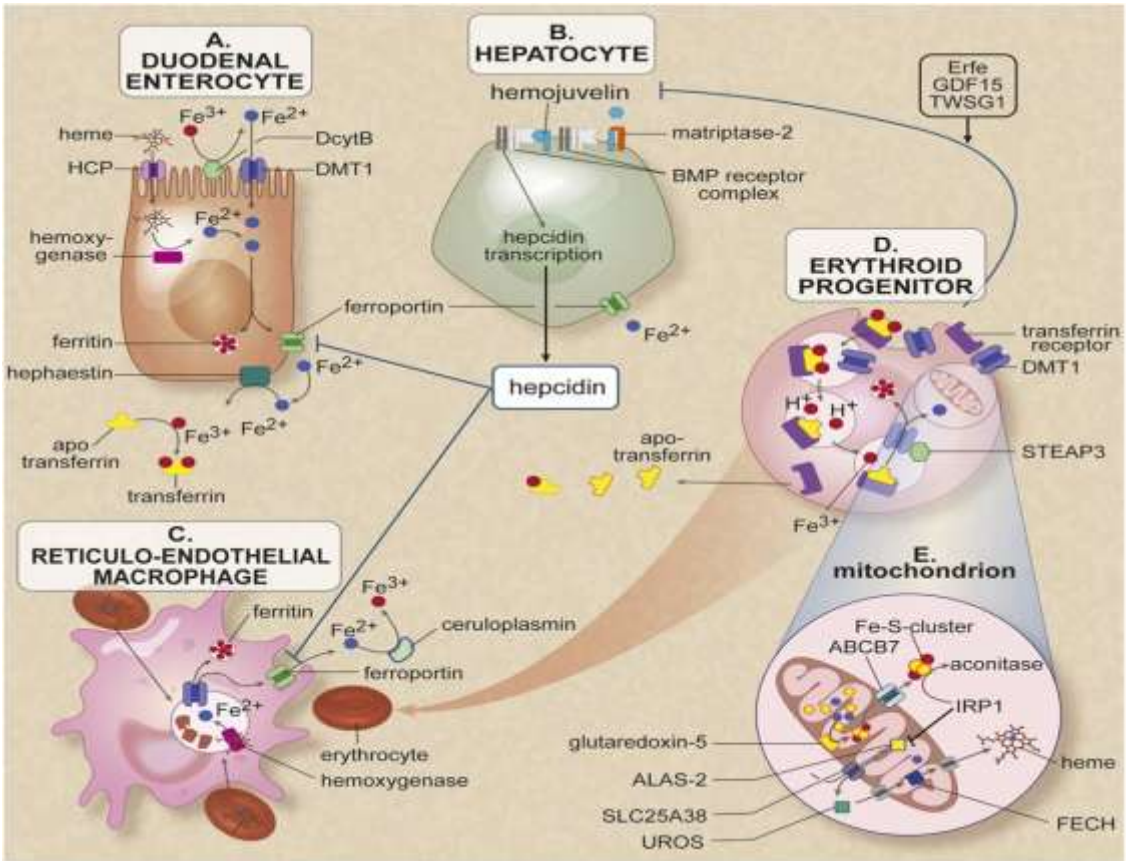
### 1.2.3. The clinical diagnosis and clinical characteristics of transfusional iron overload

Clinically, in every unit of blood transfused, contains approximately 250 mg of iron, therefore, patients having different transfusion dependent hematological disorders receiving numerous red blood cells transfusions end up having excess iron from the transfused erythrocytes gradually accumulating in various tissues, causing morbidity and mortality. (30)

A study done in Switzerland shows that, transfusional iron overload increases the risk of developing secondary diabetics mellitus, However, the controversial has been observed to be unlikely significant of high level of ferritin to sensitized insulin resistance among an individual with transfusional dependent anaemia.(31)

Patients who are repeatedly exposed to blood transfusion may slowly develop iron overload toxicity complications. In United Kingdom, a study was done among β thalassemia patients and it showed that, the main cause of death was due to anaemia, infections, and cardiac diseasedue to iron overload. (32)

### 1.2.3 Iron metabolism(35)




### 1.2.4 Diagnosing iron overload

Serum ferritin is a practical, cheap and easily accessible indicator of iron burden in the body, although additional methods like transferrin saturation, NTBI, LPI and MRI are needed for a precise diagnosis of iron overload .(33)

In a study done in Fargo, North Dakota which was looking at the prevalence of iron overload in children with solid and hematological malignancies, ferritin was done and ESR and CRP were also done to exclude inflammatory process, they concluded that serum ferritin levels were more likely to be elevated in transfused patients than non-transfused patients .(34)

