Association of Maternal factors and Iron Deficiency Anaemia in Pregnant women attending antenatal care at Chongwe Rural Health Centre in Chongwe District.

By

Elizabeth Chewe Nakanyika

"A Dissertation submitted to the University of Zambia in partial fulfilment of the requirements of the degree of Master of Science in Pathology (Haematology)"

The University of Zambia

Lusaka

© 2018

Declaration

This Dissertation represents Elizabeth Chewe Nakanyika's own work and has not been previously submitted for a degree, diploma or other qualification at this or any other University.

Candidate Name: **Elizabeth Chewe Nakanyika**

Signature:.....

Date:.....

Certificate of Approval

This dissertation of Elizabeth Chewe Nakanyika has been approved in partial fulfilment of the requirements for the degree of Master of Science in Pathology (Haematology) by the University of Zambia.

……	…… ……	……. ……
Supervisor	Signature	Month/Date/Year
……	…… ……	……. ……
Examiner 1	Signature	Month/Date/Year
……	…… ……	…….. ……..
Examiner 2	Signature	Month/Date/Year
……	…… ..……	…….. ……..
Examiner 3	Signature	Month/Date/Year

Abstract

Introduction

Iron deficiency anaemia (IDA) is estimated to occur in (18%) of pregnant women in Africa despite universal supplementation programs being implemented since 2001, as recommended by the World Health Organisation. A lack of data on iron status and maternal factors associated with iron deficiency anaemia make it difficult to manage specific patients and prevent adverse outcomes. Therefore, this study aimed to determine the prevalence and maternal factors associated with IDA in women attending antenatal care in a rural setting in Chongwe, Lusaka.

Methods: This cross-sectional study included 225 pregnant women visiting antenatal clinic at Chongwe Rural Health Centre in Chongwe District. Demographic data and information on maternal age, gestational age, educational status, socioeconomic status and clinical data were collected from all the subjects via a standard questionnaire. Complete blood count was performed, and Soluble Transferrin Receptor levels were measured to assess anaemia and iron status, respectively. Data was analysed using SPSS software version 23.

Results: The prevalence of anaemia was (39.1%) 88/225 out of which (9.1%) 8/88 presented with iron deficiency. It was found that (78.13%) 50/64 were on Iron supplements and (44.68%) of them were anaemic but none were iron deficient. Abdominal pains (OR 5.11 [1.15-22.80], $p > 0.032$) and primary education relative to secondary education (OR 2.18 [1.24-3.84], $p < 0.007$) were the maternal factors with the highest odds for IDA and anaemia without iron deficiency, respectively

Conclusion: Women not receiving iron supplementation were at a significant risk of presenting with IDA. Participants with IDA were likely to present with abdominal pain unlike other types of anaemia. A decrease in monocyte count was the only blood parameter that could potentially differentiate IDA from other types of anaemia. Therefore, pregnant women with the above features should be screened stringently to prevent and/or manage IDA.

Dedication

This dissertation is dedicated to my wonderful daughter Isabella Chansa Kabwe. I also dedicate this work to my parents Beatrice and Elicko Tobias Chewe, My Husband Mwila and my Brothers and Sisters.

Acknowledgements

Firstly, thanks and Praises goes to the Lord Almighty, Jesus Christ for He has been and always will be Faithfull in all ups and downs. In no order, I would like to thank the following for helping in my successful completion of this work. I would like to acknowledge the efforts and contributions of Dr Sumbukeni Kowa and Dr Lydia Korolova who gave me valuable support regardless of their limited and precious time. I would like to further thank the participants at Chongwe Rural Health centre as well as Chongwe District Health Office Chongwe for allowing me carry out this study. Many thanks to Lusaka Apex Medical University for the financial assistance rendered. Not forgetting the advice and support from my fellow students and family. The Lord God Almighty be with you.

Table of Contents **Page**

Declaration ii

Certificate of Approval iii

Abstract iv

Dedication v

Acknowledgements vi

Table of Contents vii

List of Tables ix

List of Figures/maps and Illustrations x

List of Abbreviations and Acronyms xi

List of Appendices xiii

Chapter 1: Introduction 1

1.1 Background 1
1.2 Statement of Problem 3
1.3 Justification of the Study 3
1.4 Literature Review 4
1.4.1 Epidemiology of Iron Deficiency Anaemia 4
1.4.2 Effect of iron supplementation on anaemia during pregnancy 4
1.4.3 Biomarkers of iron deficiency anaemia 6
1.4.4 Factors associated with maternal Iron deficiency anaemia 8
1.5 Research Questions 10

1.6 Objectives 10

Chapter 2: Methodology 11

2.1 Study Design 11

2.2 Study Site 11

2.3 Study Population 11

2.3.1 Inclusion Criteria 11

2.3.2 Exclusion Criteria 11

2.3.3 Sample Size 12

2.4 Sample collection and processing 12

2.5 Determination of iron status of pregnant women 13

2.6 Determination of Haemoglobin concentration 15
2.7 Identification of Maternal factors associated with Iron deficiency anaemia 15
2.8 Data Analysis 15
2.9 Ethical Considerations 16
Chapter 3: Results 17
3.1 Participant Recruitment and Descriptive 17
3.2 Prevalence and clinical characteristics of participants 20
3.3 Iron status of pregnant women 21
3.3.1 Relationship between Iron status at different stages of pregnancy 22
3.3.2 Clinical symptoms associated with Iron Status 23
3.4 Association of Iron supplementation with Iron deficiency anaemia 25
3.4.1 Association of Iron supplementation with maternal haemoglobin at 26
3.4.2 Association of Iron supplementation with maternal sTfR at various gestation stages 27
3.5 Maternal factors associated with IDA compared with Anaemia 28
Chapter 4: Discussions 30
4.1 Discussion 30
4.2 Conclusion 35
4.3 Limitations of the Study 36
4.4 Recommendations 37
5.0 References 38
6.0 Appendices 47
6.1 Appendix A: Information Sheet 47
6.2 Appendix B: Informed Consent For 49
6.3 Appendix C: Study Questionnaire and Clinical Examination Form 50
6.4 Appendix D: Letters of Support 53
6.4.1 Graduate Proposal Presentation Forum 53
6.4.2 Authority from Chongwe District Health Office 54
6.4.3 University of Zambia Biomedical Research Ethics Committee 55

List of Tables **Page**

Table 3.1.1: Participant key descriptive stratified by HIV status 19
Table 3.1.2: Participant key description between different gestation age groups..... 19
Table 3.2: Characteristics of participants with IDA..... 20
Table 3.3: key description of participant's iron status 22
Table 3.4: Key description of participants relative to supplementation..... 25
Table 3.5: Factors associated with IDA compared with anaemia 29

List of Figures, Maps and illustrations **Page**

Figure 2.4: Sample processing flow 13
Figure 2.5: Serum transferrin receptor analysis protocol 14
Figure 3.1: Iron status relation to anaemia at different stages of pregnancy 18
Figure 3.3.1: Iron status in relation to anaemia at different stages of pregnancy 23
Figure 3.3.2: Clinical features clinical feature correlation in iron status in pregnant women 24
Figure 3.4.1: Haemoglobin levels among pregnant women 26
Figure 3.4.2: Soluble Transferrin Receptor levels among pregnant women 27

List of Abbreviations and Acronyms

ART	Antiretroviral Therapy
ANC	Antenatal Care
BP	Blood Pressure
CBC	Complete Blood Count
CRHC	Chongwe Rural Health Centre
CDC	Centre for Disease Control and prevention
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent assay
FBC	Full Blood Count
FID	Functional Iron Deficiency
g/dl	gram per decilitre
HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
mg/dl	Milligrams per Deciliter
mm^3	Cubic milometers
NHANES	National Health and Nutrition Examination Survey
NCHS	National Centre for Health Statistics
pg	picograms
RBC	Red Blood Cells

RDW	Red Cell Distribution Width
SF	Serum Ferritin
SPSS	Statistical Package for Social Sciences
sTfR	Soluble Transferrin receptor
USAID	United States Agency for International Development
UNZA	University of Zambia
WBC	White Blood Cell
WHO	world Health Organisation
ZPP	Zinc Protoporphyrin

List of Appendices **Page**

Appendix A: Information Sheet 47

Appendix B: Informed Consent Form 49

Appendix C: Study questionnaire and Clinical Examination Form..... 50

Appendix D: Letters of Support..... 53

Chapter 1: Introduction

1.1 Background

Iron deficiency anaemia (IDA) is the most prevalent nutritional disorder affecting approximately two billion people worldwide with mortality rates reported as high as 20% in Africa (Alwan *et al*, 2015, Iannotti *et al*, 2005; Kefiyalew *et al*, 2015). Iron deficiency anaemia has been defined by the World Health Organisation (WHO) as anaemia accompanied by depleted iron stores and signs of a compromised supply of iron to the tissues (Abdelrahman *et al*, 2012). The compromised supply of iron and blood may lead to impaired mental and psychomotor development and can further lower work tolerance, productivity as well as the quality of life (Campigotto *et al*, 2015, Abu-Ouf and Jan, 2015). In pregnancy, IDA is associated with negative perinatal outcomes including premature labour, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anaemia (Nojilana *et al*, 2007, Abu-Ouf and Jan 2015). Clinical features of IDA are a result of lowered oxygen delivery to the tissues and may include but not limited to; fatigue, apathy, pallor, fainting, and breathlessness. Other features include headaches, palpitation, hair loss, and tinnitus. Iron deficiency anaemia can also impair the immune system leading to an increase in susceptibility to infections and worsening treatment outcomes (Abu-Ouf and Jan, 2015). A preliminary review of published peer reviewed literature indicates an association of IDA with poor socio-economic status, multiple gestations, parity and education status. However, most of these factors vary from one region to another. (Raza *et al*, 2011, Paliwadana *et al*, 2014 Aikawa *et al*, 2005, Raza *et al* 2014; Ononge *et al*, 2014; Obui *et al*, 2016). In Zambia, literature search yielded no studies on maternal factors associated with IDA nor where there any data on the current epidemiology in any part of the country. The generation of this data is very vital in a country were anaemia in pregnancy is the most common cause of death among women in

child bearing age and a high burden of infectious diseases. The lack of routine data on IDA and factors associated with it, has made it difficult to manage patients especially that all patients in this group may be treated equally when a certain group among them may be at more risk (WHO, 2001). Most African countries including Zambia have adopted the universal iron supplementation programme since 2001 with expectations of prevention of anaemia including IDA. However, programme managers haven't had any funding for the strategic management to improve the quality of delivery and improve outcomes. Further, there has not being any systematic monitoring of the performance of such intervention (WHO, 2001). Despite the adoption of iron supplementation programme, rates of anaemia have remained high as seen in studies done in Nigeria (Okafor *et al*, 2013; Okafor *et al*, 2017). Hence this study attempted to define the prevalence of Iron Deficiency anaemia as well as determine the maternal factors associated with it in pregnant women.

1.2 Statement of the problem

Although iron deficiency anaemia has been recognised as a major problem in pregnant women worldwide, the extent to which it affects Zambian women and the factors associated with it, have not been defined. Maternal iron deficiency anaemia increases risk of pre-term birth, low birth weight delivery and is a significant cause of morbidity in infants and perinatal as well as maternal mortality (Nojilana *et al*, 2007). Although free iron supplementation programme is available, evidence of iron deficiency anaemia in Africa remains high. (Okafor *et al*, 2013; Okafor *et al*, 2017). In Zambia, available data on anaemia as well as iron deficiency anaemia is based on haemoglobin (Hb), although it's not specific for iron deficiency anaemia. The factors associated with iron deficiency anaemia remain unknown due to a lack of preliminary data.

1.3 Justification of the study

Although substantial data on haemoglobin concentration may be available in hospital routine work, it hasn't been systematically collected in Zambia and cannot be used to infer on management of iron deficiency anaemia. Few studies in sub-Saharan Africa have reported on Iron status and iron deficiency anaemia (Lannoti *et al*, 2005) but none in Zambia. The findings from this study will inform on risks associated with iron deficiency anaemia and provide an insight into its management based on the patients' supplementation needs. Iron supplementation is universal but contingent on the availability of the supplements. Hence, identifying the factors associated with iron deficiency will increase the chances of right prioritising in supplementation in facilities such as Chongwe Rural Health Centre (CRHC).

1.4 Literature review

1.4.1 Epidemiology of IDA

Globally, Iron deficiency anaemia affects approximately 19.2% of pregnant women. The highest rates have been reported in Africa and estimated at 20.3% contributing to 20% mortality in pregnant women (Asobayire *et al*, 2001; Ahmed *et al*, 2003; Iannotti *et al*, 2005; Black *et al*, 2013; Kefiyalew *et al*, 2015). IDA is a major health problem associated with poor maternal and perinatal outcomes, such as mortality regardless of maternal age or parity (Abdelrahman *et al*, 2012; Kefiyalew *et al*, 2014). Pregnancy increases the need for iron to meet the demands of approximately 40% increase in blood volume and requirements for the growth of the foetus, placenta and other maternal tissues making most women unable to meet their own iron needs without supplementation especially during the 2nd and 3rd trimesters. Iron deficiency and IDA increases the risk of preterm birth and low birth weight (Nils Milman, 2012; Palma *et al*, 2008). Iron deficiency anaemia may also lower resistance to infections but may also reduce the risk of certain infections such as malaria. IDA has also been shown to delay cognitive and psychomotor development in infants resulting in lasting effects in learning, work productivity, health and growth (Nojilana *et al*, 2007). Infants born to iron deficient mothers are predisposed to iron deficiency during the first year of life with related consequences of developmental delays, behavioural disturbances, poor cognitive outcomes (Iannotti *et al*, 2005; Alwan *et al*, 2015), low birth weight and preterm delivery (Ahmed *et al*, 2003; Kefiyalew *et al*, 2014; Alwan *et al*, 2015).

