

**ADHERENCE TO IRON PROPHYLACTIC THERAPY DURING PREGNANCY IN AN
URBAN REGIONAL HOSPITAL IN DURBAN, SOUTH AFRICA**

by

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PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was conducted in the Optics & Imaging Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor J Moodley, and co-supervision of Professor A Naicker.



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DECLARATION

I, Princess Zinhle Mkhize declare that:

- (i) The research reported in this dissertation, except where otherwise indicated is my original work.
- (ii) This dissertation has not been submitted for any degree or examination at any other university.
- (iii) This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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Signed:  Date: 01/09/2017

DEDICATION

To my late Mother Jabu, my father Bafana and grandparents Jeslinah and William

'You may be gone from my sight but you are never gone from my heart'

Penwell-Gabel

To my family and friends

Your contribution towards my success has been amazing

Publications

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LIST OF ABBREVIATIONS

CP	Ceruloplasmin
DMT1	Divalent metal transporter - 1
Dcytb	Duodenal cytochrome b ferrireductase
HB	Haemoglobin
HIV	Human immunodeficiency virus
Hp	Hephastin
LMIC	Low-middle income countries
MCHC	Mean cell haemoglobin concentration
MCH	Mean cell haemoglobin
MCV	Mean cell volume
NMB	New methylene blue
NTDs	Neural tube defects
ID	Iron deficiency
IDA	Iron deficiency anaemia
IFA	Iron-folic acid
IRP1/2	Iron-responsive element-binding proteins
RBC	Red blood cell
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
SDR2	stromal cell-derived receptor 2
STEAP 2	Six-transmembrane epithelial antigen of the prostate 2

LIST OF ABBREVIATIONS

WHO World health organization

ZIP14 Zrt-Irt-like protein 14

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ABSTRACT

Iron and folic acid supplementation plays a major role in prevention and control of iron deficiency anaemia in antenatal care. In South Africa, although all pregnant women receive iron, folate and calcium supplementation throughout pregnancy, anaemia is still common. Low adherence may be a key contributor to the ineffectiveness of supplementation programs. Therefore, this study was conducted to examine adherence to prophylactic iron supplementation during the antenatal period. An observational clinical study was conducted in a regional hospital from January- December 2016. Women (n=100 HIV uninfected and n=100 HIV infected) were recruited and subdivided into three groups: (a) 1st attendees ≤ 34 weeks (n=33), (b) 34-36 weeks (n =34) and (c) ≥ 37 weeks /birth (n=33) respectively. A structured questionnaire was used for data collection.

Data were coded and computed onto an excel sheet for statistical analysis using SPSS software.

Data from women (n = 24) from 1st visit attendees ≤ 34 weeks and 34-36 weeks subgroups indicated that pill count and self-reported data reflected 50% adherence and 46% non-adherence, being higher in the HIV infected women (75%). Nausea was the commonest side effect in all trimesters (79, 2%). Adherence (27.8%) and non-adherence (72.1%) to iron, folic acid and calcium supplementation were observed in 176 (88%) women.

Promoting essential strategies on the importance of consumption and effectiveness of iron prophylactic therapy is essential to maintain and improve anaemia in antenatal attendees during pregnancy.

CHAPTER 1

INTRODUCTION

1.0 Overview of Iron Prophylactic Therapy

Prophylactic iron, folic acid and multivitamins are prescribed routinely for all antenatal attendees in maternity health facilities. However, the effect of supplementation to all antenatal women remains controversial (Osungbade *et al.*, 2012). There is evidence that providing prophylactic iron, folate and multivitamin supplementation prior to pregnancy is of benefit. Many women enter pregnancy with depleted iron stores (Cantor *et al.*, 2015). Nonetheless, there is uncertainty of the value of supplementation to all pregnant women irrespective of their haemoglobin levels (Parkkali *et al.*, 2013). Patient adherence to “pill taking” remains unknown. Apart from antiretroviral drugs, HIV infected pregnant women have an added burden of further supplementation of iron, folate, calcium and multivitamins.

1.1 Background and Literature

Iron deficiency is the most widespread pathologic cause of anaemia in pregnancy (Cantor *et al.*, 2015). It is the most common nutrient deficiency internationally, amongst pregnant women and children, especially in low and middle-income countries (Taye *et al.*, 2015). Iron is an essential nutrient, which is necessary for haemoglobin synthesis (Millman, 2011). Its requirement increases during pregnancy and is not adequately supplied through the regular diet, especially in low and middle-income countries (LMIC). Furthermore, a loss of appetite during pregnancy can worsen the demand for iron (Mithra *et al.*, 2014). Therefore, the most appropriate mass intervention to reduce incidence of anaemia is the provision of prophylactic iron and folic acid to all pregnant women throughout pregnancy (Mithra *et al.*, 2014). Folic acid is provided to prevent neural tube defects and macrocytic anaemia (Hodgetts *et al.*, 2014). There is strong evidence that folic acid has resulted in a significant decrease in neural tube defects especially when started two /three months prior to pregnancy and during the first trimester of pregnancy when the fetal neural system develops (Hodgetts *et al.*, 2014).

A decrease in iron stores during pregnancy is accompanied by a significant rise in the reticulocyte count and eventually an increase in erythropoiesis in the bone marrow (Ehiaghe *et al.*, 2014). Therefore, the reticulocyte count can be used to monitor progress after iron supplementation. In pregnancy the physiological demands of a growing fetus, changes in blood and red cell volume,

vomiting and increasing nutrient demands exacerbates the need for iron supplementation (Millman, 2011)

It has been shown that iron deficiency is linked with serious long-term consequences in children, such as increased vulnerability, to infections, poor physical growth, poor appetite, retardation of cognitive and psychomotor development and cardiac failure (Mamabolo and Alberts, 2014).

The efficacy and the success of intervention depends on the compliance to the Iron-folic acid tablet intake. Compliance describes the degree to which a patient accurately follows a medical instruction. Many experts believe that one of the major reasons that national iron supplementation programs have failed is women's non-adherence to pill taking. Other factors that have not been studied extensively include health systems (Mithra *et al.*, 2014). Non-adherence is defined as missing two or more doses repeatedly. Although adherence to iron- folic acid therapy is crucial, there is uncertainty affected by several social and demographic factors (Mithra *et al.*, 2014). There is evidence that older women and those with a history of miscarriage are more likely to achieve greater adherence (Nwaru *et al.*, 2015). Furthermore, it has been shown that adherence depends on the woman's behaviour during antenatal period (Nwaru *et al.*, 2015). In addition, there is a lack of evidence that the use of antiretroviral drugs during pregnancy increases the severity of anaemia (Nandlal *et al.*, 2014). The use of these drugs and other medications may increase the level of non-adherence to iron prophylactic therapy during antenatal period (Nandlal *et al.*, 2014). In addition, there is a limited focus regarding the use of other medications concurrently with iron supplements.

1.1.1. Iron

Iron is one of the most important minerals needed by the human body. It is found in every cell and is essential for haemoglobin synthesis (Milman, 2011). The human body has iron stores ready to replace iron that is lost but low iron levels over a long time may result in iron deficiency anaemia. Symptoms of low iron levels include irritability, lack of energy, weight loss, dizziness, headache and shortness of breath. Pallor of the mucus membranes such as the tongue and spoon shaped nails are two of the physical signs of low iron concentrations.

The best sources of iron are: eggs, liver, lean red meat, oysters, poultry, iron-fortified cereals, dried fruits, dried beans, fish, salmon, tuna, and whole grains. However, iron from vegetables, fruits and supplement is difficult for the body to absorb (Iron in diet. University of Maryland Medical Centre; <http://umm.edu/health/medical/ency/articles/> (accessed On 01 July 2016; pp 1-5). These sources include dried fruits, legumes, seeds, vegetables, and whole grains. Iron absorption is increased with foods rich in vitamin C.

Foods that curb iron absorption contain substances that inhibit dietary iron utilisation e.g., commercial black and pekoe teas. Excessive consumption of iron may exacerbate a genetic disorder known as hemochromatosis. This disorder affects iron absorption and leads to iron overload in the body. However, it is not likely for a person to ingest too much iron (Iron in diet. University of Maryland Medical Centre; <http://umm.edu/health/medical/ency/articles/iron-in-diet> (accessed On 01 July 2016; pp 1-5).

