

**HAE MATO-BIOCHEMICAL INDICES AND THEIR
CORRELATION WITH OBSTETRICS PROFILE AND
COMPLICATIONS AMONG PREGNANT WOMEN IN
THE NORTHERN REGION OF GHANA**

KNUST

A THESIS SUBMITTED IN
FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF

MASTER OF PHILOSOPHY

In the

Department of Molecular Medicine,
School of Medical Sciences

By

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OCTOBER, 2016

DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine-KNUST, Tamale Teaching Hospital (TTH) and Noguchi Memorial and Medical Research Institute, Legon-Accra. This work has not been submitted for any other degree and the findings have not been altered in one way or the other.

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ABSTRACT

Pregnancy is a physiological condition in which the body undergoes extensive systemic alterations and modifications to compensate for the demands of the growing fetus. However, obstetrics practice in Africa of which Ghana is not an exception; is marred by a lot of challenges and complications. Anaemia and spontaneous abortion are among the leading frontiers of pregnancy related complications. In a cross-sectional study to assess the Haemato-Biochemical indices and its correlate with obstetrics profile and complications among pregnant women in Ghana, a total of 248 pregnant women attending antenatal clinic at the Tamale Teaching Hospital (T.T.H) were assessed for this study. Complete haemogram, immunological analysis, biochemical analysis, estimation of gestation age using symphysiofundal height measurement, last normal menstrual period (LNMP), results from the first trimester ultrasound scan, parasitology analysis, serological analysis and obstetrics history such as level of parity, gravidae, history of abortion (spontaneous or induced) were retrieved from their antenatal booklet. Prevalence of spontaneous abortion among pregnant women in Ghana as shown in this study was 65.72%. High serum folic acid levels were found to be associated with spontaneous abortion. G6PD partial defect (OR= 0.447; 95%CI=0.208-0.961; $p=0.039$), Multi-parity (OR= 2.724; 95%CI=1.277-5.809; $p=0.01$) and irregular antenatal attendance (OR= 2.036; 95%CI=1.093-3.793; $p=0.025$) were risk factors for the occurrence of spontaneous abortion. The prevalence of anaemia among the pregnant women were 33.87% using ($Hb < 11g/dl$) and 43.55% using ($hematocrit < 33\%$) as criteria. Iron deficiency (ID) and iron deficiency anaemia (IDA) were the most prevalent type of