1.4.2 Effect of Iron Supplementation on Anaemia during pregnancy

Iron is an important requirement for proper development of the foetal brain as well as cognitive abilities of a new-born. During pregnancy, iron absorption is increased when menstruation stops, however, pregnant women still do not absorb sufficient additional iron

and the risk of iron deficiency increases. Consequently, it is recommended that all pregnant women are given iron supplements on their first antenatal visit (WHO, 2001; Emegoakor *et al*, 2015). Iron supplementation has been associated with lower prevalence of Iron deficiency and IDA. There is also evidence that it improves the physical well-being of pregnant women (Nils Milman, 2012). A report by the United States Agency of International Development (USAID) observed that iron supplementation in Nicaragua had led to the reduction of anaemia in pregnant women from 33% in 1993 to 16% in 2003 and similarly in India. In Nicaragua, the National level anaemia prevention program included free iron and folic acid distribution through ANC as well as wheat flour fortification which included vitamin A, while in India their program included weekly iron/folic acid distribution via ANC, deworming using Abendazole as well behaviour change communication which also covered the nutritional aspect (USAID ,2006). In Zambia, the Zambia National Strategy and Plan of Action for prevention and control of vitamin A and Anaemia supplementation programme included vitamin A supplementation, sugar fortification, maize-meal fortification, Iron and folic acid supplementation, parasite prevention and control as well as dietary diversification. This program was commenced in 1999 and ended in 2004 (USAID, 2004). However, the effectiveness and impact of this program since inception up to date has not been documented.

However, iron supplementation has been shown to increase the haemoglobin concentration in some women. Higher than normal haemoglobin concentrations are associated with poor pregnancy outcomes, for instance, high Hb concentrations may cause high blood viscosity which result in both compromised oxygen deliveries to tissues and cerebrovascular complications. High Hb concentrations are also associated with increased risk of adverse birth outcomes such as foetal death, intrauterine growth retardation, preterm delivery and low birth weight (Yip, 2000). In addition, there is also sufficient evidence that iron

supplementation is associated with glucose impairment and hypertension in mid-pregnancy. Iron supplementation significantly increases serum ferritin levels which have been positively associated with diabetes risk, hypertension, metabolic syndrome, cardiovascular risk factors and inflammation (Yip *et al*, 2000; Bo *et al*, 2009,). Moderately elevated iron stores are also believed to increase the risk of type 2 diabetes (Scholl, 2005). Iron supplementation may also lead to accelerated body iron overload in women with (undiagnosed) genetic Haemochromatosis (Milman, 2012) and increased risk of acquiring *Plasmodium vivax* in a malaria endemic setting within 30 days of supplementation (Mwangi *et al*, 2015).

1.4.3 Biomarkers of Iron Deficiency Anaemia

The progression to IDA is a stepwise process that begins with depletion of iron stores, followed by iron deficient erythropoiesis and finally reduction in Hb concentration. Its manifestation is therefore made known through Hb levels. Haemoglobin is however not a very sensitive marker as its values overlap in deficient and nondeficient subjects and is not specific for IDA as it does not provide any information about serum and / or tissue iron status (Karakochuk et al, 2015). Haemoglobin is widely driven by genetic haemoglobin disorders rather than iron deficiency (Karakochuk et al, 2015. In addition, a peripheral blood film examination provides less information with red cell distribution width (RDW) having poor performance in diagnosing IDA compared to serum ferritin (SF) as the gold standard (Abdelrahman et al, 2012). Serum Ferritin is generally considered to be a more sensitive measure of iron status and is most widely used marker for assessing iron status (Abdelrahman et al, 2012; Alwan et al, 2015). Serum ferritin, however, is unable to identify pregnant women with Functional Iron Deficiency, a state in which there is insufficient Iron incorporation into erythroid precursors even when there is adequate body iron store (Wayne et al, 2013). In addition, even when iron balance is positive, concentrations of SF are often

reduced after first trimester of pregnancy. To negate the pitfalls of SF, soluble Transferrin receptor (sTfR) can be measured and has been validated in various population groups. Soluble transferrin receptor is preferred unlike, SF which has been shown to be of poorer performance affected by inflammatory conditions a common feature during pregnancy (Alwan *et al*, 2015; Karakochuk *et al*, 2015). The ratio sTfR:SF has also been suggested to improve the ability to predict iron status, however, it is not known whether this would overcome the limitations of SF due to inflammation (Knowle *et al*, 2013). Another marker of iron stores in the body could be the determination of Iron deficiency erythropoiesis. Iron deficiency erythropoiesis is usually a result of excess formation of Zinc protoporphyrin (ZPP) that indicates the systemic supply of iron to erythrocytes in the bone marrow. Zinc protoporphyrin can be measured instantly with a low-cost assay that uses a portable haematofluorometer and may be used in combination to haemoglobin concentration in surveys aimed at assessing population iron status. However, whole blood ZPP and erythrocyte ZPP have been shown to have little diagnostic utility as a marker of iron deficiency even when combined with Hb (Mwangi *et al*, 2014). This study therefore, employed sTfR assessment as marker of iron status in pregnant women. Iron status in women with respect to soluble transferrin receptor (sTfR) has been defined with sTfR values ranging from 1.9 mg/L to 4.4 mg/L, above and below which is considered deficiency and overload respectively while anaemia in pregnant women is defined by Hb of less than 11.0g/dL (Khalafallah and Dennis 2012). Iron status assessment is not routinely performed in the care of women attending antenatal care in most African regions, however, Hb concentration is routinely performed upon which decision for management of anaemia are based. Hb is not specific to any type of anaemia and therefore, makes directed therapy challenging (Karakochuk *et al*, 2015). In addition, the physiological changes that occur during pregnancy makes some of the haematological parameters impotent as indicators for

the diagnosis of IDA (Abdelrahman *et al*, 2012). The lack of sensitivity of these markers may be due to their alteration by gestation, independent of iron status (Karakochuk *et al*, 2015). However, cellular need for iron is reflected by the amount of soluble transferrin receptor in serum (sTfR). Soluble transferrin receptor levels are increased with tissue iron deficiency and with increased erythropoiesis but unlike other biomarkers, sTfR does not appear to be influenced by inflammatory diseases and has been reported to have a low biological and analytic variability (Akesson *et al*, 1998).

1.4.4 Factors associated with Maternal Iron Deficiency Anaemia

Information on factors associated with maternal IDA is scarce as most studies look at factors associated with unspecified anaemia, defined by low Hb. A review in Brazil by Campigotto and his colleagues found that most of the maternal factors associated with iron deficiency anaemia fail under three main categories. These included socio-economic factors, obstetric factors and health indicators (Campigottto *et al* 2015). However, these vary depending on the region. The socio-economic factors associated with iron deficiency anaemia mainly include the education status of the mother, particularly lower education level. A study in Nigeria found that there was a negative correlation between lower education level of the mother and Iron deficiency anaemia (Olatunbosun *et al*, 2014). Similar findings have been reported else-where world-wide (Raza *et al*,2011, Ononge *et al* 2014, Obui *et al* 2016). Other socio-economic factor associated with IDA were marital status in particular, being married, maternal age and residing in rural areas (Campigotto *et al*, 2015, Chowdhury *et al* 2015). In addition, obstetric factors have also been associated with IDA, these include multiple pregnancies, advanced gestational age, history of previous miscarriage and late on-set pre-natal hospital visits (Campigotto *et al* 2015, Banga *et al*, 2016). The main obstetric factors are, an increase parity and gravida that have been related

to anaemia causing exhaustion and exacerbating IDA (Bangal *et al*, 2016). Obesity as an indicator of health has also been a factor associated with IDA, however, its association have either been positive or negative, or non-existent in different regions (Kordas *et al*, 2013; Campigotto *et al*, 2015).

1.5 Research questions

1.5.1 What are the maternal factors associated with iron deficiency anaemia in pregnant women attending antenatal care at Chongwe rural health centre (CRHC)?

1.6 Objectives

1.6.1 General Objective

To identify maternal factors that could aid in screening for iron deficiency anaemia in pregnant women attending antenatal care at Chongwe rural health centre, in Chongwe, Lusaka.

1.6.2 Specific Objectives

1.6.2.1 To determine the iron and haemoglobin levels of pregnant women at different gestational ages attending antenatal care.

1.6.2.2 To establish the prevalence of iron deficiency anaemia among pregnant women attending antenatal care.

1.6.2.3 To determine the maternal factors associated with IDA in pregnant women attending antenatal care.

Chapter 2: Methodology

2.1 Study design

This was a cross-sectional study that recruited pregnant women of different ages and stages of pregnancy. Demographic and clinical data was collected to determine the association between maternal factors and IDA using sTfR as a biomarker for iron status and Hb to define anaemia.

2.2 Study site

Since most Zambians (61.5%) (2010 census of population and housing) live in rural areas, this study was carried out at a primary antenatal clinic in Chongwe, a rural district in Lusaka province of Zambia.

2.3 Study population

All pregnant women attending antenatal clinic at Chongwe Rural Health Centre in Chongwe District where approached to participate in this study. The study participants were recruited only once on their first ANC visit during the study period. Pregnant women below the age of 18 years were excluded from this study.

2.3.1 Inclusion criteria

All pregnant women above 18 years attending antenatal care were approached to provide written consent to take part in the study.

2.3.2 Exclusion criteria

The participants with a history of blood transfusion within the last 3 months and those below 18 years of age were excluded from the study.

2.3.2 Sample size calculation

A study in a similar setting in Africa has indicated prevalence of IDA as 17.9% (Okafor et al, 2013). Using this rate and estimating that the actual prevalence to be within 5%, with 95% confidence level. Sample size was calculated using the Kish Leslie formula for cross-sectional studies.

Where;

n = minimum sample size

Z = statistic for 95% confidence interval, 1.96

P = expected prevalence, and

d = precision, 0.05

Minimum sample size was calculated at 225 participants and 225 were recruited in the study.

2.4 Sample collection and processing

2ml of blood was collected by venepuncture from each consenting participant in an ethylenediaminetetraacetic acid (EDTA) container for Complete blood count (CBC) and preparation of a thin smear for malaria parasite examination. Another 2mL in a plain container for measurement of sTfR and Rapid Plasmin Reagen (RPR) was collected. The 2ml EDTA blood collected for CBC and thin smear was also used for HIV testing. All tests except sTfR are done routinely as part of standard care practice. The serum for sTfR was collected by centrifugation of the plain container at 3000 rpm for 15 minutes at 4^0C and placed in a 2ml cryovial which was stored at -20^0C before being transported to the University Teaching Hospital (UTH) in Lusaka where it was stored at -80 ^{0}C and later

processed after 3 months using a Human sTfR Quantikine In Vitro diagnostic (IVD) ELISA kit was used from R&D systems). The processing flow is shown in the figure 2.5 below.

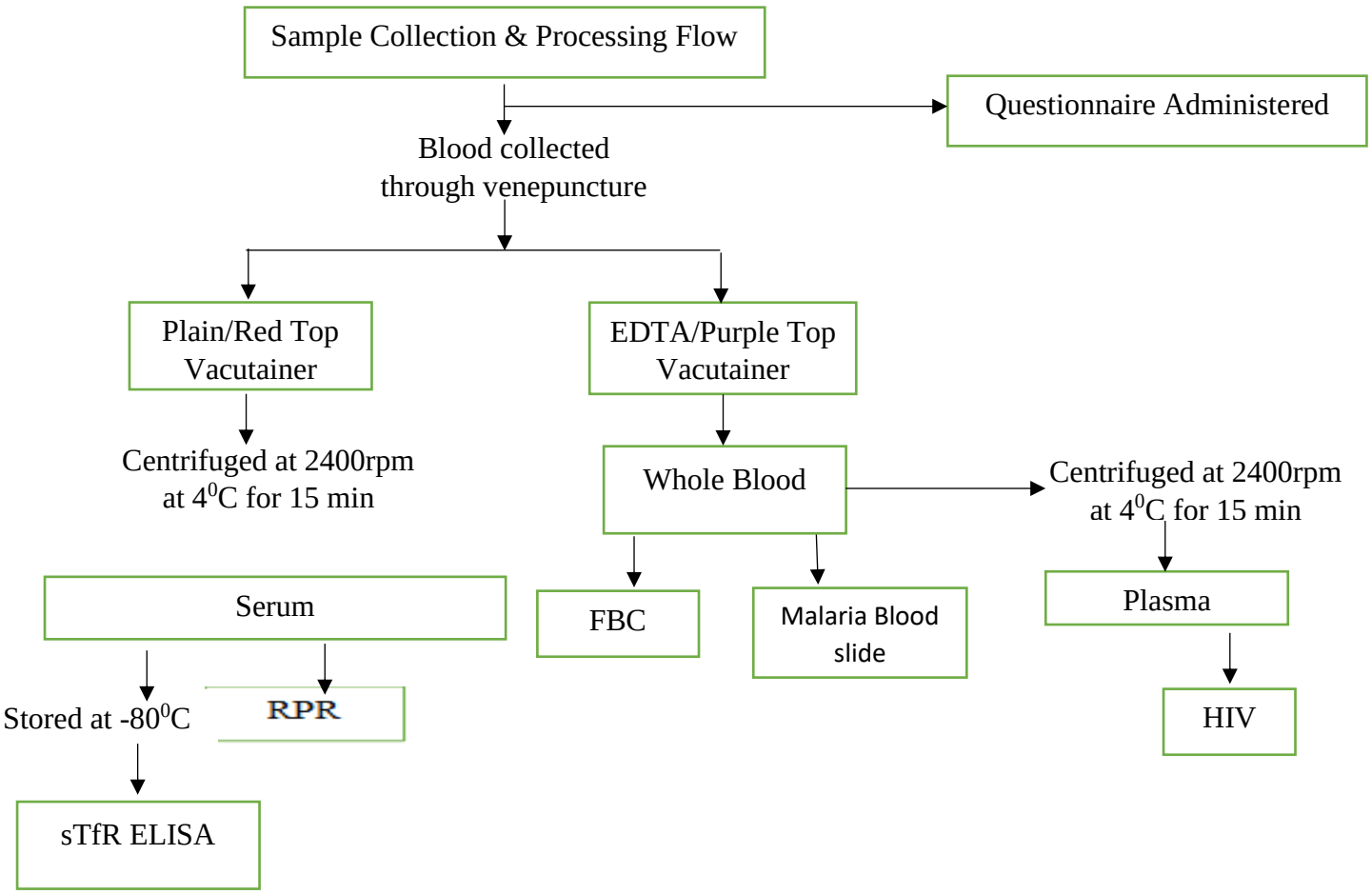

Fig 2.4: Sample processing flow.