Table 1.0: Recommendation for daily iron requirement (The Food and Nutrition Board at the Institute of Medicine) (adapted from University of Maryland medical centre)

FEMALES	AMOUNT PER/DAY	INFANTS & CHILDREN	AMOUNT PER/DAY	MALES	AMOUNT PER/DAY
9 to 13 years	8mg	Younger than 6 months	0.27mg	9 to 13 years	8mg
14 to 18 years	15mg	7 months to 1 year	11mg	14 to 18 years	11mg
19 to 50 years	18mg	1 to 3 years	7mg	19 & older	8mg
51 & older	8mg	4 to 8 years	10mg		
Pregnant women-all ages	27mg				
Lactating women 19 to 30 years	9mg				

1.1.2 Iron Metabolism

Iron metabolism is essential for understanding diseases of iron such as iron deficiency anaemia and iron overload (WHO, 2015). Iron importation can take place through endocytosis of transferrin receptor 1 or through ferrous iron importers DMT1 and ZIP14, which needs the activity of iron reductases such as STEAP2, SDR2 and Dcytb (WHO, 2015). Intracellular iron can be stored in ferritin, used for protein biosynthesis, produce reactive oxygen species (ROS) and control transcription using iron-responsive element-binding proteins (IRP1/2). Export occurs through ferropoetin, frequently aided by hephastin (Hp) and ceruloplasmin (Cp), and reserved by hepcidin (WHO, 2015).

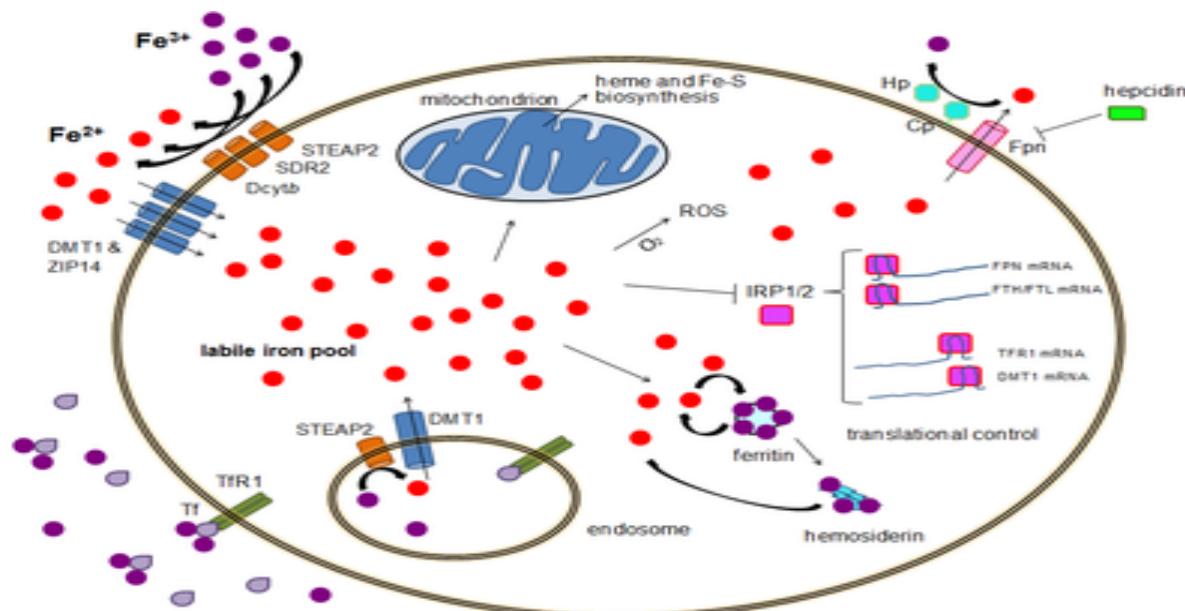


Figure: 1.0 Diagram showing a generalised view of cellular iron homeostasis in humans (adapted from human iron metabolism (https://en.wikipedia.org/wiki/Human_iron_metabolism) accessed on 01 July,2016: pp 1-11)

1.1.3. Haemoglobin

Haemoglobin is a molecule comprised of four subunits. Each subunit has an iron containing pigment (heme) and a protein (globulin). The subunits consist of two types, alpha and beta and each gram of subunit can take 1.34 ml of oxygen. Therefore, the oxygen carrying ability of blood is directly proportional to its haemoglobin concentration. Because some cells may contain more haemoglobin than others, the number of blood cells cannot indicate blood oxygen content. Haemoglobin determination is used to screen for anaemia, to classify the severity of anaemia, and to aid in evaluating the patient's response to anaemia therapy). Total haemoglobin content is lowered by the blood loss and bone marrow suppression, which reduces total red blood cell count (RBC). Haemoglobin levels are also lowered in patients who have abnormal types of haemoglobin or haemoglobinopathy (Haemoglobin - Complete Blood Count – <http://www.rnceu.com/cbc/cbchg.html> 2013; accessed 1 July 2013: 1-2). Red blood cells with abnormal types of haemoglobin are often brittle and or destroyed easily in the vascular system. Some patients have normal red blood cell count but a low haemoglobin level. Normal Haemoglobin values are: Adult (males):13.5–17g/dl, females: 12-15g/dl, Pregnancy: 11- 12g/dl, Newborn: 14-24g/dl. 77 of this value is fetal haemoglobin, which drops to approximately 23% of the total at 4 months of age, children: 11-16g/l (RnCeus.com, 2013).

1.1.4. Ferritin

Ferritin is a protein that originates from cells that stores iron for later use. The Ferritin test measures the amount of iron in the blood. The amount of ferritin (serum ferritin level) is directly linked to the amount of iron stored in human body. Low ferritin levels even within normal ranges may indicate inadequate iron. However, normal value ranges may differ slightly among different laboratories. Normal ranges are, Male: 20 to 200ng/ml, female: 15 to 150ng/ml. (Gersten, 2016). Ferritin levels can rise with inflammatory disorders and a higher than normal level may be due to too much iron in the body, frequent transfusion of packed red blood cells and alcoholic liver disease. A lower than normal level may be caused by heavy menstrual bleeding, high physiological demand during pregnancy, intestinal conditions that cause poor absorption of iron, iron deficiency anaemia and long term digestive tract bleeding (Gersten, 2016).

1.1.5. Reticulocyte

Reticulocytes are defined as immature red cells seen in the peripheral blood that contains at slightest two dots of reticulin material that reflect with new methylene blue (NMB) in their cytoplasm (a case of iron deficiency anaemia; <http://www.meddean.luc.edu/lumen/meded/mech/cases/case7/> accessed 09June 2016: pp 1-7). More forms that are immature have various dots and tiny networks of skeins of bluish material. These remnants are residual ribosomal RNA used for haemoglobin synthesis in the developing erythrocyte. The RNA is thinly distributed to form networks on Wright's stain; a supravital stain causes precipitation and aggregation of the RNA and creates the dots and skeins of reticulin. RNA containing red cells are usually greyish on Wright's stain and contrast well with mature, ortho-chromic or pink red cells, providing an indication to the presence of reticulocyte reaction (a case of iron deficiency anaemia; <http://www.meddean.luc.edu/lumen/meded/mech/> accessed; 09June 2016: pp 1-7). Three ways to express reticulocyte response are: reticulocyte count, corrected reticulocyte count and absolute reticulocyte count. They are counted as the number of NMB-reactive cells per 1,000 red cells and expressed as a percentage of reticulocytes (absolute number per 100 red cells). A reticulocyte count assists in categorising anaemia into hypo or hyper-proliferative types. The normal count is 0.5-1.5% (a case of iron deficiency anaemia; <http://www.meddean.luc.edu/lumen/meded/mech/cases/case7/>: accessed 09 June 2016: pp 1-7) The hypoproliferative type indicates reduced reticulocytes, the bone marrow not able to produce requisite number of red blood cells (RBC's) and lack of essential substances (iron, B12, and folate).