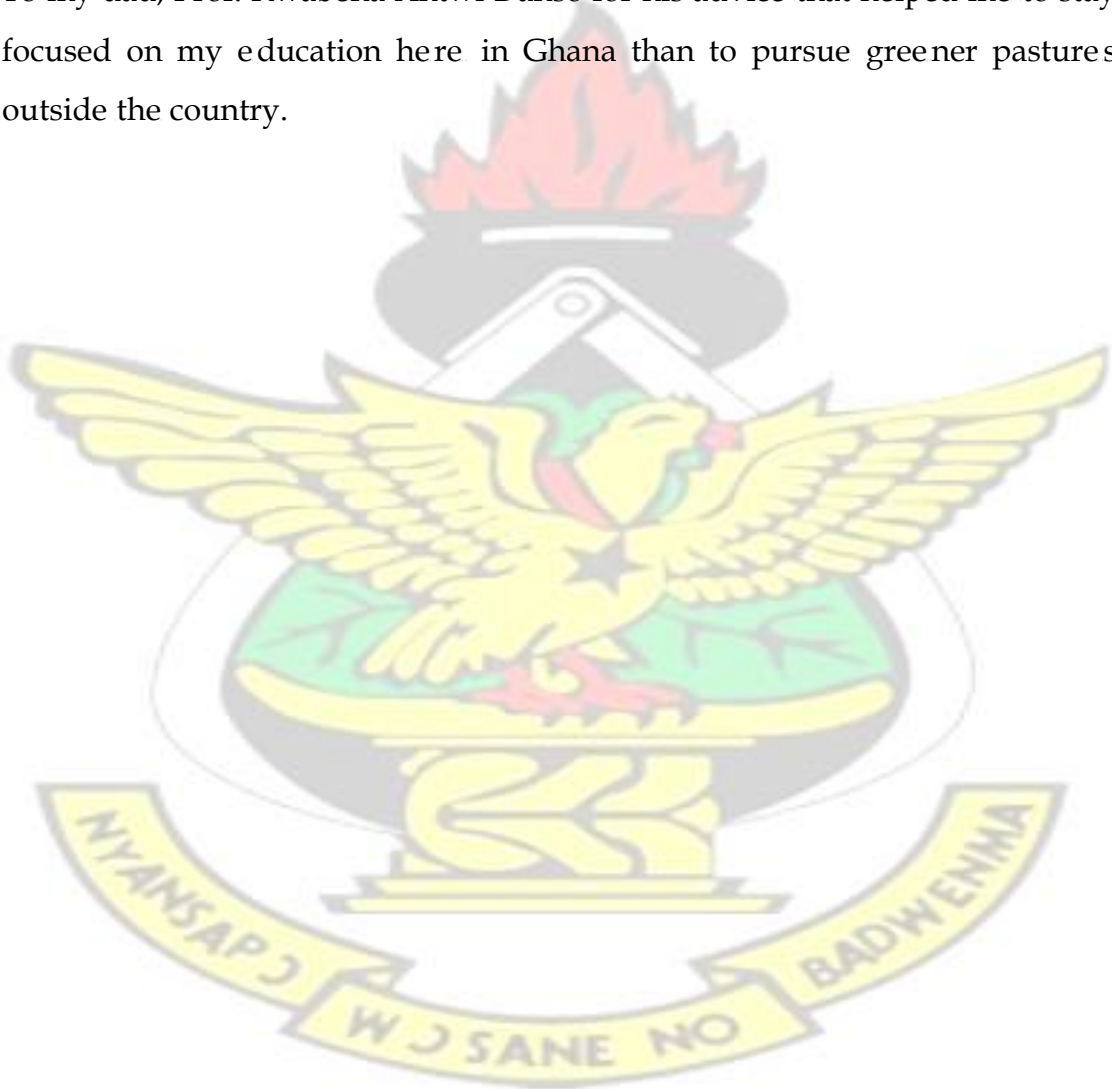
anaemia. The prevalence of iron deficiency (ID) and iron deficiency anaemia (IDA) in this current study were 16.94% and 4.44% respectively using the conventional markers of assessing iron status. With the introduction of the modern indicators of ID and IDA, prevalence of iron deficiency and iron deficiency anaemia were 50% and 16.13% respectively using transferrin:log ferritin as a marker. When transferrin receptor was used as an index for the assessment of iron status, 77.42% and 27.8% prevalence was obtained for ID and IDA respectively. Low ferritin concentration, advanced gestational age (2nd and 3rd trimesters), non-intake of haematinics and poor appetite for food (anorexia) were the predisposing factors for the development of anaemia in pregnancy. There is the need for the introduction of the modern markers of assessing iron status which are not influenced by inflammation or diurnal variabilities to augment the conventional markers in our clinical practice since ID and IDA are being underdiagnosed. Policy on folic acid and hematinic supplementation should be urgently and critically reviewed in obstetrics practice.



DEDICATION

This work is dedicated to two wonderful ladies I met in the course of my study; Abigail Abenaa Nyamful, the young intelligent lady who supported me in all aspects throughout this project, and Emelia Nani Puorideme for her financial assistance to make this dream a reality. Not forgetting my lovely brother, Joseph Owusu Boateng who has provided me the platform to explore academically.

To my dad, Prof. Kwabena Antwi Danso for his advice that helped me to stay focused on my education here in Ghana than to pursue greener pastures outside the country.



ACKNOWLEDGEMENT

“Do not be afraid, I will save you. I have called you by name, you are mine. When you pass through deep waters, I will be with you– Isaiah 43 v 1b-2”.

I wish to acknowledge with gratitude the Almighty God, for His grace and mercy, wisdom and strength He has given me to undertake this project when troubles almost overwhelmed me. His grace saw me through all the great difficulties, frustrations and the turbulence. I also wish to express my profound gratitude to my supervisor Dr. W.K.B.A. Owiredu of the Department of Molecular Medicine, KNUST, for the patient and excellent supervision, the fatherly role and above all the inspiration that made this project a reality.

To Dr. Nafiu Amidu, of the Department of Medical Laboratory Sciences, UDS, you have been the pillar that holds me and the strength that keeps me moving, the voice that inspires me that it is possible. I am deeply grateful.

I wish to express my heartfelt appreciation to Mr. Peter Paul M. Dapare for his enormous contributions towards this work. At moments when I almost gave up, when going forward was almost impossible your strength held me up. I owe it all to you bro; you are a brother from a different mum

