PREVALENCE AND DIAGNOSTIC DYNAMICS OF IRON DEFICIENCY ANAEMIA IN ADULTS WITH SICKLE CELL ANAEMIA ATTENDING MUHIMBILI NATIONAL HOSPITAL

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By

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A dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences
October, 2015

CERTIFICATION

The undersigned certify that they have read and hereby recommended for acceptance by Muhimbili University of Health and Allied Sciences entitled: ***Prevalence and diagnostic dynamics of iron deficiency anaemia in adult sickle cell patients at Muhimbili National Hospital*** in partial fulfillment of the requirements for the degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences.

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_____

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(**Second Supervisor)**

Date: _____

DECLARATION AND COPYRIGHT

I, **Dr. Mbonea S. Yonazi** 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.

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DEDICATION

To my beautiful wife **Mariam** and my beloved son **Joshua Yonazi** for their patience and understanding as treasurable family time was devoted to the completion of this work.

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ABSTRACT

Background: The prevalence of iron deficiency anaemia (IDA) and sickle cell disease (SCD) is high in Tanzania. Evidence from other countries, suggests that the prevalence of IDA in SCD is low. There is limited information available on the prevalence of IDA in SCD in Tanzania. This is compounded by the challenges in the diagnosis of IDA in SCA.

Aim: To determine the prevalence, diagnostic models and factors associated with IDA in adults with SCD at Muhimbili National Hospital (MNH).

Methods: This was a descriptive cross-sectional hospital based study involving steady-state adult individuals with SCD who attended the SCD Clinic at MNH, between May and December 2014. A structured questionnaire was used to collect socio-demographic and clinical information. Venous blood samples were taken for blood count and biochemical iron studies. Bone marrow iron status was determined amongst a subset selected using low mean cell volume (MCV) and mean cell haemoglobin (MCH). Six diagnostic models for IDA in SCD were proposed and compared with bone marrow iron status. Chi-square test was used for categorical variables, and student *t*-test for independent sample means. Logistic regression was used to analyse factors associated with IDA in SCA.

Results: A total of 170 individuals with SCA aged 18 to 51 years (65.3% females) were enrolled. Prevalence of IDA ranged between 16.5% - 48% using the seven diagnostic models. The sensitivity and specificity for diagnosis of IDA using transferrin saturation (TSat) was 47.6% and 81% respectively, whilst for serum ferritin were 71.4% for both parameters. The highest specificity, 95.2% was observed using TSat+Ferritin and TSat+ferritin+MCV models. However overall performance in the receiver operating curves (ROC) curves was greater for serum ferritin than any other tests (AUROC =0.765, p=0.003, 95% CI 0.615-0.915). Low MCV was found to be the predictor of IDA in SCA. Age, sex or history of blood transfusion was not found to be associated with IDA.

Conclusion: The prevalence of IDA in adults with SCD is high. Different models showed a variation in detection and accuracy of diagnosis of IDA. Serum ferritin below 100µg/L was found to be the best test for diagnosis of IDA. Further research is needed to determine the feasibility and cost of determining iron status in SCD.

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ABBREVIATIONS

AUC	-	Area under the Curve
BM	-	Bone Marrow
BMA	-	Bone marrow aspiration
CRP	-	C - reactive protein
DMT1	-	Divalent Metal Transporter
FBP	-	**Full Blood Picture**
FEP	-	Free Erythrocyte Protoporphyrin
HbS(S)	-	Sickle Haemoglobin
HBSC	-	Haemoglobin SC
HGB	-	Haemoglobin
Hgb	-	Haemoglobin
HIV	-	Human Immunodeficiency Virus
HPLC	-	High Performance Liquid Chromatography
IDA	-	Iron Deficiency Anaemia
MCHC	-	Mean Cell Haemoglobin Concentration
MCHC-S	-	Mean Cell Haemoglobin-sickle Concentration
MCV	-	Mean Cell Volume
MNH	-	Muhimbili National Hospital
MRI	-	Magnetic Resonance Imaging
MUHAS	-	Muhimbili University of Health and Allied Sciences
MWP	-	Muhimbili-Welcome Programme
RBCs	-	Red Blood Cells
RDW	-	Red blood Cell Distribution Width
ROC	-	Receiver Operating Characteristics
SCA	-	Sickle Cell Anaemia
SCD	-	Sickle Cell Disease
SCT	-	Sickle Cell Trait
Tf	-	Transferrin

TIBC	-	Total iron binding capacity
TSat	-	Transferrin saturation
WHO	-	World Health Organization
WHO	-	World Health Organisation

1.0 INTRODUCTION

Sickle cell disease (SCD) is the commonest inherited disorder worldwide with largest toll in Sub-Saharan Africa [1]. In equatorial Africa, the prevalence of sickle cell trait (SCT) ranges between 10% and 40%, with Uganda reported to have a prevalence of up to 45% in some ethnic regions [1]. The prevalence of SCT in Tanzania is estimated to be around 13% [1].

Generally the World Health Organisation (WHO) estimates that 50% of the cases of anaemia are due to iron deficiency anaemia (IDA) [2], being more prevalent in countries of low socio-economic status. Worldwide anaemia affects one quarter of the population [2,3]. Greater burden has been established in Asia and Sub-Saharan Africa [4]. However the magnitude differs in different age groups and sex, the young children and pregnant women, being the most affected groups [5].

Surveys in Tanzania estimate prevalence of about 30% - 55% of the population is anaemic [6,7] and the prevalence iron deficiency anaemia has been estimated to be around 40-61% [7–9].

Anaemia and particularly IDA is associated with various diverse complications. Heightened risk of maternal and child morbidity and mortality has been known to be a serious consequence of anaemia [5,10], moreover IDA impairs physical and cognitive growth in children as well as reducing physical performance in adults resulting in reduced productivity [5,6]. Anaemia has as well been found to be a risk factor for mortality in SCD [1] and in TB patients [11].

Patients with SCD in developing countries may possess similar risk factors for IDA and in addition may have excessive urinary loss of iron [12]. Other known risk factors for IDA in general population include, Age (being seen more in young children, and women of reproductive age), gender (females being more affected than males), dietary deficiencies, hook worms infestation and other gastrointestinal conditions like variceal bleeding, peptic ulcers and cancers. The identification of iron deficiency in SCD is important, as it is associated with low work performance in adults and impairment of cognitive performance at all ages in children [13].

Despite the burden of SCD in Africa and particularly Tanzania, limited studies have been conducted to explore the prevalence of Iron deficiency Anaemia in Sickle Cell Disease.

1.1 Literature review

1.1.1 Sickle Cell Disease- Pathophysiology

Sickle cell disease is an inherited, chronic haemolytic condition. It results from substitution in the sixth codon the β-globin gene, GAG>GTG, leading to the substitution of valine for glutamic acid (Glu6Val) [14,15]. The resultant HbS chain has a tendency to reversibly polymerize in deoxygenated states. Polymerization renders the red blood cell rigid and with a deformed shape, assuming the characteristic sickle-like shape. The latter being attributed to many of the SCD clinical manifestations [15].

In the post-capillary venules, with low oxygen tension, deoxygenation occurs, leading to conformational change in the haemoglobin molecule. Interactions between hydrophobic valine residues of deoxy-HbS results in a polymer formation. Further lengthening and hardening of the helical polymers precipitate the characteristic SS-erythrocyte change in shape. The latter also results in reduced RBC deformability and membranal damage following repeated sickling [14].

The process of fibres lengthening and polymerization and the resultant SS-RBC deformed shaped is known to require time for priming, referred to as “delay time”. The more the intracellular concentration of HbS, the shorter the delay time, and vice versa [14,15]. The biochemical changes in SCD are far beyond the RBC sickling effect and SCD has been described to be a pro-inflammatory condition owing to elevated inflammatory cytokine upon crises [16–18].

1.1.2 Iron metabolism

Iron forms a crucial component in various structural and functional proteins and it is thus widely and differentially distributed in the body. A greater portion of total body iron (about 2.1 g in adult humans) is contained in the haemoglobin pool of RBCs and erythroid precursors, and thus functions in oxygen transport. Tissue macrophages harbour up to 600 mg of Iron, myoglobin of muscles and bone marrow (BM) contains 300mg and 150mg respectively. A greater portion (up to 1g) of excess body Iron is stored in the liver [15,19]. The human body possesses no known mechanism for iron excretion. Iron is lost from the body (1-2mg/day), only through sloughing of senescent enterocytes and skin cells or upon minor bleeding [19]. The lost amount is replaced by intestinal absorption (10% of the iron content in Western diet containing 15mg of elemental iron). Hence iron balance is achieved by tight control of the absorption of the dietary iron. **Figure 1 (A).**

Absorption of nutritional iron involves conversion (reduction) of Ferric form (Fe3+) to ferrous form (Fe2+). The latter is then taken across the apical membrane of enterocytes through a Divalent Metal Transporter (DMT1). Ferrous iron is then processed and excreted across the basolateral membrane of the enterocytes into the bloodstream, across a Fe2+ transporter, ferroportin. The export of Fe2+ through the latter is coupled with oxidation to ferric form (inert state), catalysed by a membrane bound ferroxidase hephaestin enzyme**. Figure 1 (A).**

The exported iron is picked and transported by Transferrin (Tf) to all the of the body tissues. Transferrin iron constitutes a labile pool of about 3 mg of total body iron (0.1%), capable of escalation to support increased erythropoiesis demand. The transferrin iron pool is replenished mainly by iron recycled from senescent RBCs. The effete RBCs are removed from circulation by the reticuloendothelial macrophages (mainly in the spleen), that degrade the haemoglobin yielding iron into the bloodstream. Similar to intestinal enterocytes, macrophages excrete Fe^{2+} through a ferroportin channel on their plasma membrane, a route coupled with oxidation of Fe^{2+} to ferric form (Fe^{3+}). The latter is then taken by Tf.

The efflux of ferrous iron (Fe^{2+} through a ferroportin channel across macrophages and enterocytes plasma membranes constitute a vital step in systemic iron regulation. This step is negatively controlled by hepcidin [19,20].

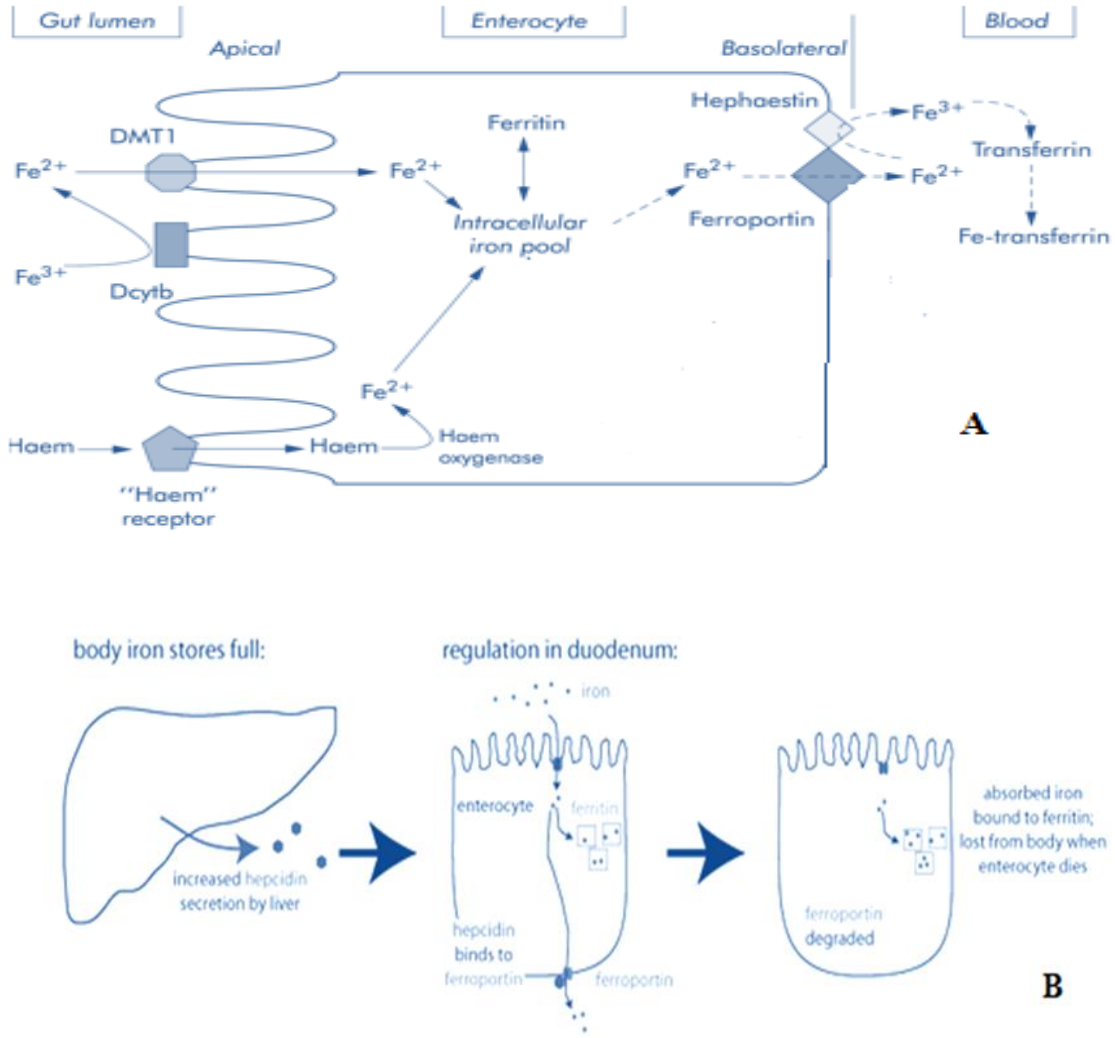


Figure 1: Iron metabolism

Figure 1 (A) shows absorption of iron across an enterocyte. Duodenal cytochrome b (Dctyb), DMTI1 (divalent metal transporter-1). Figure 1 (B) shows the inhibitory role of the hepcidin on the ferroportin channel of the enterocyte.