2.5 Determination of Iron status of pregnant women attending antenatal care

A Human sTfR Quantikine In Vitro diagnostic (IVD) ELISA kit was used from R&D systems to determine the iron status of the pregnant women. The Absorbance was read on a BioTeck EL800 spectrophotometer available at UTH, Tropical Gastroenterology and Nutrition (TROPGAN) laboratory. The ELISA kit was received with diluted wash buffer, stop solution, sTfR specimen diluent, sTfR assay diluent, sTfR conjugate, substrate,

standards, controls and microplates. All were kept in the refrigerator at 2-8°C. Frozen serum samples and reagents were brought to room temperature. Wash buffer concentrate was prepared mixing gently to dissolve any crystals at room temperature before diluting 20mL of it in deionized miliQ water to make a 500mL wash buffer (working solution). sTfR controls were also reconstituted with 200µL of deionised MilliQ water, vortexed and allowed to sit for a minimum of 30 minutes before use. All other reagents were ready for use without requiring prior preparation. The procedure is illustrated in figure 2.5 below.

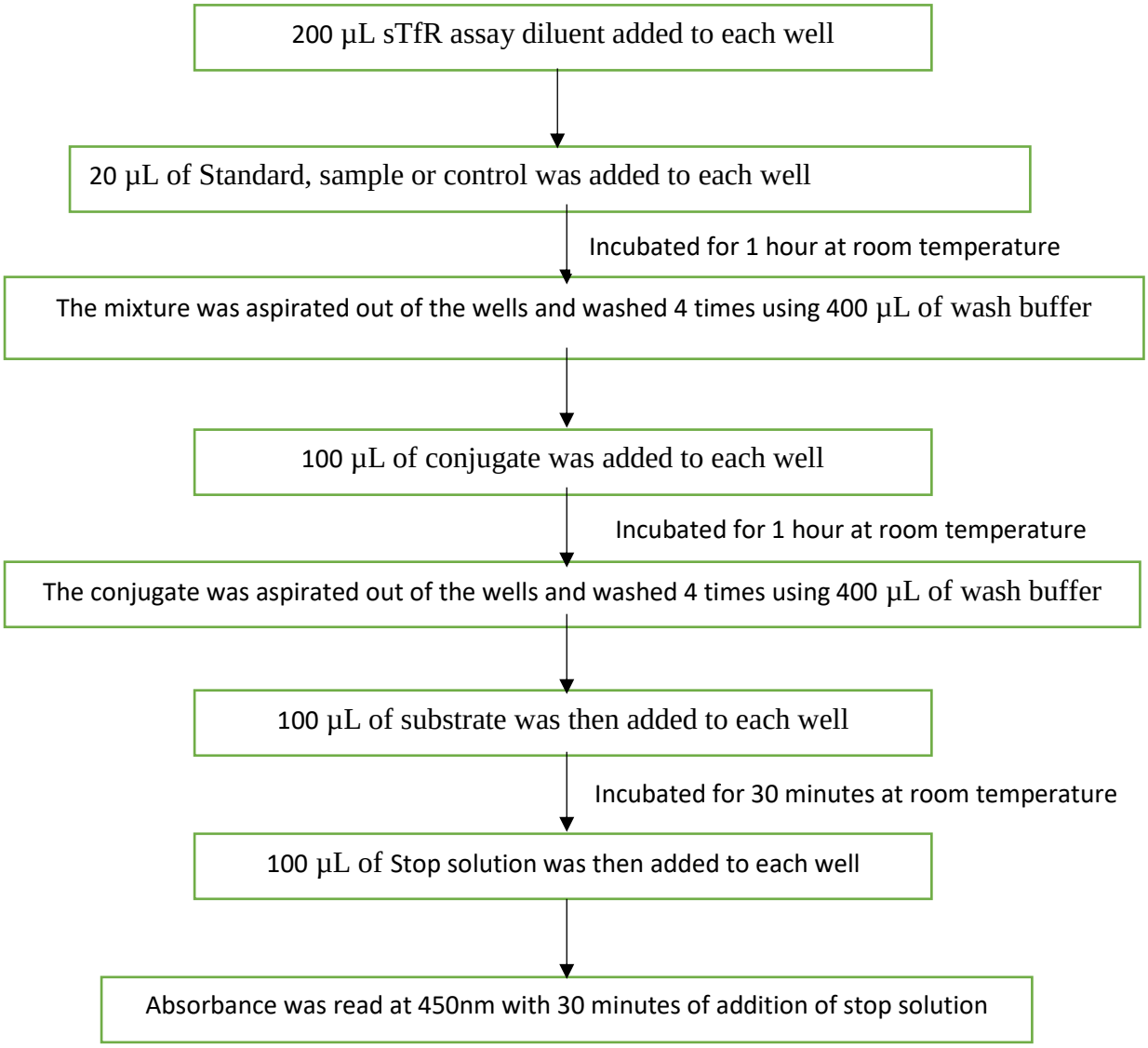

Figure 2.5: Serum transferrin receptor analysis protocol.

2.6 Determination of Haemoglobin concentration

Anaemia was determined using the concentration of Hb. Blood in EDTA container was placed in an automated haematological blood analyser (Sysmex Poch 100i) available at CRHC and processed according to manufacturer's instruction and laboratory protocol. This was achieved by placing the vacutainer in the analyser vacutainer and reading complete blood counts.

2.7 Identification of maternal factors associated with Iron Deficiency Anaemia

Demographic and clinical data was collected through a standard questionnaire administered by the researcher. Clinical and demographic data were collected, entered on a Microsoft office 13 excel spreadsheet. This data was then transferred to the Statistical Package for Social sciences (SPSS) version 22 and analysed. Testing for infections as maternal clinical factors involved only those done routinely as standard of care practice. These include, malaria (blood slide), syphilis (RPR) and Human Immunodeficiency virus (HIV) (rapid antibody test). HIV and RPR were done using antibody test that uses capillary action to indicate a reactive or non -reactive result with an inbuilt control whereas malaria testing was done by a blood slide examination. Briefly, a thin blood smear was made from EDTA blood and left to dry at room temperature. The dried specimen was stained with giemsa for 15 minutes. The giemsa was washed off with distilled water and the washed slide was further dried at room temperature. Fifteen minutes later, the slide was examined under oil emersion at x100 magnifications for malaria parasite identification.

2.8 Data analysis

Descriptive statistics were used to characterize study participants and their Hb and Iron levels. IDA was determined by anaemia (Hb less than 11.0g/dl) and ID with sTfR more

than 4.4mg/L. Descriptive data of each covariate were compared using chi squared and Mann Whitney U test for categorical and continuous variables, respectively. Analysis of variance was used to compare between different gestational age groups and iron status groups. Odds of having IDA and/or anaemia were performed through binary and ordinal logistic regression models whose data fit was analysed using Hosmer–Lemeshow test. Missing values were excluded list wise. Data that was collected through the excel spreadsheet was exported and analysed using SPSS version 22 supported on Windows 8. For the Chi-square and multivariate analysis, a p-value of less than- or equal to 0.05 was considered significant

2.9 Ethical considerations

The study was approved by the Biomedical Research Ethics Committee of the University of Zambia School of Medicine, Lusaka, Zambia (REF. No. 003-07-16). All participants were above 18 years and gave written informed consent. This study involved pregnant women who fall under special population, however, it did not pose any harm or threat to the unborn child or the mother. The participants did not undergo any additional invasive procedures they would not normally go through during their routine antenatal visits. Permission was sought from the management at Chongwe District Health Office as well as Chongwe Rural Health centre for the study to be conducted at this facility.

Chapter 3: Results

3.1 Participant Recruitment and Descriptive

A total of 302 pregnant women attending antenatal care at CRHC were approached for the purposes of participating in this study and to give consent. Forty-six (46) of them did not consent while 29 of those that gave consent were deemed not eligible to take part in the study. Therefore, this study comprised a total of 225 participants whose data was analysed as shown in figure 3.1. The study's mean maternal age was 25.29 years and ranged from 18 to 44 years old. The older participants were more likely to be HIV positive with a P-value of 0.0001. Stratified by HIV status, only maternal age and presence of Anaemia were statistically different between the HIV positive and negative participants as shown in Table 3.1.1. There was no difference between their number of pregnancies, children, gestational age, body mass index (BMI), education status as well as their blood pressure. The mean values of the participant description are shown in Table 3.1.1 below. At the time of recruitment, 41.78% (94/225) of the participants were already on iron supplements, however, the period under which they had been on these supplements was not recorded. Anaemia was found across all categories with the highest prevalence (43.02%) seen in participants who were on Iron supplements and were HIV negative. Out of the 225 participants, IDA was found in (3.56%) (8/225). All of whom were not supplemented with Iron as shown in the figure 3.1 below.

.

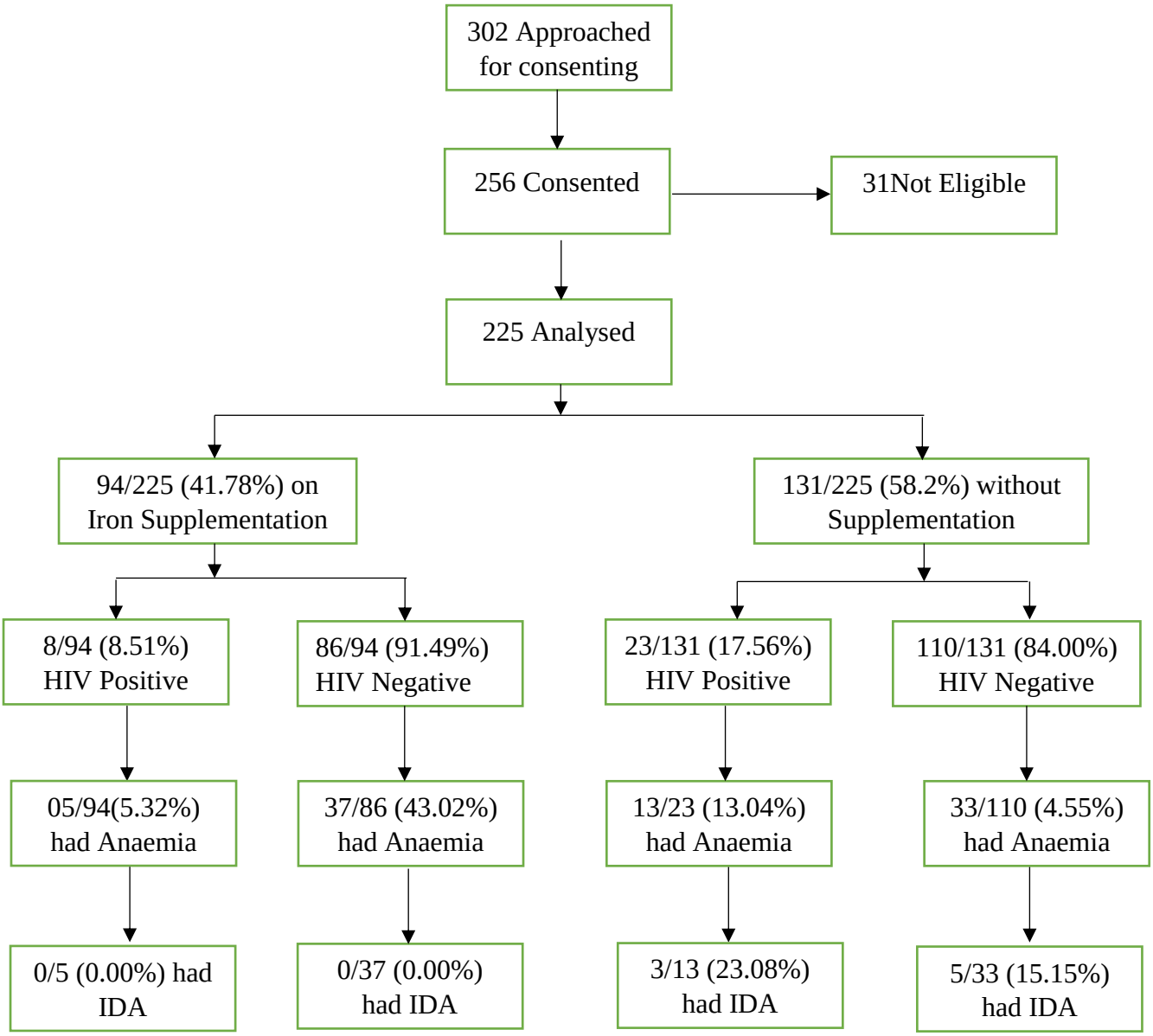

Figure 3.1: Iron status in relation to anaemia at different stages of pregnancy

Table 3.1.1: Participant Key Description stratified by HIV status

		Mean (Range)	SD	HIV POS (31)	HIV NEG (196)	P value
Age (Yr)		25.29(18-44)	6.1	29(19-38)	24.7(18-44)	<0.0001
Gravida		2.74 (1-10)	1.64	3.27(1-7)	2.66(1-10)	0.058
Parity		1.67(0-9)	1.6	2.1(0-6)	1.6(0-9)	0.110
Gestational Age (Wk)		23.58 (4-40)	7.6	22.7 (10-38)	23.71	0.526
BMI		26.97(18.43-46.87)	4.9	27.29(20.13-46)	26.91(18.43-45.70)	0.690
Systolic BP		111.93(55-185)	14.37	111.39(78-135)	112.02(55-185)	0.820
Diastolic BP		70.88(16-117)	10.88	71.48(53-90)	70.78(16-117)	0.739
Education	None			1(3.23%)	4(2.04%)	0.851
	Primary			11(35.48%)	85(43.37%)	
	Secondary			18(58.06%	102(52.04%)	
	Tertiary			1(3.23%)	5(2.55%)	
Anaemia				18(58.06%)	70(35.71%)	0.018

The study participants were further described according to their gestational age groups. The first trimester was described as the first 12 weeks of gestation, second trimester being between 13 and 26 and above this as 3rd trimester. Participants in their third trimester were more likely to be on Iron supplements, have a positive malaria blood slide and present with a complaint as shown in the table 3.1.2 below. The table 3.1.2 below further shows no statistical differences between the participants HIV status, anaemia, iron deficiency anaemia, RPR status and their education status between the three different gestational categories.