### 1.3 Conceptual frame work

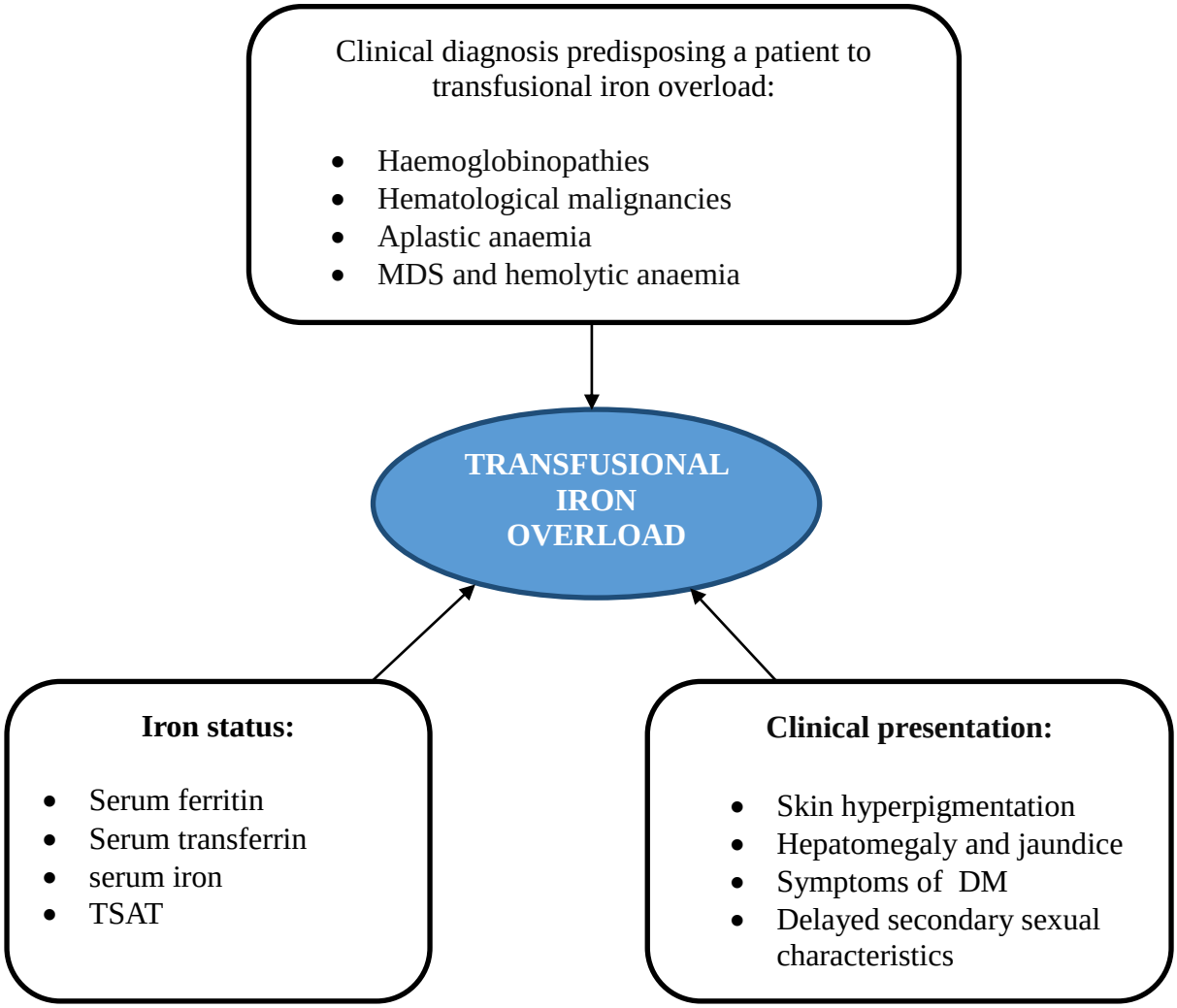



**Figure 1: Conceptual frame work**

### 1.3.1Variables:

Figure 2: Shows the study variables of which Dependent variable were TIO, serum ferritin, serum transferrin, serum iron, TSAT and independent variables were Haemoglobinopathies, Hematological malignancies, aplastic anaemia, MDS and hemolytic anaemia and Skin hyperpigmentation, Hepatomegaly and jaundice, symptoms of diabetes mellitus and delayed puberty.

**1.4 Problem statement:**

Transfusion therapy in transfusion dependent anaemia patients improves both the quality of life and prolongs survival.

A consequence of chronic transfusion therapy is secondary iron overload which adversely affects the function of the heart, liver and other organs hence increasing mortality and morbidity.

Globally, leading cause of death in patients with transfusion dependent anaemia is cardiac disease from myocardial iron deposition.

In MNH, despite transfusing patients with transfusion dependent anaemia, screening for iron overload and iron chelation therapy is not provided.

Several researchers have been published on the subject worldwide, but there is a very limited data on prevalence of transfusional iron overload among transfusion dependent anaemiapatients in Africa.

This creates an obvious gap in knowledge that this study intended to fill.

**1.5 Rationale:**

The limited information in the form of research in our country has prevented patients with transfusion dependent anemia from being provided with iron chelation therapy as part of their management.

Although iron overload is a predictable consequence of recurrent blood transfusion therapy, many clinicians especially in developing countries have little or no experience with such patients and therefore are unfamiliar with transfusional iron overload and the associated risk of iron toxicity.

Despite the fact that this is the first study in Tanzania pertaining transfusional iron overload amongst patients with transfusion dependent anaemia, the study findings will be expected to form the protocols for therapy initiation and the basis for further larger iron overload studies in transfusion dependent anaemia patients in Tanzania and worldwide.

## 1.6 Research objectives

### 1.6.1 Broad objective:

To determine the prevalence, clinical presentation, diagnosis and iron status among patients with transfusion dependent anaemia attending Muhimbili National hospital.

### 1.6.2 Specific objectives:

1. To determine the prevalenceof transfusional iron overload among patients with transfusion dependent anaemia attending at MNH.
2. To document the primary diagnoses associated with transfusion dependent anemiaat MNH.
3. To describe the clinical presentationscharacterizedby transfusion dependent anaemia in patients with transfusional dependent anemia attending MNH
4. To describe the iron status among patients with transfusional dependent anaemia attending MNH.

# CHAPTER TWO

## 2.0 METHODOLOGY:

### 2.1 Study design:

This is a prospective descriptive hospital based cross sectional study.

### 2.2 Study duration:

The study was conducted over a period of four months.

### 2.3 Study Area and Study Population:

The study was conducted at Muhimbili National Hospital (MNH) located in Dar es Salaam which is the largest city, in the united republic of Tanzania. According to the 2012 national population census, the city has a total population of about 4.3 million.

MNH serves as the national referral hospital and university teaching hospital with 1500 bed facility, attending 1200 outpatients per week, admitting 1200 inpatients per week.

MNH receives referred patients from Dar es Salaam district hospitals as well as other hospitals from within the city. Regions nearby or far from Dar es Salaam also refer some patients to MNH.

MNH offers services in different departments like surgery, obstetrics and gynecology, paediatrics, psychiatry and internal medicine. Internal medicine has bed capacity of 210 beds and it receives total admissions of 40-60 patients per day. Internal medicine department is divided into units namely, pulmonology, gastroenterology, endocrinology, nephrology and hematology units.

Muhimbili National Hospital has a hematology unit which offers diagnosis and multidisciplinary treatment of many hematological diseases such as malignant blood diseases, hereditary haemoglobinopathies,bone marrow failure syndromes and myeloproliferative diseases. Hematology unit admits about 80 patientsand about 200 patients attend out-patient hematology clinic in a period of one month.

Paediatrics department has units including general paediatrics, paediatric oncology, diarrhoea, malnutrition and neonatal. General paediatric ward receive about 9-15 patients per day of which 2-5 out of admissions are hematological diseases mostly sickle cell disease, hemophilia and aplastic anaemia.Paediatric hematology clinic is run every Thursday and it receives about 50 patients in a week.

Paediatric oncology unit admits about 0-5 patients per day whereby about 2-3 patients out of 5 admissions are of hematological malignancies.Patients who are discharged are followed up weekly for a period of more than two years.

The central pathology laboratory (CPL) is the biggest laboratory at MNH, consisting of six units, namely, parasitology, microbiology, histopathology, hematology and blood transfusion, clinical chemistry and mortuary. Most of the tests including the rare tests from MNH and the nearby hospitals including the private hospitals are done in CPL. It is the leading diagnostic laboratory service provider in Tanzania and is operating in 24 hours. Units in this department are computerized and results are posted in the Jeeva system for clinicians to view online in the wards or clinics.

Clinical chemistry unit has fully automated machines including the combined state of Art Architec 4100 machine which wasused to test serum ferritin, iron, transferrin and CRP. The machine is capable of analyzing both immunological, hormonal assays, metabolic tests and it has the capability of testing 1200 samples per hour.