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LIST OF ABBREVIATIONS

ACD- Anaemia of chronic disease AIDS-

Acquired Immune Deficiency Syndrome aPTT

- Activated partial thromboplastin time

CBS - Cystathionine beta synthase

DNA-Deoxyribonucleic acid

FDP- Fibrin degradation products

G6PD-Glucose-6-Phosphate Dehydrogenase

GFR- Glomerular filtration rate

GSH- Glutathione

GSTM1 - Glutathione S-transferase class

1 HbSC- Haemoglobin SC hCG -

Human chorionic gonadotropin HCT-

Hematocrit

HIV-Human Immunodeficiency Virus

hPL - human placental lactogen

IDA-Iron deficiency anaemia

ID-Iron deficiency

LBW- Low birth weight

LDL-c - Low-density lipoprotein cholesterol

LNMP - last normal menstruation period

MCV-Mean Cell Volume

MTHFR- methylenetetrahydrofolate reductase

NADPH-Nicotinamide adenine dinucleotide phosphate

NSAIDs- Non-steroidal anti-inflammatory drugs

NTDs- Neural tube defects

PGE2-Prostaglandin E2

PT-Prothrombin time

RBC-Red blood cells ROS-

Reactive Oxygen Species

sTfR - Soluble

transferrin receptor THFA-

tetrahydrofolic acid

TIBC-Total iron binding deficiency

TSH-Thyroid stimulating hormone

TT3- tri-iodothyronin

TT4-Total tetra-iodothyronin

TT-Thrombin time

WHO- World Health Organization

Chapter 1**INTRODUCTION****1.1 GENERAL INTRODUCTION**

Obstetrics practice in developing countries has a higher risk of complications such as maternal morbidity, mortality and perinatal deaths. Factors responsible for these misfortunes include poor health care delivery system, cultural norms, poor nutrition, low level of education, gender discrimination, teenage pregnancies, high parity, anaemia and other complications (WHO, 2009).

Pregnancy is a physiological condition characterized by systemic adjustments and adaptations to cater for the increasing demands of the growing foetus (Flick and Kahn, 2013).

After fertilization and implantation of the embryo in the stratum functionalis of the uterus (decidua), the corpus luteum, placenta, and developing embryo (mainly syncytiotrophoblast) release hormones, growth factors, and other substances into the maternal bloodstream. These substances trigger a series of events that causes a modification in function of the maternal cardiovascular, respiratory, renal systems, nervous, gastrointestinal, musculoskeletal and other systems. These maternal adaptations aim to establish four important physiological functions that support foetal growth. First, there is increased availability of substrates and precursors for foetal-placental metabolism. Hormone production is enhanced by increases in dietary intake above the usual intake. Also, an alteration in the endocrine system increases the availability of glucose and low-density lipoprotein (LDL) cholesterol concentration in the maternal circulation. Lastly, there is an increase in cardiac output which facilitates the transport capacity of substrates and precursors to the placenta, and foetal waste products to maternal organs for disposal in the maternal-foetal circulation system through the umbilical arteries and veins.

Maternal-foetal exchange is regulated by the placenta after 10-12 weeks gestation, but it may occur through histiotrophic mechanisms before this time. Disposal of additional waste products (heat, carbon dioxide, and metabolic by-products) occurs through peripheral vasodilation and increases in skin blood flow, ventilation, and renal filtration. These four critical maternal physiological changes must meet the combined demands of maternal exercise and fetal growth and development (Weissgerber and Wolfe, 2006).

Maternal blood volume increases with increasing plasma volume, as well as increase in red cell and white cell counts (Lund and Donovan, 1967). The plasma volume increases by 40% to 50%, whilst red cell volume increases by 15% to 20%, this disproportionate increment results in a condition often described as “physiological anemia of pregnancy” (Ueland, 1976).

Anaemia in pregnancy (haemoglobin level $<11\text{g/dl}$ and hematocrit (HCT) lower cut-off of 33%) as defined by World Health Organization (WHO, 1992) is a major public health concern, especially in developing countries; for which pregnant women and children are the most vulnerable (de Benoist *et al.*, 2008).

Anaemia affects 41.8% of pregnant women globally, with the highest prevalence in Africa (WHO, 2006). Overall, fifty seven percent (57%) of pregnant women in Africa have anaemia, which corresponds to approximately 17 million affected women, with severe consequences on health, social, and economic development (Allen, 2000; de Benoist *et al.*, 2008; Levin, 1986). Studies in Africa by various researchers have shown a high prevalence of anaemia in pregnancy ranging from 41-83% in different settings (Antelman *et al.*, 2000; Haggaz *et al.*, 2010; Kidanto *et al.*, 2009; Meda *et al.*, 1999; TDHS, 2005; Uneke *et al.*, 2007).

Anaemia in pregnancy has negative consequence for both the mother and neonate. It may result in foetal anaemia, low birth weight (LBW), preterm

delivery, intra uterine growth restriction and perinatal mortality due to the impairment of oxygen delivery to the placenta and the foetus (Allen, 2000; Haggaz *et al.*, 2010; Kidanto *et al.*, 2009; Scholl and Hediger, 1994). Anaemia causes low physical activity and increased risk of maternal morbidity and mortality, high rate cardiac failure especially in severe anaemia (Allen, 2000; de Benoist *et al.*, 2008; Scholl and Hediger, 1994).