Table 3.1.2: Participant Key description between different gestational age groups

		Total (225)	First (14)	Second (142)	Third (69)	P value
HIV Positive		31(13.71%)	2(14.29%)	22(15.38%)	7(10.14%)	0.580
Anaemia		88(38.9%)	4(28.57%)	53(37.06%)	31(44.93%)	0.390
IDA		8(3.54%)	0(0.00%)	8(5.59%)	0(0.00%)	0.090
RPR		12(5.30%)	0(0.00%)	12(8.39%)	0(0%)	0.118
Fever		4(1.77%)	0(0.00%)	4(2.80%)	0(0.00%)	0.310
Supplements		93(41.15%)	0(0.00%)	27(18.88%)	66(95.65%)	<0.0001
Malaria Parasite		13(5.75%)	0(0.00%)	4(2.80%)	9(13.04%)	0.010
Education	None	5(2.21%)	1(7.14%)	3(2.10%)	1(1.45%)	0.250
	Primary	95(42.04%)	3(21.43%)	60(41.96%)	32(46.38%)	
	Secondary	120(53.10%)	10(71.43%)	74(51.75%)	36(52.17%)	
	Tertiary	6(2.65%)	0(0.00%)	6(4.20%)	0(0.00%)	
Complaints		130(57.52%)	7(50.00%)	62(43.36%)	61(88.41%)	<0.0001

3.2 Prevalence and clinical characteristics of Pregnant Women with Iron deficiency anaemia

The prevalence of IDA was 8/225(3.56%) as shown in the table 3.1.2 above, all of whom were not supplemented with iron as shown in the figure 3.1 above. 3/8(37.50%) were HIV positive. The clinical characteristics of the participants with IDA have been shown in the table 3.2 below. Table 3.2 below shows their clinical, social and laboratory features. Clinically, 5/8(62.5%) presented with a complaint of which abdominal pain was the commonest complaint with 3(37.5%) participants having this complaint. Back pain was seen in 2 participants with IDA and nausea, headache and join pains were seen in only one participant. Patients with IDA had a mean age of 25, where in their 21st week of their gestation and in their 2nd trimester. The mean number of pregnancies was 2.5 with the highest as much as 7 while the mean number of children was 1 and maximum was 3. Their mean BMI was 25.43 and mean blood pressure was 109.63/69.13. Only one participant with IDA had no education while 4 had been to secondary school and 3 to primary school.

Table 3.2: Characteristics of patients with Iron Deficiency Anaemia

Clinical Characteristics		Social Characteristics [Mean(Range)]			Full blood count characteristics[Mean(Range)]	
HIV	3/8(37.5%)	Maternal Age (Yrs.)		25 (20-35)	WBC $10^3/mm^3$	5.29(2-9)
RPR	2/8(25%)	Gestational age (Wks.)		21(17-24)	Lymphocyte %	43.7(20-72)
Any Complaint	5/8(62.5%)	2nd Trimester		8/8(100%)	Monocyte%	8.43(3-17)
Joint Pains	1/8(12.5%)	Gravida		2.5(1-7)	Granulocyte%	61.94(48-77)
Headache	1/8(12.5%)	Parity		1(0-3)	RBC ($10^6/mm^3$)	5.89(3-17)
Abdominal pain	3/8(37.5%)	Weight		59.38(53-77)	Hb (g/dl)	9.88(8-11)
Back pains	2/8(25%)	Height (CM)		153.13 (148-162)	HCT%	30.88(26-35)
Leg Pains	1/8(12.5%)	BMI		25.43(22.64-35.15)	MCV (μm^3)	74.3(66-90)
Nausea	1/8(12.5%)	Systolic BP		109.63(89-139)	MCH (pg)	23.85(20-31)
		Diastolic BP		69.13(54-93)	MCHC (g/dl)	32.06(30-37)
		Education	None	1/8(12.5%)	Platelet($10^3/mm^3$)	227.75(157-336)
			Primary	3/8(37.5%)	RDW (%)	16.51(13-20)
			Secondary	4/8(50%)	sTfR(mg/l)	5.06(3.66-6.21)

The laboratory features shown in the table 3.2 above indicates the mean white cells counts of 5.29x10^3/mm^3, of which the majority were granulocytes (61.94%) whereas lymphocytes and monocytes accounted for 43.7% and 8.43 % respectively. The red cell indices in IDA participants has also being listed in the table 3.2 above together with the sTfR of 59.54 mmol/l and ranged from 43 to 73 mmol/l. The red cell count was 5.89(3-17) $x10^6/mm^3$ while Hb one of the parameters used for definition of IDA had a mean of 9.88 and ranged from 8 to 11 and was related to Haematocrit (HCT) of 30.88. The MCV measured a micro-m^3 had a mean of 74.3μm^3 and ranged from 66 μm^3 to 90μm^3 while MCH and Mean Corpuscular Haemoglobin Concentration (MCHC) which were measured in pg and g/dl respectively, had means of 23.85pg and 32.06g/dl ranging from 20-31pg and 30-37g/dl respectively. RDW was 16.51% and ranged from 13% to 20% and platelet count had a mean of $227.75x10^3/mm^3$.

3.3 Iron Status of pregnant women Attending antenatal care at CRHC

Twenty-eight percent (63/225) of the study participants had iron overload, (63%) 143/225 had normal iron status and (8.9%) 20/225 were iron deficient. The participants with an increasing gestational age were significantly associated with iron overload and so was supplementation and being in the third trimester with P-values of 0.0001 in these cases as shown in the table 3.3 below. Participants who were HIV positive were more likely to be iron deficient while complaints had a significant association with iron overload with p-values of 0.001 and 0.023 respectively. Other factors such as maternal age, para, gravida, BMI, BP, RPR, education status, fever, or any indication of malaria had no significant association with the iron status of the study participants as shown in the table 3.3 below.

Table 3.3: Key Description of the participants' Iron status

		Iron Status				
		Iron Overload	**Normal Iron**	**Iron Deficient**	**Total**	**P Value**
Total		63(28.19%)	142(63.56%)	20(8.9%)	225	
mean Age (SE)		25.23(0.78)	25.04(0.52)	27.20(1.23)	25.29(0.41)	0.338
Mean gestational Age (SE)		27.21(0.97)	22.30(0.62)	21.35(1.14)	23.58(0.51)	<0.0001
Mean Gravida (SE)		2.84(0.22)	2.66(0.133)	2.95(0.38)	2.74(0.11)	0.633
Mean Parity (SE)		1.77(1.32)	1.61(1.36)	1.75(1.06)	1.67(1.46)	0.788
Mean BMI (SE)		27.91(0.68)	26.66(0.40)	26.11(0.79)	26.97(0.32)	0.170
Mean Systolic BP (SE)		112.95(1.72)	111.13(1.20)	114.40(3.77)	111.93(0.95)	0.510
HIV		5(7.8%)	18(12.6%)	8(40.00%)	31(13.66%)	0.001
RPR		4(6.25%)	6(4.20%)	2(10.00%)	12(5.29%)	0.510
Education	None	2(3.13%)	2(1.40%)	1(5.00%)	5(2.20%)	0.598
	Primary	25 (39.06%)	63(44.06%)	8(40.00%)	96(42.30%)	
	Secondary	37(57.81%)	73(51.05%)	10(50.00%)	120(52.86%)	
	Tertiary	0(0.00%)	5(3.50%)	1(5.00%)	6(2.64%)	
Illness		0(0.00%)	1(0.70%)	0(0.00%)	1(0.44%)	0.745
Fever		1(1.56%)	3(2.10%)	0(0.00%)	4(1.76%)	0.792
Supplements		50(78.13%)	43(30.07%)	1(5.00%)	94(41.41%)	<0.0001
Malaria Hx		2(3.13%)	4(2.80%)	0(0.00%)	6(2.64%)	0.736
TxMalaria		2(3.13%)	2(1.40%)	0(0.00%)	2(0.88%)	0.886
Malaria Parasite		1(1.56%)	11(7.69%)	1(5.00%)	13(5.73%)	0.212
Trimester	First Trimester	3(4.69%)	10(6.99%)	1(5.00%)	14(6.17%)	<0.0001
	Second Trimester	27(42.19%)	99(69.23%)	17(85.00%)	143(63.00%)	
	Third Trimester	33(51.56%)	34(23.78%)	2(10.00%)	69(30.40%)	
Complaints		45(70.31%)	77(53.85%)	8(40.00%)	130(57.27%)	0.023

3.3.1 The relationship between Iron status and Anaemia in Pregnant women attending antenatal at CRHC

A relationship between study participants who had anaemia and those who didn't, basing on their iron status and respective gestational age categories was also assessed. Iron overload and normal iron status was seen in all groups regardless of being anaemic or not. However, those who were iron deficient with anaemia were only seen in the 2nd trimester and those without anaemia but were iron deficient were seen in all gestational categories as shown in figure 3.3.1. below.

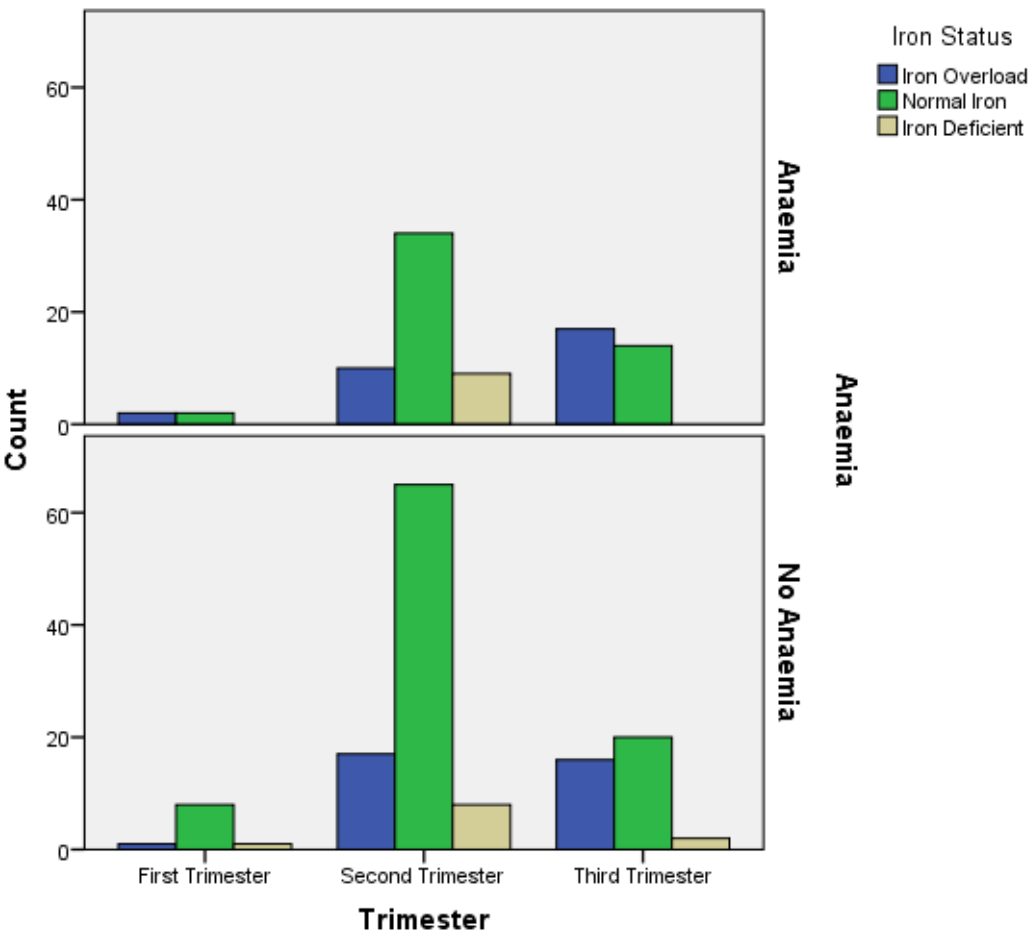

Figure 3.3.1: Iron status in relation to anaemia at different stages of pregnancy

3.3.2 Clinical features associated with Iron status

Headache was the most common feature (42%) in the study population but was mostly seen in iron overload but less so in those who were iron deficient. Those who were iron deficient presented mostly with abdominal pains. Other features whose prevalence increased in iron overload were oedema and dizziness while back pain and nausea increased in participants who were iron deficient as shown in the figure 3.3.2 below. The features aggregated as ‘others’ in the figure 3.3.2 below were relatively comparable among the iron status categories. These included joint pains and leg pains while one participant had increased heart rate had also an overload of iron in their blood.