Hematology unit is responsible for analyzing samples such as Complete Blood Count (CBC) and other tests for blood related diseases including hematological malignancies whereas blood Transfusion section deals with pre and post donation counselling of blood donors.

Blood Transfusion section screens all internally donated blood for HIV, Hepatitis B and C and Syphilis from donors.It isalso responsible for issuing safe and screened blood to all patients in need of blood within the hospital.It is capable of issuing about 2075 units in all departments per month whereas about 269 units are issued in the internal medicine department and 150 units in the paediatric ward in a period of one month.

### 2.3.1 Inclusion criteria

**All patients who have consented** and are two years old or above with history of recurrent blood transfusion and have received 10 units RBCs or more and/or ≥100mls per kilogram body weightin his or her lifetime.

### 2.3.2 Exclusion criteria

Patients with anaemia due to acute blood loss like in postpartum hemorrhage, post trauma and patients who have received multiple blood transfusion but having a non-hematological clinical diagnosis like solid tumors.

### 2.4 Sample size calculation

All patients with transfusion dependent anaemia who have received ≥ 10 units or ≥ 100mls/kg of red blood cells, attending MNH were included in the study.

### 2.5 Recruitment of study subjects:

Convenience sampling technique was used to obtain study subjects.

Study subjects were recruited by the principalinvestigator, whereby adults were recruited from the medical wards and general hematology clinic.

Children were recruitedfrom the general paediatric wards, paediatric hematology clinic, paediatric oncology clinic and paediatric oncology wards during five working days Monday to Friday.

Patients in the adult ward were recruited on Monday from 9:00 am to 1pm and patients who attended the general hematology clinic were recruited on Friday from 9 am to 5 pm.

Patients in paediatric hematology ward were recruited on Tuesday from 8:00 am to 11 am, thereafter those from paediatric oncology ward were recruited from 11:30 am to 2 pm on the same day. Patients from paediatric oncology clinic were recruited on Wednesday from 9am to 1pm. Patients from paediatric hematology clinic were recruited on Thursday from 9 am to 2 pm.

All the information regarding the study objectives and procedures were explained to the patients, parents and guardians. Patients were required to provide written informedconsent before enrollment into the study.

**2.6Procedures and data collection:**

Data collection commenced after the obtaining the ethical clearance.Patients (or parents/guardians) were provided with written, informed consent/assent before being recruited. A structured questionnaire was used by the principle investigator to obtain patient's information from the patients themselves and from the parents or guardians on behalf of paediatric patients, Case files and laboratory results were also used.

The informationfilled in the questionnaire included patient's name, his/her file number, age, sex, level of education and occupation of the patients or parents/guardians, history of blood transfusion, number of blood transfusions received, history of iron chelation use, and clinical diagnosis.

Clinical history including age at puberty was obtained. Ingirls, delayed puberty was marked by lack of menstruation at the age of 16 years old andlack of breasts by age of 13 years old. Lack of testicular enlargement at 14 years old in boys was considered as delayed puberty. Symptoms of diabetes mellitus like polyuria, polyphagia, polydipsia and blurred vision were recorded. Patients were examined for pallor, jaundice, pulse rate,skin changes, and and liver size was also recorded. Body weight was measured by using Secca weighing scale in children. This was done by the principle investigator and the information was put in the structured questionnaire.

Data was collected from the admitted patients in adult medical wards, general pediatric wards, pediatric oncology ward and outpatients.

**2.6.1 Sample collection:**

Blood for laboratory analysis was collected by venipuncture.

The venipuncture site depended on the accessibility of the vein. The most frequently used venipuncture sites like the median cubital and cephalic veins of the arm. Preparation of the puncture site includes application of tourniquet and vein palpation, sterilizing the site by the methylated spirit. Then a 10cc needle and 5cc needle in adults and paediatrics was inserted respectively and blood was drawn while releasing the tourniquet. Thereafter pressure was applied over the vein with the help of bandage, the used needle was disposed into a sharps container and the specimen waslabeled.For each of the study participant, the principle investigator drew 8mls of venous blood in adults and 5mls in paediatric patients and 3mls and 2mls of bloodwas put into an EDTA tube with purple top for determination of complete blood count in adults and paediatrics respectively and 4mls/3mls for adults/paediatrics was put into a plain bottle with red top for determination of C- reactive protein, serum ferritin, serum iron and serum transferrin. The samples were sent to clinical chemistry and hematology laboratory for processing.

**2.6.2 Sample processing**:

Collected samples were put in the storage box for transportation to the central pathology lab within 2 hours after collection at temperature of 20-25$^0$C. For the serum ferritin, serum iron, serum transferrin and CRP, the samples were centrifuged at 3500 rpm for ten minutes within 6 hours of samples arrival. The sample in the same plain bottle having the serum and the clotted RBCs was put in the machine to be run in the Architectplus machine module ci 4100. It takes 15 to 20 minutes for the results to be out. The results were automatically entered in the Jeeva system.

Samples collected for complete blood count were transported to the central pathology laboratory within room temperature and by the help of the laboratory technician3700 celldyn machine was used to run the test within six hours after collection.Results were displayed in the Jeeva system so as to be viewed by the principle investigator.

**2.6.3 Principles and procedures of the tests:**

**2.6.3.1 Hematology tests**

Specimens for complete blood count wereanalyzed using a CellDyne 3700 automatic machine within 6 hours of sample arrival in the laboratory by the laboratory technician.

**2.6.3.2 Chemistry tests**

The architect ferritin assay is a type of immunoassay used to determine the presence of ferritin in human serum and plasma by using a Chemiluminescent Microparticle Immunoassay technology.

At a pH of 4.8, iron is released from transferrin to which it is bound, and then reduced to a ferrous state. Then iron forms a stable colored complex of which the color intensity is proportional to the amount of iron in the sample tested.

Transferrin is a beta globulin synthesized in the liver primarily, it is the protein responsible for iron transport.

The formation of insoluble immune complexes by addition of transferrin antibody, makes the transferrin concentration able to be measured by turbidity function.

Indication for serum iron assay in this study is to screen for iron overload by comparing it with serum ferritin, making sure that elevated ferritin levels are not due to defensive mechanism like reacting to inflammation or infection as acute phase reactant protein. Normal range is 4.4-27.9µmol/l

Indications for ferritin assay include; screening for iron overload, iron deficiency anaemia and acute phase reactant in inflammatory process. The range of 20-300ng/ml is considered normal. Iron overload will be considered when patient has a serum ferritin of ≥ 1000ng/ml regardless of the CRP level.