The causes or aetiology of anaemia in pregnancy is multi-faceted. It includes genetic causes such as G6PD deficiency, sickle cell disease, thalassemia etc., and acquired which include nutritional deficiencies (iron deficiency, folate, vitamin B12 etc.), infectious agents (malaria, hookworm, HIV etc.), hemolytic, hemorrhagic, bone marrow suppression/aplasia (van den Broek, 1996b).

Of the nutritional causes of anaemia, iron deficiency is the most prevalent and life threatening because the physiological changes associated with pregnancy exert a demand for extra iron needed for transfer to the foetus (Letsky, 1995b).

Iron is an essential mineral found in hemoglobin, an important molecule required for transport of oxygen and metabolism. Almost two-thirds of the iron in the human body is found in hemoglobin which is responsible for transporting oxygen from the lungs to our body's tissues) (Ursel, 2001).

During pregnancy, through puerperium and during the peri-natal periods, a total amount of about 700-850mg of iron is needed to meet the iron requirements of a mother and foetus. The gravid mother requires about 300-500mg additional iron to maintain this iron balance during pregnancy and this additional iron requirement has to be obtained from the mother's iron stores or from iron supplements. 25-30% of the populace in developing countries has no iron reserves, and this situation has more adverse effects on pregnant teenagers (Harrison, 1982).

Malaria, hookworm and other helminthes infections are implicated in the pathogenesis of anaemia in pregnancy. Pregnant women are highly susceptible to malaria (Brabin, 1983). The malaria parasites are able to sequester into the placenta to avoid detection and clearance by the reticulo-endothelial system more especially the spleen. This phenomenon explains the susceptibility of pregnant women to malaria parasites. The parasites cause damage to the placental tissues thus triggering inflammatory cascades, causing a thickening in the walls of the placenta. Subsequently, there is a decreased flow of nutrients from the mother to the foetus resulting in the low birth weight infants (Fried and Duffy, 1996).

Hookworm infections on the other hand, cause damage to the terminal ileum where Vit B12 and other micronutrients are absorbed, thereby impairing their absorption thus increasing the susceptibility of pregnant women to anaemia (Bondevik *et al.*, 2000; Stephenson *et al.*, 2000).

Infection with HIV has emerged as an additional important risk factor for anaemia in pregnancy (WHO, 1992). Sickle cell disease is prevalent among 1-2% of infants born in sub-Saharan Africa, hemoglobin SC (HbSC), β -thalassaemia are also prevalent which are all commonly associated with anaemia. With improved health care delivery, a larger segment of these patients are able to reach reproductive age and conceive (Rust and Perry, 1995).

The most morphological form of anaemia seen in these patients is normocytic normochromic with haemoglobin values ranging from 6 to 9 g/dL (Letsky, 1995b).

G6PD deficiency, the commonest red blood cell enzymopathy, is also one of the forces to reckon with when it comes to haemolytic anaemia. A shortened red cell lifespan and severe haemolytic anaemia is seen in association with certain drugs such as sulphur containing drugs (sulfadoxine pyrimethamine

S.P) given as prophylaxis for malaria during pregnancy. Haemolysis as a result of co-infection may play a major role in the clinically significant haemolysis seen in patients with G6PD deficiency (Beutler, 1994).

The impact of these aetiological factors in the pathogenesis and pathophysiology of anaemia will vary by geographical area and by season (WHO, 1989).

Spontaneous abortion is the termination of pregnancy (expulsion of foetus) before 20 completed gestational weeks from the last menstrual period, or less than 500 g fetal weight (WHO, 1977).

The rate of spontaneous abortion among North American women is about 15%– 20% and prevalence varies in different age categories, 15% in women younger than 25 years and increases to 35% in women older than 38 years (Tallia *et al.*, 2001).

In a study by Jibril *et al* in Nigeria on women admitted to the gynaecological ward, the prevalence of spontaneous abortion among the pregnant women was 53.2% (Jibril *et al.*, 2014).

Meseret *et al.* (2015) in Northwest Ethiopia found 10.9% prevalence rate of spontaneous abortion among rural women in Gonji district.

Wilcox *et al* conducted a prospective study of 221 women who were trying to conceive in Northern America. The rate of spontaneous abortion was 12% among clinically diagnosed pregnancies (Wilcox *et al.*, 1988).

There are two types of spontaneous abortion: sporadic and recurrent. Recurrent miscarriage affects about 1% of couples (Stirrat, 1990) and it is highly frustrating for both patients and physicians. Classically, recurrent pregnancy loss is defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation.