1.1.3 Hepcidin Control

Hepcidin hormone is derived from the hepatocytes. It accumulates upon iron intake and in inflammatory conditions. It acts by binding to ferroportin channel enhancing its phosphorylation, endocytosis and lysosomal lysis. Hence hepcidin hinders the transfer of dietary iron from duodenal enterocytes to plasma, the mobilization of recycled iron from macrophages to plasma, and the yielding of stored iron from hepatocytes (Figure 1B). On the contrary hepcidin concentrations diminish in iron deficiency states or hypoxia, hence promoting intestinal iron uptake and release from macrophages [20,21].

1.1.4 Diagnosing iron deficiency anaemia

Anaemia is defined as haemoglobin concentration below the reference range with respect to age and sex, and further characterized by either type or cause, based on red blood cell (RBC) indices and iron studies respectively. The WHO defines anaemia as a Hb level below 13.0 g/dL in men, 12.0 g/dL in women, and 11.0 g/dL in pregnant women and preschool children (13). Sickle Cell Disease is associated with chronic haemolysis and reduced haemoglobin level, ranging from 7-9g/dl in steady state. The type of anaemia being normocytic normochromic due to haemolysis, with higher WBC and platelets values compared to normal controls [22–24].

In normal population, IDA is identified by lower than normal values for mean cell volume (MCV) (<80fl), mean cell haemoglobin MCH (<27pg) and elevated red cell distribution width RDW (>14%) [25,26]. However microcytosis has been associated with thalassaemic states [12,27], thus Hb level and MCV alone cannot be used to screen for IDA in SCD, registering the need for usage of more tests with background knowledge of the genotype.

The reticulocyte index describes the reticulocyte count corrected for haemoglobin, given by the following formula: Reticulocyte index = (% reticulocytes × patient's Haematocrit)/normal haematocrit. In patients with anaemia, a reticulocyte index ≥ 3 implies an appropriate bone marrow response as in blood loss or haemolysis, where as in clinical practice a reticulocyte index ≤ 2 denotes inadequate bone marrow response (hypoproliferation) [17,25]. In the study by Kassim et al [28], using a more rigid definition

of reticulocyte index <1 yielded sensitivity and specificity of 80% and 90% respectively, a less rigid definition for reticulocyte index (<2) for bone marrow hypoproliferation, as used in clinical practice (<2) would have the increased sensitivity and specificity of this test [28].

Classical biochemical markers for diagnosing IDA in normal population include reduced serum Iron (<45), low serum Ferritin <15µg/L), elevated total iron binding capacity (TIBC) (>450µg/dl) and low Transferrin saturation (TSat) (<16%) [25]. Several studies have demonstrated IDA in SCD with varied magnitude. However owing to the variability of the iron biomarkers in SCD, different screening tests have been proposed to reach the diagnosis, ranging from a combination of haematological parameters to iron biomarkers [12,28–30].

Other newer biochemical tests include reticulocyte haemoglobin (CHr), percent hypochromic red blood cell (%HYPO), soluble transferrin receptor and erythrocyte zinc Protoporphyrin (ZPP). Despite of individual variability, these test may have no agreed cut-off points corresponding to IDA; however useful in combination in instances of detailing functional iron deficiency anaemia [31,32].

Serum iron is reduced in IDA and inflammatory conditions. It also exhibits a diurnal variation and readily affected by recently ingested iron thus its use alone limited application [25].

Serum ferritin being an active phase reactant is elevated in inflammatory conditions like acute SCD crises, infection, malignancy and in elderly (33,34). Various studies have suggested a higher levels (60-100µg/L) of serum ferritin as sufficient in detecting IDA in coexisting inflammation, infection and malignancy (35,36).

In a retrospective study of anaemic veterans with concomitant inflammatory conditions, by Kis et al (35), they revealed that, serum ferritin compared to, MCV, TSat, and TIBC far better correlates to bone marrow iron status in anaemic patients with a wide range of

other inflammatory conditions. Levels of serum ferritin ≤100µg/L was 100% accurate in separating iron-deficient from iron-sufficient patients, with positive and negative predictive values of 97% and 79% respectively (35). This value is relatively higher than the one commonly suggested by medical literature of ≤ 50µg/L (15,37).

In a cross-sectional study amongst steady state sickle cell disease patients by Akinbam et al (38) in Lagos Nigeria, they reported serum ferritin was within the normal range (15-300ng/ml) in 90% of the participants. This suggests the applicability of serum ferritin in steady state sickle cell disease. Iron Deficiency was found in 7.76% (ferritin below 15ng/ml) and only 2% had above normal ferritin levels (38). Bone marrow iron status was however not determined in this study.

In a study of IDA amongst SCD patient by Vichinsky et al (39), they used response to iron therapy to define a state of iron deficiency to determine the sensitivity and specificity of the TSat, Serum ferritin, MCV and free erythrocyte protoporphyrin (FEP). In this study patients with TSat of <16%, Serum Ferritin <25ng/ml and low MCV, were eligible for Iron trial. They reported IDA in 9% of the study population who responded to iron therapy. Transfusion history and age were found to be of predictive importance. They further reported Serum ferritin (<25ng/ml) was found to be the most specific test, followed by MCV.

In a study to determine iron status amongst SCD in Yemen, by Kassim et al [(28)], they reported a prevalence of 13.3%. They employed a battery of tests (TSat <16%, Serum iron <45µg/dL, TIBC>450µg/dL, low MCV, reticulocyte count, and reticulocyte index) to define the iron status. Majority of the iron-deficient patients were relatively older than those described by Vichynsky et al (39), age was not found to be a predictive value in this group. However as in Vichynsky's group, the non-transfused composed majority (90%) of the iron-deficient patients.

In this group, haematological and biochemical parameters differed significantly between the transfused and non-transfused patients. Comparing Reticulocyte count and the

battery of biochemical parameters, the former produced a sensitivity and specificity of 40% and 98% respectively [28].

Bone marrow iron stain with Prussian blue (Perl's Stain) is considered the gold standard for assessment of body Iron stores (40,41). The procedure is invasive and not deemed suitable for regular screening purposes. In a study by Hughes et al (41) in St. Mary's Hospital London, they reported, that at least seven bone marrow particles need to be examined to establish the absence of iron stores, needing a review of additional slide(s). In this study they had not examine iron granules in the erythroblast. The later has been reported as useful in delineating functional iron deficiency in the presence of adequate bone marrow iron stores (particles and macrophages) particularly in conditions chronic diseases and inflammation (41,42).

1.1.5 Factors associated with IDA in SCD

Different factors have been associated with IDA in SCD; However with variations depending on the methods of diagnosis of IDA and the composition of the study participants. In a study by Vichinsky *et al* comprising of all age groups with SCD, IDA was found in paediatric participants only. This was not the case in a report by Kassim *et al* using a similar diagnostic criterion. Male sex has been shown to be a risk factor for IDA in SCD[12], and this is postulated to be due to increased urinary iron loss(43). Furthermore history blood transfusion has been reported to associate with iron replete status[30]. Iron in the transfused RBC mass seems to ameliorate the deficiency state. Red blood cell parameters have as well been reported to predict a state of IDA in SCD. Low MCV, low reticulocyte count and higher RBC counts in patients IDA and SCD, have been reported by Vichinsky *et al* [39], Kassim *et al*[28] and King *et al*[29] respectively.

1.1.6 Conceptual framework

The factors (age, sex etc.) associated with IDA are crosscutting in all populations including those with SCA. However the clinical and laboratory presentation and thus its diagnosis have been found to be complicated by the concurrent inflammatory processes in SCA. Assessing the sensitivity and specificity of the common methods of diagnosing IDA either singular or as a battery of tests vis-à-vis the bone marrow iron status is deemed helpful.

The following conceptual framework illustration describes the various variables addressed in this study.

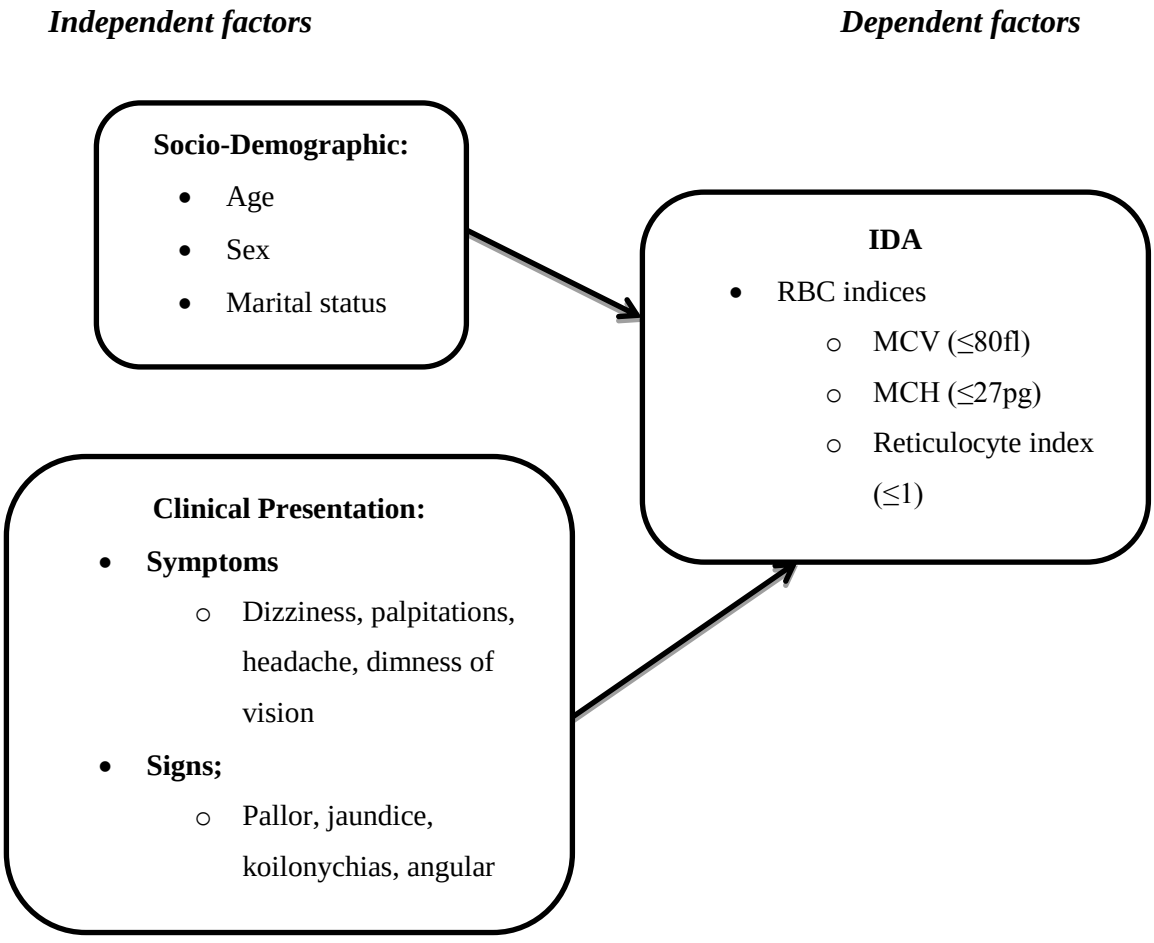


Figure 2: Conceptual framework

1.1.7 Problem statement

Sub-Saharan Africa possesses a growing burden of Sickle Cell Disease and Tanzania is among the leading countries in Africa with high burden of the disease.