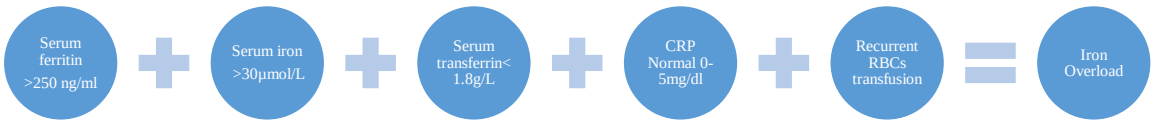
Serum transferrin being below the lower limit of normal indicates the presence of iron overload, hence it is used as one of the screening tests for iron overload. The normal range is 1.8-3.91g/l.

C - reactive protein is an antigen-antibody reaction between CRP in the sample and polyclonal anti-CRP antibody, presented as agglutination which is detected as an absorbance change with the magnitude of the change being proportional to the quantity of CRP in the sample.Its main indication in this study is to rule out the inflammatory process that could interfere with the results of serum ferritin. The normal range is 0-5 mg/dl.

**2.6.4 Diagnostic criteria for iron overload:**

A ferritin level of ≥250 ng/mL, the transferrin level of ≤1.8g/L with the increased levels of serum iron of ≥30µmol/L and in the absence of clinical picture or signs of inflammation such as increased CRP is consistent with iron overload. Serum ferritin being the practical measure of body iron as it has been observed in many studies, its elevation with normal C - reactive protein signifies iron overload.

Serum ferritin of ≥ 1000ng/m/L, history of receiving ten transfusions or more in adults and ≥ 100mls/kg of red blood cells in paediatrics, regardless of the level of CRP, it signifies iron overload.




**OR**

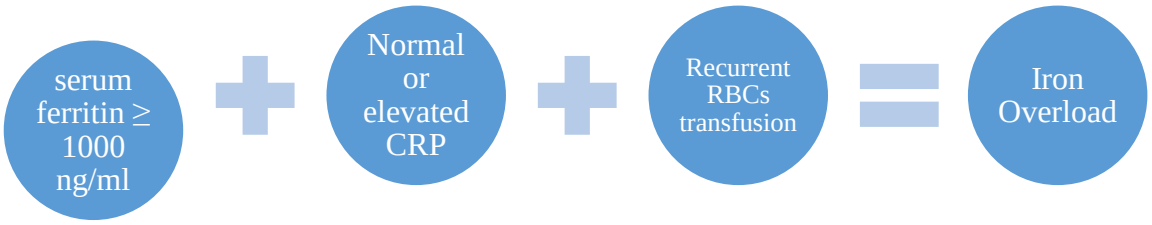



**Figure 2: Criteria for diagnosing iron overload.**

**2.7Data management and statistical analysis.**

The collected data was checked for quality and coding, thiswas done prior entering into the computer (SPSS version 20) statistical programme, which was used for analysis.

Categorical data were assessed by Chi-square analysis test. P-value of less than 0.05 was considered statistically significant**.**

**2.8 Ethical consideration.**

Ethical clearance was sought from Research and Publications Committee of Muhimbili University of Health and Allied Sciences. The permission to conduct this study was sought from authorities of MNH.A formal written informed consent for those aged 18 years and above,assent for those aged 7 to 17 years old and informed permission from the parents/guardians for those aged below 7 years of age in Swahili was sought from participants. Patients' information was kept confidential and information collected in the questionnaire was entered into computer using identification numbers to maintain confidentiality. Patients/parents who werefound to have iron overload were well educated on the complications which may result, away of managing the condition by use of iron chelation therapy. Deferiprone and/or deferoxamine were prescribed to be used as a single drug or in combination for at least one month for patients who have stopped receiving blood transfusion and for the patients who were required to receive more blood. The iron chelators were prescribed for life.The patients were supposed to get these medications outside MNH because they are not available inside MNH.

# CHAPTER THREE

## RESULTS

A total of 112 participants were recruited, 60 (53.6%) were males and 52(46.4%) were females. Most of participants belong to the age group between 2 and 17 years 59 (52.7%). between 18 and 50 years were 43 (38.4%). Those > 50 years were 10 (8.9%). Sixty (53.6%) were under eighteen and among adults 24 (21.4%) were unemployed. Majority had primary level of education 69 (61.6%) and only 15.2% attained higher education.

**Table 1: Socio-demographic data of transfusion dependent anaemia patients**

| | | **Male** | **Female (%)** | **Total (%)** |
|---|---|---|---|---|
| | | 60 (53.6) | 52 (46.4) | 112 (100) |
| **Age** | 2 - 17 years | 22 | 37 | 59 (52.7) |
| | 18 - 35 years | 16 | 12 | 28 (25.0) |
| | 36 - 55 years | 9 | 6 | 15 (13.4) |
| | > 55 years | 5 | 5 | 10 (8.9) |
| **Occupation** | Under eighteen | 22 | 38 | 60 (53.6) |
| | Public servant | 6 | 4 | 10 (8.9) |
| | Peasant | 9 | 9 | 18 (16.1) |
| | Petty business | 15 | 9 | 24 (21.4) |
| **Marital status** | Married | 21 | 8 | 29 (26) |
| | Single | 36 | 47 | 83 (74.1) |
| **Education** | None | 13 | 15 | 28 (25) |
| | Primary level | 32 | 37 | 69 (61.6) |
| | Secondary level | 2 | 3 | 5 (4.5) |
| | Higher Education | 5 | 12 | 17 (15.2) |
| **Body weight** | 10-30 kg | 17 | 13 | 30 (26.8) |
| | >30kg | 35 | 47 | 82 (73.2) |

Table 2 shows that, the most frequent clinical presentation observed was pallor 93 (83%) followed by cardiac arrhythmias 64 (57.1%). Skin color change was observed in only 15 (13.4%) patients. Delayed puberty was noted in 6 patients (5.4%) only.

**Table 2: Description of clinical presentation characterized by TDA**

| | **Male (%)** | **Female (%)** | **Total (%)** |
|---|---|---|---|
| **Pallor** | | | |
| Yes | 51 | 42 | 93(83) |
| No | 9 | 10 | 19(16.9) |
| **Jaundice** | | | |
| None | 46 | 37 | 83(74.1) |
| Tinge | 14 | 13 | 27(24.1) |
| Deep | 0 | 2 | 2(1.8) |
| **Skin colour change** | | | |
| Normal | 49 | 48 | 97(86.6) |
| Skin hyperpigmentation | 11 | 4 | 15(13.4) |
| **Liverspan** | | | |
| Normal | 50 | 41 | 91(81.3) |
| Hepatomegaly | 10 | 11 | 21(18.8) |
| **Cardiac manifestation** | | | |
| Tachycardia | 29 | 23 | 52(46.4) |
| Bradycardia | 6 | 6 | 12(10.7) |
| Normal | 25 | 23 | 48(42.9) |
| **Endocrine features** | | | |
| Delayed puberty | 2 | 4 | 6(5.4) |
| Diabetes Mellitus | 1 | 0 | 1(0.9) |
| None | 53 | 52 | 105(93.8) |