The hypoproliferative type indicates increased reticulocytes, the cause of anaemia being outside the bone marrow (haemolytic anaemia, haemorrhage and post anaemia treatment), reduced survival of

RBC's and a normal bone marrow that responds effectively by increasing the output. A RBC has a lifespan of about 120 days and less than 1% is replaced every day (a case of iron deficiency anaemia; <http://www.meddean.luc.edu/lumen/meded/mecases/case7/anaemia.html>: accessed 09June 2016: pp 1-)

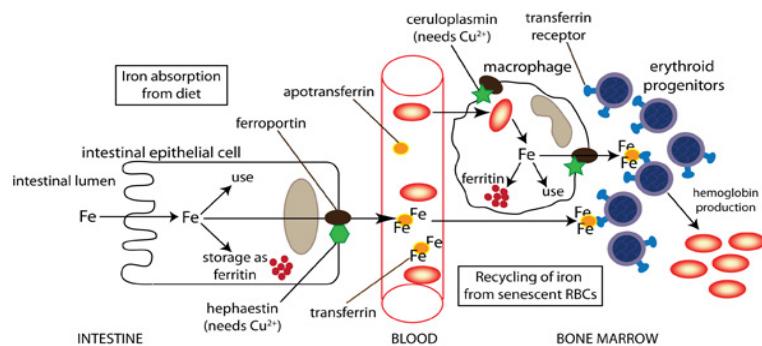


Figure: 1.1: Diagram illustrating iron absorption, storage and recycling. (adapted from human iron metabolism (https://en.wikipedia.org/wiki/human_iron_metabolism), accessed on 01 July,2016:)

1.2. Iron deficiency anaemia

Iron deficiency (ID) is a worldwide problem with infants and their mothers been more vulnerable to both the development and the consequences of iron deficiency. In concert with pregnancy, the crucial period of “1000 days” is associated with adverse effects later in life, such as increased risk of non-communicable diseases, as well as decreased capability and economic productivity (Burke *et al.*, 2014). Furthermore, entering pregnancy with low iron status results in serious life consequences to both mother and the infant. It is considered to be the main common cause of iron deficiency anaemia (IDA) in pregnancy (Mithra *et al.*, 2014). Iron deficiency in pregnancy is due to the fact that iron stores are inadequate to meet the increasing demands of pregnancy. It has been reported that IDA is associated with a rise in maternal mortality, stillbirths, low birth weights babies, neonatal sepsis and intrauterine growth restriction (Tunkiyi and Moodley, 2016).

Anaemia is a public health problem affecting both low and high-income countries resulting with major outcomes on human health as well as social and economic development (Bekele *et al.*, 2016). The World Health Organization (WHO) defines anaemia as a haemoglobin (HB) concentration of $\leq 11\text{g/dl}$ (Tunkiyi and Moodley, 2016). Furthermore, IDA is characterised by microcytic, hypochromic erythrocytes and decreased iron stores. A deficiency in iron is the most common cause of microcytic

anaemia. A patient with less than normal microcytic and hypochromic cells is deficient to iron. Therefore the mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) are significant indicators/markers of IDA and are used to diagnose and observe recovery (Erhabor *et al.*, 2013).

1.2.1. Anaemia Prevalence

The World Health Organization (WHO) estimates anaemia prevalence at 9% in high income countries and 43% in LMIC , 47% in children younger than 5 years, 42% in pregnant women, and 30% in non-pregnant women aged 15-49 years with Africa and Asia accounting for more than 85% of the total anaemia burden in high risk groups (Bekele *et al.*, 2016). A study conducted in the United States of America (USA) concluded that prevalence of iron deficiency in that country was approximately 18%. (Bekele *et al.*, 2016).The rates of iron deficiency increases across the trimesters of pregnancy from 6.9% to 14.3% to 28.4% (Cantor *et al.*, 2015). Furthermore, anaemia in pregnancy is linked with substantial health and economic cost implications. The Saving Mothers Report (2010-2013) revealed that 40% of maternal deaths in South Africa (SA) were associated with anaemia, irrespective of micronutrient (prophylactic iron, folic acid and multivitamins) supplementation. Anaemia prevalence at first antenatal visit in a study conducted in South Africa reports 42.7%. These results are consistent with prevalence rates of 40% in Kenya, 38.2% in Ethiopia and 47.4% in Tanzania (Tunkyi and Moodley, 2016). Anaemia ranges from mild, moderate and severe and the WHO states that the haemoglobin level in pregnancy at 10.0-10.9g/dl is (mild anaemia), 7-9.9 g/dl (moderate anaemia) and ≤ 7 g/dl (severe anaemia). Iron deficiency anaemia detected early in pregnancy is linked to loss of energy and low iron intake resulting to a decrease in gestational weight gain over the entire pregnancy (Panda *et al.*, 2015).

1.2.2 Human immunodeficiency virus (HIV) infection and Anaemia in pregnancy

Sub-Saharan countries have a high prevalence of anaemia associated with HIV infection. However, there is a lack of data on anaemia amongst South African pregnant women. The prevalence of anaemia in SA is 19.7% (Van Bogaert, 2006).

Furthermore, prevalence from African countries show varying rates possibly reflecting varying sample sizes of the study populations, geographical area (rural or urban), rates of parasitic infestations and levels of education (Tunkyi and Moodley, 2016). In 2012, The Saving Mothers Report in South Africa stated that HIV infection (70%) and anaemia (30%) were the commonest conditions amongst pregnant women who died during pregnancy and in the puerperium phase

(Nandlal *et al.*, 2014). The consequences of the effect of anaemia in association with HIV infection on both maternal and birth phase is unclear.

1.3. Iron and Folic supplementation

Oral iron supplementation clinically prevents iron deficiency in pregnancy (Jasti *et al.*, 2005). According to WHO recommendations daily oral iron and folic acid supplementation is recommended to reduce the risk of low birth weight, maternal anaemia and iron deficiency in all antenatal care clinics (WHO guidelines, 2012). One of the national health objectives designed by the U.S Department of Health and Human Services was to decrease anaemia in the third trimester to 20% by the year 2010. Prevention for average risk populations includes sufficient dietary iron intake and oral low dose (30mg) iron supplements early in pregnancy and suggested prophylaxis for iron deficiency anaemia (IDA) in high risk populations is elemental iron of 60 to 100mg daily. Prenatal screening for IDA was reviewed by the U.S. Preventive Services Task Force (USPSTF) in 2006 and recommended routine screening on the basis of fair quality evidence. There was evidence that treating asymptomatic IDA in pregnancy results in moderate health benefits (Cantor *et al.*, 2012). The most appropriate mass intervention for iron supplementation is to administer iron along with folic acid in a tablet form to all pregnant women. The aim is to improve the level of haemoglobin for anaemia to be reduced as highly as possible at term (Mithra *et al.*, 2014). However, in public health settings the supply of oral iron therapy to pregnant women has been less effective.

1.3.1. Oral Ferrous Sulphate

Ferrous sulphate is supplied to all antenatal settings. It is a white, circular, biconvex, sugar-coated tablet used for prevention and treatment of iron deficiency anaemia. Each tablet contains 200mg dried ferrous sulphate equivalent to 65mg ferrous iron. It originates from a group of medicines called iron supplements whose work is to replace lost iron. There are products that increase or decrease the absorption of iron. For example, it is reduced with drinking tea, coffee, milk, eggs and wholegrain and increases with meat products containing high levels of vitamin C. Like any other medication, ferrous sulphate can cause side effects which are constipation, diarrhoea, abdominal pain and “blackened/dark stools” (Patient information leaflet: ferrous sulphate 200mgms {28 pack} <https://www.medicines.org.uk/emc/medicine/18081> accessed 05 July 2016 pp 1-4).

1.3.2. Oral Folic Acid

Folic acid is highly significant for the foetal development, as it can decrease the incidence of spinal bifida caused by neural tube defects (NTDs). A recommendation by the department of health states

that women should take a daily supplement of 400 micrograms of folic acid while they are trying to fall pregnant, and this dose should continue for the first 12 weeks of pregnancy for baby's spine to develop (Hodgetts *et al.*, 2014). Dietary sources of folic acid consist of green leafy vegetables, brown rice, granary bread, and breakfast cereals prepared with folic acid (Health questions: Why do I need Folic Acid in pregnancy ? <http://www.nhs.uk/chq/pages/913.aspx?categoryid=54> accessed 05 July 2016). It is not possible to get folic acid from food. Therefore, it is important to get it from taking supplements. An exact amount of folic acid is contained in each tablet and is also safe to use after 12 weeks (Health questions: do I need Folic Acid in pregnancy ? <http://www.nhs.uk/chq/pages/> accessed 05 July 2016). However, there is a high rate of unplanned pregnancy especially in low-income countries. Therefore, folic acid is supplied to all antenatal attendees from the first clinic visit until delivery. In addition in many countries including South Africa, bread a staple food is fortified with folia acid. Furthermore, all pregnant women in South Africa receive folia acid alone or in combination with folic acid (pregamal) tablets.