The occurrence of IDA has been found as well to be prevalent in the same locality, as SCD.

Although both conditions are debilitating and may worsen the clinical picture in affected individuals, only few studies have been done to explore their dual occurrence and possible outcomes.

High mortality rate has been associated with low haemoglobin amongst patients with SCD. And IDA being prevalent, it is thus important to exclude it amongst the patients, as part of overall efforts in improvement in quality of care. Current challenges in clinical practice entail properly diagnosing IDA in SCD by the prevailing methods.

This study intended to determine the prevalence and the diagnostic dynamics of Iron Deficiency Anaemia among adult sickle Cell patients attending Sickle Cell clinic at Muhimbili National Hospital.

1.1.8 Research questions

This study aims to answer the following questions;

1. What is the prevalence of IDA in individuals with (SCD)?
2. What is the sensitivity and specificity of iron indices in diagnosing IDA in patients with SCA?
3. What are the factors which are associated with IDA in SCA?

1.1.9 Rationale

The findings of this study will highlight the extent of the problem of IDA amongst patients with SCD. This will assist in raising the awareness of the possibility of IDA and its resultant effects even in patients with SCD, contrary to common belief amongst care givers. And thus lead into improvement in the quality of care amongst SCD patients.

It is expected that the findings of the study will highlight the challenges of diagnosing IDA in SCD, and thus advocate for readily availability of various biochemical tests needed to circumvent the challenges.

This study will help to find patients with IDA and thus appropriate treatment. Moreover, the study findings will aid to form a basis for further larger Iron studies in SCD, in Tanzania and elsewhere.

1.2.0 Objectives

1.2.1 Broad Objective:

To determine the prevalence and diagnostic models of IDA among patients with SCD attending Clinic at MNH.

1.2.2 Specific Objectives:

1. To determine the prevalence of IDA in adults with SCD patients attending SCD at Muhimbili National Hospital.
2. To assess the sensitivity/specificity of biochemical iron indices in detection of IDA in adults with SCD patients attending SCD at Muhimbili National Hospital.
3. To describe the factors associated with IDA in adults with SCD patients attending SCD at Muhimbili National Hospital.

2.0 Methodology

2.1 Study design

This was a hospital based descriptive cross-sectional study.

2.2 Study Duration

The study was conducted for a period of nine months, May 2014 - January, 2015

2.3 Study area and Populations

The study was conducted at the Sickle Cell Clinic at MNH in Dar es Salaam. MNH is the largest referral hospital in the country, catering to the population in Dar es Salaam and surrounding regions. The SCD clinic at MNH, the largest in the country, caters for a cohort of 4,000 paediatric and adults patients. Clinic days are Thursday and Friday, for paediatric and adult patients respectively. An adult clinic has an average attendance of 50 (40-50) per day, of regular stable patients and those in crises, anaemia and other SCD associated conditions.

2.4 Inclusion criteria

Participants were enrolled into the study if were of age ≥ 18 years, consenting to participate in the study and at steady-state.

Steady state was defined as absence of history of an acute painful crisis that necessitated emergency treatment or admission in the hospital for the past four weeks prior to recruitment date, absence of history of blood transfusion in the past 3 previous months, no history of concurrent illness such as inflammation, acute infection, in the past previous 4 week and no history of use of medications like antibiotics that may alter the blood counts in the past 3 weeks (44).

2.5 Exclusion criteria:

These included history of taking iron supplementation at the time of recruitment or use in the previous 12 months. Other possible causes of severe anaemia in SCD requiring investigation such as menorrhagia and individuals with a drop in haemoglobin of more than 2g/dl since their last visit. Patients with concurrent chronic conditions like hypertension, heart failure, diabetes, HIV, avascular necrosis and tuberculosis were

excluded. Newly enrolled patients, who had come for screening purposes, were as well excluded.

2.6 Sample size calculation

Iron deficiency anaemia has been found in 13.3 % of steady-state patients with SCD(28). Allowing for a maximum error of 5%, sampling frame i.e. Patients above 18 years so far registered in the sickle cell clinic cohort is 1,275 (as of August 2014).

The minimum sample size will be determined using the Open Epi, open source calculator, (Version 2, Updated in September 2010).

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Where;

N = Sampling frame

DEFF = Design effect (for complex surveys) will be 1 (for random sample)

Z = Standard normal deviation corresponding to two sided specified significant level will be 1.96 (at 95% confidence interval)

d = Margin of error (precision) will be 0.05

P = Proportional of patients - 13.3%

Putting P= 13.3% d=0.05

n= Sample Size = 154

The minimum sample size was 154 participants, 170 were recruited.

2.8 Recruitment of study subjects, procedure and Data collection

All adult SCD patients attending the clinic were consecutively recruited into the study until the sample size was met, whilst considering the inclusion and exclusion criteria.

Data collection commenced after attainment of ethical clearance. Consenting adult patients attending the SCD clinic and meeting the inclusion and exclusion criteria were recruited. Data collection entailed focused history taking on socio demographic characteristics, number of admissions, blood transfusion history. Physical examination was done detailing relevant physical signs for anaemia, haemolysis and IDA, those being, pallor, jaundice, koilonychias, brittle nails and angular stomatitis. All patients

were examined for Height, weight; blood pressure and O_2 saturation were measured as part of routine good clinical practice at the clinic.
The SCD status SS was obtained from the patients' booklets and Muhimbili Wellcome Programme Database, as approved by the Principal Investigators.

Ten millilitres of venous blood was drawn for laboratory tests (hematologic, biochemical, and iron studies). The blood was collected into sterile vacutainers for haematological tests (EDTA anticoagulant) and biochemical tests (vacutainers without additives). Haematological tests were run at the MWP Laboratory at MNH and the iron studies at MUHAS-HAVARD Laboratory (Cobalt-Integral) at MUHAS.
Full blood count and automated reticulocyte count were done using Sysmex-XT 2000i (Cobe Japan) at Muhimbili Wellcome Program laboratory.

Bone marrow aspiration (BMA) was done in 43 patients, selected sequentially amongst those with features of microcytic hypochromic anaemia (MCV ≤ 80fl and MCH≤27) on FBP. Consent for BMA was sought from the eligible study participants. A sample was obtained from posterior superior iliac crest. The BMA slides were stained with Prussian blue and categorized for the iron status. The BMA iron stain was used as a gold standard for Iron stores, and compared against other parameters, i.e. RDW and Iron studies, in determination of iron deficiency state. The examination of the BMA slides for iron stain was done by the investigator under supervision of a consultant haematologist. Gale's method for bone marrow iron examination was employed to determine the iron status. In reporting the bone marrow iron status, a series of seven particles were examined to confirm a negative status, if not found in one slide, a series of slides were examined. Quality control procedures were observed as per Central Pathology Laboratory, Muhimbili National Hospital protocols. (Appendix II and III).

2.9 Definition of key terms

Anaemia, was defined using The World Health Organization (WHO) criteria, haemoglobin concentration lower than 13.0 g/dl in men and lower than 12.0 g/dl in non-pregnant women defined anaemia and haemoglobin 10-12.9 g/dl for men and 10 - 11.9

g/dl for women was used to define mild anaemia, haemoglobin 7-9.9 g/dl for both genders defined moderate anaemia and haemoglobin < 7 g/dl for both genders defined severe anaemia.

Symptoms of anaemia: Lassitude/fatigue, breathlessness, palpitation, dizziness, headaches and dimness of vision.

Signs of IDA: Koilonychia, angular stomatitis and brittles nails

Red blood cell (RBC) indices: These include RBC parameters (mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and red cell distribution width) that are used to describe the RBC features. RBC indices are characteristically deranged certain anaemias e.g. microcytic hypochromic anaemia in IDA.

Bone Marrow Iron Status was determined by microscopic examination of smears of bone marrow aspiration stained with Prussian blue (Perl's Stain).

Biochemical iron indices implied: serum ferritin, Serum iron, total iron binding capacity and transferrin saturation (TSat).

Steady State: Absence of history of an acute painful crisis that necessitated emergency treatment or admission in the hospital for the past four weeks prior to recruitment date, absence of history of blood transfusion in the past 3 previous months, no history of concurrent illness such as inflammation, acute infection, in the past previous 4 week and no history of use of medications like antibiotics that may alter the blood counts in the past 3 weeks (44).

Transferrin saturation (TSAT), the following formula TSAT = SI/TIBC x 100, where TIBC was calculated by multiplying transferrin concentration (g/l) by 25(mathematical constant) was used to obtain percentage transferrin saturation (45).

Diagnosis of iron deficiency state

Six diagnostic models for diagnosing IDA were employed. BM iron status was used as the gold standard, reported as negative to imply deficiency. Amongst the participants who underwent BM aspiration, this model (BM iron status) was compared with six other

diagnostic modalities namely: 1. Low MCV (<80fl) and low MCH (<27pg), 2. Serum ferritin <100µg/L, 3. Transferrin saturation (TSat) <16%,
4. Serum ferritin <100µg/L and TSat <16%, 5. Serum ferritin <100µg/L or TSat <16%. The 6th being the bone marrow (BM) iron status. The 6 models were compared across with gender and age groups. Model 2 to 5 were compared with BM iron status to determine their sensitivity and specificity (test characteristics).

Receiver operating characteristic (ROC) curve:

It is a plot of the true positive rate against the false positive rate for the different possible cut-off points of a diagnostic test. It graphically displays the trade-off between sensitivity and specificity and is valuable in ascertaining the best cut-offs for clinical use. The overall accuracy of a test is expressed as area under the ROC curve (AUC) and provides a useful index for comparing test performances. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test.(46)

2.9 Key Variables

In this study the dependent variable was IDA. A categorical variable grouped either as those with IDA and those without IDA, as diagnosed by bone marrow Iron Status (being either negative or positive). This gold standard test was then compared with serum ferritin and transferrin saturation and the RBC indices (RDW and reticulocyte index). The history of transfusion and the severity-number of units transfused (categorical variables), as well as the number of hospital admissions were compared with the bone marrow iron status for the likelihood of being of predictive value.

2.10 Data management and statistical analysis

The collected data was checked for quality and coding was done prior to entering into the computer (SPSS ver. 20.0) statistical program, which was used for analysis.

Continuous data was summarized using, means in descriptive statistics. Chi-square test was used to determine an association between categorical variables. Student t test was used to compare means of two groups of patients. The sensitivity and specificity of RBC indices and iron studies were calculated using BMA iron status as standard test. Receiver operating curves (ROC) were used to compare the overall function of the

serum ferritin and TSat in assessment of IDA in SCD. Logistic regression analysis was used to assess the association between the socio-demographic factors and IDA, and clinical characteristics/presentation and IDA. Differences were considered significant if p value was less than 0.05.

2.11 Ethical Consideration

Ethical clearance was sought from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (MUHAS). The permission to conduct this study was obtained from authorities of Muhimbili National Hospital. A formal written informed consent, in Swahili, was sought from the participants (*Appendix II*). Non-consenting patients and those not eligible for the study were attended by the investigator as part of the SCD clinical team. As the study entails collecting venous blood and bone marrow samples (for some), aseptic technique was employed to avoid contaminations. Patients' names were not used; information collected on questionnaires (*Appendix I*) was entered into computer using identification numbers to maintain confidentiality.

3.0 RESULTS

In this study 170 adult sickle cell anaemia (SS) patients in steady state were recruited between May 2014 and January 2015.