Chromosomal abnormalities are implicated in about 50% of all spontaneous abortions, the remaining 50% of the causes remains to be established (Cramer and Wise, 2000). Oxidative stress due to Glucose-6-Phosphate Dehydrogenase deficiency and loss of other antioxidant defenses have been implicated and shown to be associated with spontaneous abortion (Simsek *et al.*, 1998)

Oxygen is useful for sustaining life in cells, it undergoes extensive metabolism that can result in the production of toxic derivatives in the electron transport chain in the mitochondria during the generation of ATP. The end products of oxygen metabolism may include molecules in an activated electronic state with unpaired electrons that are highly reactive with molecules found in biological systems. These activated derivatives are referred to as reactive oxygen species (ROS) (Halliwell and Gutteridge, 1988). ROS extensively damage cellular organelles including the mitochondria, nuclear and mitochondrial DNA, and cell membrane, leading to failure to repair and subsequently, cell death (Kowaltowski and Vercesi, 1999; Pierce *et al.*, 1991; Ronnenberg *et al.*, 2002).

To prevent ROS-induced damage, there are cellular antioxidant systems which maintain a balance between ROS production and antioxidant activity in cellular homeostasis. When this balance is shifted by excess ROS production, oxidative stress occurs leading to cell damage and cell dysfunction (Agarwal and Allamaneni, 2004; Agarwal *et al.*, 2005).

The etiology of spontaneous abortion includes chromosomal abnormalities, uterine anatomic anomalies, immunologic disorders such as antiphospholipid antibody syndrome, clotting disorders such as hyperhomocystinemia, and sperm DNA fragmentation (Cramer and Wise, 2000). Jauniaux *et al.* suggested that early pregnancy loss may result from premature oxygenation of the early embryonic environment (Jauniaux *et al.*, 2000)

There is evidence implicating oxidative stress in the aetiology of spontaneous abortion as biochemical markers of ROS-induced membrane damage such as

lipid peroxidation products are found and reaching high levels immediately before pregnancy loss (Sane *et al.*, 1991)

Antioxidants when present at even lower concentrations than an oxidizing agent, is able to significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1995). These agents are present in cells in enzymatic and nonenzymatic forms. Common enzymatic defenses include superoxide dismutase (SOD), catalase, and glutathione peroxidase and glutathione reductase (G6PDH). Nonenzymatic agents are ferritin, ceruloplasmin, transferrin, ascorbic acid (vitamin C), and α -tocopherol (vitamin E), lycopene, metallothionein, and bilirubin also have antioxidant properties (Carbone *et al.*, 2003)

Decreased concentrations of plasma ascorbic acid, α -tocopherol, total thiols, and erythrocyte reduced glutathione (GSH) results in an increase in oxidative stress and spontaneous abortion (Vural *et al.*, 2000)

1.2 STATEMENT OF PURPOSE

Pregnancy is associated with several complications which include pregnancy induced hypertension, gestational diabetes, pre-eclampsia, anaemia and spontaneous abortion. Anaemia is more prevalent and contributes significantly to maternal mortality and reproductive health morbidity but little or no data exist about the true picture of anaemia in pregnancy; that is the prevalence, type of anaemia, causes and the aetiological factors responsible for their pathogenesis (de Benoist *et al.*, 2008). Some authorities attribute anaemia in pregnancy to nutritional deficiency, especially of iron and/or folate, but comparatively few data exist to substantiate this allegation (van den Broek, 1996a).

No single biochemical marker or hematological Index exists to effectively diagnose anaemia in pregnant women despite a wide range of available laboratory test (Cardoso *et al.*, 1994). The conventional markers of assessing

iron status (serum ferritin, serum iron, TIBC, TSAT%, transferrin) are influenced by inflammatory conditions or infections as well as diurnal variations thereby masking the diagnosis of iron deficiency (ID) and iron deficiency anaemia (IDA) (Burns *et al.*, 1990b; Cazzola and Beguin, 1992; Harju *et al.*, 1984).

Published rates of prevalence for developing countries range from 35% to 56% for Africa, 37% to 75% for Asia and 37% to 52% for Latin America (WHO, 1992). This is in sharp contrast to industrialized countries where anaemia in pregnancy occurs in less than 20% of women. Exact population prevalence figures are usually not available for developing countries for which Ghana is not an exemption (WHO, 1992).

Spontaneous abortion is associated with psychological morbidity and highly frustrating for both patients and physicians. A third of women attending specialist clinics are clinically depressed, and one in five have levels of anxiety that are similar to those in psychiatric outpatient populations (Adolfsson *et al.*, 2004).

Many conventional/cultural beliefs about the occurrence and treatment of women with spontaneous abortion have not withstood scrutiny. There is paucity of in-depth scientific research in this study area in this part of the world, so mostly, occurrence of spontaneous abortion is largely attributed to witchcraft or sorceries, traditional beliefs and customs.

It is because of the lack of data on this very important subject matter which affects the lives of women and neonates that this research has been designed to generate scientific information aimed at helping to address these lapses.

1.3 AIM OF THE RESEARCH

The aim of this study is to determine the biochemical markers and hematological indices in pregnancy and their correlation with obstetrics profile and complications.