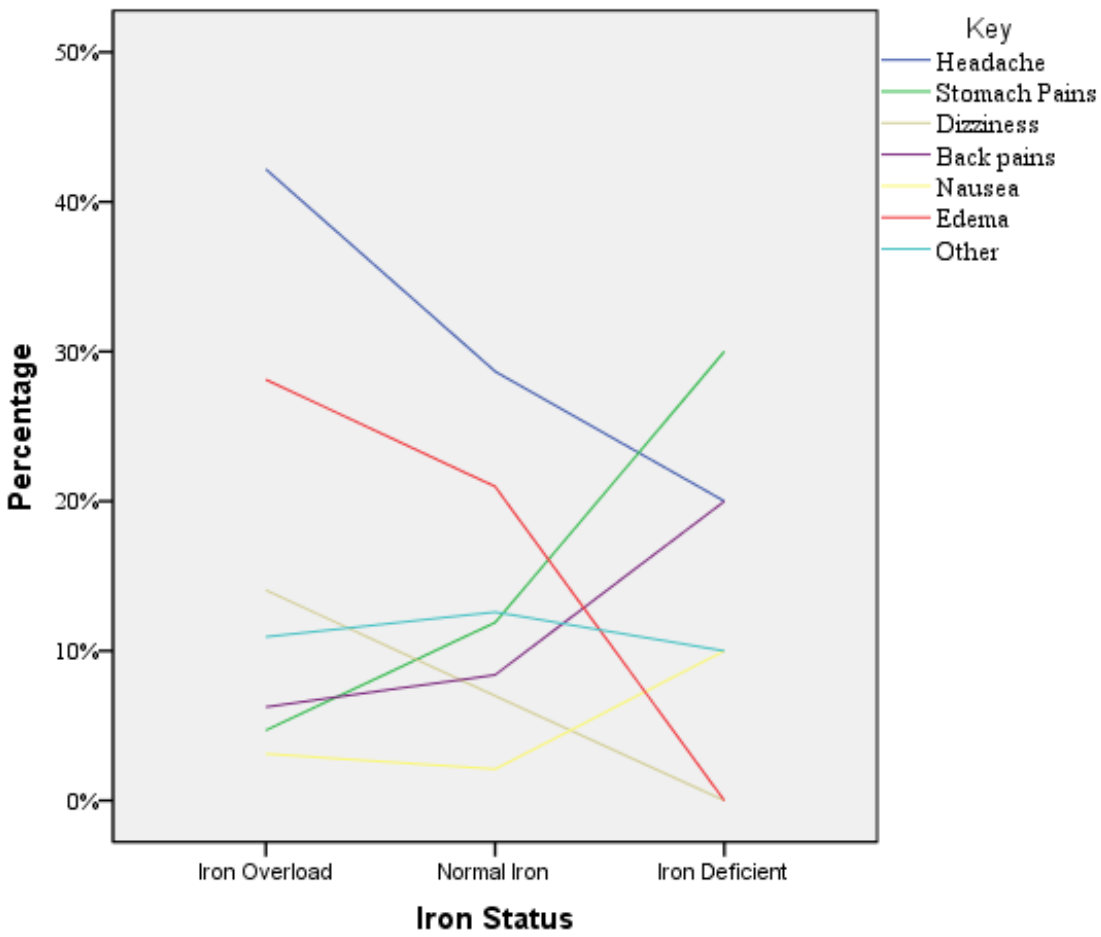

Figure 3.3.2: clinical feature correlation in iron status in women attending antenatal clinic at CRHC

3.4 Association of Iron supplementation with Iron deficiency anaemia parameters

Study participants with a higher gestational age, those who were HIV negative and those that presented with complaints were more likely to be on iron supplements with statistically significant P values of 0.0001, 0.036 and 0.0001 respectively as shown in the table 3.4 above. The mean gestational age for those who were supplemented with iron was 30.01 weeks whilst their mean maternal age 25.24 years old. Other factors including gravida, para, BMI, BP, RPR and education status did not differ significantly between iron supplemented and non-supplemented participants also shown in the table 3.4 above.

Table 3.4: Key description of patients relative to supplementation

			Supplements		**Total**	**P value**
			No	**Yes**		
		SD	131	94	225	
Mean Age		6.13	25.32 (18-44)	25.24 (18-38)	25.29	0.932
Mean gestational age		7.60	19.09 (4-30)	30.01 (14-40)	23.58 (4-40)	<0.0001
Mean Gravida		1.64	2.64 (1-8)	2.88 (1-10)	2.74 (1-10)	0.279
Mean Para		1.60	1.56 (0-6)	1.83 (0-9)	1.67 (0-9)	0.213
Mean BMI		4.87	26.15 (18.43-46.87)	28.12(19.82-45.70)	26.97 (18.43-46.87)	0.003
Mean Systolic BP		14.37	113.23 (55-185)	110.11 (81-153)	111.93 (18.43-46.87)	0.107,
Mean Diastolic BP		10.88	71.87 (16-117)	69.47 (53-95)	70.88 (16-117)	0.101
HIV	HAART		11 (8.30%)	7 (7.40%)	18 (7.90%)	0.036
	NR		110 (82%)	86 (91.50%)	196 (86.30%)	
	Reactive		12 (9.00%)	1 (1.10%)	13 (5.70%)	
RPR			9 (6.80%)	3 (3.20%)	12 (5.30%)	0.236
Education	None		4 (3.00%)	1 (1.10%)	5 (2.20)	0.091
	Primary		51 (38.30)	45 (47.90%)	96 (42.30%)	
	Secondary		72 (54.10%)	48 (51.10%)	120 (52.90%)	
	Tertiary		6 (4.50%)	0 (0.00%)	6 (2.60%)	
Complaints			59 (44.40%)	71 (75.50%)	130 (57.30%)	<0.0001

3.4.1 Association of Iron Supplementation and maternal haemoglobin at various gestational stages

The correlation of iron supplementation on the Hb level, a marker of anaemia is shown in the figure 3.4.1 below. Hb levels were also compared between the three different gestation groups and among those who were under iron supplementation and those who weren't as shown in figure 3.4.1. The box plots show a relative decline of the mean Hb from the first to third trimester among both iron supplemented and non-supplemented participants. However, this decline is not statistically significant. Further, it shows that none of the participants in the first trimester were supplemented. The variation in Hb seen in the first trimester is similar to that in the 2nd trimester with the lowest variation in Hb seen in the third trimester. The difference between those who were on iron supplements and those who were not, however, was not significant.

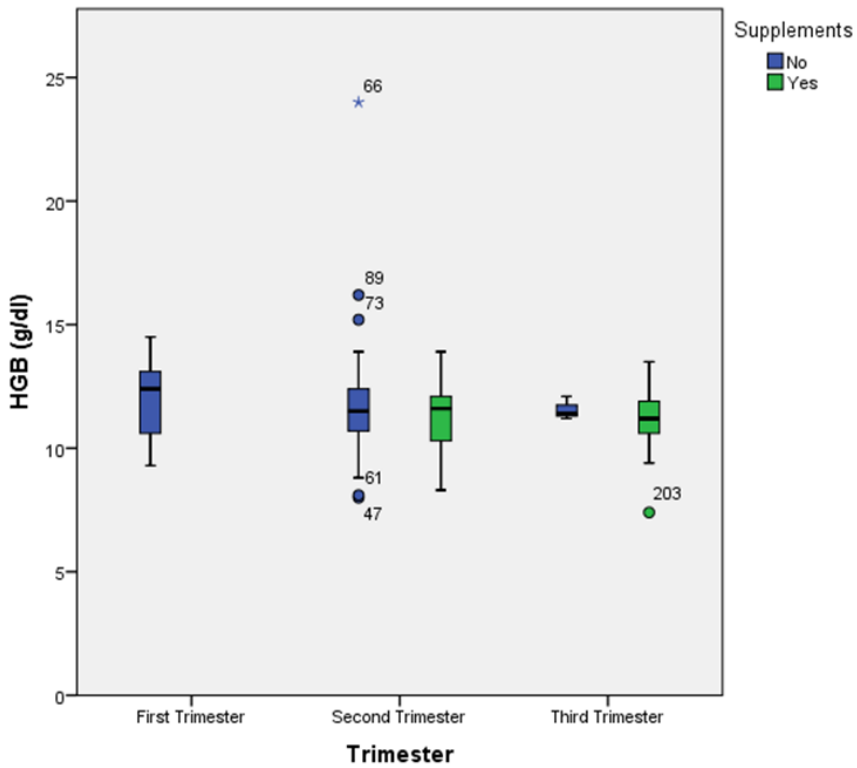

Figure 3.4.1: HB levels among Iron supplemented and Un-supplemented women at different gestational ages attending antenatal care at CHRC.

3.4.2 Association of Iron Supplementation and maternal sTfR at various gestational stages

The level of sTfR was shown to have a relative increase from first to third trimester but this increase was not statistically significant in both the supplemented and none-supplemented groups (p value = 0.267). However, there was a statistical significance of 0.001 between supplemented and non-supplemented participants independent of the gestational age group as shown in the figure 3.4.2 below.

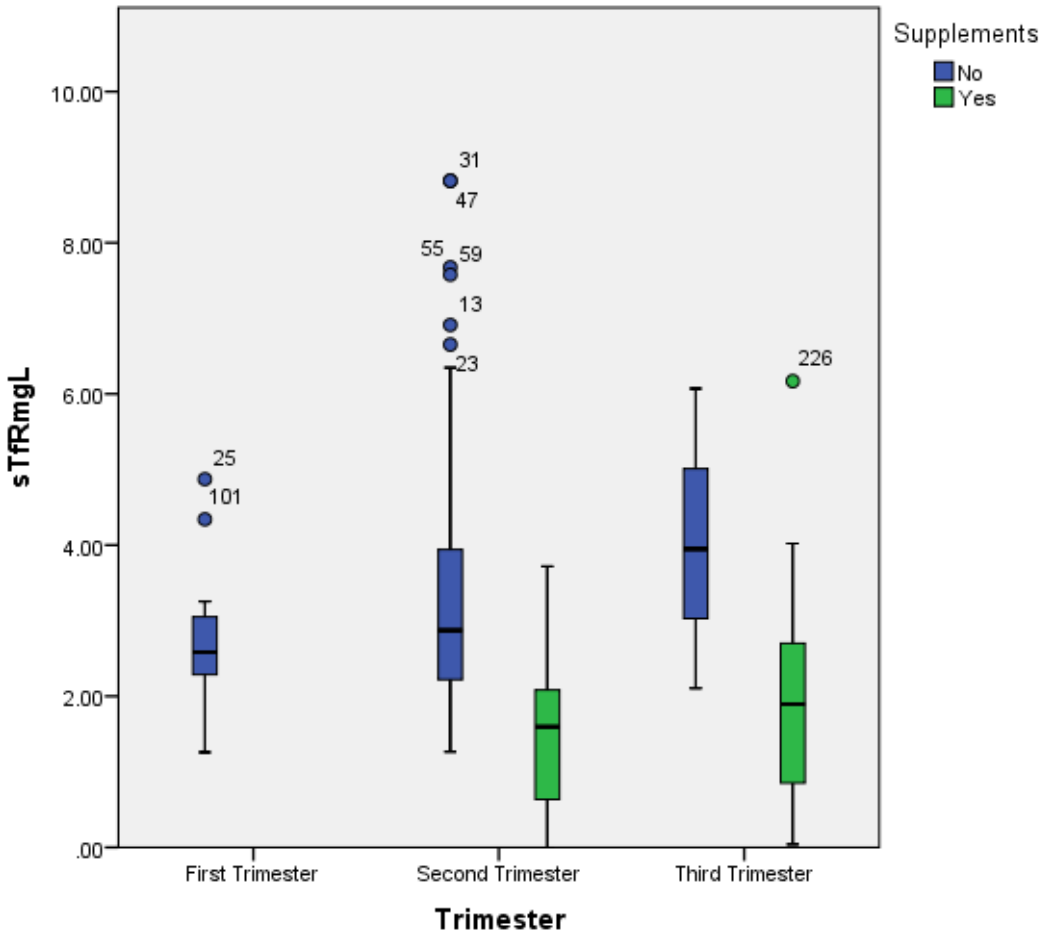

Figure 3.4.2: sTfR levels among Iron supplemented and Unsupplemented women at different gestational ages attending antenatal care at CHRC

3.5 Maternal Factors associated with Iron Deficiency Anaemia

The odds of IDA significantly increased in pregnant women with abdominal pains (OR 5.11 [95%CI 1.15-22.80], p = 0.032) and reduced in those with increased MCH (OR 0.74 [95%CI 0.61-0.88], p = 0.001, independent of RPR, Monocyte %, MCV, and RDW which were significant in univariate analysis. In anaemia other than IDA, only univariate analysis yielded significant changes in odds with HIV, Parity, primary education, RBC count, MCV, MCH and RDW (Table 3.5). Therefore, only three factors had common odds of affecting IDA and other types of anaemia, i.e. MCV, MCH and RDW. An increase in monocyte % was associated with reduced odds of IDA whereas no leukocyte flagged for anaemia. This may indicate the potential impact of infections on IDA, although those tested (RPR and HIV) had split effects.