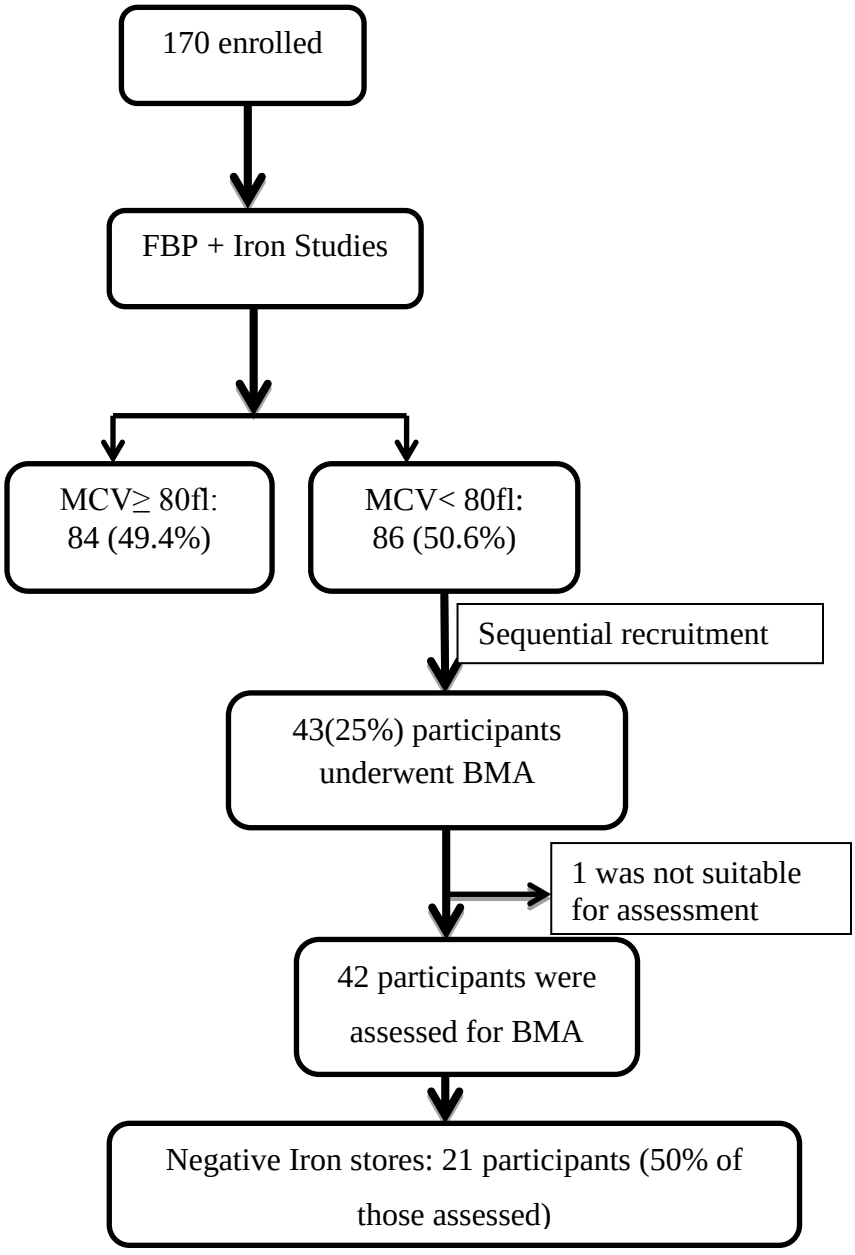


Figure 3: Flow chart

3.1 Socio-demographic and clinical characteristics

Majority (77%) of the study population were youths of 25 years of age and below. Grouping into those above 20 and below or equal to 20 years, the findings were 104 (61.2%) and 66 (38.8%) respectively. Females constituted 65.3% of the study population. Only 3 (1.8%) of the participants had no formal education, others had completed primary education or higher. Forty three per cent of the study participants had some form of employment; whilst the rest who were unemployed constituted of students (43%).

3.2 Clinical presentation among SCD patients

Among the study participants, 161 (94.7 %) had no symptoms of anaemia. Pallor and jaundice were found in 93.5% and 91.8% of the study participants respectively. One hundred and fifty two (91.8) participants had tinge of jaundice, deep jaundice was observed in 2.5% of the participants.
In this study, stigmata of iron deficiency (angular stomatitis, koilonychia and brittle) were found in only 2 (1.18%) participants, amongst the 170 recruited.

One hundred and fourteen (67.1%) participants had received blood transfusion, and amongst these majority reported to have had received 1-5 units (41.8%) and more than 10 units (50.6%).

Amongst the study participants, 148 (87.1%) had history of hospital admission, and amongst these, majority reported to have been admitted between 1-5 times. Thirteen per cent reported not to have ever been admitted. The socio demographic and clinical characteristics of study participants are summarized in table 1.

Table 1: Socio-demographic characteristics of the study participants (n=170)

Variable	**All**	**By gender**		
Demographic characteristics		**Male**	**Female**	**P-value**[#]
Number of Participants	170	59 (34.7%)	111 (65.3%)	
Age (years)				
Mean (±SD)	23.3 ± 6.02	22.39 ± 5.1	23.83 ± 6.4	0.139*
Median (IR)	22 (6)	21(6)	22(6)	0.16**
Age groups				
Below 25 years	131 (77.1%)	46 (35.1%)	85 (64.9%)	
26 to 34	27 (15.9%)	11 (40.7%)	27(59.3%)	
Above 35 years	12 (7.1%)	2 (16.7%)	12 (83.3%)	0.338
Employment				
Employed	73 (42.9%)	24 (32.9%)	49 (67.1%)	0.745
Level of education				
Primary-college (formal education)	167 (98.2%)	59 (35.3%)	108 (64.7%)	0.316
Clinical characteristics				
Symptoms of anaemia				
None	161 (94.7%)	56 (34.8%)	105 (62.5%)	1.0
Pallor				
Pale	159 (93.5%)	51 (32.1%)	108 (67.9)	0.009
Jaundice				
Jaundiced	156 (91.8%)	52 (33.3%)	104 (66.7%)	0.246
Stigmata of IDA				
Number of signs				
None	168 (98.8%)	59 (35.1%)	105 (62.5%)	0.425

Blood Transfusion				
Received	114 (67.1%)	42 (36.8%)	72 (63.2%)	0.493
Units of blood ever received				
More than 10 units	86 (50.6%)	28 (32.6%)	58 (67.4%)	0.629
Hospital admission				
Admitted	148 (87.1%)	53 (35.8%)	95 (64.2%)	0.482
Number of admissions				
≤10 admissions	117 (68.8%)	44 (37.6%)	73 (62.4%)	
More than 10 admissions	53 (31.2%)	15 (28.3%)	38 (71.7%)	0.297

#Test statistic was Pearson Chi-square test, unless otherwise stated

* Student T-test, **Man-Whitney test

3.3 Haematological and biochemical parameters

The range of Haemoglobin (Hgb) was 3.3 – 13.3g/dl and the mean was 8.14 ± 1.41g/dl. One hundred and thirty three (78.2%) participants had moderate (Hgb of 7.0-9.9 g/dl) anaemia, while 26 participants (15.3%) had severe (Hgb below 7g/dl) anaemia. The anaemia was found to be of microcytic in 86 (50.6%) of the participants, with mean MCV and MCH of 79.81 ± 9.46 and 27.14 ± 3.76 respectively. Majority had elevated high RDW, above 14.8% with mean of 21.17 ± 4.43%. Reticulocyte count was elevated in majority of the participants.

Amongst the participants with MCV<80fl, 43 underwent bone marrow aspiration for iron confirmation. In one of these, assessment it was not possible due to absence of marrow particles. Iron deficiency was confirmed in half of the 42 remaining participants.

The median for serum ferritin was 129.15 (203.2), and 40% of the study participants had serum ferritin below 100µmol/L. Transferrin saturation below 16% was found in 29.4% of the study population. Both serum ferritin and transferrin saturation showed a statistically significant association with the age groups. (Table 2)

Table 2: Haematological and biochemical parameters (n=170)

Variable	All	By gender		
		Male	Female	P value
RBC x 10^{12}/L	3.11 ± 0.874			
	< 3.38	44(31.2%)	97 (68.8%)	
	≥ 3.88	15 (51.7%)	14 (48.3%)	0.052
HB (g/dl)	8.14 ± 1.41	8.64 ± 1.5	7.8 ± 1.3	0.001
Non-anaemic	2 (1.2)	2	0	
Mild anaemia	9 (5.3%)	6 (66.7%)	3	
Mod. anaemia	133 (78.2%)	46 (34.6%)	87 (65.4%)	
Severe anaemia	26 (15.3%)	5 (19.2%)	21 (80.8%)	0.01
MCV (fl)	79.81 ± 9.46	77.87 ± 8.0	80.85 ± 10.0	0.05
<80	86 (50.6%)	38 (44.2%)	48 (55.8%)	0.01
MCH (pg)	27.14 ± 3.76	26.5 ± 3.2	27.4 ± 3.9	0.13
MCHC (g/dl)	33.86 ± 1.58	34.0 ± 1.2	33.7 ± 1.7	0.28
RDW (%)	21.17 ± 4.43	22.4 ± 2.9	20.5 ± 4.5	0.007
RETIC%	10.16 ± 4.80	9.85 ± 4.1	10.3 ± 5.1	0.545
RETIC INDEX	1.66 ± 1.66	5.35 ± 2.0	4.48 ± 1.3	
				0.001
WBC x 10^9/L	11.62 ± 3.01			
>11	88 (51.8)	32 (36.4%)	56 (63.6%)	0.747
PLT x 10^9/L	434 ± 157			
	> 450 70 (41.2%)	27 (38.6)	43 (61.4%)	0.415
BM Iron Status[#]				
Negative	21(50%)	13 (61.9%)	8 (38.1%)	0.062
Iron Studies				
Serum iron (µmol/L)	14.48 ± 5.99	13.56 ± 5.6	14.9 ± 6.1	0.147
Serum ferritin (µg/L)	129.15* (203.2)			
<100	68(40%)	29 (42.6%)	39 (57.4%)	0.1
Ser transferrin	2.57 ± 0.55			
TIBC (µmol/L)	64.13 ± 13.74			
> 75	35 (20.6%)	16 (45.7%)	19 (54.3%)	0.163
TSat (%)	23.29 ± 10.41			
< 16	50 (29.4%)	25 (50%)	25 (50%)	0.008

*Median, # n=42

Figure 3a shows prevalence of IDA, as diagnosed by various models. Bone marrow iron status ranked highest with prevalence of 50% amongst the subset of the study population. In the whole study group, the "SerORTsat" modality had the highest prevalence of all the other five modals. Notably "MCV+MCH" modal had the lowest prevalence.

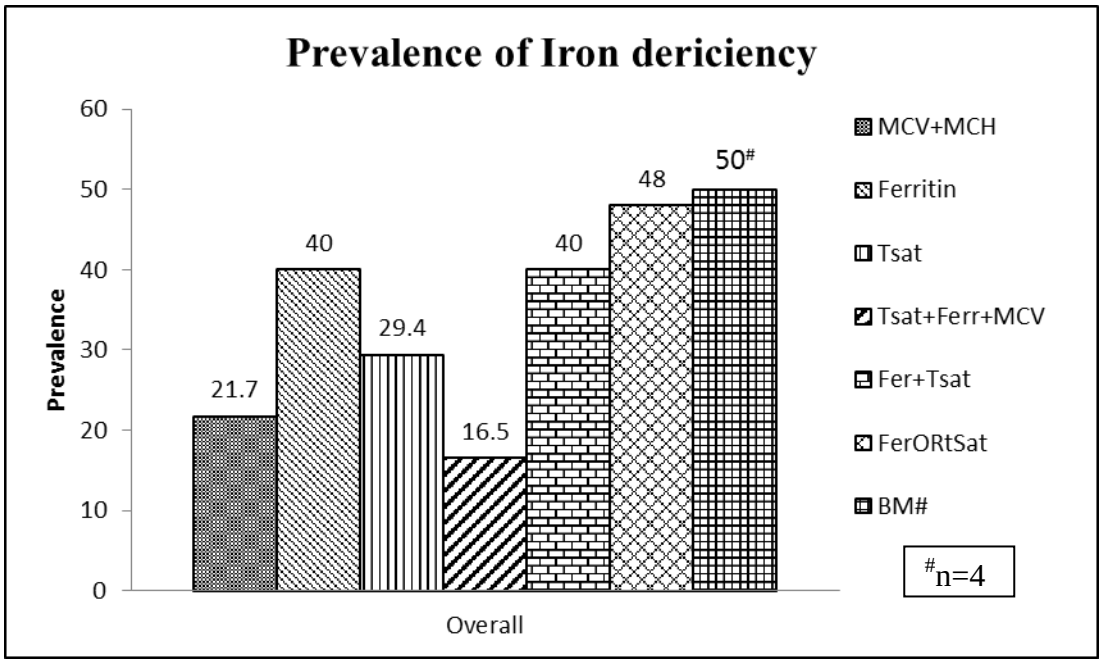


Figure 4a: Overall prevalence IDA by various diagnostic models (n=170)

'MCV+MCH'= low mean cell volume and low mean cell haemoglobin, 'Ferritin'= ferritin <100µg/L

'Tsat'= transferrin saturation <16%, 'Fer+Tsat'= ferritin <100µg/L and transferrin saturation <16%,

'FerORtSat'= ferritin <100µg/L or transferrin saturation <16%, and 'BM'= bone marrow iron status

Figure 3b shows the distribution of prevalence of IDA by sex, as diagnosed by different diagnostic models. It reveals that there was a statistically significant association between sex various models, namely, low MCV and low MCH, low transferrin saturation and, low TSat or low serum ferritin model.