1.3.1 SPECIFIC OBJECTIVES

1. To determine the biochemical markers and hematological Indices in pregnant women who are;
 - a) hematinic naïve (no hematinic intake) and those on hematinics
 - b) anaemic
 - c) having obstetrics history of spontaneous abortion
2. To determine the prevalence, types and risk factors of anaemia in pregnant women in the Northern region of Ghana.
3. To determine the correlates of these biochemical markers and haematological indices in certain obstetric profiles such as parity, gravidarum, gestational age, spontaneous abortion, anaemia in pregnancy.
4. To determine the prevalence of Spontaneous abortion and risk factors among pregnant women in Northern Ghana.

Chapter 2**LITERATURE REVIEW****2.1 BACKGROUND**

Pregnancy is a physiological condition characterized by systemic alterations to meet the nutritional and metabolic requirements for the growing and developing fetus (Flick and Kahn, 2013)

2.2 PREGNANCY AND ASSOCIATED PHYSIOLOGICAL CHANGES

The various physiological modifications that occur during pregnancy include;

2.2.1 Hematological Changes**2.2.1.1 Changes in Blood Volume**

Maternal blood volume increases during pregnancy, and this involves an increase in plasma volume as well as in red cell and white cell volumes (Lund and Donovan, 1967)

Ueland (1976) found an increase in plasma volume by 40% to 50%, whereas the red cell volume goes up by only 15% to 20%. This disproportionate increase causes a situation known as “physiological anemia of pregnancy” (normal hemoglobin, 12 g/dL; hematocrit, 35%).

Because of this hemodilution, blood viscosity decreases by approximately 20%. The exact mechanism of this increase in plasma volume is unknown but several hormones such as reninangiotensin-aldosterone, atrial natriuretic peptide, estrogen, and progesterone plays critical role in this interesting phenomenon. Two hypothesis offers to explain this mechanism; first, there is an underfill state caused by initial vasodilation, which stimulates the stretch receptors causing the release of hormones such as renin by the juxtaglomerular apparatus, angiotensin, and aldosterone. The second mechanism is an overfill state characterized by an early increase in sodium retention (due to an increase

in mineralcorticoids) that retains fluid, causing an increase in blood volume. There is also increase in plasma levels of clotting factors I, VII, VIII, IX, X, and XII, and fibrinogen concentration during pregnancy (Fay *et al.*, 1983)

A study by Frolich *et al.* (1998) also observed an increase in thrombopoietin, levels increasing with advance gestation as well as slight increase in systemic fibrinolysis.

2.2.1.2 Red Blood Cells

During pregnancy, there is significant increase in total blood volume by about 1.5 liters, mainly to supply the demands of the neovasculature and to cater for the blood loss occurring at delivery (Ramsay, 2010). This change in plasma volume alters the red blood cell indices in pregnancy and causes a small increase in mean corpuscular volume (MCV), 4 fl by average. There is increased production of RBCs and a hemoglobin concentration of 9.5 g/dL to meet the demands of pregnancy (Crocker *et al.*, 2000).

2.2.1.3 White Blood Cells

White blood cell count is increased in pregnancy with the lower limit of the reference range being 6,000/cumm. Fleming explained the increase in white blood cell count (Leucocytosis), during pregnancy and attributed to the physiologic stress induced by the pregnant state (Fleming, 1975).

Neutrophils are the main type of white blood cells seen on differential counts (Gatti *et al.*, 1994; Konijnenberg *et al.*, 1997) and this due to impaired neutrophilic apoptosis in pregnancy (Gatti *et al.*, 1994).

In pregnancy state, neutrophil cytoplasm shows toxic granulation on staining, chemotactic and phagocytic activities are depressed as a result of inhibitory factors present in the serum of pregnant women (Jessica *et al.*, 2007).

There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. Immature forms as myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance (Karalis *et al.*, 2005). They simply indicate adequate bone marrow response to an increased drive for erythropoiesis occurring during pregnancy.

There is monocytosis occurring in the early first trimester in pregnancy during pregnancy, but decreases as gestation progresses. The increase monocytes levels help in preventing fetal tissue rejection by infiltrating the decidual tissue (7th– 20th week of gestation) possibly, through PGE2 mediated immunosuppression (Kline *et al.*, 2005)

However, Eosinophil and basophil counts do not change significantly during pregnancy (Edelstam *et al.*, 2001).

2.2.1.4 Platelets

Large cross-sectional studies done among healthy pregnant women have shown that platelet count decrease during pregnancy, particularly in the third trimester. This is referred to as “gestational thrombocytopenia”, partly due to hemodilution and increased platelet activation and accelerated clearance occurring in pregnancy state (Shehata *et al.*, 1999)

Gestational thrombocytopenia does not have any complications on both the mother and foetus (Ramsay, 2010). The platelet volume distribution width increases significantly and continuously as gestation advances, for reasons already stated above. This implies, mean platelet volume is not a sensitive measure of platelet size during advance gestational age of pregnancy.