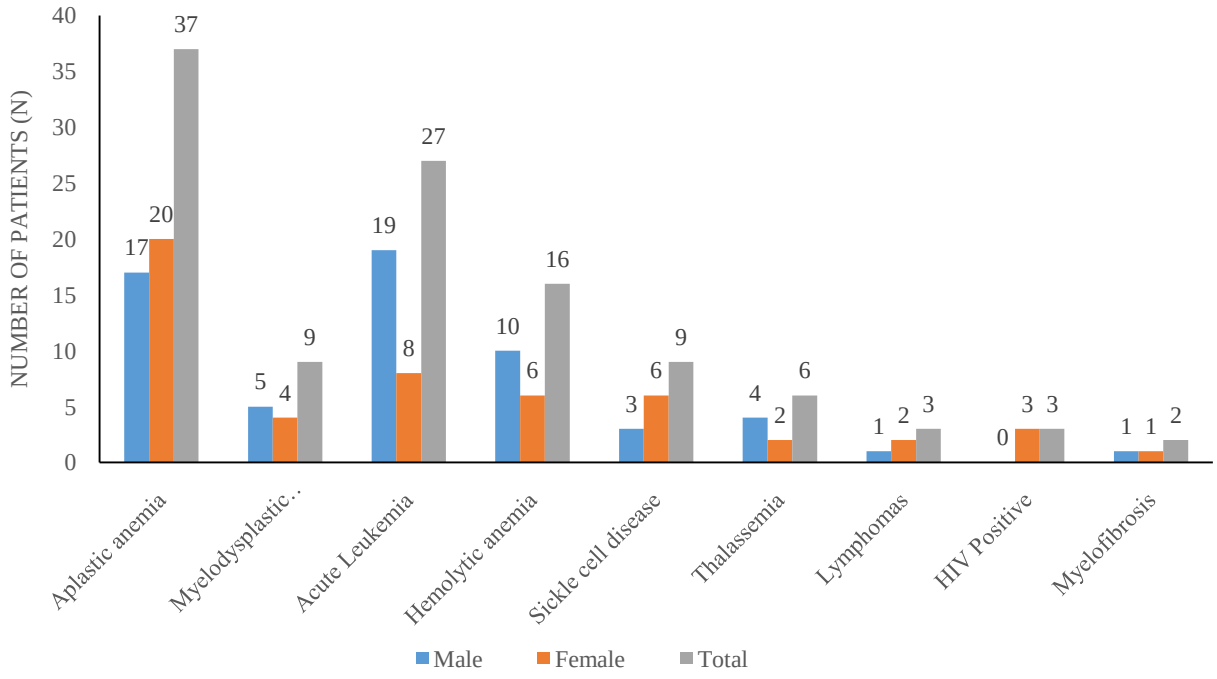



**Figure 3: Description of clinical diagnosis among transfusion dependent anaemia patients**

Figure 3 shows that, among the several diagnosis observed, aplastic anaemia was leading 37 (33%) followed by acute leukemia 27 (24.1%) patients, mostly were men 19(17%).

Table 3shows that 83 (74.1%) had ferritin levels of > 1000ng/ml and 80 (71.4%) had TSAT levels of > 45%. Forty six (41.1%) patients had elevated serum iron levels above 30 µmol/l. Transferrin levels of < 1.7g/l were observed in 50 (44.6%). Sixty seven (59.8%) had normal CRP levels.

**Table 3: Description of Iron status among patients with transfusion dependent anaemia**

| | **Male (%)** | **Female (%)** | **Total (%)** |
|---|---|---|---|
| **Serum ferritin level (ng/mL)** | | | |
| 250-1000**ng/mL** | 18(16.1) | 11(9.8) | 29(25.9) |
| >1000**ng/mL** | 44(39.3) | 39(34.8) | 83(74.1) |
| **Serum Iron (µmol/l)** | | | |
| 5-10**µmol/l** | 5(4.5) | 5(4.5) | 10 (8.9) |
| 11-30**µmol/l** | 29(25.9) | 27(24.1) | 56 (52.9) |
| >30 **µmol/l** | 26(23.2) | 20(20.0) | 46 (41.1) |
| **Transferrin (g/l)** | | | |
| 1.7-3.8**g/l** | 32(28.6) | 28(25.0) | 60 (53.6) |
| < 1.7-3.8 **g/l** | 28(25.0) | 22(19.6) | 50 (44.6) |
| > 3.8**g/l** | 0(0.0) | 2(1.8) | 2 (1.8) |
| **CRP (mg/dl)** | | | |
| 0-5 **mg/dl** | 36(32.1) | 31(27.7) | 67 (59.8) |
| 6-25**mg/dl** | 18(16.1) | 14(12.5) | 32 (28.6) |
| >26**mg/dl** | 6(5.4) | 7(6.3) | 13 (11.6) |
| **TSAT (%)** | | | |
| < 16-45**%** | 5(4.5) | 4(3.6) | 9 (8.1) |
| 16-45**%** | 13(11.6) | 10(8.9) | 23 (20.5) |
| > 45**%** | 40(35.7) | 40(35.7) | 80 (71.4) |

Table 4 shows that serum ferritin of 250 to more than 1000ng/ml under severity of anaemia had a p-value of 0.563 which was not significant and TSAT ranging from <45% to >45% had a p-value of 0.686 which was not significant. Serum ferritin and TSAT levels were observed to be statistically significant with a p-value of <0.01 in relation to duration of blood transfusion, whereas regarding number of units ferritin and TSAT p-values were <0.01 and 0.01 respectively and this was statistically significant.

**Table 4: Relation between duration of blood transfusion, number of BT, severity of anaemia and iron status in patients with transfusion dependent anaemia.**

| Variable | Characteristics | Ferritin(ng/ml) | | | TSAT | | |
|---|---|---|---|---|---|---|---|
| | | 250-1000 | >1000 | P Value | <45% | >45% | P Value |
| Severity of anaemia | Mild | 2 | 5 | 0.563 | 2 | 5 | 0.686 |
| | Moderate | 13 | 37 | | 14 | 36 | |
| | Severe | 14 | 41 | | 16 | 39 | |
| Duration of transfusion | Less than 1 year | 11 | 30 | <0.01 | 12 | 29 | <0.01 |
| | 1 year and above | 18 | 53 | | 20 | 51 | |
| Number of units | >100mls/kg | 6 | 18 | <0.01 | 7 | 17 | 0.01 |
| | 10 -20 units | 15 | 41 | | 16 | 40 | |
| | >20 units | 8 | 24 | | 9 | 23 | |

Table 5 shows that number of males (60) is more than females. (52) The number of those having serum ferritin > 1000ng/ml is 70% for males and 78.8% for females. Out of thirty seven patients with aplastic anaemia, 83.8% had serum ferritin level of >1000ng/ml, out of twenty seven patients with acute leukaemia, 66.7% had serum ferritin of >1000ng/ml and five patients out of 9 with SCA had serum ferritin of >1000ng/ml.