1.3.3. Oral Calcium

Calcium is a rich mineral that is essential for many various processes, such as bone information, muscle contraction, and enzyme and hormone functioning. Most of the body's calcium originates in bones and teeth and about 1% is present in the intracellular structures, cell membranes and extracellular fluids (WHO guidelines, 2012). Some studies have revealed that calcium supplementation during pregnancy has a significant effect on reducing the risk of pre-eclampsia (WHO guidelines, 2012). Insufficient consumption of this nutrient by pregnant women may result in adverse effects to both the mother and the fetus. However, too much consumption of calcium may enhance the risk of urinary stones and urinary tract infection and absorption of other essential micronutrients might be reduced. The WHO in conjunction with the Food and Agriculture Organization reports that a dietary intake of 1200 mg per day is recommended for pregnant women (WHO guidelines, 2012). In addition, for the purpose of WHO guidelines, two systematic reviews were combined to particularly to investigate if calcium supplementation in pregnancy safely improves maternal and fetal outcomes. Pre-eclampsia, eclampsia, gestational hypertension with or without proteinuria, complications at delivery and any adverse events were considered as critical maternal outcomes by the Nutrition Guidance Advisory Group (WHO guidelines, 2012). Furthermore, adverse infant outcomes were preterm birth, low birth weight (≤ 2500 g), stillbirth, death during the neonatal period and any adverse effects including small for gestational age or admission to a neonatal care unit (WHO guidelines, 2012). Therefore, the WHO suggested a scheme for calcium supplementation in pregnant women. A daily dosage of 1.5 – 2.0 g elemental calcium

should be supplied to all pregnant women, mostly those at higher risk of gestational hypertension (WHO guidelines, 2012). The standard clinical practice in South Africa is to give calcium to all antenatal attendees until the end of pregnancy (Maternity Care guidelines for clinics and district hospitals in South Africa, 2016).

1.4. Adherence to Oral Iron Prophylactic Therapy

Limited adherence to iron supplementation is considered to be a main reason for the low effectiveness of anaemia prevention programs. Iron supplementation has been a therapeutic approach by tradition, initiated after the diagnosis of iron deficiency. Traditionally, iron supplementation was based on therapeutic approach of using high dose iron salt or elixir that provides between 60 mg and 180 mg Fe/d (Beard, 2000). High doses could cause disturbance in normal absorptive routes and are associated with side effects such as gastrointestinal upset, constipation and nausea.

The 1990 Institute of Medicine Committee and the 1991 Life Sciences Research Office guidelines suggested that non- anaemic women be supplied with 30 mg Fe/d starting in the 12th week of pregnancy (Beard, 2000). Furthermore, if anaemia is present low plasma ferritin level confirms iron deficiency; the daily dose should be 120 or 180 mg Fe/d. It was in 1993 when the Institute of Medicine report recommended that women be screened for anaemia at the first antenatal visit (Beard, 2000). In addition, the WHO/Food and Agriculture Organization calculated iron requirements for pregnant women based on the need for iron that could be absorbed and the need for increased heme iron in the diet. Therefore, the WHO advocated supplementation of 60mg Fe/d along with 250 ug folate (Beard, 2000). Iron deficiency Anaemia prevalence and incidence is still significantly high among pregnant women despite routine oral iron supplementation (Tunkyi and Moodley, 2016).

1.4.1. Adherence and side effects

Several studies have shown that side effects appear to be one of the causes of non- adherence to iron prophylactic treatment. A study conducted in Bangladesh in 2001 compared side effects of iron supplementation and their impact on adherence amongst pregnant women (Hyder *et al.*, 2001). The presence of nausea and vomiting reduced the level of adherence in the study group (Hyder *et al.*, 2001). Also, a study conducted in Malawi in 2009 reports nausea (43.6%) as the single most important reason for non-compliance in the study group (Kalimbira *et al.*, 2009). Studies conducted in India also reports nausea and vomiting as the most common side effects observed, accompanied by heartburn, diarrhoea, constipation and abdominal pain (Sangwan *et al.*, 2014). Forgetfulness and

bad taste were also given as reasons for non-adherence (Panda *et al.*, 2015). The main reason for skipping doses is forgetfulness (48.8%) followed by gastritis, constipation, vomiting, and travel (Mithra *et al.*, 2014).

Compliance level is again influenced by age, education, parity, socio – economic status and number of clinic visits especially in low and middle-income countries. A study by Sangwan *et al.*, (2014) showed the association of age, parity, education, socio – economic status, early registration and number of clinic visits were associated with Iron – Folic Acid (IFA) intake.

Furthermore, a study by Roy *et al.*, (2013) reports influence of education, parity, timing of registration and number of antenatal clinic visits were proportional to IFA, whilst age, socio – economic status and family type were not directly associated. In contrast, a study by Mithra *et al.*, (2013) has shown that age, socio economic status and parity are major contributors to IFA adherence.

The study adherence in their group was 64.7%, which is similar to the findings (67.9%) by Sangwan *et al.*, (2014). These results were again comparable to a study conducted in Senegal in 2008 (Sengwan *et al.*, 2014). Anaemic women have a lower level of adherence whereas those above 25yrs and in second pregnancy are more compliant (Sengwan *et al.*, 2014). Few studies have addressed the challenges of the socio economic climate on iron supplement adherence (Sangwan *et al.*, 2014).

1.4.2. Pill count

A few studies have used the “pill count” to observe adherence of iron – folic acid consumption. A study conducted in the United State in 2005 among non – Hispanic white and Non-Hispanic Black women revealed that “pill count” is more significant than self- reported compliance (Jasti *et al.*, 2005). Adherence was monitored using an electronic counting device to record date and time at which the bottle was opened at the time of pill taking. Study groups were unaware of this device. Pill count was more accurate than self-reports (Jasti *et al.*, 2005). Another study in Bangladesh utilised a similar method of monitoring pill count to record adherence of iron-folic acid adherence (Heyder *et al.*, 2002).

In South Africa, no studies have been conducted on adherence to iron prophylactic supplements during pregnancy. African- based studies have revealed that antenatal attendees have complex reasons for not taking iron supplements that involves socio-cultural context that intensely shapes one’s behaviour (Sambili *et al.*, 2016). Therefore, this study includes other factors that can reduce the level of adherence to iron supplements among pregnant women attending antenatal clinic.

This study establishes adherence to prophylactic iron supplementation during the antenatal period, in an urban setting in South Africa.

1.4.3. Aims and Objectives of the study

The use of iron supplements by pregnant women is still uncertain, therefore the aim of this study is to establish adherence to prophylactic iron supplementation during the antenatal period, in an urban setting in South Africa.

The specific objectives include assessing adherence to prophylactic iron supplementation and determination of haemoglobin levels; reticulocyte count and serum ferritin after oral supplementation in HIV uninfected and infected pregnant women.

CHAPTER 2

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Adherence to Iron Prophylactic Therapy during pregnancy in an Urban Regional Hospital in Durban, South Africa.

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Abbreviations: Hemoglobin (Hb), Human Immune Deficiency Virus (HIV), Antiretroviral Therapy (ARV), Zidovudine (ZDV), Tuberculosis (TB), Low and Middle- Income Countries (LMICs), World Health Organization (WHO), Antenatal clinic (ANC).

SYNOPSIS

This study set out to establish whether antenatal attendees adhere to prophylactic iron therapy throughout pregnancy in a low socio-economic population.

ABSTRACT

Objective:

To assess adherence to prophylactic iron supplementation during the antenatal period in South Africa.

Methods:

An observational clinical study was conducted in a regional hospital from January-December 2016. Women (n=100 HIV uninfected and n=100 HIV infected) were recruited and subdivided into three groups: (a) 1st visit attendees ≤ 34 weeks (n=33), (b) 34-36 weeks (n =34) and (c) ≥ 37 weeks /birth (n=33) respectively. A structured questionnaire was used for data collection. Data were coded and computed onto an excel sheet for statistical analysis using SPSS software.

Results:

Data from women (n = 24) from 1st visit attendees ≤ 34 weeks and 34-36 weeks subgroups indicated that pill count and self-reported data reflected 50% adherence and 46% non-adherence, being higher in the HIV infected women (75%). Nausea was the commonest side effect in all trimesters (79, 2%). Adherence (27.8%) and non-adherence (72.1%) to iron, folic acid and calcium supplementation were observed in 176 (88%) women.

Conclusion:

Adherence to iron, folic acid and calcium supplementation was low in pregnant women receiving antenatal care at the study site.

Keywords: Iron, folic acid, calcium, Adherence, Anemia, Pregnancy, Supplements.