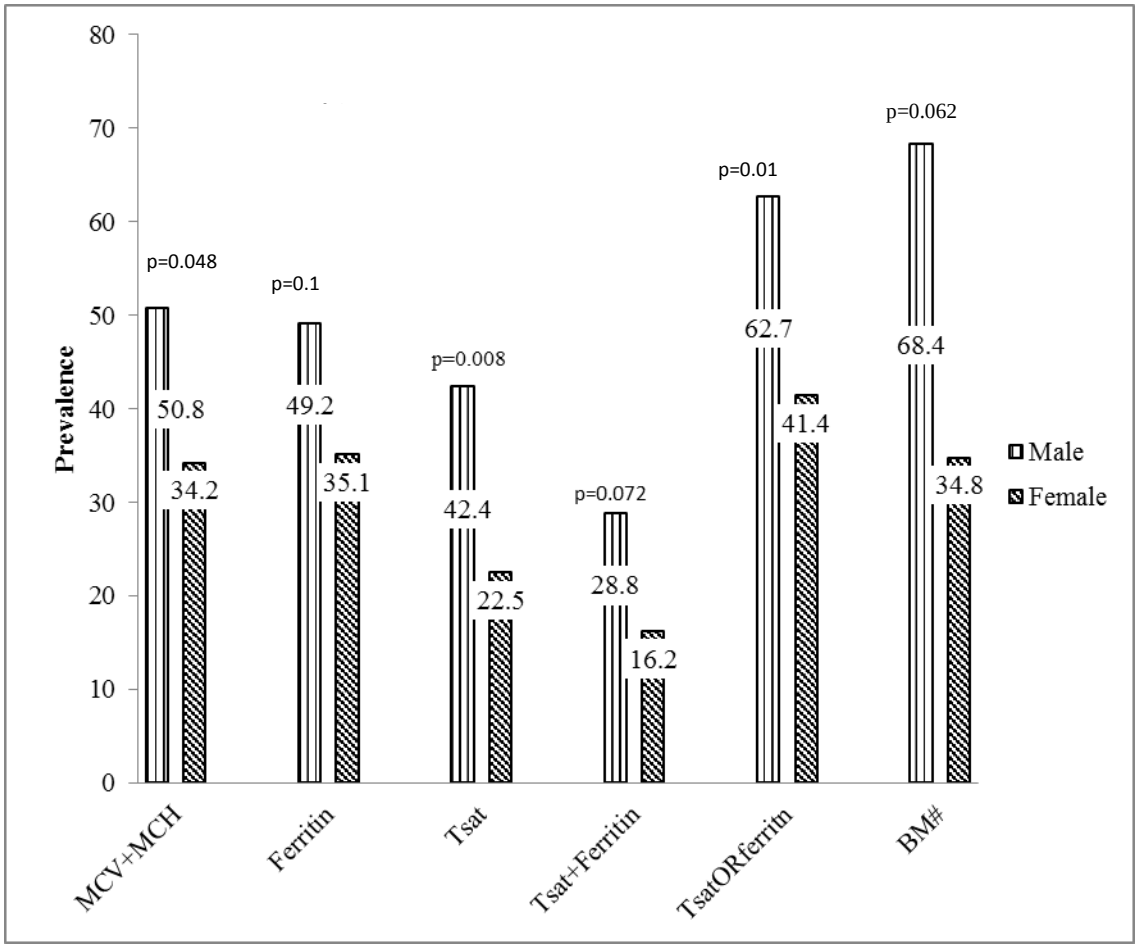


Figure 4b: Prevalence of IDA by various models and sex (n=170)

'MCV+MCH'= low mean cell volume and low mean cell haemoglobin, 'Ferritin'= ferritin <100µg/L

'Tsat'= transferrin saturation <16%, 'Fer+Tsat'= ferritin <100µg/L and transferrin saturation <16%,
'FerORtSat'= ferritin <100µg/L or transferrin saturation <16%, and 'BM'= bone marrow iron status

Figure 3c shows the distribution of IDA amongst the age groups as diagnosed by different diagnostic models. There was no statistically significant association between age groups and any of the diagnostic models.

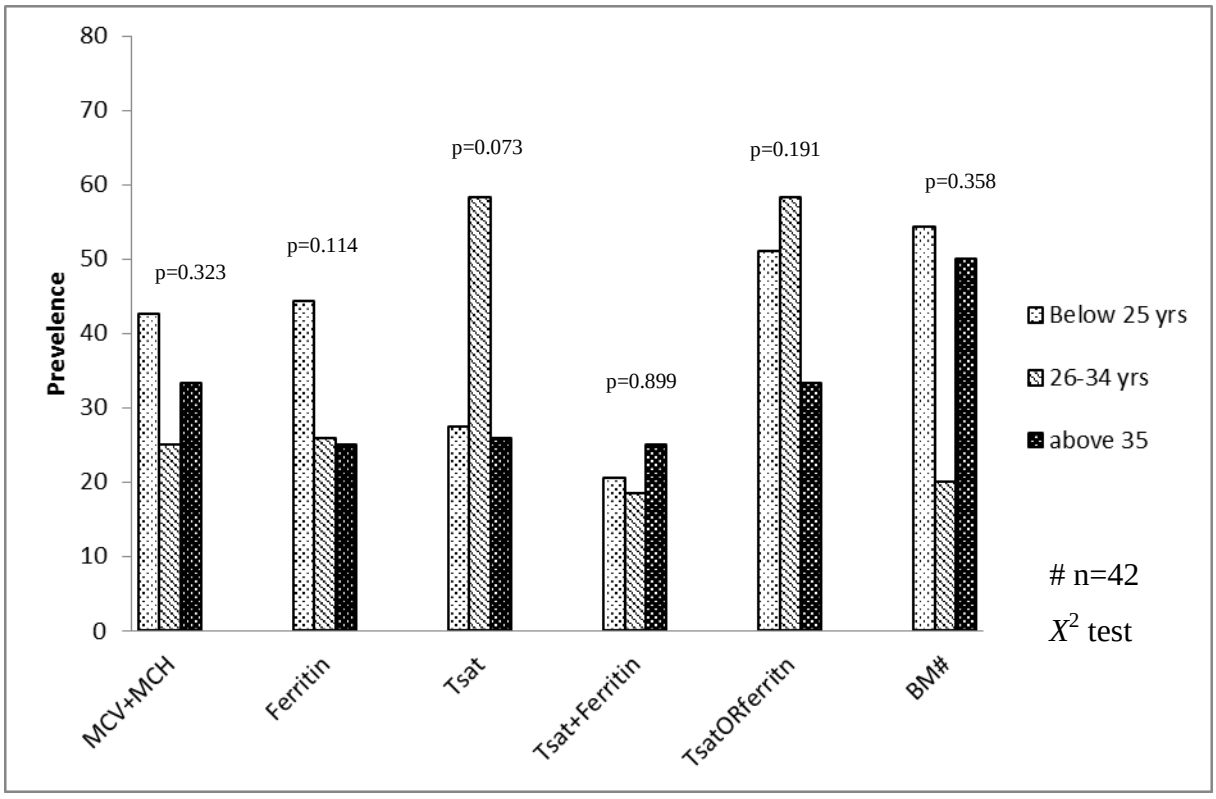


Figure 4c: Prevalence of IDA by various methods in different age groups (n=170)

3.4 Sensitivity and specificity of biochemical iron indices in detection of IDA in SCA

In this study, a statistically significant association was found the between serum ferritin below 100µmol/L and the bone marrow iron status. The sensitivity, specificity and the predictive values were all at 71.43%. The positive likelihood ratio (LR+) and negative likelihood ratio (LR-) values suggested minimal increase in the likelihood that one will be diagnosed with IDA. (Table 3)

When the distribution of transferrin saturation (TSat) was compared with the bone marrow (BM) iron status, it was found that, the former (TSat) had sensitivity and specificity of 47.6% and 81% respectively. And the distribution was not statistically significant. The likelihood ratios suggested minimal increase in the likelihood that one will be diagnosed with IDA. (Table 3)

When transferrin saturation (TSat) and serum ferritin were serially combined, the resulting diagnostic model had a sensitivity and specificity of 33.33% and 95.2%. The likelihood ratios indicated that a positive test for this model is highly conclusive of the likelihood of IDA. There was a statistically significant association between this model and BM iron status. (Table 3)

In a parallel diagnostic modal of TSat and serum ferritin, a statistically significant association with the BM iron status was evident. This model had a relatively high sensitivity (85.7%) and a low sensitivity of 52.4%. The positive (PPV) and negative predictive (NPV) values were both 87.5% and 58.8% respectively. The likelihood ratios that the model minimally modifies likelihood that one will be diagnosed with IDA. (Table 3)

Table 3: Test characteristics of various diagnostic models in the diagnosis of IDA compared to BM iron status (n=42)

Diagnostic model	IDA	No IDA	Sensitivity	Specificity	PPV	NPV	LR+	LR-	X^2	p-value
	21 (50%)	21 (50%)								
Serum ferritin										
< 100 µg/L	15 (71.4%)	6 (28.6%)								
≥100 µg/L	6 (28.6%)	15 (71.4%)	71.4%	71.4%	71.4%	71.4%	2.5	0.4	6.1	0.029
TSat										
<16%	10 (71.4%)	4 (28.6%)								
≥16%	11(39.3%)	17 (60.7%)	47.6%	81%	71.4%	63%	2.51	0.65	3.86	0.1
Serum ferritin <100µg/L and TSat<16%										
Yes	7 (87.5%)	1 (12.5%)								
No	14 (41.2%)	20 (58.2%)	33.3%	95.5%	87.5%	58.8%	20.83	0.70	5.56	0.045
Serum ferritin <100µg/L OR TSat<16%										
Yes	18 (64.3%)	10 (35.7%)								
No	3 (21.4%)	11 (78.6%)	85.7%	52.4%	64.3%	21.4%	1.8	0.27	6.86	0.02

Figure 4 shows serum ferritin to be a good indicator of bone marrow iron status. At level below 100µg/L, the sensitivity and specificity are 71.4%, area under ROC (AUROC) being 0.765. p = 0.003 (95% CI 0.615-0.915).

At suggested level of TSat below 16%, the sensitivity is 47.6% and specificity of 76.2%, area under ROC (AUROC) being 0.719, p = 0.015 (95% CI 0.562-0.876).