Table 3.5: Factors associated with Iron Deficiency Anaemia and anaemia other than IDA

	Odds of IDA				Odds of Anaemia other than IDA			
	Odds Ratio (OR)	95% C.I. for OR		P Value	OR	95% C.I. for OR		P Value
		Lower	Upper			Lower	Upper	
AGE (Yrs.)	0.992	0.883	1.115	0.893	0.977	0.933	1.022	0.304
GESTATIONAL AGE (Wk.)	0.953	0.865	1.05	0.331	1.024	0.988	1.061	0.189
HIV	0.244	0.055	1.079	0.063	0.401	0.186	0.867	0.020
RPR	0.144	0.026	0.803	0.027	0.432	0.133	1.406	0.163
GRAVIDA	0.905	0.565	1.448	0.676	0.842	0.706	1.004	0.055
PARITY	0.699	0.387	1.26	0.234	0.814	0.677	0.977	0.027
WEIGHT (Kg)	0.949	0.874	1.031	0.219	0.991	0.969	1.014	0.458
HEIGHT (M)	0.96	0.85	1.085	0.511	1.025	0.980	1.071	0.279
BMI	0.918	0.762	1.105	0.364	0.962	0.908	1.019	0.188
SYSTOLIC BP (mmHg)	0.988	0.938	1.04	0.641	0.990	0.971	1.009	0.289
DIASTOLIC BP (mmHg)	0.985	0.923	1.05	0.640	0.984	0.959	1.009	0.208
Education* None	0.131	0.012	1.456	0.098	0.569	0.092	3.521	0.569
Primary	1.016	0.222	4.652	0.983	2.180	1.237	3.840	0.007
WBC Count ($x10^3/mm^3$)	0.86	0.594	1.244	0.423	0.984	0.924	1.047	0.602
Lymphocyte (%)	1.019	0.968	1.073	0.472	0.989	0.969	1.009	0.274
Monocyte (%)	0.832	0.693	0.999	0.049	0.994	0.961	1.028	0.708
Granulocyte (%)	1.003	0.993	1.014	0.541	0.999	0.991	1.007	0.787
RBC count ($x10^6/mm^3$)	1.109	0.976	1.26	0.111	0.517	0.298	0.897	0.019
Haematocrit (%)	0.904	0.76	1.077	0.259	0.686	0.618	0.762	<0.0001
MCV (μm^3)	0.922	0.862	0.987	0.020	0.958	0.924	0.992	0.017
MCH (pg)	0.735	0.611	0.883	0.001	0.845	0.776	0.921	<0.0001
MCHC (g/dL)	0.896	0.8	1.003	0.057	0.935	0.873	1.001	0.053
Platelet ($x10^3/mm^3$)	1.000	0.989	1.012	0.939	1.002	0.997	1.006	0.439
RDW	1.574	1.177	2.107	0.002	1.643	1.324	2.039	<0.0001
Complaints (General)	1.253	0.292	5.376	0.761	0.773	0.451	1.326	0.350
Joint pains	7.679	0.757	77.9	0.085	1.054	0.173	6.439	0.954
Headache	0.298	0.036	2.467	0.261	1.097	0.619	1.944	0.750
Abdominal pains	5.113	1.146	22.804	0.032	0.818	0.347	1.925	0.645
Back pains	3.722	0.7	19.8	0.123	1.058	0.415	2.702	0.906
Nausea	5.071	0.536	47.967	0.157	1.191	0.260	5.454	0.822

*Education status significant in relation to Secondary Education

Chapter 4: Discussion

4.1 Discussion

The key findings from this study included a higher than expected rate of iron overload, high rates of anaemia but lower rate of Iron deficiency anaemia (IDA). Rapid plasmin reagen (RPR) and HIV antibody reactivity may play separate roles in iron deficiency anaemia and anaemia.

The mean age was 25.25 years and was comparable to other similar studies in the region (Michelo *et al*, 2007; Raza *et al*, 2011). The participants in this study did not have any differences between the HIV infected and un-infected women except that the older participants with a mean of 29 years were more likely to be HIV positive. A study in Ethiopia looking at sero-prevalence of syphilis and HIV also found that pregnant women between 25 to 29 years old, were more likely to be HIV positive (Mulu *et al*, 2007). This difference may be due to social education and effective HIV control measures reducing the incidence of HIV in the younger population and reduced mortality due to effective HAART therapy. Sexual activity may not significantly alter the HIV distribution in this population as the study did not show any differences in the number of children or pregnancies between HIV positive and negative participants. The distribution of HIV, however, in this study was representative of all gestational age groups, that is, first, second and third trimesters. Anaemia was not more or less prevalent in any of the trimesters. Similarly, there was no statistical difference in the education status, RPR test result or presence of fever between these gestational age groups. However, Participants in the third trimester were more likely to be on iron supplements and were also likely to present with a *Plasmodium falciparum*, a causative agent of malaria. *P. falciparum* is a known iron chelating parasite and may explain an association with taking iron supplements (Scholl *et al* 2005). In addition, studies have shown that host iron Deficiency is protective against *P. falciparum* and hence iron supplementation may increase the risk of malaria infection especially in malaria endemic areas (Clark *et al* ,2014). Other studies however found

that iron supplementation particularly increased the risk of infection with *P. vivax* and not *P. falciparum* (Mwangi *et al* 2015). Another significant association was the presence of any complaint with participants in the third trimester more likely to present with a complaint. Whether this was due to having an older foetus or related to supplementation of iron and/or presence of malaria parasite is not known. In this study, the iron status of the pregnant women and Hb levels regardless of supplementation status was assessed and is discussed below. The period of supplementation was not considered due to the study design, therefore, only studied the factors that were associated with iron status. The iron supplementation policy entails that supplementation upon first antenatal visit take place, this may explain why the participants in their first trimester were less likely to be on supplements compared to those in the third trimester as these would have had multiple visits to the clinic before being recruited in the study.

The study found that 3.56% (8/225) of women who attended ANC had IDA and all of whom were not supplemented with iron. This prevalence is lower compared to other reports in Africa (Ifeyinwa *et al*, 2013). The lower prevalence of IDA in our study could be attributed to an increase in the proportion of pregnant women taking iron supplements as well as an increased awareness of the need to offer iron supplements during pregnancy. Furthermore, it can also be attributed to the free antenatal policy that is currently being practised in government institutions in Zambia (Reproductive health policy, 2000). No previously published information on the prevalence of IDA among pregnant women in Zambia was found. The commonest clinical features were abdominal pains (3/8), followed by back pains (2/8) and (1/8) headache, (1/8) pain in the legs and (1/8) nausea. The National Heart, Lung and blood institute, NHLBI has listed some of the clinical features associated with IDA as brittle nails, swelling or soreness of tongue and fatigue. Other studies have indicated cardiovascular and cerebrovascular associations that including pallor of mucous membranes, fatigue, general

weakness, decreased appetite, dizziness, headache, shortness of breath, increased heart rate and palpitations (Hasim *et al*, 2007). Reduced iron may lead to a decrease in haemopoiesis because iron forms a core component of haemoglobin in the red blood cells. It is however, not clear whether this reduction is likely to be associated with reduced oncotic pressure or volume of blood, imbalances to which may lead to oedema, seen in some patients as swelling. All the clinical features observed in IDA cases may be attributed to oedema and reduced oxygen carrying capacity of RBCs. Nevertheless, the list of clinical features observed above should be interpreted with caution as there were only 8 cases of IDA. The commonest clinical feature in participants with IDA was abdominal pain. However, a clinical review looking at the incidence of abdominal pain in pregnancy showed that abdominal pain was a common feature of pregnancy in all pregnant women regardless of their iron status. They further observed that it could be caused by normal physiological changes that occur in the body to accommodate a growing foetus or could be due to pathological causes (Gyampoh *et al*, 2009). Other studies have found that abdominal pain in pregnancy can be due to parasitic infections which have been reported at a high prevalence in this region (Modjarrad *et al*, 2005; Siwila *et al*, 2010) and not a result of IDA. A study in Nepal found that parasitic infections such as Hookworm and malaria were the root cause of Iron Deficiency Anaemia (Dreyfuss *et al*, 2000) however, a study by Abdu-Hassira found no significant association between Hookworm, syphilis and Malaria infection with Iron Deficiency Anaemia (Hassira *et al*, 2014). In this study, stool was not collected to identify parasites that cause abdominal discomfort and/whether these parasites may contribute to iron status reduction in pregnant women. Hence, whether these abdominal pains that were observed in these pregnant women were due to parasitic infections or other causes, it is not clear. Another clinical feature that was common in IDA was back pain. It has been approximated that 50% of women experience back pains particularly lower back pain during pregnancy, this could be due to various reasons. One of them could be due to weight

gain brought about because of the growing foetus causing pressure on the spinal nerve and IDA. Iron deficiency anaemia has been known to cause back pain by causing a reduction of oxygen supply to tissues including muscles leading to muscle fatigue and consequently back pain (Hussey, 2017). Participants who came for ANC complaining of headache and swelling in their extremities in this study were more likely to have iron overload. It is approximated that 35% of pregnant women present with a headache during their antenatal visit. Headaches can be benign and go away after administering an analgesia, however others can predispose patients to higher risk of mortality and morbidity (Natasha *et al*, 2015). Hence, iron status of women need to be assessed before supplementation (Zafar and Iqbal 2008).

Iron status was significantly influenced by iron supplementation (P value ≤ 0.000) agreeing with findings of Makhoul *et al* in Nepal (Makhoul *et al* 2010). Studies have shown that iron supplementation can correct ID and IDA, However, its need and beneficial effects on pregnancy has been questioned of late. For instance, in the United Kingdom (UK), they have advised against universal supplementation in their clinical guidelines (Palihawadana *et al* 2014). The findings from this study indicated a very high prevalence of iron overload as shown in table 3.3. Participants in their third trimester were more likely to be have iron overload, whereas iron deficiency was more prevalent among HIV positive participants. It was also observed that those who were receiving HAART were less likely to be supplemented with iron. Whether, this is policy related or clinicians are more swayed to look at the patients' HAART therapy performance than their micronutrient needs remain unknown. The participants taking iron supplements were also likely to present with an older gestational age and complaints. It was hypothesised that most of the participants had late antenatal booking, instead of booking in their first trimester, they would book in their second or third trimesters. Late antenatal booking has been cited in Zambia and other African countries (Gudayu *et al*, 2014; Sinyangwe *et al*, 2016). Although IDA occurred only in the participants that were not on iron supplements,

prevalence of anaemia could not be split between those on iron supplements and those that were not. This may indicate that other factors other than iron supplementation were cardinal in determining whether one has anaemia or not. Paliwadana *et al*, 2014 also approximated the prevalence of ID to be twice as that of IDA. Serum transferrin receptor levels were shown to increase from the first to third trimester in both participants who were on iron supplements and those not, although this was not statistically significant with a P value of 0.267. However, Scholl and colleagues found that iron supplementation did not increase the levels of serum iron in the first and second trimesters but was significantly raised in the third trimester although SF was used as a biomarker for iron status (Scholl et al, 2005). Other studies have contradicted Scholl as well (Paliwadan *et al*, 2014 and Okafor *et al*, 2013). Although the findings on the differences in sTfR levels were not significant, the median sTfR in the third trimester of 4.4mg/L indicated that pregnant women needed iron supplementation, otherwise up to 50% will be iron deficient. It also indicates that the gain in iron stores upon supplementation is gradual. The Mean Hb levels on the other hand showed relatively gradual decline from the first trimester to third trimester regardless of iron supplementation which might indicate a lack of association between iron supplementation and Hb levels.

Abdominal pains and reduced monocyte % were found to increase the odds for IDA and not anaemia. A positive RPR, however, reduced the odds of IDA with no significant change in odds on anaemia. Although none of the IDA cases were supplemented with iron, this finding may show an interplay of infections, especially those that can cause abdominal pains. Rapid plasmin reagin is a proximal test for exposure to syphilis but should be interpreted with caution as it doesn’t segregate between active infection and treated cases and would need repeated testing to confirm state of disease (Kenyon *et al*, 2017). During active infection of syphilis, *Treponema pallidum pallidum* can get iron from the host interacting with transferrin and lactoferrin (Jovanovic 2000; Lafond and Lukehart, 2006). Some studies, however, have found a

very low prevalence of syphilis and no association of RPR with IDA (Baingana *et al*, 2015) posing further questions on the role its prevention would play. A higher number of children, increased RBC count and HCT were significantly associated with lowering the odds of anaemia but had no influence on IDA. Being HIV positive also reduced odds of anaemia, but as discussed above, may be related to HAART, and needs further research. Education status may be correlated with social economic status but only increased odds of anaemia but not IDA. Other studies have shown education status to impact both IDA and anaemia. They have also shown gestational age to affect IDA (Raza *et al*, 2011), however, in a rural setting were sources of food are limited and being in secondary school may not entirely translate into a better socio-economic status or diet. The social demographic findings have been different in different regions (Aikawa *et al*, 2006; Raza *et al*, 2011; Ononge *et al*, 2014; Palihawadana *et al*, 2014) and hence, determining risk factors for IDA in each setting may be vital to improving community ANC and prevent IDA.