INTRODUCTION

Iron deficiency is the most widespread pathologic cause of anemia in pregnancy [1]. Globally, particularly in low and middle-income countries (LMICs), it is the commonest nutrient deficiency affecting both pregnant women and children [2]. Iron is necessary for hemoglobin synthesis and its requirement increases during pregnancy. Therefore, to reduce the incidence of anemia, women receive prophylactic iron and folic acid supplementation throughout pregnancy and the puerperium [1-3]. Folic acid prevents neural tube defects and macrocytic anemia, especially when initiated prior to conception and continued in early pregnancy during the neural system development [4].

In pregnancy, the physiological demands of a growing fetus, changes in red cell volume, vomiting and increasing nutrient demand support the need for iron supplementation [5]. A decrease in iron stores during pregnancy is accompanied by a significant rise in the reticulocyte count (RC) that eventuates in an increase in erythropoiesis [6]. Therefore, the RC may be used to monitor progress after iron supplementation.

The efficacy and success of any medical intervention such as prophylactic iron-folic acid therapy throughout pregnancy is dependent on compliance and adherence to the regimen of pill usage. Compliance describes the degree to which an individual accurately follows a medical instruction [3].

The major reason that national iron supplementation programs fail is women's non-adherence to pill taking and other factors including health systems [3]. Non-adherence is defined as missing two or more doses repeatedly [3].

Furthermore, adherence depends on the woman's behavior during the antenatal period [7]. The concurrent use of other medication affect adherence thereby increasing the severity of anemia during pregnancy. It is unknown whether the use of antiretroviral drugs during pregnancy increases the severity of anemia [8]. However, a study by Odhiambo et al., [9] showed that anti-retroviral therapy including zidovudine improved hemoglobin levels over time.

The World Health Organization (WHO) states that approximately 56% and 23% of pregnant women in LMICs and in high-income countries are respectively anemic [10], and defines anemia as a hemoglobin (HB) concentration of $\leq 11\text{g/dl}$ [11]. In South Africa, 42.7% of maternal deaths is associated with anemia, irrespective of micronutrient (prophylactic iron, folic acid and multivitamins) supplementation [12]. The aim of the present study was to establish adherence to prophylactic iron, folic acid and calcium supplementation among pregnant women in a regional hospital in Durban, South Africa.

METHODS

This observational clinical study received institutional ethical (BE485/15) and regulatory hospital approval. The study was conducted from January- December

2016 in a regional hospital that serves mainly low socio-economic Black South African population group.

A study population (n=200) consisting of HIV infected (n=100) and uninfected (n=100) women were recruited and stratified into: - a) 1st visit attendees ≤ 34 weeks (n=33), b) 34-36 weeks (n=34) and c) ≥ 37 weeks /birth (n=33) gestation respectively. Inclusion criteria included Black South African women attending their first antenatal clinic, who provided written informed consent and were ≥ 18 years of age. Women with medical complications such as cardiac, diabetes, hypertension and hematological disease were excluded.

It is a standard clinical practice in South African public sector hospitals that all pregnant women receive prophylactic iron and folate supplementation throughout pregnancy. All participants received a month's supply of iron "pregamal" tablets (a combination of ferrous fumarate 200mg and folic acid 100mg) at every antenatal visit. For anemic women, ferrous sulphate (FeSO_4 ; 200mg) and folic acid (5mg/day) was prescribed. In addition, women received calcium carbonate supplementation (1250mg/day) as a preventative measure against the development of pre-eclampsia.

Women recruited were followed-up at their next scheduled antenatal visit for adherence. They were requested to return the balance of their supplements. Adherence was assessed via self-reporting and by manual pill count. Self-reporting referred to women who reported without carrying their containers. Whereas, pill

count refers to the number of pills remaining in the container. All adherence and non-adherence information, including side effects were obtained at each follow-up visit.

Full blood count, reticulocyte and serum ferritin tests were analyzed. Demographics including clinical data (age, area of residence, social status, HIV and TB information, parity, gestation age, blood pressure, maternal height and weight, urine dipsticks, blood results, birth details, dietary habits (types of food, smoking, alcohol consumption, recreational drugs and herbal medication), feeding choice and family planning were recorded.

The Statistical Package for the Social Sciences (SPSS Statistics version 24, IBM Corporation, New York) was used for analysis. Independent sample t-test and ANOVA were used for parametric data. Categorical data are presented using Fisher's exact Chi-squared test. Parametric data are expressed as mean \pm standard deviation. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical and demographic data are outlined in Table 1. Birthweight was significantly different between HIV infected and uninfected groups ($p = 0.004$). Birthweight at 1st visit ≤ 34 weeks (3.06 ± 0.56 , 3.21 ± 0.45 Kg) and ≥ 37 weeks /birth (3.41 ± 0.43 , 3.15 ± 0.40 Kg) appropriately matched gestational age albeit that it was lower at 34-36 weeks (2.98 ± 0.52 vs 2.86 ± 0.62 Kg).

The hematological, reticulocyte profiling and serum ferritin (SF) levels taken at antenatal visits (1st visit attendees ≤ 34 weeks, 34-36 weeks and ≥ 37 weeks/birth groups) are shown in table 2. Hemoglobin levels were below the normal reference range for pregnancy at 52%, 58%, 50%, 56%, in the HIV uninfected 1st visit attendees ≤ 34 weeks, HIV infected 1st visit attendees ≤ 34 weeks, HIV uninfected 34-36 weeks and HIV infected 34-36 weeks groups respectively. The uninfected and infected ≥ 37 weeks /birth groups showed lower percentages (18%, 33%). Based on HIV status, SF levels were similar across all groups. In the HIV uninfected 1st visit attendees ≤ 34 weeks, the RC percentage was higher (6%) than normal compared to the other groups (2%, p=0.44). The reticulocyte production index (RPI) was significantly higher and within the reference range for the HIV uninfected ≥ 37 weeks /birth (1 ± 0.34) compared to the other groups ($0.78\% \pm 0.41$ vs 0.62 ± 0.24 vs 0.88 ± 0.26 vs 0.82 ± 0.33 vs 0.81 ± 0.37 : p=0.0001). Maternal hemoglobin levels at delivery were significantly different between groups (p = 0.006).

After the initial enrolment visit, a loss to follow-up was noted at subsequent visits, particularly in the 34-36 weeks group (Table 3). Hematological profiles such as hemoglobin and SF as well as reticulocyte data did not differ across groups and were within the normal reference range (Table 3). However, the mean RPI for the infected 1st attendees ($0.77 \pm 0.30\%$) were below the normal reference range (1-2%).

The percentage of pill count, self-reported adherence and reasons for non-adherence across the study groups are outlined in table 4.

In the groups that were followed-up, a higher percentage returned empty containers (50%) compared to those returning 2 or more pills (20.8%) and to those that did not know the number of pills remaining in the container (29.1%). These women reported an unknown pill number remaining in the container and gave no reason for their non-adherence. However, in the HIV infected groups there was a higher percentage that were unaware of the pills remaining (41.7%) compared to the HIV uninfected groups (16%). The number returning empty containers were lower in the HIV infected (25%) compared to the uninfected (75%) groups. Moreover, the groups that were followed-up at ≥ 37 weeks/birth gestation, the percentage of women unaware of the number of pills remaining was higher (67.6 %) compared to those that returned 2 or more pills (10.5%) and to those that reported empty containers (27.8%). Women reporting unknown number of pills were higher in the HIV uninfected groups compared to the infected (70.2% vs 64.7%). In contrast, those that reported no pills remaining were higher in the HIV infected (31.7%) compared to the HIV uninfected group (24.1%). In the HIV uninfected groups, there was 5.4% of two or more pills remaining compared to 3.5% in the HIV infected groups.

Table 4 shows the reasons for non-adherence within all groups. In the women that were followed-up at scheduled visits, nausea was highest (40%), followed by skipped doses (32%), dark stools (16%), constipation (16%) and vomiting (4%). Nausea was highest in the 1st attendees group compared to 34 – 36 weeks groups

irrespective of HIV status. Similarly, the women followed-up at ≥ 37 weeks /birth gestation had higher percentage of nausea (39.2%) followed by dark stools (34%), skipped doses (15.9%), constipation (14.8%), vomiting (13.6%), heartburn and forgetfulness (2.8%) and pill overload (1.7%).