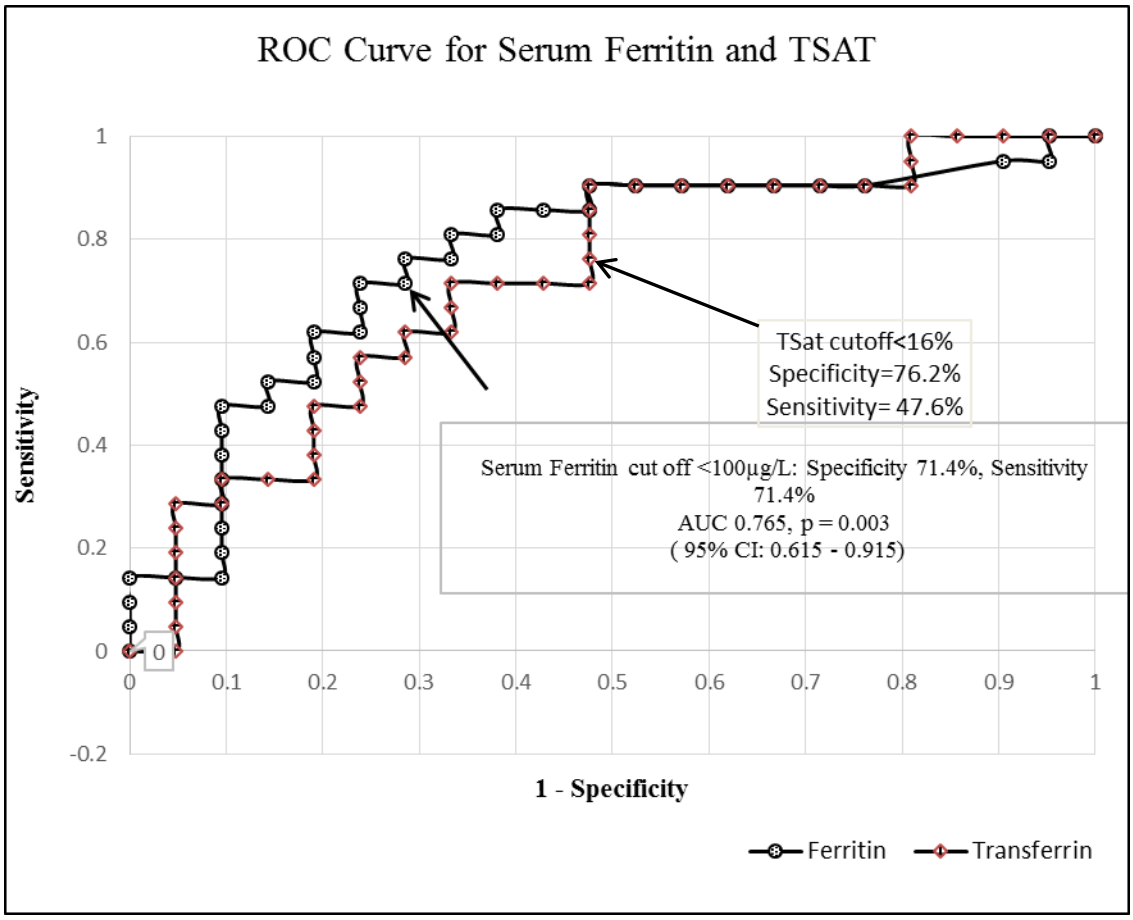


Figure 5: Receiver operating characteristics (ROC) curve for serum ferritin and Transferrin saturation

3.5 Factors associated with diagnosis of IDA in SCD

Table (4a) shows results of univariate analysis. This was done using serum ferritin model which was chosen owing to its overall superior performance compared to TSat in the ROC curves.

Those of age 20 years and below were twice as much likely to be diagnosed with IDA [OR=2.187, (1.16- 4.12), p = 0.015].

Male sex was associated with increased likelihood of diagnosis of IDA however was not statistically significant [OR=1.79 (0.94- 3.39), p = 0.077].

History of hospital admissions and transfusion conferred increased likelihood of the diagnosis in this model.

Determining the associations using the bone marrow iron status model has not been shown here; it however produced non-significant erratic results, with a single exception of a strong association between sex and IDA. Male sex was associated with increased risk of IDA [OR=4, (1.12- 14.8), p = 0.034].

Table 4-a: Factors associated with diagnosis of IDA in SCD - Univariate logistic regression analysis

Variable	IDA by Serum Ferritin			
	IDA n=68	**Non-IDA n=102**	**OR (95%CI)**	**p-value**
Age (>20yrs)	22.34 ± 5.3	23.99 ± 6.4	2.19 (1.16-4.12)	**0.015**
Sex (F)	-	-	1.79 (0.94- 3.39)	**0.077**
Employment (not employed)	-	-	2.09 (1.10-3.96)	**0.024**
History of blood transfusion (None-0)	-	-	1.5	**0.01**
Number of units of blood (≥10)	3.59 ± 3.8	4.16 ± 3.9	0.79 (0.43- 1.46)	0.453
History of admission (None-0)	-	-	1.5	**0.01**
Number of admissions (>10)	4.3 ± 3.7	5.2 ± 4.6	2.1 (1.04- 4.22)	**0.038**
Hgb (g/dl) (mild-moderate anaemia)	8.01 ± 1.1	8.2 ± 1.5	1.11 (0.7- 2.61)	0.82

In the multivariate analysis (Table 4-b), none of the factors was confirmed to be the predictor of IDA in the tested model of interest (Serum ferritin).

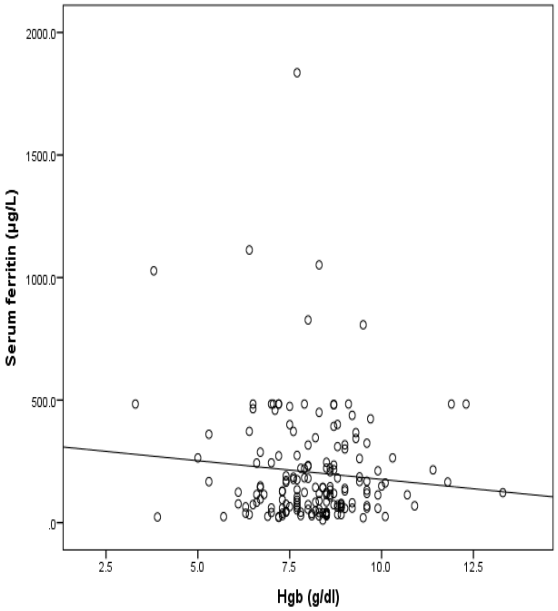
Table 4-b: Factors associated with diagnosis of IDA in SCD by various diagnostic models - Multivariate logistic regression analysis

Variable	IDA by Serum Ferritin	
	OR (95%CI)	**p-value**
Age	0.600 (0.29- 1.252)	0.174
Sex	0.60 (0.31- 1.18)	0.432
Employment	0.653 (0.31- 1.38)	0.653
Number of hospital admissions	1.85 (0.90- 3.79)	0.094

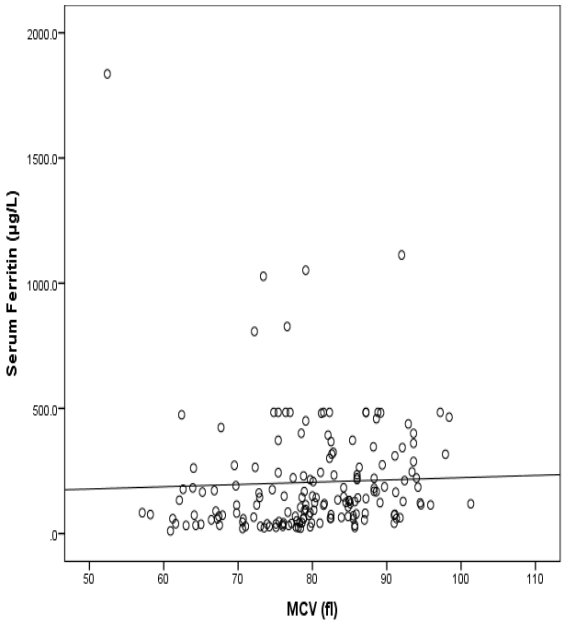
Correlation between serum ferritin and RBC indices among study participants

Figure 5 (A-D) shows scatter plots show no evidence of correlation between serum ferritin and Hgb, MCH and RDW, ($r = -0.093$, p value $=0.23$, $r = 0.011$, p value $= 0.88$, and $r = 0.012$, p value $= 0.88$ respectively). A positive correlation existed with MCV (5-B) ($r = 0.037$, p value $= \leq 0.001$).

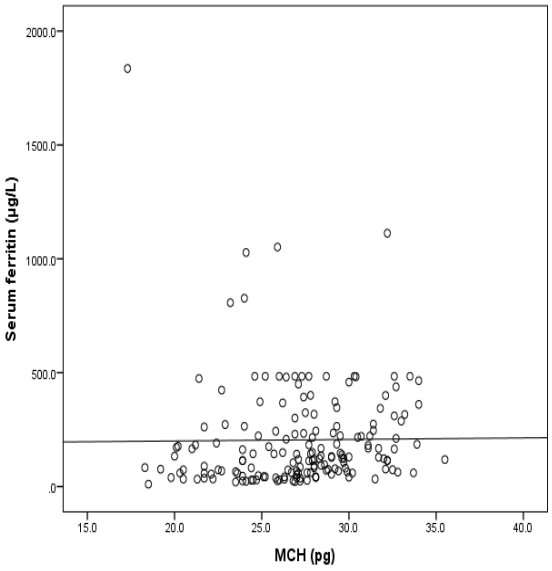
A: Correlation of serum ferritin and MCV



B: Correlation of serum ferritin and MCV



C: Correlation of serum ferritin and MCH



D: Correlation of serum ferritin and RDW

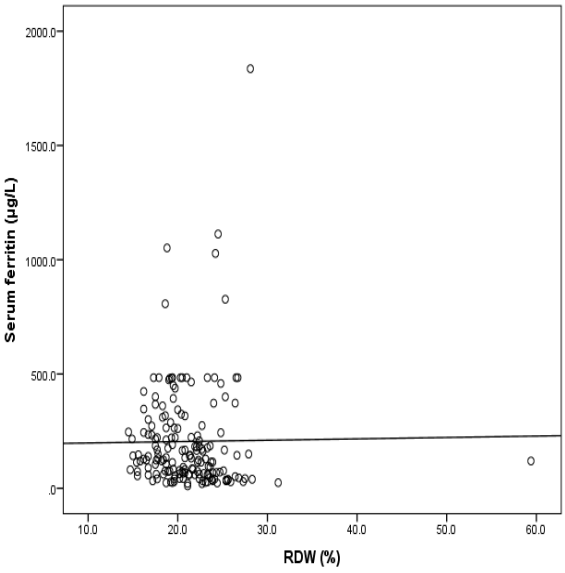


Figure 6: Scatter Plots showing correlation between serum ferritin and RBC indices

4.0 DISCUSSION

This study sought to determine the prevalence of IDA amongst adults with SCA. Diagnosing IDA in a pro-inflammatory background of SCA is bound to be met with false negatives from simplest the tests for haematological parameters to the biochemical iron indices like serum ferritin and transferrin saturation (TSat). Owing to the aforementioned challenges in ascertaining IDA in SCA, various diagnostic have been models tried.

Prevalence of IDA in SCD

In this study, prevalence of IDA is SCA amongst a subset of the recruited patients with both low MCV and low MCH (low MCV+MCH), was found to be 50%, using bone marrow iron status model. The prevalence varied from 22%-48% across the other 5 models that were used. This is in accordance with other previous authors who found varied prevalence, (8.5% to 67%) using different battery of tests (12,28,29,39,47,48).

Kassim *et al* [28] in 2009 employing a combination of low serum ferritin, TSat<16%, increased TIBC and low MCV for age as a diagnostic criteria, reported 13.6% prevalence of IDA among SCA patients comprising of both children and adults.

Using a similar criteria King *et al* [29] in 2005, amongst a group under-five years with HBSS and HBSC, reported a prevalence of 8.5%. Using a similar diagnostic model (Low TSat, Low Ferritin and low MCV) in the current study the prevalence found was 16.5%, almost twice that of King *et al*. This is almost twice as much and can be ascribed to a strict selection criterion, recruiting only patients in steady state as opposed to just patients who had not been transfused in the past 3 months in the former study. Reticulocytosis and increased serum ferritin are known to accompany sickle cell crisis (34,49–51). These would be expected to lessen the prevalence in a lesser in a diagnostic model which includes participants in crisis on near crisis. The higher prevalence in a current study with stricter inclusion criteria can be ascribed to factors commoner in an older population such as menstrual blood loss, gastrointestinal bleeding and urinary iron loss.

Employing BM iron status as a gold standard Rao *et al*[30] found prevalence of IDA in 60 patients with SCA to be 28%. In another study by Nkurumah *et al*(47) using BM iron status as a reference model the reported prevalence for IDA amongst 33 patients was 42%. This is consistent with our findings with the BM iron status diagnostic model. However in their study Nkurumah *et al* only studied SCA patients with severe anaemia (Hgb below 5g/dl) and in crisis, whilst this study used low MCV and MCH as a selection criteria for BM the same, for patients in steady state.