4.2 Conclusion

The study therefore reports lower rates of IDA, comparable rates of anaemia and increased rates of iron overload compared to other studies in the region. Iron supplementation has been shown to be important in elevation of iron status, however, no association with anaemia was observed. Iron deficiency and iron deficiency anaemia where associated with abdominal pains and backache, with abdominal pains increasing the odds of IDA 5-fold while education status and HIV status were the maternal factors associated with anaemia but not IDA.

4.3 Study Limitations

The impact of iron supplementation could not be evaluated because of weaknesses in the study design. However, associations were measured to determine its potential outcome at the point of participant recruitment in the study. This therefore, may give relevant information to the clinician and to what the state of the patient might be in efforts to manage them.

Baseline data before supplementation was not measured. Data such as iron status and anaemia were not available making it difficult to assess the impact of micronutrient supplementation in this population. This may be important in determining exactly which patient needs supplementation and by what dosage and frequency.

Further, risk posed by parasitic infections especially intestinal worms was not evaluated as the study did not collect any stool samples or carry out other intestinal parasitic infection tests. The study collected only blood for RPR which is part of the standard routine examination done at the ANC unit.

The sample size was too small to determine the social, clinical or haematological characteristics associated with IDA, hence a larger study may be required to make the appropriate analysis. This will aid in quick diagnosis and improve patient care.

4.4 Recommendations

To overcome some of the limitations above, a prospective cohort study is needed to assess the impact of iron supplementation and define factors that lead to the development of IDA and anaemia. In this regard, the impact of supplementation should be assessed in detail including its impact on the unborn child as well as morbidity after their birth.

Further, the impact of treatment of intestinal worms and blood parasites is needed to assess their importance in controlling IDA and anaemia. Efforts in prevention of such infections actively and through educating the community may be explored.

Anaemia prevalence was very high regardless of micronutrient supplementation, therefore, other efforts that may reduce this prevalence need to be explored.

5.0 References

Abdelrahman E.G., G.I. Gasim, I.R. Musa, L.M. Elbashir, and I. Adam, 2012. Red blood cell distribution width and iron deficiency anemia among pregnant Sudanese women. Diagnostic Pathology.;7:168.

Abu-Ouf, N. M. and Mohammed M. Jan. 2015. The impact of maternal iron deficiency and iron deficiency anemia on child's health. Saudi Med J 2015; Vol. 36 (2): 146-149 doi: 10.15537/smj.2015.2.10289

Ahmed F. I. Mahmuda, A. Sattar, and M. Akhtaruzzaman., 2003. Anaemia and vitamin A deficiency in poor urban pregnant women of Bangladesh. Asia Pacific Journal of Clinical Nutrition; 2012(4):460-6.

Aikawa R, N. C Khan, S. Sasaki and C. W. Binns, 2006. Risk factors for iron-deficiency anaemia among pregnant women living in rural Vietnam. Public Health Nutrition: 9(4), 443–448

Alwan N.A., J.E. Cade, H.J. McArdle, D.C. Greenwood, H.E. Hayes, and N.A. Simpson, 2015. Maternal iron status in early pregnancy and birth outcomes: insights from the Baby's vascular health and Iron in Pregnancy study. British Journal of Nutrition. 113(12):1985-92.

Asobayire F.S, P. A. L Davidsson, J.D Cook, and R. F Hurrell, 2001. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. American Journal of Clinical Nutrition; 74:776–82.

Aulakh R, I. Sohi, T. Singh and N. Kakkar, 2009. Red Cell Distribution Width (RDW) in the Diagnosis of Iron Deficiency with Microcytic Hypochromic Anemia. Indian Journal of Pediatrics; 76 (3: 265-267.

Baingana, R.K., J.K. Enyaru, H. Tjalsma, D.W. Swinkels, and L. Davidsson, 2015. The aetiology of anaemia during pregnancy: a study to evaluate the contribution of iron deficiency and common infections in pregnant Ugandan women. Public Health Nutrition. 18(8): p. 1423-35.

Bangal VB, K Gupta, K Aher, H Tuse and K Bhosale, 2016. Study of prevalence, socio-

epidemiological factors and clinical profile of iron deficiency anaemia among pregnant rural population. Saudi J. Med. Pharm. Sci.; 2(2):30-39.

Berrak S.G., M. Angaji, E. Turkkan, C. Canpolat, C. Timur, and E. Eksioglu- Demiralp .2007. The effects of iron Deficiency Anaemia on neutrophil/monocyte apoptosis in children. Cell proliferation; 40(5):741-54.

Black R.E., C.G. Victora, S.P. Walker, Z.A. Bhutta, P. Christian P, M. de Onis, M. Ezzati, S. Grantham-McGregor, J. Katz, R. Martorell, R. Uauy; and, Maternal and Child Nutrition Study Group., 2013. Maternal and child undernutrition and overweight in low-income and middle-income countries. The Lancet. 3;382(9890):427-451.

Bo S, G. Menato, P. Villois, R. Gambino, M. Cassader, I. Cotrino, and P. Cavallo-Perin. 2009. Iron supplementation and gestational diabetes in mid-pregnancy. American Journal of Obstetric Gynecology. 201:158. e1-6.

Campigotto A.C, M.C.A Duarte de Farias, DC. F Pinto, F.G.F Albuquerque.2015. Factors Relating to Iron Deficiency Anemia in Pregnancy: An Integrative Review. International Archives of Medicine Section: Obstetrics & Gynecology. 8(159)

Chan K, K. Chan, K. Lam, S. Tam, and T. Lao. 2009. Iron supplement in pregnancy and development of gestational diabetes—a randomised placebo-controlled trial. BJOG: An international journal of Obstetrics and Gynaecology. 116:789–798. discussion 797-8

Chandra S. A. K. Tripathi, S. Mishra, M. Amzaru and A. K. Vaish. 2012. Physiological changes in haematological parameters during pregnancy. Indian Journal of hematology and blood transfusion. ;28(3): 144-146.

Choi HJ, HJ Lee, HB Jang, JY Park, JH Kang, KH Park and J Song, 2011. Effects of maternal education on diet, anaemia and Iron deficiency anaemia in Korean school-aged children. BMC Public health 201111:870 https://doi.org/10.1186/1471-2458-11-870

Clark M A, M. M. Goheen and C. Cerani. 2014. Influence of Host Iron status on plasmodium infection. Frontier in pharmacology; 5: 84.

Dreyfuss M.L., R.J. Stoltzfus, J.B. Shrestha, E.K. Pradhan, S.C. LeClerq, S.K. Khatry, S.R. Shrestha, J. Katz, M. Albonico, and K.P. West Jr. 2000. Hookworms, Malaria and Vitamin A Deficiency Contribute to Anemia and Iron Deficiency among Pregnant Women in the Plains of Nepal. Journal of Nutrition. October;130(10):2527-36

Emegoakor F. J, C. Chukwemeka, A. Iyoke, H. U. Ezegwui, F. Okechukweu, O. U. Umeora, and I. and O. Ibeagha. 2015. Rate and predictors of low serum ferritin levels among healthy parturient women in Enugu Nigeria. Journal of Blood Medicine. ;6 :261-267.

Gyampoh B, A Antonoiu, and N Agarwal, 2009. Clinical review- Abdominal pain in pregnancy.GPonline.com; accessed on 02/09/2017

Hamiel OP, RS Newfield, I Koren, A Agmon, P Lilos and M Philip, 2003. Greater prevalence of iron deficiency in overweight and obese children and adolescents. International Journal of Obesity (2003) 27, 416–418 (2003)

Healthy People 2010, Operation Definition: objective 19-12c.

Hussey D., 2017. Lower back pain, pregnancy and Iron Deficiency. Root cause medicine, 2017.

Iannotti L.L., K.O. O'Brien, S.C. Chang, J. Mancini, M. Schulman-Nathanson, S. Liu, Z.L. Harris, and F.R. Witter., 2005. Iron deficiency anemia and depleted body iron reserves are prevalent among pregnant African-American adolescents. Journal of Nutrition; 135(11):2572-7

Jovanovic, T., C. Ascenso, K.R. Hazlett, R. Sikkink, C. Krebs, R. Litwiller, L.M. Benson, I. Moura, J.J. Moura, J.D. Radolf, B.H. Huynh, S. Naylor, F. Rusnak., 2000. Neelaredoxin, an iron-binding protein from the syphilis spirochete, Treponema pallidum, is a superoxide reductase. Journal of Biological Chemistry. 275(37): p. 28439-48.

Karakochuk C.D., K.C. Whitfield, S.I. Barr, Y. Lamers, A.M. Devlin, S.M. Vercauteren, H. Kroeun, A. Talukder, J. McLean, and T.J. Green., 2015. Genetic hemoglobin disorders rather than iron deficiency are a major predictor of hemoglobin concentration in women of reproductive age in rural prey Veng, Cambodia. Journal of Nutrition; 145(1):134-42.

Kefiyalew F., E. Zemene, Y. Asres, and L. Gedefaw., 2014. Anemia among pregnant women in Southeast Ethiopia: prevalence, severity and associated risk factors. BMC Research Notes.; 7:771.

Kenyon, C.R., et al., What Is the Role of Paired Rapid Plasma Reagin Testing (Simultaneous Testing of Acute and Convalescent Samples) in the Diagnosis of Repeat Syphilis and the Follow-up of Syphilis? Sex Transm Dis, 2017.

Khalafallah A.A.,and A.E. Dennis., 2012. Iron deficiency anaemia in pregnancy and postpartum: pathophysiology and effect of oral versus intravenous iron therapy. Journal of Pregnancy; 2012:630519.

Knowles J, D.I. Thurnham, B. Phengdy, K. Houamboun, K. Philavong, I. Keomoungkhone, and K. Keovilay., 2013. Impact of inflammation on the biomarkers of iron status in a cross-sectional survey of Lao women and children. British Journal of Nutrition; 110(12):2285-97.

Kordas K, Z Y F Centeno, H Pach´on,and A Z Jimenez Soto, 2013. Being Overweight or Obese Is Associated with Lower Prevalence of Anemia among Colombian Women of Reproductive Age. Journal of Nutrition. 143: 175–181, 2013.

Lafond, R.E. and S.A. Lukehart, Biological basis for syphilis. Clin Microbiol Rev, 2006. 19(1): p. 29-49.

Maheshwari B K, Raut P, Agarwal S.K, Joshiu, Dhirhet T. C, Gahine R, 2011. A study on the iron status in Iron Deficiency Anaemia one month before and after Iron Therapy in school going children. Journal of Clinical and Diagnostic Research. April:5;2: 324-327

Makhoul Z., D. Taren, C. Thompson, B.Duncan, I. Hakim, M.Miranoto. (2007). Anaemia and Iron Deficiency in Rural Nepali pregnant women, risk factors, effects of vitamin A

supplementation and their association with birth outcomes. University of Arizona. http://hdl.handle.net/10150/193930. Accessed on 02/09/2017

Melgert B.N., F. Spaans, T. Borghuis, P.A. Klok, B. Groen, A. Bolt, P. de Vos, M.G. van Pampus, T.Y. Wong, H. van Goor, W.W. Bakker, and M.M. Faas, 2012. Pregnancy and Preeclampsia Affect Monocyte subsets in humans and rats. Plos one;7(9):e45229.

Milman N., 2012. Oral Iron prophylaxis in pregnancy, Not too little and Not too much. Journal of Pregnancy. 514.

Modjarrad K, I. Zulu, D.T. Redden, L. Njobvu, D.O. Freedman, S.H. Vermund., 2005. Prevalence and predictors of intestinal helminth infections among human immunodeficiency virus type 1-infected adults in an urban African setting. American Journal of Tropical Medicine and Hygiene. Oct;73(4):777-82

Mulu A., A. Kassu, B. Tessema, G. Yismaw, M. Tiruneh, F. Moges, Y. Wondmikun, T. Nishikawa, and F. Ota., 2007. Seroprevalence of Syphilis and HIV-1 during Pregnancy in a Teaching Hospital in Northwest Ethiopia, Japanese Journal of infectious diseases:60, 193-195.

Mwangi M.N, Johanna M. Roth, Menno R. Smit, Laura Trysburg, Alice M. Mwangi, Ayse Y. Demir, Josp .M. Wielders, Petra F. Mens, Jaco j. Verweij, Sharon E. Cox, Andrew M. Prentice, F medsci, Inge D. Brouwer, and A. Hansvernoef. 2015. Effects of daily antenatal Iron supplementation on plasmodium infection in Kenyan women. Jama.14(10): 1009-1020.

Mwangi M.N., S. Maskey, P.E. Andang, N.K. Shinali, J.M. Roth, L. Trijsburg, A.M. Mwangi, H. Zuilhof, B. van Lagen, H.F. Savelkoul, A.Y. Demir, and H. Verhoef., 2014. Diagnostic utility of zinc protoporphyrin to detect iron deficiency in Kenyan pregnant women. BMC Medicine; 12:229.

Ng N., S. Cox and S. Maiti. 2015. Headache in pregnancy: an overview of differential diagnosis. Journal of pregnancy and child health: 2.130.

Nojilana B., R. Norman, M.A Dhansay, D. Labadarios, M.E Van Stuijenberg, D. Bradshaw and The South African risk assessment collaborating group, 2007. Estimating the burden of disease attributable to Iron Deficiency anaemia in South Africa in 2000. S African medical Journal; 741-746.

Obai G, P. ondongo and R. Wanyama, 2016. Prevalence of anaemia and associated risk factors among women attending antenatal care in Gulu and Hoima Regional Hospitals in Uganda: a cross sectional study. Pregnancy and child birth; 16:76.