DISCUSSION

The present study assessed compliance and adherence of pregnant women to iron, folic acid and calcium supplementation at a regional hospital. Our methods of assessment of adherence have been utilized by other studies [13-15]. In our study, adherence of pill intake was 50% in the follow-up groups. Non-adherence was noted in 20.8% of women who had ≥ 2 pills remaining whilst 29.1% reported an unknown number of pills at follow-up visits. We report a higher percentage of women with an unknown number of pills remaining (41.7%) in the HIV infected compared to the uninfected women. Off note, most of the adherence data collected at ≥ 37 weeks/birth gestation was self-reported rather than by pill count. A reason for this factor is that some women consulted at 1st visit were only followed-up for pill taking at ≥ 37 weeks /birth gestation. Moreover, in the women that were consulted at recruitment and birth the percentage of the number of pills remaining in both the HIV uninfected and infected women was low (5.4%, 3.5%). However, the percentage of women reporting no pills remaining was higher in the HIV infected women (31.7%) compared to the uninfected women (25.1%).

There was a high percentage of unknown number of pills remaining in both the HIV uninfected and infected women (70.2%, 64.7% respectively). Self-reporting may overestimate compliance compared to pill count or biochemical methods [14]. In contrast, Ibrahim *et al.*, [13] observed that self-reporting (41.1%) was a better indicator of adherence compared to pill count (36.7%). However, Bondarianzadeh *et al.*, [15] demonstrated that women may falsely report pill taking, confirmed by positive stool tests.

Off note, we observed that women were occasionally issued inadequate iron tablet supplements until the follow-up visit. This may be attributed to poor antenatal attendance, incorrect follow-up dates and the absence of an effective logistic system to dispense the supply, or the shortage of drug supply from the institution. A study from another LMIC viz. Ethiopia also reported inadequate iron supplementation at antenatal clinic visits due to poor iron tablet supplies and the lack of an effective distribution system [14].

In the current study, the reasons for non-adherence to iron prophylactic therapy included nausea, vomiting, dark-colored stools, constipation, heartburn, skipped doses, forgetfulness and pill overload. In the HIV infected compared to the uninfected group, nausea (40% vs 39.2%) was the most common side effect followed by dark stools (16% vs 34%), constipation (16% vs 14.8%), skipped doses (32% vs 15.9%), vomiting (4% vs 13.8%). Similarly, other studies have corroborated our findings of the commonest adverse event being nausea [16-18]. In contrast to

our study, dark stools were not frequently noted in similar studies [16-18]. A high level of pregnancy induced nausea and/or vomiting may exacerbate a women's adherence during the first trimester [19]. In our study, the incidence of nausea was high across all trimesters. This may be a side effect attributed to the poor quality of iron supplements or due to the high level of iron intake on an empty stomach; the latter is corroborated by the low socio-economic status of the women in our study [20]. Notably, side effects are not associated with non-compliance [21].

In the current study, anemia was higher in the HIV infected groups compared to the uninfected groups, being higher at 34-36 weeks compared to the term/birth group. Similarly, Tunkyi and Moodley [10] observed a high incidence of anemia in HIV infected pregnant women.

The significant improvement of hemoglobin level at delivery from 1st visit attendees to ≥ 37 weeks/birth groups in the current study is corroborated by a study conducted in India [20]. Anemia prevalence in the latter study was reduced from 48% at 1st visit attendees, to 10% at 4th visit. Our findings also reveal that women enter pregnancy having inadequate bone marrow response. There was a significant decrease in the production of red blood cells within our study population. The RPI levels were significantly different ($p=0.0001$) across groups. In addition, in the HIV uninfected 1st visit group, the mean RC was higher compared to the other groups. These data indicates an immature release of red blood cells from the bone marrow and probably accounts for the premature labor (13%) and iron deficiency anemia. Despite a

similar reticulocyte levels across the four groups ($p >0.05$), RPI in the HIV infected 1st visit follow-up groups suggest an inadequate bone marrow response.

In the current study, age and socio economic income did not correlate with adherence to iron supplementation. Similarly, Roy et al.,[22] showed that age and socio-economic status did not correlate whilst Mithra et al., [3] suggest parity as a key contributor in iron supplementation compliance.

In addition, the current study demonstrates that iron supplementation is not associated with poor birth outcomes such as low birth weight, prematurity and small-for-gestational age infants. A study conducted in Tanzania [23] also demonstrates its non-correlation with low birth weight, prematurity and small-for-gestational age babies.

The limitation of the current study was the lack of follow-up visits. After the initial enrolment visit, many women did not return, particularly in the 34-36 weeks group hence adherence data was collected only at the time of delivery. A few women delivered in other health centers and some may have visited other health facilities or missed scheduled dates during antenatal period. Furthermore, incomplete laboratory results had an impact on adherence data, mainly during follow-up visits. Thirty three percent of women with incomplete results were eliminated at follow-up visit. Lastly, we relied mostly on self-reported data than pill count and a loss to follow-up contributed to this limitation.

The findings from the current study were similar to those from other LMIC countries; side effects were the most common reasons for non-adherence to iron prophylactic treatment. Furthermore, the RPI might be useful in measuring the bone marrow response and response to treatment.

Health education sessions are recommended to improve women's understanding of the importance of iron prophylactic therapy including visiting the clinic on scheduled dates.

Further research should include an electronic counting device for accurate pill count including the addressing of concurrent usage of iron prophylactic therapy with other treatments.

Author contributions

All authors contributed to aspects of the research proposal and submission of article.

P Z Mkhize: Initiation of the study, writing of protocol, conduction of the research including recruitment, informed consent, data collection, patient follow-up and data analysis

T Naicker: Assistance with data analysis, and writing of the paper

O Onyangunga: Assistance with writing and comment on research proposal

J. Moodley: Initiation of the study, research proposal and assistance with writing.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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Table 1: Clinical and demographic data of all study groups.

Parameters	1 st attendees ≤ 34 weeks HIV uninfected	1 st attendees ≤ 34 weeks HIV infected	34-36 wks. HIV uninfected	34-36 wks. HIV infected	≥ 37 weeks /birth HIV uninfected	≥ 37 weeks /birth HIV infected	P value
	N = 33	N = 33	N = 34	N = 34	N = 33	N = 33	
Residence:							
Urban (%)	100	100	88	91	79	82	0.01
Rural (%)	0	0	12	9	21	18	
Maternal Age (yrs.)	24 ± 5	28 ± 7	25 ± 5	31 ± 6	27 ± 7	30 ± 6	0.0001
Maternal height (cm)	158 ± 6	159 ± 7	159 ± 7	157 ± 5	160 ± 7	158 ± 7	0.54
Maternal wt. (kg)	69 ± 16	66 ± 12	76 ± 13	76 ± 17	80 ± 16	84 ± 18	0.0001
BMI (kg/m ²)	28 ± 6	27 ± 5	30 ± 5	30 ± 6	32 ± 6	34 ± 6	0.0001
Systolic pressure (mmHg)	120 ± 13	115 ± 14	112 ± 10	111 ± 12	112 ± 9	111 ± 11	0.01
Diastolic pressure (mmHg)	70 ± 10	70 ± 12	70 ± 9	70 ± 11	71 ± 8	70 ± 7	0.1
Parity	0 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	0.0001
Gravidity	2 ± .755	2 ± 2	3 ± 2	3 ± 0.9	3 ± 2	3 ± 1	0.0001
GA (wks.) current visit	19 ± 7	20 ± 7	35 ± 0.72	35 ± 2	39 ± 2	38 ± 1	0.0001
GA at birth	38 ± 2	39 ± 2	39 ± 2	39 ± 2	40 ± 2	39 ± 2	0.019
Baby wt. (kg)	3.06 ± 0.56	3.21 ± 0.45	2.98 ± 0.52	2.86 ± 0.62	3.41 ± 0.43	3.15 ± 0.40	0.004
NVD (%)	54.5	33.3	50	32.3	27.2	27.2	
Scheduled C/S (%)	18.1	18.1	14.7	14.7	15.1	9	
Emergency C/S (%)	0	3.0	9	23.6	42.4	48.4	0.67
Male/Female ratio (%)	45:27	24:30	28:44	29:44	52:33	48:39	0.23
Stillbirth: FSB-MSB-NND (%)	0-3-3	0-0-0	3-0- 0	0-3- 0	0-0-0	0-0-0	0.14
Complications:							
Severe PE (%)	4	0	4	0	0	0	0.97
CPD (%)	8	0	4	0	4	0	
FD (%)	0	20	8	13	4	4	
Preterm labor (%)	13	0	0	0	0	0	
Contraceptives:							
Injectable (%)	66	79	68	76	79	64	0.53
Oral (%)	16	0	18	3	9	3	
Tubal ligation (%)	9	12	9	15	12	30	
Feeding:							
Breast (%)	79	79	76	59	91	52	0.003
Formula (%)	21	21	24	41	9	48	

Mean ±SD; NVD – Normal vaginal delivery, C/S – Caesarian section, FSB – Fresh still birth, MSB – Macerated still birth, NND – Neonatal death, CPD – Cephalopelvic disproportion, FD – Foetal distress, PE - Preeclampsia, GA – Gestational age, Kg – Kilograms, Wks. – Weeks CM – Centimeters, YRS - years .