Moreover similar to findings in the current study; in a study by Okeahialam *et al* (52) using BM iron status to diagnose IDA amongst 45 children with SCA, prevalence of IDA was reported to be 47%.

It has thus been observed that use BM iron status as a diagnostic model has yields higher prevalence that the haematological and iron indices models. This can be explained by the relative stability of the former in pro-inflammatory conditions like SCA. However it is limited by its invasive nature as evidenced by the numbers of participants studied the various reported groups and the current study.

In a population based study, on the other extreme Mohanty et al reported a prevalence of IDA in 67% SCA persons (48). This is higher than our findings and the rest of the previous reports. This can partly be attributed to the use of Zinc protoporphyrin-heme ratio test for diagnosis of IDA, which poses high sensitivity but low specificity (53). The study was however done at a specific tribal sect that might have had prevailing factors for IDA.

Contrary to reports in the medical literature that SCA is associated with iron overload, this study asserts the fact that IDA is common among patients with SCA.

Table 5: Summary of various studies of Iron deficiency anaemia is Sickle cell anaemia

Prevalence of IDA (%)	Number (n)	Diagnostic criteria	Year	Age	Reference
3.1	97	Hgb and TSat, Hgb and ferritin	2013	Children (<5 years)	Akodu *et al (*50)
13.6	75	Low serum iron + TSat< 16% + High TIBC + low MCV	2009	All age groups	Kassim *et al* (28)
67	62	Response to iron treatment, Zinc protoporphyrin/haem ratio	2008	All age groups	Mohanty *et al* (45)
8.5	121	TSat<16 + Serum Iron + MCV	2005	Children <5 years	King *et al* (29)
13	100	Serum ferritin+ Serum Iron +MCV	2007	Children (6 months – 10 years)	Mangosongo *et al* (56)
28	60	BM iron status, Serum ferritin, TSat	1984	Adults	Rao *et al* (30)
42.7	33	BM iron status	1984	Children (5 months – 12 years)	Nkurumah *et al* (44)
27	27	Low MCV, TSat, TIBC, serum iron, serum ferritin	1983	Teenage-adult	Davies *et al* (12)
9	70	Response to iron therapy, serum ferritin, TSat, MCV and FEP	1981	All age groups	Vichinsky *et al* (37)
47	45	BMA, serum iron and TIBC	1981	Children	Okeahialam *et al* (49)

Sensitivity and specificity of tests to diagnose IDA

When the diagnostic models 2-5 (Table 3) were compared to BM iron status model (6^{th} model) sensitivity and specificity varied across the diagnostic models. Stringent models employing serial pattern have shown higher specificity and likelihood rations than their parallel counterparts at the expense of sensitivity and vice versa. The only parallel 5^{th} diagnostic model (low TSat or Low Ferritin) had the highest sensitivity, exceeding that of ferritin alone (2^{nd} model- serum ferritin below 100µg/L). This implied that chances of one being diagnosed with IDA were high if either of the two tests was employed independently.

In this study serum ferritin below 100µg/L (2^{nd} model); scored modestly in the both sensitivity and specificity. The sensitivity of serum ferritin exceeded that of TSat. The specificity of the TSat (3^{rd} model) exceeded that of serum ferritin. This is contrary to what was reported by Vichinsky *et al*[39] who reported specificity of ferritin and TSat to be 91% and 87% respectively. They compared a response to iron treatment (gold standard model) against individual (TSat<16%, serum ferritin<25mg/uL, FEP and MCV) tests; in 70 SCA patients. Transferrin saturation is known to be sensitive to IDA however non-specific, as it is lowered in inflammatory processes[45] like SCA. This reversal of the expected in the current study could be attributed to the small number [42] of participants who underwent BM aspiration.

Akodu *et al*[54] determined the prevalence of IDA in SCA children using two different diagnostic models, of Hgb + TSat and Hgb + ferritin, reported greater diagnostic success in the former model (TSat). This can be ascribed to a much lower cut-off point for serum ferritin (<25ng/ml) was much lower than in the current study, and a few number of affected individuals [3].

In a study by King et al, who compared the performance of tri-test (TSat<16%, serum iron and MCV) gold-standard model and individual tests; amongst 121 SCA children, specificities of TSat, serum iron and low MCV for age were, 64.3%, 46% and 95% respectively. These results revealing high specificity for MCV and low for TSat were as well reported by Davies *et al*[12] and Vichinsky *et al*[39].

We further assessed the efficacy of various biochemical iron indices in diagnosing IDA in patients with SCA. Serum ferritin concentration was the most reliable index for the detection of depleted iron stores with the largest area under the ROC curve. When sensitivity and specificity were considered together, serum ferritin values below 100 µg/L could be suitable to exclude iron deficiency, with a sensitivity and specificity of 71.4% for a cut point of 100µg/L, (p=0.003, 95% CI 0.615-0.915). The area under the curve (AUC) for serum ferritin (0.765) was greater than that of TSat (0.719) implying the former has a better overall performance than the latter in the diagnosis of IDA in this group.

Studies employing the ROC curves in ascertaining the usefulness of different biochemical iron indices in diagnosing IDA in various other inflammatory associated conditions have been conducted in patients with kidney disease, heart failure and acute care hospital. Similar to this study, these studies by Ong K *et al* [(55)], Fernández-Rodríguez *et al*[(56)] and a meta-analysis by Guyatt *et al*[(57)] confirmed the superiority of serum ferritin in diagnosing IDA in amidst concurrent disease conditions.

This study indicates that TSat alone is not a reliable index in the diagnosis of IDA. Use of diagnostic models with parallel pattern for TSat and ferritin risks high rate of false positives (Table 3). Serum ferritin at a level less than 100 µg/L may be used as a diagnostic parameter of depleted iron stores. Mean cell volume has been shown to associate well with the serial models implying it is of a high indicative value. Thus patients with low MCV found to have low serum ferritin are highly likely to benefit from iron treatment. It is our opinion however that the decision ought to be individualized taking into account benefits/hazards of iron treatment and other risk factors for IDA. A trial with iron treatment has as well been advocated.

Prospective studies to determine IDA in SCA involving iron treatment, and monitoring with newer diagnostic tests such as serum transferrin receptor, reticulocyte-haemoglobin, and inflammatory markers such as hepcidin and C-reactive protein (CRP), are warranted.

In the light of importance of the biochemical iron indices, we did a survey of the current cost (in Tanzanian currency) for the same. At the national referral hospital, public and private categories existed. For serum ferritin, transferrin saturation, TIBC and serum iron, the cost was 35,000/-, 15,000/-, 100,000/-, and 5,000 respectively in the private category (total 55,000/- excluding TIBC). Whereas in the public category it was 30,000/-, 12,000/- and 6,000/- (total 48,000/-) for serum ferritin, serum transferrin and serum iron respectively. The same set of tests was priced at 106,000/- at private laboratory in the same city. The authors find it unnecessary for a highly priced TIBC test whilst the needed TSat can be calculated from relatively cheaper serum iron and serum transferrin. However this calls for clinicians to be informed of the available alternatives. The affordability of total cost of the tri-tests, for public category at the possibly only public facility is as well debatable in a country in country with a reported poverty rate of 65.6% [58]. Surveys are warranted to establish demand rate, utility and affordability of the iron related tests in relation to iron related cost-of-illness.

Factors associated with IDA

This study also sought to determine the factors associated with the diagnosis of IDA in SCA.

Age was not found to be associated with the diagnosis of IDA. Age correlation in the diagnosis of IDA was also reported by Vichinsky *et al*[39]. They reported IDA only in children less than 5 years of age amongst their study group comprising of children and adults. Kassim *et al*[28] using a similar set diagnostic models reported IDA across all age groups (children and adults) of their study population.

This study recruited adults only, majority being young adults. The narrow age range [18-51] and commonness of factors within the group might have modified the overall picture.

Moreover sex was not confirmed to associate with the diagnosis of IDA in SCA, in this study. Mangosongo *et al*[59] reported similar findings in their study amongst children in Tanzania. This is however contrary to what was reported by Rao *et al*[30] and Davies *et al*[12]. The former reported IDA in 11 (65%) males of the 17 iron deficient patients whilst the latter (Davies et al) reported IDA in 8 males of their 10

patients with IDA. Hormones are thought to influence higher MCHC in males and sustained RBC survival in females [60]. Increased MCHC and thus MCHC-S heighten haemolysis in males with SCA. As one third of haemolysis occurs in the intravascular compartment, this leads to greater urinary iron loss in males than female [43,61].

In previous report by Rao *et al*[30] and Vichinsky *et al* [39], history of previous transfusion was found to predict a state of iron replete. This was not the case in the current study. Transfusion history and number of units transfused were not found to correlate with IDA. This can perhaps be explained by the fact that chronic transfusion programme (such as recurrent top-up transfusion to suppress HBS) is not a feature at our hospital. Transfusion of blood is offered on ad hoc basis when required as in severe anaemia. Thus despite majority (67.1%) reported to had been transfused, and amongst these 50.6% received >10 units perhaps still this was not enough alter their iron stores to be detectable in the analysis. On the other extreme, repeated or regular simple (non-erythrocytapharesis) transfusion has been documented to lead to iron overload [62,63], a serious consequence associated with vital organ dysfunction.

In the current study we could not establish a statistically significant correlation between diagnosis of IDA (by ferritin) and haematological parameters namely Hgb, MCH and RDW. Conversely, King *et al* [29]showed that IDA was associated with higher RBC counts and lower reticulocyte count. It has been postulated that in individuals with iron deficiency, erythropoiesis is impaired thus reducing the erythrocytes turn over whilst the reduced mean cell sickle haemoglobin concentration (MCHC-S) confers a relatively longer RBC life span, than in iron replete sickle cell individuals, thus allowing for a fair increase of the RBC mass.

Mean cell volume was found to correlate with the serum ferritin in this study. Implying those with low serum ferritin had low MCV. However the correlation is deemed a poor ($r = 0.296$). This corroborates with the findings previously reported by Adekile *et al*[64] and Tiwari *et al*[65]. The later (Tiwari *et al*) reported a positive correlation ($r = 0.496$, $p = \leq 0.001$) between serum ferritin and MCV amongst pregnant women in 2nd and 3rd trimester. They further reported a statistically

significant correlation between serum ferritin and Hgb and RDW, contrary to our findings in this study. In a study by Kassim *et al*[28] and Vichinsky *et al* [39], low MCV was found in patients confirmed with IDA. Iron deficiency anaemia is known to result in reduced intracellular concentration of haemoglobin (MCHC), causing an overall RBC volume reduction [66].

Iron deficiency anaemia in SCA is thought to be associated with reduced haemolysis rate and increased RBCs survival [43,67]. This is hypothesized to result from reduced MCHC-S, thus reduced rate of HBS polymerisation upon deoxygenation [43,68]. This theoretical evidence of benefit of IDA in SCA was demonstrated in case series of 4 patients by Castro *et al,* upon iron restriction, there was improved anaemia, reduced MCHC, reticulocyte count, RDW, dense cells and haemolytic rate; reduced hospitalisation duration and improved RBC survival, and rapid healing of chronic leg ulcer [68]. Despite of the reported benefits, non-haematological complications of IDA were not explored in their limited case series.

The known non-haematological complications IDA include impaired intellectual performance, growth retardation, abnormal behaviour, anorexia, poor weight gain (in children), reduced work, physical performance and productivity [6,13,69]. These are significant and should be prevented. However empirical treatment with iron salts in patients with chronic haemolysis (SCA) and recurrent transfusion may pose a risk for iron overload. Larger controlled studies exploring the benefits vis-a-vis risks of IDA in SCA are warranted to guide clinical management of patients, controlled iron restriction has postulated to as therapeutic strategy in alleviating sickling crises and disease burden [68].

In this study other clinical risk factors namely, presence of anaemia symptoms, pallor, stigmata of IDA and jaundice were not found to be associated with the diagnosis of IDA.

6.0 CONCLUSIONS

This study asserts that iron deficiency anaemia is common in adults with sickle cell anaemia.

The prevalence varied (16.5% - 50%) depending on the diagnostic methods employed, underscoring the variation in detection capability.