**Table 5: Relationship of gender and clinical diagnosis with serum ferritin level.**

| **Variable** | **Serum Ferritin Level** | | **Total** | **P-value** |
|---|---|---|---|---|
| | **250-1000ng/ml (N %)** | **>1000ng/ml (N %)** | | |
| **Genders** | | | | |
| Male | 18(30) | 42(70) | 60 | |
| Female | 11(21.2) | 41(78.8) | 52 | 0.198 |
| **Clinical Diagnosis** | | | | |
| **Aplastic anaemia** | | | | |
| YES | 6(16.2) | 31(83.8) | 37 | |
| NO | 23(30.7) | 52(69.3) | 75 | 0.076 |
| **Acute leukemia** | | | | |
| YES | 9(33.3) | 18(66.7) | 27 | |
| NO | 20(23.5) | 65(76.5) | 84 | 0.221 |
| **Sickle cell disease** | | | | |
| YES | 4(44.4) | 5(55.6) | 9 | |
| NO | 25(24.3) | 78(75.7) | 104 | 0.174 |

**Table 6: Prevalence of transfusional iron overload among transfusion dependent anaemia**

| | | |
|---|---|---|
| **Prevalence of iron overload among 112 patients who were recruited in the study** | Prevalence based **ferritin (ng/mL)** | $\frac{83 \times 100}{112} = 74\%$ |
| | Prevalence based **TSAT** | $\frac{80 \times 100}{112} = 71\%$ |
| | | **$\underline{72.5\%}$** |

# CHAPTER FOUR

## DISCUSSION

In this study, a total of 112 participants fulfilled the criteria for inclusion in the study. Aplastic anaemia and acute leukemia were the commonest diagnosis observed. This study suggests that both serum ferritin and transferrin saturation are well standardized, inexpensive and widely used tests for assessment of iron overload. There was no patient on chelation therapy.

The prevalence of transfusional iron overload in this study was 72.5% which is significantly high compared to the studies in USA and Nigeria of which the prevalence has been reported to be 40.4% and 36.7% respectively(18)(19). The variations of prevalence could be due to geographical and racial differences. The small number of patients recruited in this study could also be contributory.

The majority of the study subjects in the study aged between 2 and 17 years 59 (52.7%). This is similar to the study done in Europe by Y. Aydinok which reported the median age of 18 years, depicting the fact that most of the hematological disorders which are transfusion dependent affect young people more(19).This can be explained by the presence of genetic transfusion dependent hematological disorders that manifests early or later in life is also a contributing factor(20). This study revealed that middle aged participants were also exposed to the transfusional dependent anaemia. The observed finding was similar to the study done in Spain whereby transfusional dependent anaemia was observed mainly among the 3rd and 6th decade.(21)

The majority of study subjects in the present study were males (53.6%), in line with several other studies similar to this study(19). This finding is similar to the studies done in developed and developing countries whereby the observed similarity of the male gender being higher than female was based on the inherited and acquired hematological disorders being more common in males with unknown reason .(19)(22) This also takes in account that most of the recruited patients falling in that age group had acute lymphoblastic leukemia which is reported to be higher in men than females.(23)

The most clinical sign upon diagnosis is liver enlargement. As a result of time and if left untreated liver cirrhosis and liver failure may occur. In our study 18.8% of patients had hepatomegaly, this cannot be compared with other studies because of the advanced ways that are used to detect iron deposition in the liver (24)(25)(10). In most of the patients, hepatomegaly and jaundice was observed to be related to the clinical diagnoses patients had.

It has been observed in this study, 52 (46.4%) had tachycardia and 12 (10.7%) had bradycardia, similar findings were observed in a study done by Koonrungsesomboon N et al which showed the presence of heart rate variability in thalassemia patients who were transfusion dependent(26). Heart rate variation may be considered to be used as a potential indicator of an iron overload and an early marker of cardiac involvement in patients with transfusion dependent anaemia.Currently there is a use of modern technology in assessment of cardiac iron involvement therefore heart rate variability is mainly used in LMIC(27).

Six patients (5.4%) were found to have delayed puberty, this is similar to the study done in Pakistan by Hira Tahir(28). Delayed puberty can be due to the iron deposition on gonadotrophin cells of the pituitary gland leading to disruption of gonadotrophin production**.**(29)

Only one patient was found to have features of diabetes mellitus. This can be due to the fact that the loss of insulin secretory capacity and insulin resistance plays an important role in the pathogenesis of diabetes mellitus secondary to transfusional iron overload in transfusion dependent anaemia patients.(26)

Skin hyperpigmentation was observed in 15 (13.4%) participants. Chong Gao et al also observed similar findings in his study, this could be explained by the possibility of one having hemosiderin accumulation in the skin due to the multiple blood transfusions(2).

Chong Gao assessed the clinical outcome of transfusional iron overload in patients with refractory anaemia and observed aplastic anaemia being the commonest diagnosis(2), similar to what this study have found. These findings are different from those found in a study done by Yesim Aydinok et al, which was conducted in UK, Canada, Thailand and Turkey, thalassemia major was observed in 99.1% indicating that distribution of hematological disorders differ according to the geographical regions(19). The main reason for aplastic anaemia and acute leukemia being the most occurring haematological disorders was due to the fact that there were the common cases attended during the study period. Furthermore, in this study, there was a male 19(70.4%) predominance affected by acute leukemia than female 8(29.6%) similar finding was reported by Nottage et al(33,50), this can be described by the acute lymphoblastic leukemia which is the type of leukemia observed in this study, as it is known to have increased male to female ratio.

This study revealed that most of the transfusion dependent anaemia patients had received transfusion in a period of more than one year (57%) and most (28.6%) had severe anaemia. This is similar to several studies.(30)(31)With sickle cell anaemia patients found in our study. Similar counts are reported in study done in USA.(32)

Serum ferritin, iron and transferrin saturation levels were observed to be high whereas transferrin levels were found to be low. 83 (74.1%) patients had serum ferritin levels of >1000ng/mL and 80 (71.4%) had TSAT of >45%. This is in support with several studies which have shown similar findings.(9)(33)(34)(35) C-reactive protein levels in 67(59.8%) patients was found to be normal, only 11.7% were found to have elevated C-reactive protein levels, this is in keeping with the study done by Gurram in North Dakota(18) who observed that C-reactive protein levels were normal or slightly elevated in patients with elevated serum ferritin levels. This suggests that the inflammatory contribution to the elevated ferritin level was minimal as most of the patients with elevated ferritin levels had normal or slightly elevated levels of C-reactive protein.