2.2.1.5 Hemostatic Profile

Pregnancy is associated with significant changes in the hemostatic profile. Fibrinogen and clotting factors VII, VIII, X, XII, vWF and ristocetin cofactor activity increase remarkably as gestation progresses. Increased levels of coagulation factors are due to increased protein synthesis mediated by the rising estrogen levels (de Boer *et al.*, 1989).

Thus, pregnancy is a prothrombotic state. In pregnancy, aPTT is usually shortened, by up to 4 seconds in the third trimester, largely due to the hormonally influenced increase in factor VIII. However, no marked changes in PT or TT occur (Ramsay, 2010).

There are changes in the levels and activity of the natural anticoagulants but no changes in levels and activity of Protein C has been observed. Levels of total and free Protein S, reduces progressively with advance gestation. Antithrombin levels and activity are usually stable throughout the pregnancy, fall during labor and rise again soon after delivery (Clark *et al.*, 1998).

Coagulation factors remain elevated for up to 8-12 weeks post-partum. Markers of hemostatic activity which are clinically significant when measured are thrombin-antithrombin complexes (TAT) and prothrombin fragments (F1 + 2), which reflect in vivo thrombin formation since others may be negative when measured. TAT levels increase with gestation; in early pregnancy the upper limit of normal is similar to the adult range of 2.63 g/L, whereas by term, the upper limit normal level is 18.03 g/L. D-dimer levels are markedly increased in pregnancy, with typical reference range being tenfold higher in late pregnancy than in early pregnancy or in the non-pregnant state (Ramsay, 2010)

The increase in D-dimers reflects the overall increase in total amount of fibrin during pregnancy consequent to increased thrombin generation, increased fibrinolysis or a combination of both (Eichinger, 2004)

2.3 ANAEMIA IN PREGNANCY

2.3.1 Definition

Anaemia in pregnancy is defined as haemoglobin level $<11\text{g/dl}$ and hematocrit (HCT) lower cut-off of 33% by World Health Organization (WHO, 1992)

Verhoeff et al defined anaemia in pregnancy as haemoglobin level below 11g/dl for which pregnant women show signs and symptoms indicating tissue and organ hypoxia causing reduced metabolism, physical and mental capacities as a result of tissue hypoxia (Verhoeff *et al.*, 1999)

Around the world, prevalence and epidemiology of anemia differs; between developed and developing nations thus, about 52% in developing countries versus 23% in the developed countries (WHO, 2001a)

About half of pregnant women in Africa have anaemia with the highest prevalence and effect occurring in the West African sub region (WHO, 1992; WHO, 2001a).

2.3.2 Anaemia Prevalences as reported by various authorities

Varying prevalence of anemia has been reported from developing countries such as 85% in India (Amel-Ivan and Mangaiarkkarasi, 2013), 42.6% in Nepal (Marahatta, 2007), 20% in Brazil (Fujimori *et al.*, 2011), 32% in Ghana (Stephens *et al.*, 2014), 56.6% in Malawi (van den Broek and Letsky, 2000) 47.4% in Tanzania (Msuya *et al.*, 2011) and 64% in Nigeria (Ezugwu *et al.*, 2013). A rece

nt study by Dim and Onah showed a relatively high prevalence of 40.4% among antenatal clinic attendees (Dim and Onah, 2007).

Iron deficiency is the most common cause of anemia in the general population and in pregnancy state (van den Broek and Letsky, 2000; WHO, 2001a)

The risk factors for the development of anaemia in pregnancy include grand multiparity, low socioeconomic status, malaria infestation, late booking to antenatal, human immunodeficiency virus (HIV) infection, and inadequate child spacing amongst others (Adinma *et al.*, 2002; Aimakhu and Olayemi, 2003; Aluka *et al.*, 2001; Amadi *et al.*, 2000)

2.3.3 Consequences of anaemia in pregnancy

Anaemia in pregnancy affects both the pregnant woman and foetus negatively.

Anemia in pregnancy causes foetal anaemia, low birth weight (LBW), preterm birth, intra uterine growth restriction and increased perinatal mortality. This occurrence is as result of impaired oxygen delivery to the placenta and the foetus (Allen, 2000; Haggaz *et al.*, 2010; Kidanto *et al.*, 2009; Scholl and Hediger, 1994)

On the health of the pregnant women themselves, anaemia causes low physical activity and increased risk of maternal morbidity, mortality, high rate cardiac failure especially in situations where the anaemia is severe (Allen, 2000; de Benoist *et al.*, 2008; Scholl and Hediger, 1994).