Table 2: Hematological results of all study groups

Parameters	1 st attendees ≤ 34 weeks HIV uninfected	1 st attendees ≤ 34 weeks HIV infected	34-36 wks. HIV uninfected	34-36 wks. HIV infected	≥ 37 weeks /birth HIV uninfected	≥ 37 weeks /birth infected	Reference range	P value
	(n = 33)	(n = 33)	(n = 34)	(n = 34)	(n = 33)	(n = 33)		
Hemoglobin at recruitment (g/dL) %	11 ± 2 (51.5)	11 ± 2 (57.5)	11 ± 2 (52.9)	11 ± 2 (55.8)	12 ± 2 (18.1)	12 ± 2 (33.3)	11 - 12	0.0001
Serum ferritin (µg/L)	34 ± 35	28 ± 29	30 ± 42	23 ± 15	33 ± 20	30 ± 22	10 - 291	0.68
Reticulocyte C (%)	6 ± 23	2 ± 0.46	2 ± 0.48	2 ± 0.50	2 ± 0.43	2 ± 0.59	0.5 - 2	0.44
Reticulocyte C A (X 10 ¹² /L)	0.06 ± 0.03	0.06 ± 0.04	0.07 ± 0.02	0.08 ± 0.06	0.09 ± 0.1	0.07 ± 0.03	0.05 - 0.1	0.09
Corrected R C (%)	2 ± 0.45	0.10 ± 0.34	2 ± 0.33	2 ± 0.44	2 ± 0.34	2 ± 0.50		0.0001
Reticulocyte P I	0.78 ± 0.41	0.62 ± 0.24	0.88 ± 0.26	0.82 ± 0.33	1 ± 0.34	0.81 ± 0.37	1 - 2	0.0001
Hemoglobin at birth (g/dL)	11 ± 0.59	11 ± 2	11 ± 2	11 ± 2	13 ± 0.91	12 ± 0.89	11 - 12	0.006

Mean ± SD

RC – Reticulocyte count, RCA – Reticulocyte count absolute, CRC – Corrected reticulocyte, RPI – Reticulocyte production index.

Table: 3: Clinical data collected during pregnancy follow-up

Parameters	1 st attendees ≤ 34 weeks HIV uninfected	1 st attendees ≤ 34 weeks HIV infected	34-36 wks. HIV uninfected	34-36 wks. HIV infected	Reference range	P value
	N = 10	N = 9	N = 2	N = 3		
Maternal Age (yrs.)	24 ± 5	27 ± 6	21 ± 0.71	31 ± 3	-	0.06
GA (wks.)	26 ± 8	30 ± 11	38 ± 3	37 ± 2	-	0.08
Maternal wt. (kg)	65 ± 13	77 ± 13	81 ± 37	79 ± 9	-	0.16
Maternal ht. (cm)	158 ± 7	160 ± 6	159 ± 10	153 ± 2	-	0.31
BMI (kg/m ²)	26 ± 4	30 ± 5	33 ± 13	33 ± 3	-	0.04
Hemoglobin (g/dL)	11 ± 0.72	11 ± 2	11 ± 0.15	11 ± 0.94	11 - 12	0.96
Reticulocyte C (%)	2 ± 0.59	2 ± 0.45	2 ±	2 ± 0.35	0.5 – 2	0.31
Reticulocyte CA (X 10 ¹² /L)	0.08 ± 0.03	0.14 ± 0.3	0.19 ±	0.06 ± 0.02	0.05 - 0.1	0.66
Corrected R C (%)	2 ± 0.47	2 ± 0.4	2 ±	2 ± 0.31	-	0.34
Reticulocyte PI	2 ± 0.32	0.77 ± 0.30	1 ±	2 ± 0.31	1 - 2	0.20
Serum ferritin (µg/L)	50 ± 52	23 ± 14	18 ±	27 ± 29	10 - 291	0.53

Mean ±SD

BMI – Body mass index

Table 4: Pill count and self - reported adherence.

	HIV uninfected N = 12	HIV infected N = 12	Total %	Total No. of women: 24
Follow up at scheduled dates: 1st attendees ≤ 34 weeks / 34 – 36 weeks gestation				
Parameters:				
Number of women returned 2 or more pills	1 (8.3%)	4 (33.3%)	5 (20.8%)	Adherence (50%)
Number of women returned zero or no pills	9 (75%)	3 (25%)	12 (50%)	
Number of women with unknown number of pills remaining	2 (16.7%)	5 (41.7%)	7 (29.1%)	Non-adherence (46%)
Adherence at term birth and at recruitment: 1st attendees ≤ 34 weeks , 34 -36 weeks, ≥ 37 weeks /birth gestation				
	HIV uninfected N = 91	HIV infected N = 85	Total % of all groups	Total no. of women: 176 (88%)
Parameters				
Number of women returned 2 or more pills	5 (5.4%)	3 (3.5%)	8 (10.5%)	Adherence (27.8%)
Number of women returned zero pills	22 (24.1%)	27 (31.7%)	49 (27.8%)	
Number of women with unknown number of pills remaining	64 (70.2%)	55 (64.7%)	119 (67.6%)	Non-adherence (72.1)
Reasons for non – adherence: follow – up groups: 1st attendees ≤ 34 weeks / 34 – 36 weeks gestation				
	HIV uninfected N = 12	HIV infected N = 12	Total %	Total no. of women: 24
Factors	1 st visits	34-36 wks.	1 st visits	34–36 wks.
Side effects (%)				
Nausea	4 (33.3)	1 (8.3%)	5 (38.4%)	0
Vomiting	0	1 (50)	0	0
Dark stools	2 (16.7%)	0	1 (7.7%)	1 (7.7%)
Constipation	1(8.3%)	0	1 (7.7%)	2 (15.3%)
Skipped doses (%)	30 (3)	50 (1)	30 (3)	33 (1)
				8 (32%)
Reasons for non – adherence at birth and at recruitment: 1st attendees ≤ 34 weeks / 34 – 36 weeks, ≥ 37 weeks /birth gestation				
Factors	HIV uninfected N = 91	HIV infected N = 85	Total % of all groups N= 176; 88%	
Side effects (%):				
Nausea (%)	35 (38.4%)	34 (40%)	69 (39.2%)	
Vomiting (%)	16 (17.9%)	8 (9.4%)	24 (13.6%)	
Dark stools (%)	31 (34%)	29 (34.1%)	60 (34%)	
Constipation (%)	12 (13.2%)	14 (16.5%)	26 (14.8%)	
Heartburn (%)	1 (1%)	4 (4.7%)	5 (2.8%)	
Other (%)	-	7 (8.2%)	7 (3.9%)	
Skipped doses (%)	18 (19.8)	10 (11.8%)	28 (15.9%)	
Forgetfulness (%)	2 (2.2%)	3 (3.5%)	5 (2.8%)	
Pill overload (%)	-	3 (3.5%)	3 (1.7%)	

CHAPTER 3

SYNTHESIS AND CONCLUSION

Anaemia is a major public health problem globally, and pregnant women are particularly vulnerable. Iron deficiency is the most common cause of anaemia and daily intake of iron supplements during pregnancy considerably reduces the incidence of maternal anaemia and the risk of low birth weight babies. In low-middle income countries (LMIC), the incidence of anaemia is 56%, while it is 23% in high-income countries (WHO, 2015). In the context of LMIC, nutritional iron deficiency is the leading cause of anaemia in pregnancy (Hoque *et al.*, 2009). In Africa, the prevalence of anaemia is estimated at 52% (Jafar and Zwiebe, 2014). The Saving Mothers Report (2010 - 2013) states that 40% of maternal deaths in South Africa (SA) were associated with anaemia, while Tunkyi and Moodley, 2016 report similar figures in a regional hospital in Durban, South Africa. .

According to WHO (2011) anaemia is defined as a condition in which the number of red blood cells and their oxygen-carrying capacity is inadequate to meet the body's physiologic needs. It is present when the haemoglobin (Hb) concentration in the peripheral blood is ≤ 11 gm/dl (Sabina *et al.*, 2016). Our study demonstrates a decrease in haemoglobin levels of above 50% in women that were < 37 weeks gestation whereas pregnant women ≥ 37 weeks gestation had less than a 35% decrease. Similar to our study, a Cochrane review by Rukuni *et al.*, 2015 found that there is good evidence of improvement in haematological indices with iron supplementation in pregnancy.