The observed findings in this study have been observed to be not statistically significant using p value as the major analytical significant study report findings. The observed difference in analytical significant was due to the lack of enough participants to support p value as analytical statistical finding.(36)

Serum ferritin was found to be helpful in screening for transfusional iron overload in a study done by P.T Telfer.(23) This is similar to our study findings which has shown the significant correlation of the ferritin levels to the clinical diagnoses observed in this study since 83 (74.1%) patients were found to have ferritin level of >1000ng/ml. Furthermore, duration of blood transfusion was observed to have a strong association with the ferritin level and TSAT, p value of 0.000 and 0.001 respectively, whereby the more the patient is exposed to blood transfusion, the higher risk of acquiring iron overload. It was observed that a number of ten or more transfusions was enough to cause the elevation of ferritin levels.(37)(38)Distinguishing elevated serum ferritin due to iron overload from elevated serum ferritin not due to iron overload can be done by measuring serum transferrin saturation since there is a strong correlation between serum ferritin and serum transferrin saturation levels.(38)(9)(34)

We observed that number of women recruited in this study is slightly higher than men, however there is no difference on the marked elevation of the serum ferritin for both men and women. This can be compared with the study done by Rushton in UK which showed that regardless of the repeated blood loss through menstruation, pre-menopausal women who are receiving multiple RBC units are equally affected with iron overload as compared to men.(39)

Among the clinical diagnoses that were observed, we isolated aplastic anaemia, acute leukemia and SCA to see how they relate with the level of serum ferritin, it was observed that majority of patients in each diagnosis had serum ferritin level of >1000ng/ml. This is to show

that the number of RBC units one has received in a certain period of time has a major contribution to iron overload.

# CHAPTER FIVE

## STUDY LIMITATIONS

Although the research has reached its aims, there were some unavoidable limitations.

First, because of time limit and single area of research, this research was conducted only on a small size of recruited patients who attended MNH during the study period. Therefore to generalize the results for larger groups, the study should have involved more participants at different levels.

Second, many participants failed to remember the exact number of units especially if the number exceeded 20 units, although this has not affected this study, it is a point to be considered when further studies are to be done.

## CONCLUSION

The prevalence of transfusional iron overload in patients with transfusion dependent anaemia at Muhimbili National Hospital is 72.5%.

Serum ferritin and transferrin saturationwere observed to be significantly elevated in all clinical diagnoses observed in this study.

## RECOMMENDATIONS

Having confirmed that iron overload is common among transfusion dependent anaemia patients, there is a need of screening for iron overload in a patient with a history of recurrent blood transfusion of ≥ 10 units or ≥ 100mls/kg in lifetime.

Ferritin level of > 1000ng/ml and TSAT of > 45% in a patient with the history of receiving blood transfusion of ≥ 10 units or ≥ 100mls/kg in life time, chelation therapy should be initiated since it is effective at reducing iron burden and preventing organ damage.

Serum ferritin and transferrin saturation are the easily available tests that can be used to diagnose transfusional iron overload in our setup.

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**Appendix I: PROFORMA FOR IRON OVERLOAD AMONG TRANSFUSION DEPENDENT ANAEMIA PATIENTS.**

- **Study No.....|__|__|__|__|**
- Today's Date (DD-MM-YY) .....|__|__|-|__|__|-|__|__|

**SOCIAL DEMOGRAPHIC HISTORY (**mark with a tick on the appropriate box**)**

- Hospital ID number (HID).....|__|__|__|__|__|__|__|
- Age 1. 2-17 years 2.18-35 3. 36-55 3.>55…..... |___|
- Gender1. Female 2. Male…..... |___|

**MARITAL STATUS (**mark with a tick on the appropriate box**)**

1. Married2.Single 3.Scholar4.Pre Scholar ….....……|__|

**EDUCATION LEVEL (**mark with a tick on the appropriate box**)**

1.None 2.Primary level 3.Secondary4. High Education ….....……|__|

**OCCUPATION (**mark with a tick on the appropriate box**)**

1. Under eighteen 2.Public servant3.Peasant 4. Unemployed..……|__|

**MEDICA HISTORY (**mark with a tick on the appropriate box**)**

- Clinical diagnosis1. Aplastic 2.MDS 3.A/Leukaemia 4.Haemolytic anaemia 5.SCA 6.Thalasaemia 7.Lymphoma 8.HIV 9.Myeofibrosis…..... |___|
- Blood transfusion ever received 1. > 100ml/kg 2.10 units 3.10-20 units 4. .>20units..... |___|
- Duration of blood transfusion1. <6/12 2.6-12 3.> 12…..... |___|
- History of chelation therapy use1. Yes 2. No.....……|__|
- Endocrine features1. Delayed puberty 2. D/M 3.None.....|___|

**THE CLINICAL PRESENTATION CHARACTERIZED BY TDA**

- Pallor 1. Yes 2. No…….....……|__|

- Jaundice 1.None 2. Tinge3.Deep….....……|__|
- Skin colour 1.Normal 2.Bronze hyperpigmentation……|__|
- Liver 1. Normal 2. Hepatomegaly….....……|__|
- Cardiac manifestation 1.Tachycardia 2.Bradycardia 3.Normal……|__|

**OTHERS BLOOD INVESTIGATIONS**

- WBC level (K/uL) 1. Normal 2.Low 3.High…..... |___|
- Platelet Level (K/uL) 1. Normal 2.Low 3.High…..... |___|
- Haemoglobin Level (g/dl) 1. Mild anaemia 2.Moderate anaemia 3.Severe anaemia…|___|

**THE INVESTIGATIONS OF IRON STATUS**

- **Serum ferritin level**1. 10-250**ng/mL** 2.250-1000**ng/mL** 3.>1000**ng/mL** …..... |___|
- **Serum Iron** 1. 5-10**µmol/l** 2.11-30**µmol/l** 3.>30 **µmol/l** …..... |___|
- **Transferrin** 1. 1.7-3.8**g/l** 2.< 1.7-3.8**g/l** 3.> 3.8**g/l** …..... |___|
- **CRP** 1. 0-5**mg/dl** 2.6-25**mg/dl** 3.>26**mg/dl**…..... |___|
- **TSAT**1. 16-45**%**2. < 16-45**%**3.> 45**%**…..... |___|

**Appendix II:CONSENT/ASSENT FORM (ENGLISH VERSION)**

Consent to participate in the study of the prevalence of transfusional iron overload among patients with transfusion dependent anaemia attending Muhimbili national hospital.

Dear Sir/Madam,

Greetings!