Various diagnostic models have been shown to possess varied accuracy indices. Serum ferritin provides below 100µg/L a good screening test for IDA for SCD.

MCV was found to correlate with the diagnosis of iron deficiency anaemia in adults with SCA.

5.0 STUDY STRENGTHS AND LIMITATIONS

Strengths

This study has determined the prevalence of IDA in adult with SCA. Most studies have dealt with paediatric groups. Diagnostic models comprising of the common tests have been amply compared. It has thus well exemplified the diagnostic dynamics and challenges of encountered diagnosing IDA amongst patients with SCA.

Limitations

Small number of patients enrolled for bone marrow aspiration (BMA), might have affected the precision and significance of the associations. A few participants opted out of aspiration procedure. This was mitigated by use of inferior diagnostic models of serum ferritin and transferrin saturation.

Use of red blood cell indices to determine eligibility for BMA limited exploring their association with BM iron status. Nonetheless this allowed for ethical consideration.

Financial constraints prevented measurements of haemolytic and inflammatory markers; this would have elucidated the current findings.

In this study recall bias may have affected the participant’s ability to recollect information such as the last time painful crisis was experienced and number of units of blood ever transfused.

7.0 RECOMMENDATIONS

Patients with sickle cell anaemia should be screened for IDA and treated accordingly.

Importance of availability of tests such as FBP and iron studies cannot be overemphasised.

Health providers are hereby called to attention on the prevalence of IDA in SCA.

A case control study design to determine the iron status in sickle cell anaemia would be exceedingly informative.

This study found severe anaemia in 26% of the study population, larger causal study for severe anaemia and IDA in SCA is recommended.

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APPENDICES

Appendix I: Questionnaire (English version)

IDS Study No |__|__|__|

SCD Study No|__|__|__|__|

PROFORMA FOR IRON DEFICIENCY IN SCD STUDY-

SCREENING (registration station)

- Today's Date (DD-MM-YY) ……..|__|__|-|__|__|-|__|__| **DATE TOD**

DEMOGRAPHIC HISTORY (registration station)

- Hospital ID number HID)……..…..|__|__|__|__|__|__|**HID**
- Name…….….|_____| **NAME**
- Date of birth……|__|__|-|__|__|-|__|__| **DOB**

MARITAL STATUS (registration station)

	N/Y		
MARRIED	|__|	**WIDOWED**	|__|
SINGLE	|__|	**COHABITING**	|__|
DIVORCED	|__|	**OTHERS**	|__|

HIGHEST LEVEL OF EDUCATION (registration station)

	N/Y		
INFORMAL/NONE	|__|	**UNIVERSITY**	|__|
PRIMARY	|__|	**STUDENT**	|__|
SECONDARY	|__|	**NOT EMPLOYED**	|__|

CURRENT EMPLOYMENT (registration station)

	N/Y		
CIVIL SERVANT	|__|	**SELF EMPLOYED**	|__|
PRIVATE SECTOR	|__|	**OTHERS**	|__|
FARMER/PEASANT	|__|		

LABORATORY INVESTIGATIONS (phlebotomy station)

SAMPLES TAKEN (Please tick)

2ml EDTA	
2 ml Red Top (Serum)	

DIAGNOSIS OF ANEMIA AND OTHER HAEMATOLOGICAL PARAMETERS (clinical station)

1. Lassitude/Fatigue (N/Y- details)…… |__|-|_____|ANAEFATIG
2. Breathlessness (N/Y- details)…….…… |__|-|_____|ANAEBREATH
3. Palpitation (N/Y- details)…….…… |__|-|_____|ANAEPALP
4. Dizziness (N/Y- details)……..…… |__|-|_____|ANAEDIZZ
5. Tinnitus (N/Y- details)…….. ..…… |__|-|_____|ANAETINN
6. Headache (N/Y- details)……..…… |__|-|_____|ANAEHEAD
7. Dimness of vision (N/Y- details)…… ……..…… |__|-|_____|ANAEDIMVIS

PAST MEDICAL HISTORY ***(clinical station)***

- Ever received blood transfusion (N/Y***) If yes go to next question*****…...|__| BTR
- Units of blood transfusion ever received……|_____|| UBT
- Ever admitted to hospital (N/Y)) If yes go to next question……|__| HADM
- Number of hospital admissions ever……..…..|_____|| NHADM

PHYSICAL EXAMINATION ***(clinical station)***

- Pallor (**N/Y**) ……|__| **PALL**
- Jaundice (**N**il / **T**inge/**M**oderate/**D**eep) ……..|__| **JAUN**
- Koilonychia (**N/Y**) ……...……...…….....|__|**KOI**
- Brittle nails (**N/Y**) …….. ……....|__|**BRI**
- Angular stomatitis (**N/Y**) ……..……..…|__|**ANG**

HAEMATOLOGICAL PARAMETERS

- WBC level (K/uL)…….……. |_____|**WBC**
- Platelet Level (K/uL)……. |_____| **PLT**
- Haemoglobin Level (g/dl) … …… ……|_____| **HAE**
- RBC Indices

MCV (fl)		**MCHC**	
MCH (pg)		**RDW (%)**	

Reticulocyte count (%)	
Haematocrit (HCT %)	
Reticulocyte Index (Calculated)	

BONE MARROW IRON STATUS

Positive	[ ]	Gale's Grade [ ]
Negative	[ ]	
Not done	[ ]	

BIOCHEMICAL PARAMETERS

Serum ferritin level……ug/L	[ ]
Serum Iron	[ ]
TIBC	[ ]
Transferrin Saturation	[ ]

Appendix II: Bone marrow iron staining Procedure (Perl's Prussian-blue reaction) and quality assurance procedures

4 air-dried smears were prepared for each patient (smeared immediately after aspiration). Followed by fixing in absolute methanol for 20 minutes at room temperature. The slides were thereafter kept in a mixture of equal volumes of hydrochloric acid (0.2N) and potassium ferrocyanide (20g/l) for 10 minutes. Followed by washing in tap water for 20minutes and counterstain with aqueous neutral red (10g/l) for 30 seconds, rinsed with tap water and allowed to air-dry.

In each batch a positive control slide and a fresh prepared stain was used. If the control slides did not prove satisfactory, the staining procedure is not accepted.

To prevent contamination, the glassware and slides were thoroughly washed by soaking in 2mol/l HCL followed by washing with ionized water.

Appendix III: Gale's Method of histological grading of bone marrow iron

Adopted from Phiri KS et al, J Clin Pathol. 2009 (40)

Grade 0	**None**	**No visible iron under high power magnification (x1000)**
Grade 1	**Very slight**	Small iron particles just visible in few reticulum cells under high power magnification (x1000)
Grade 2	**Slight**	Small, sparsely distributed iron particles just visible under low power magnification (x100)
Grade 3	**Moderate**	Numerous small iron particles present in reticulum cells throughout the marrow fragment (x100)
Grade 4	**Moderate heavy**	Larger iron particles throughout the fragment with tendency to aggregate into clumps (x100)
Grade 5	**Heavy**	Dense, large clumps of iron throughout the fragment (x100)
Grade 6	**Very heavy**	Very large deposits of iron, both intra- and extra-cellular, obscuring cellular detail in the fragment (x100)

Appendix IV: Consent form (English)

Consent to participate in the study of Prevalence and diagnostic diagnostics of Iron Deficiency Anaemia in Adult Sickle Cell Patients attending MNH.

Dear Sir/Madam,

Greetings!

My Name is Dr. Mbonea Yonazi, a resident doctor in the Department of Haematology and Blood Transfusion at MUHAS. I am conducting a study regarding the prevalence of Iron Deficiency Anaemia among Sickle Cell Disease Patients attending MNH. I am requesting your participation.

PURPOSE OF THE STUDY:

The aim of this study is to determine the prevalence of Iron Deficiency Anaemia among sickle cell patients.

Anaemia is reduction of the level of haemoglobin in the blood that can occur due to nutritional deficiencies, blood loss or destruction of red cells. Iron deficiency anaemia occur as a result of deficiency of iron in the body.

HOW TO PARTICIPATE:

Patients who will be ready to participate will sign a consent form to approve his/her willingness.

Short interview will be done and sample for investigation such as blood and Bone Marrow Aspiration will be taken.

CONFIDENTIALITY:

Information obtained from you will be confidential and will be of help in this study and better care for patients with sickle cell disease in the future.

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 of 10mls will be drawn from your arm for laboratory tests. And that for bone marrow aspiration, 2mls will be drawn from the pelvic bone, after

anaesthetising the region well; this will reduce the pain. We do not expect risk by drawing blood although you will feel some pain when the needle pierces your skin for drawing this blood (on the arm), and when bone marrow sample is aspirated. The skin on your arm and over you pelvic will be thoroughly cleaned prior to prevent infections.

BENEFITS:

Your participation in this study will help you know whether or not you have Iron deficiency and its associated anaemia, and thus appropriate treatment of the same.

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 Sickle Cell Disease patients.

CONTACT PERSONS:

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

Dr. Mbonea Yonazi
Principal Investigator
Muhimbili University of Health and Allied Sciences (MUHAS)
Department of Haematology and Blood Transfusion
P.O. Box 65001 Dar es Salaam.
Tel. 0713 215 930

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

Professor Mainen J. Moshi
The Chairman
Senate Research and Publication Committee 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 IV: Consent form (Swahili Version)

FOMU YA MAKUBALIANO YA KUSHIRIKI KATIKA UTAFITI

Habari! Mimi ni Dk Mbonea Yonazi na ni Daktari katika shahada ya Uzamili katika Chuo Kikuu Cha Sayansi Za Tiba cha Muhimbili. Nafanya utafiti kuhusu upungufu wa damu unaotokana na upungufu wa madini ya chuma miongoni mwa wenye Seli hai Mundu (Sickle cell) hapa Muhimbili hospitali.

Ninaomba ushirikiano wako.

Nia ya Utafiti;

Dhumuni ni kujua ni wagonjwa wangapi wana upungufu wa damu unaotokana na upungufu wa madini ya chuma miongoni mwa wagonjwa wa sickle cell wanaotibiwa hapa Muhimbili.

Upungufu wa damu (anemia) unasabishwa na kupungua kwa wekundu wa damu, unaoweza kutokana upungufu wa viinilishe, kupoteza damu au kuharibiwa kwa chembe nyekundu za damu. Upungufu wa madini ya chuma mwilini ni moja ya sababu zinazosababisha upungufu wa damu.

Jinsi ya Kushiriki:

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

Usiri:

Taarifa ya magonjwa yako hazitatangazwa kwa yoyote zaidi ya mtafiti. Matokeo ya utafiti kwa ujumla yatasaidia kuboresha huduma ya tiba kwa kwa wagonjwa wa sickle cell.

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.

Madhara:

Kiasi cha mililita 10 za damu kitachukuliwa kutoka kwenye mkono wako kwa ajili ya vipimo vya kuangalia wingi wa damu, na viashiria vya madini ya chuma kwenye damu/mwilini. Na kwa damu ya mfupa, kiasi cha mililita 2 ndicho

kitakachochukuliwa, kwenye mfupa wa kiuno, baada ya kupasafisha vema na kukuchoma sindano ya ganzi. Hatutegemei hatari/madhara yoyote kwa utoaji huu wa damu, ni kawaida kusikia maumivu kwa mbali, ya muda mfupi.

Faida:

Kushiriki kwako katika utafiti huu, kutakusaidia kujua uwepo wa upungufu wa damu unaotokana na upungufu wa madini ya chuma.

Pia utafaidika kupata matibabu halisia kama itakavyokuwa inahitajika ili kutibu upungufu wa madini ya chuma unaoleta upungufu wa damu.

Ni tumaini letu kuwa utafiti huu utasidia kuboresha huduma kwa wagonjwa wa sickle cell hapa kwetu na penginepo.

Nitakushukuru kwa kushiriki kwako utafiti huu. Aksante.

Iwapo utakuwa na swali lolote kuhusu utafiti huu wasiliana na Dr. Mbonea Yonazi, Chuo kikuu Cha Afya Na Sayansi za Tiba Muhimbili; Idara ya Tiba; S.L.P 65001 Dar Es Salaam. Simu 0713215930.

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

Professor Mainen J. Moshi

Mkurugenzi wa kamati ya tafiti na matoleo chuoni. Chuo Kikuu Cha Afya na Sayansi za Tiba 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……..