Moreover, we report a significant decrease in the production of red blood cells in the pregnant women studied. The reticulocyte production index (RPI) shows a significance difference of $p=0.0001$ across all the groups. Women enter pregnancy having inadequate bone marrow response. A low reticulocyte count with low RBCs, low hemoglobin, and low hematocrit may be associated with iron deficiency anemia (American Association for Clinical Chemistry, 2001-2017 <https://labtestsonline.org/understanding/> accessed 12th June 2017).

In addition, in the HIV uninfected 1stvisit \leq 34 weeks group the mean reticulocyte count was higher compared to the other groups (6 ± 23 units). The red blood cells are prematurely released from the bone marrow into the blood stream, due to a high physiologic demand for iron. Only a limited number of women in our study population were followed-up at scheduled visits hence our results display no statistical differences on haematology and reticulocyte levels between the four groups ($p >0.05$). However, the mean reticulocyte production index of the HIV infected 1st visit \leq 34 weeks follow-up groups indicated an inadequate bone marrow response. The reticulocyte production index in the other follow-up groups was within the normal reference range and indicate a response to iron prophylactic therapy. Additionally, we noted non-significant difference in serum ferritin levels across our study population probably suggesting the presence of underlying anemia of chronic diseases or inflammation. Sahoo *et al.*, 2016 indicated that 50% patients had increased serum ferritin, 40% normal and 10% decrease. Therefore, it is recommended that serum ferritin should not be used in a single measurement as a marker for iron stores.

The need for iron and folic acid increases during pregnancy because of the demands of a growing fetus. In 2012, the World Health Assembly Resolution recommended a broad implementation strategy for maternal, infant and young child nutrition, which stated six global nutrition targets that covers a 50% reduction of anaemia in women of reproductive age by 2025 (WHA, 2014). The current World Health Organization (WHO) guidelines endorse provision of a daily prophylactic oral dose of iron (30-60 mg) and folic acid (400 mg) to all pregnant women, initiated at an early stage of pregnancy. The use of iron and folic acid supplements during pregnancy are reported to have an impact in reducing maternal anaemia (Nisar and Dibley, 2016).

Numerous studies have shown that despite the use of a supplementation program, iron deficiency anaemia is still common during pregnancy (Cogswell *et al.*, 2014; Ogundipe *et al.*, 2012; Bilimale *et al.*, 2010; Ibrahim *et al.*, 2011).

However, other studies disagree with this statement (Abdullahi *et al.*, 2014; Nisar *et al.*, 2016). A major reason for the failure of national iron supplementation programs is said to be a woman's non-adherence to pill taking. In our study, adherence was assessed via self-reports and manual pill count by the women in the study. Similarly, Ibrahim *et al.*, 2011; Lutsey *et al.*, 2007; Gebremedhin *et al.*, 2014 adopted methods for adherence via self-reporting and pill counting. We also found that women were intermittently issued supplements that were inadequate to cover a one –month period or until their next clinic date. This could be due to their poor attendance to clinic appointments, the absence of effective logistic systems to dispense the supply or the lack of drug supplies. Furthermore the poor quality of iron tablets from some pharmaceutical suppliers, may lead to nausea and vomiting and therefore poor adherence to pill taking. Gebremedhin *et al.*, 2014 observed that nearly half of antenatal care attendees were not given iron supplements due to poor iron supply and lack of effective distribution system. We found that adherence of pill intake was 50% in the follow-up groups. Non-adherence was noted in 20.8% of women who had ≥ 2 pills remaining whilst 29.1% reported an unknown number of pills at follow-up visits.

We also report a higher percentage of women with an unknown number of pills remaining (41.7%) in the HIV infected compared to the uninfected women. However, the percentage of women reporting no pills remaining was higher in the HIV infected group (31.7%) compared to the uninfected group (25.1%). Moreover, there was a higher percentage of unknown pills remaining in both uninfected and infected women (70.2%, 64.7% respectively). Most of the adherence data collected at ≥ 37 weeks/birth gestation was self-reported rather than by pill count. A reason for this feature is that some women recruited at their 1st visit: ≤ 34 weeks were only followed-up for pill taking at ≥ 37 weeks /birth gestation. Several studies found that self-reporting overestimates compliance as compared with pill counting or biochemical methods (Gebremedhin *et al.*, 2014).

These studies report a 75% level of compliance based on the self-reporting method. A study by Ibrahim *et al.*, (2011) reflected 58.9% non-adherence and 41.1% adherence (self-reporting) whereas 63.3% of non-adherence and 36.7% adherence reflected by pill count. In addition, Lutsey *et al.*, (2007) also confirms 85% consumption of pills that were self-reported whereas pill count suggested 70%.

The present study also assessed reasons for non-adherence to iron prophylactic supplementation in antenatal care attendees. Nausea (40% vs 39.2%) was found to be a common problem across all trimesters, followed by dark-coloured stools (16% vs 34%), constipation (16% vs 14.8%), skipped doses (32% vs 15.9%) and vomiting (4% vs 13.8%) in the HIV infected and uninfected women respectively; heartburn, forgetfulness and pill overload had lower percentages . . Similarly, Bresani *et al.*, 2013, report that the three commonest adverse events were nausea (25.8%) and constipation (17.2%). In addition, Kalimbiri *et al.*, (2009), report that nausea (43.6%) was the commonest problem in their study. In a study by Panda *et al.*, 2015, nausea (24%) and vomiting (26%) were the most common reasons observed followed by heartburn (10%), diarrhea (8%), constipation (6%), abdominal pain (4%) and forgetfulness (24%). In contrast, Mithra *et al.*, 2014 found forgetfulness (48.8%) followed by vomiting (47.69%), constipation (21.54%) and gastritis (13.84%) as common. In contrast, Ibrahim *et al.*, (2011) report frustration from the large pill intake (54.35%) as the most common reason for non-adherence.

The current study revealed that iron supplementation is not associated with poor birth outcome such as low birth weight, prematurity and small for gestational age infants. The mean birth weight at 1st visits \leq 34 weeks and \geq 37 weeks/birth groups matched the gestational age regardless of HIV status, whilst the 34-36 weeks groups were slightly lower for gestational age (2.98 ± 0.52 vs 2.86 ± 0.62 Kg). Other authors have also found no association of iron supplementation to low birth weight, prematurity and small-for-gestational-age neonates (Etheredge *et al.*, 2015).

In addition, demographic characteristics such as age and socio economic income do not correlate with adherence to iron prophylactic supplementation. Similar to our study, age and socio-economic status are not associated with iron prophylactic supplementation (Roy *et al.*, 2013). However, Mithra *et al.*, (2014), report that age, socio-economic status and parity are key contributors towards iron supplementation compliance.

Summary and Recommendations

The findings from the current study were similar to those from other LMIC countries; side effects were the most common reasons for non-adherence to iron prophylactic treatment. Furthermore, the RPI might be useful in measuring the bone marrow response and response to treatment.

Health education sessions are recommended to improve women's understanding of the importance of iron prophylactic therapy including visiting the clinic on scheduled dates.

Further research should include an electronic counting device for accurate pill count including the addressing of concurrent usage of iron prophylactic therapy with other treatments.

CHAPTER 4

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APPENDIX



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22 March 2016

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Protocol: Iron Prophylactic therapy during pregnancy in an urban regional hospital in Durban, South Africa.

Degree: MMedSc
BREC reference number: BE485/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 24 November 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 15 March 2016 to queries raised on 29 January 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from 22 March 2016. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its meeting taking place on 12 April 2016.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

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Biomedical Research Ethics Committee

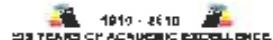
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11 May 2017

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Protocol: Iron Prophylactic therapy during pregnancy in an urban regional hospital in Durban, South Africa.

Degree: MMedSc

BREC reference number: BE485/15

NEW TITLE: Adherence to Iron Prophylactic therapy during pregnancy in an urban regional hospital In Durban, South Africa.

We wish to advise you that your letter received on 24 April 2017 submitting an application for amendments to change the title to the above has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee.

This approval will be ratified at the next BREC meeting to be held on 13 June 2017.

Yours sincerely

A handwritten signature in blue ink, appearing to read "Anusha Marimuthu".

Anusha Marimuthu
Senior Admin Officer: Biomedical Research Ethics Committee

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cc postgrad: mglenchock@ukzn.ac.za