Okafor I. M., Enosakhare A. A, Antai A. B, and A.U. Usanga., 2013. Prevalence of Iron Deficiency Anaemia among Pregnant Women in Calabar, Cross River State Nigeria. Journal of Pharmacy and Biological sciences.; 7(2); 60-64.

Olatunbosun O.A., A.M. Abasiattai, E.A. Bassey, E.S. James, G. Ibanga, and A. Morgan, 2014. Prevalence of anaemia among pregnant women at booking in the University of Uyo Teaching Hospital, Uyo, Nigeria. Biomed Research International; 2014:849080.

Ononge, S, O. Campbell and F. Mirembe, 2014. Haemoglobin status and predictors of anaemia among pregnant women in Mpigi, Uganda. BMC research notes 7:712

Palihawadana TS, G.I., Motha MBC, Williams HSA, Iron Deficiency Anaemia in pregnancy: Diagnosis, prevention and treatment. Sri Lanka journal of Obstetric and Gynaecology, 2014(September).doi:10.1038/ijo.2015.3.

Palma S, R. Perez-Iglesias, D. Prieto, R. Pardo, J. Llorca, and M. Delgado-Rodriguez. 2008. Iron but not folic acid supplementation reduces risk of low birth weight in pregnant women

without anaemia: a case-control study. Journal of epidemiology and Community Health. 62: 120-124.

Raza, N., et al., Assessment of iron deficiency in pregnant women by determining iron status. J Ayub Med Coll Abbottabad, 2011. 23(2): p. 36-40.

Saroja C.N., S. Alapaty, and M. Jeyan, 2014. Efficacy of Red cell distribution width (RDW) as a screening test for diagnosing children with Iron Deficiency anaemia. Scholars Journal of Applied Medical Sciences; 2(6H):3412-3415

Scholl P.F, A.K Tripathi, and D.J Sullivan. 2005. Bioavailable Iron and Heme metabolism in Plasmodium Falciparum. Current Topics in Microbiology and Immunology.;295:293-324.

Siu, A.L. and U.S.P.S.T. Force, Screening for Iron Deficiency Anemia and Iron Supplementation in Pregnant Women to Improve Maternal Health and Birth Outcomes: U.S. Preventive Services Task Force Recommendation Statement. Annals of Internal Medicine, 2015. 163(7): p. 529-36.

Siwila J, I.G. Phiri, H.L. Enemark, M. Nchito, and A. Olsen, 2010. Intestinal helminths and protozoa in children in pre-schools in Kafue district, Zambia Transactions of the Royal Society of Tropical Medicine and Hygiene. Feb;104(2):122-8

Sultana G.S., S. A. Haque, T. Sultana, Q. Rahman and A.N.Ahmed, 2011. Role of Red Blood Cell Distribution Width (RDW) in the detection of Iron Deficiency Anaemia in pregnancy within the first 20 weeks of gestation. Bangladesh Medical Research Council Bulletin; 37(3): 102-5

Sultana GS, S.A. Haque, T. Sultana, and A.N. Ahmed, 2013. Value of Red Blood cell distribution Width (RDW) and red cell indices in detection of Iron Deficiency Anaemia. Mymensingh Medical Journal; 22(2): 370-6

Thomas D.W., R.F Hinchliffe, C. Briggs, I.C. Macdougall , T. Littlewood and I. Cavill. 2013. Guidelines for Laboratory Diagnosis of Functional Iron Deficiency. British Journal of Haematogy, 161,639-648.

USAID micronutrient Programe, 2004. Zambia National Strategy and Plan of Action for the prevention and control of Vitamin A Deficiency and Anaemia 1999-2004.

USAID's A2Z Micronutrient and Child Blindness Project, ACCESS Program, and Food and Nutrition Technical Assistance (FANTA) Project.2006. Maternal Anemia: A Preventable Killer.

Valdes LG, C Campoy, J Florido, I Rusanova, MT Miranda and HJ McArdle, 2015. The impact of maternal obesity on iron status, placental transferrin receptor expression and hepcidin expression in human pregnancy. International Journal of Obesity (2015) 39, 571–578 (2015)

Wilczynski C. and E.B. Staros,2014. Transferin Receptor, Reference ranges, interpretation, collection and panels. Emedicine.medscape.com

Yip R., 2000. Significance of an abnormally low or high haemoglobin concentration during pregnancy: special consideration iron nutrition. American Journal of Clinical Nutrition.: 72(suppl): 272S-9S.

Zafar T and Z Iqbal. 2008. Iron Status in Preeclampsia. Professional Med J Mar 2008; 15(1): 74-80.

6.0 Appendices

6.1 APPENDIX A: Information Sheet

My name is Elizabeth Nakanyika Chewe, am a student at the University of Zambia pursuing a master's degree in Pathology (Hematology). Am doing a research on maternal Iron Deficiency anemia which is widespread in developing countries like Zambia. This study is collecting information from pregnant women in Chongwe attending antenatal care. The study will identify the women who have low blood volume. This study will identify women with iron deficiency anaemia to know who is at greatest risk of anaemia to help the care givers to know the correct supplement to give to pregnant women who will present with similar problems in future and therefore save their lives.

I am inviting you to be part of this study. You do not have to decide today whether or not you will participate. Before you decide, you are free to talk to anyone you are comfortable with about this study.

There may be some words that you may not understand, please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask.

Participant selection: I am inviting all pregnant women at different stages of pregnancy attending antenatal care at Chongwe Rural Health center to come and participate in this study. This includes all those on iron supplements and those who are not.

Participation is voluntary: Participation in this study is voluntary. Feel free to answer any questions and ask any question where you are not clear. You are also free to withdraw from this study at any point

Procedures and protocols: I will collect blood from your arm using a syringe and needle. I will collect about 4mls of blood only once.

Description of process: You will only make one visit. During this visit, blood will be collected and tested for complete blood count and your iron status will be measured. I will also ask you a few questions about your general health and measure your height and weight.

Possible disadvantages to participants: The recruitment questionnaire will take roughly 10 minutes. I will not collect any extra blood or other specimens for purposes of this study.

Confidentiality: I will not use your names for any purpose of this study, hence, you are not obligated to provide it or any information that you do not wish to be given.

Benefits to participants:

All pregnant women are benefiting during the course of the study, which is providing blood collection containers and other laboratory reagents which are often in short supply, hence ensuring that good health care continues despite the reagent supply shortages. The study personnel are also working closely with the laboratory staff, to ensure that test results are reported promptly. Participants will receive the same standard of care as all other patients. Participants also benefit in knowing whether they are iron efficient or not, which will determine whether they are supplemented with iron or not.

Benefits to the community:

The study will define the prevalence of iron deficiency anaemia locally, and will also define the age of pregnancy at risk of IDA to inform on policy.

Your participation in the study will help us prevent deaths because of anaemia. For further information about the study, you are free to contact the following on their mobile numbers:

Principal Investigator- Elizabeth Chewe, Study Coordinator, MIDA study Mobile: 0976209410, University of Zambia, School of Medicine, Department of Pathology and Microbiology and,

The Chairperson, UNZABREC on.

Tel: 256067 or Email: unzarec@zamtel.zm, Ridgeway Campus, Lusaka.

6.2 APPENDIX B: CONSENT FORM

TITLE: Association of maternal factors and Iron Deficiency Anaemia in pregnant women attending antenatal care in Chongwe District, Lusaka.

Name of researcher: Elizabeth Nakanyika Chewe

Position: masters of Science (haematology) student

Address: University of Zambia, School of Medicine,

Department of Pathology and Microbiology, Lusaka Zambia

1. I confirm that I have read and understand the information sheet for the above study and I have had the opportunity to ask questions
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving reason.
3. I agree to take part in the above study

	Signature	Date	Thumb Print
Participant	_____	|__|__|/|__|__|/|__|__|	
Witness	_____	|__|__|/|__|__|/|__|__|	

6.3 APPENDIX C: Questionnaires.

Study Questionnaire and Clinical Examination Form

	Date of Interview	|__|__|/|__|__|/|__|__|	
	Study ID Number	|__|__|__|__|__| □ □ □	
	Hospital Number	|__|__|__|__|__| □ □ □	
	Phone	**Tel:** |__|__|__|__|__|__|__|__|__|__|__|	
	Physical Address	_____	
	Date of Birth	|__|__|/|__|__|/|__|__|	
	Age of Pregnancy	□ □ **Months_____**	
	HIV status	**Positive_____ Negative_____**	

GENERAL INFORMATION

	What is the level of education	**1=Primary Education** **2=Secondary Education** **3=Tertiary** **9=None**	
	Any obstetric, gynaecological illness during pregnancy?	**1=No** **2=Yes**	
	If obstetric, gynaecological illness present, specify the illness	**Illness/s_____**	
	Parity (number of pregnancies)	**Gravida Para**	

MAIN COMPLAINTS

	Do you have any of the symptoms below?	1 = yes 2 = no	How long have you had the complaint? 1 = < 1 week 2 = >1 week 3= > 2 weeks
	Fever	1 2	1 2 3
	Hypothermia	1 2	1 2 3
	Lethargy	1 2	1 2 3
	Jaundice	1 2	1 2 3
	Eye Infection	1 2	1 2 3
	Cough	1 2	1 2 3
	Night sweat	1 2	1 2 3
	Weight loss	1 2	1 2 3
	Headache	1 2	1 2 3
	Body weakness	1 2	1 2 3
	Irritable	1 2	1 2 3
	Drowsiness	1 2	1 2 3
	Nausea/Vomiting	1 2	1 2 3
	Diarrhoea	1 2	1 2 3
	Dysuria	1 2	1 2 3
	Joint pains / muscular pains	1 2	1 2 3
	Oedema	1 2	1 2 3
	Skin lesions / rashes	1 2	1 2 3
	Oral thrush	1 2	1 2 3
	Restless	1 2	1 2 3
	Seizures	1 2	1 2 3
	Other symptom _____	1 2	1 2 3

Past medical history

	Have you had blood transfusion before	**Yes=1** **No=2**	
	If yes, How long ago was the transfusion performed?	**1= < 3 Month** **2= > 3 Month**	
	Have you had Malaria during Pregnancy?	**Yes=1** **No=2**	
	Have you suffered from any form of fever since your pregnancy?	**Yes=1** **No=2**	
	Did the patient take any antibiotics at home before going to clinic?	**2** **If Yes, Name the antibiotics** **.....** **.....** **.....**	
	How long were the above drugs taken?	**1= < 1 week** **2= > 1 week**	
	Did the patient take any nutrient supplement	**2** **If Yes, Name the supplements** **.....** **.....** **.....**	
	If 'yes' when were the supplements commenced?	**1=before pregnancy** **2=First trimester** **3=second trimester** **4=Third trimester**	

Physical Examination

	Weight	□□,□**kg**	
	Height	□□□,□**cm**	
	Temperature (ear)	□□,□**°C**	
	Respiration Rate	_____ **/ min**	
	Blood pressure	|__||__||__|/ |__||__||__| mmHg	

6.4 APPENDIX D: Letters of Support

6.4.1 Graduate Proposal Presentation Forum (GPPF)

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

Telephone : +260211252641
Telegram: UNZA, Lusaka
Telex: UNZALU ZA 44370
Email: assistantdeanpgmedicine@unza.zm

P.O Box 50110
Lusaka, Zambia

18th April, 2016

Ms. Elizabeth N. Chewe
Department of Pathology and Microbiology
School of Medicine
UNZA
LUSAKA

Dear Ms. Chewe,

RE: GRADUATE PROPOSAL PRESENTATION FORUM

Following the presentation of your dissertation entitled **"Correlation of Serum Transferrin Receptor with Iron Deficiency Anaemia in Pregnant Women attending Antenatal Care in Chongwe District, Lusaka",** your supervisor has confirmed that the necessary corrections to your research proposal have been done.

You can proceed and present to the Research Ethics.

Yours faithfully,

Dr. S.H. Nzala
ASSISTANT DEAN, POSTGRADUATE

cc: HOD, Pathology and Microbiology

6.4.2 Permission from Chongwe District Health Office

Chongwe Rural Health centre,

P.O.Box, 25

Chongwe.

The District Medical Officer,

Chongwe District Health Office,

P.O.Box, 25

Chongwe

Dear sir/ madam

Ref: PERMISSION TO CONDUCT MSC RESEARCH PROJECT AT CHONGWE RURAL HEALTH CENTRE.

The matter above refers, I am an employee at Chongwe Rural Health centre pursuing a master's of Science Degree in Heamatology. With your permission, I would like to conduct my research project in titled;Correlation of Serum Transferrin Receptor with Iron Deficiency Anaemia inpregnant women attending antenatal care inChongwe District, Lusaka, at Chongwe Rural Health centre.

Kindly find attached my concept of the proposal. Your kind consideration to this letter will be highly appreciated.

Yours faithfully,

Elizabeth C. Kabwe

19/04/16

6.4.3 Ethic approval from University of Zambia Biomedical Research Ethics Committee

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

20th October, 2016.

Our Ref: 003-07-16.

Ms. Elizabeth N. Chewe,
University of Zambia,
School of Medicine,
Department of Pathology,
P. O Box 50110,
Lusaka.

Dear Ms. Chewe,

RE: RESUBMITTED RESEARCH PROPOSAL: **"THE CORRELATION OF SERUM TRANFERRIN RECEPTOR WITH IRON DEFICIENCY ANAEMIA IN PREGNANT WOMEN ATTENDING ANTENATAL CARE IN CHONGWE DISTRICT, LUSAKA" (REF. No. 003-07-16)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 28th September, 2016. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. S.H Nzala
VICE-CHAIRPERSON

Date of approval: 20th October, 2016. **Date of expiry:** 19th October, 2017.