My Name is Dr. Helena Tom Kakumbula, a resident doctor in the Department of Haematology and Blood Transfusion at MUHAS. I am conducting a study regarding the **prevalence of transfusional iron overload among patients with transfusion dependent anaemia**at MNH. I am kindly requesting for your participation.

**PURPOSE OF THE STUDY:**

The aim of this study is **to determine the prevalence of transfusional iron overload among patients with transfusion dependent anaemia,describe the clinical diagnosis predisposing the patient to transfusion dependent anaemia, to describe the clinical presentation associated with transfusional iron overload and to describe the iron status among patients with transfusional dependent anaemia** attending at MNH.

**HOW TO PARTICIPATE:**

Patients who will be ready to participate will sign a consent/assent form to approve his/her willingness.Short interview will be done and blood sample for investigation will be taken.

**CONFIDENTIALITY:**

Information obtained from patients will be confidential and will help in improving the care of transfusion dependent anaemia patients.

**COSTS:**

You will not be required to pay anything for your participation.

**VOLUNTARY PARTICIPATION & RIGHTS TO WITHDRAW:**

Your participation is voluntary and you have the right to withdraw from participating in our study at any time. Whatever your decision may be, it will not affect in any way your rights to care and treatment.

**RISKS**

Blood sample will be drawn from your arm. We don't expect any complications from drawing blood although you will feel some pain when the needle pierces your skin and mild bleeding (on the arm) which will be controlled by compression to the injection site and replacement therapy as per protocol. The skin on your arm will be thoroughly cleaned prior to the procedure so as to prevent infections.

**BENEFITS:**

Your participation in this study will help you know whether or not you have transfusional iron overload secondarily to multiple blood transfusions you have received and whether it is associated with other risk factors as well as the way forward regarding the treatment.
You will as well get the benefit of getting appropriate treatment as per need.
We hope that the information from this research will be useful in contributing to improve the quality of care in transfusion dependent anaemia patients.

**CONTACT PERSONS:**

If you have any inquiries about this study, please do not hesitate to contact:

Dr. Helena Tom Kakumbula,
Principal Investigator
Muhimbili University of Health and Allied Sciences (MUHAS)
Department of Haematology and Blood Transfusion
P.O. Box 65001Dar es Salaam.
Tel. 0713721288
Email: helentom2002@yahoo.co.uk

OR in case of any information about your rights as a participant in this study please contact:

**DoctorJoyce Masalu;**
The Chairperson**Senate** Research and Publication Committee
Muhimbili University of Health and Allied Sciences (MUHAS)
P.O. Box 65001 Dar es Salaam
Tel. 2151489

I will be grateful if you willingly agree to participate in this study.

I _____

Have understood the above information and my questions have been answered by the investigator to my satisfaction. I willingly agree to take part in this research.

Name of the participant: _____

Signature of the participant: _____Date _____

Signature of Investigator _____ Date: _____

**Appendix III:CONSENT FORM (SWAHILI VERSION)**

Habari! Mimi ni Dk. Helena Tom Kakumbula ni Daktari katika shahada ya Uzamili katika Chuo Kikuu Cha Sayansi Za Tiba cha Muhimbili. Nafanya utafiti kuhusu kujaa kwa wingi wa madini ya chuma mwilini yatokanayo na sababu za kuwekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na mengineyo katika hospitali ya Muhimbili.Ninaomba ushirikiano wako.

**NIA YA UTAFITI**:

Lengo la utafiti huu ni kujua wagonjwa wangapi miili yao ujaa wingi wa madini ya chuma mwilini yatokanayo na sababu za kuwekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na mengineyo katika hospitali ya Muhimbili. Utafiti huu utafanywa miongoni mwa wagonjwa wote wanaowekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na Mengineyo wanaofika hospitali ya Muhimbili.

**JINSI YA KUSHIRIKI**

Mgonjwa ambaye yuko tayari kushiriki ataweka sahihi yake , ili kuonyesha utayari. Yatafuata maswali machache ya Utangulizi, kisha vipimo vya damu vitachukuliwa

**USIRI**

Taarifaza mgonjwa hazitatangazwa kwa yeyote zaidi ya mtafiti**,** matokeo ya utafiti kwa ujumla yatasaidia kuboresha huduma kwa wagonjwa ambao miili yao ujaa wingi wa madini ya chuma mwilini yatokanayo na sababu za kuwekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na mengineyo.

**GHARAMA**

Hutatakiwa kulipa gharama yoyote kwa kushiriki kwako.

**UTAYARI WAKUSHIRIKI AU KUJITOA**

Kushiriki kwako ni hiyari na waweza kujitoa. Lakini haitakunyima haki ya kupata tiba zingine.

**ATHARI**

Damu kwa ajili ya vipimo itatolewa kwenye mkono. Hatutegemei athari yoyote damu itakapovutwa, isipokuwa waweza kusikia maumivu kidogo, bomba lenye sindano ndogo litatumika ili kupunguza kutoka damu nyingi baada ya kipimo. Ili kuepuka kusababisha maambukizi, mara zote ngozi yako itasafishwa vema na dawa kabla ya kuchomwa sindano yoyote.

**FAIDA**

Kushiriki kwako katika utafiti kutakusaidia kujua iwapo mwili wako umejaa kwa wingi madini ya chuma mwilini yatokanayo na sababu za kuwekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na mengineyo.Ni tumaini letu kuwa utafiti huu utasidia kuboresha huduma kwa wagonjwa wenye miili yao kujaa wingi wa madini ya chuma mwilini yatokanayo na sababu za kuwekewa damu mara nyingi na mara kwa mara kutokana na matatizo mbalimbali yasababishwayo na magonjwa ya damu yakiwemo kansa ya damu na mengineyo hapa Muhimbili na kwingineko nje ya Tanzanaia.

Nitakushukuru kwa kushiriki kwako utafiti huu. Aksante.

Iwapo utakuwa na swali lolote kuhusu utafiti huu wasiliana

Dr. Helena Tom Kakumbula,

Chuo kikuu Cha Afya Na Sayansi za Tiba Muhimbili;

Idara ya Tiba; S.L.P 65001 Dar es Salaam.

Simu 0713721288

**AU** endapo utakuwa na swali lolote kuhusu haki zako kama mshiriki katika utafiti huu wasiliana na:

**Daktari: Joyce R Masalu**;

Mwenyekitii wa Kamati ya Tafiti na Matoleo Chuoni.

Chuo Kikuu Cha Afya na Sayansi Kishirikishi Muhimbili;

S.L.P 65001 Dar Es Salaam.

Simu 2151489.

Mimi……nimeelezwa/ nimesoma yaliyomo katika fomu hii na nimeelewa maana yake. Nakubali kushiriki katika utafiti huu.

Sahihi……..(Mshiriki) Tarehe……..

Sahihi…….. ( Mtafiti) Tarehe……..