Anaemia may result from defects at any stage of red cell and haemoglobin production or when an increased rate of red cell destruction (haemolysis) exceeds the capacity of the bone marrow to make up for the loss. Red cell and plasma volume changes may also cause reduced haemoglobin concentration, such as those that occur physiologically in pregnancy, where red cell volume is increased less markedly than plasma volume. Generally, anaemia in pre

gnancy occurs at a haemoglobin level below 11g/dl and all anaemic patients show signs and symptoms indicating tissue and organ hypoxia and the ensuing reduced metabolism. Without adequate oxygen, the physical and mental capacities of individuals are reduced (Verhoeff *et al.*, 1999).

According to WHO report on anaemia in 2001, most persons suffering from anaemia are found in developing countries, where high anaemia prevalence is seen, particularly in pregnant women, young children, female adolescents, and women of childbearing age (WHO, 2001a). Per their findings, overall anaemia contributes to about 20 percent of maternal and perinatal deaths in developing countries. Anaemia and iron deficiency can result in delays in mental and psychomotor development, reduce cognition and limit a child's learning ability. It can also cause infant mortality and morbidity (Walter *et al.*, 1989).

2.3.4 AETIOLOGY, CLASSIFICATION AND TYPES OF ANAEMIA

Anaemia is consistently higher among pregnant women compared with their non-pregnant counterparts (WHO, 1992). The aetiology of anemia in pregnancy is multi-factorial. Increased demands for nutrients, especially iron, increased susceptibility to malaria as a result of decreased immunity in primiparous women, multi-gravida occurring too frequently and beginning at too early age has been implicated in the pathogenesis of anemia (Baker, 1981; Fleming, 1989b; Masawe, 1981; van den Broek, 1996a; Watson-Williams, 1968) (WHO, 1992). Aetiological factors include nutritional deficiencies, haemoglobinopathies, infections with hookworm and HIV (WHO, 1992)

Suharno *et al.* found Vitamin A deficiency to be associated with anaemia (Suharno *et al.*, 1993). The different causes interact to form a complex and interrelated cascade cascade (e.g. haemolysis as a result of malaria may result in folate deficiency and associated splenomegaly may result in sequestration with further destruction of red cells).

The relative contribution of each of these aetiological factors will vary by geographical area and by season hence the need for careful study of the various aetiological factors in varying locations (WHO, 1989)

Most authors attribute anaemia in pregnancy to nutritional deficiency, especially of iron and/or folate, but few data exist to substantiate this fully and assessment is complicated by difficulty in getting an ideal marker to assess both iron and folate status in pregnancy (van den Broek, 1996a)

2.3.5 Classification of Anaemia by etiology

Anaemia in pregnancy may be classified based on etiology as;

- I. Physiological anaemia of pregnancy (hemoglobin level $< 112\text{g/dl}$)
- II. Acquired
 - a. Acquired form of anaemia can be sub-categorized as;
 - b. Nutritional (Iron deficiency, folate deficiency, B-12 deficiency, etc.)
 - c. Infections (Malaria, hookworm infection, HIV etc)
 - d. Hemorrhagic (Acute or chronic blood loss)
 - e. Bone marrow suppression (Aplastic anaemia, drugs, etc.),
 - f. Renal disease
- III. Genetic - haemoglobinopathies - sickle cell disease, thalassemia, enzymopathies etc

Anaemia in pregnancy can also be classified as mild, moderate or severe, with WHO classifying mild anaemia as Hb level of 10.0-10.9 gm/dL, moderate anaemia as 7-9.9 gm/dL and < 7gm/dL as severe anaemia (Idowu *et al.*, 2005)

2.3.6 Types of Anaemia

2.3.6.1 Nutritional deficiencies

Nutritional deficiencies occur as a result of an imbalance in low nutrient intake, poor absorption and increased nutrient loss in gastrointestinal disorders or increased demand as in the cases of pregnancy.

2.3.6.2 Iron deficiency anaemia (IDA)

Iron deficiency anaemia is a condition where there is a reduced amount of haemoglobin and decreased number of red blood cells in the body due to iron deficiency. IDA leads to less oxygen getting to the cells and tissues, thus affecting their function. (Lanerolle and Atukorala, 2006)

In the developing world, nearly half of the population (almost 50%) has iron deficiency compared to about 11% iron deficiency prevalence in the industrialised world (Fleming, 1970). Roughly 47% of non-pregnant women and 60% of pregnant women have anaemia worldwide. In the industrial world, anaemia prevalence during pregnancy averages 18%, and out of this, over 30% is attributable to iron deficiency (Fleming, 1970)

2.3.6.3 Causes of iron deficiency (ID) and iron deficiency anaemia (IDA)

Iron deficiency anemia (IDA) occurs when iron deficiency is severe to reduce erythropoiesis causing a decrease in hemoglobin concentration below the lower normal limit. This type of anemia is the most prevalent and may result from either excessive loss or, less frequently, decreased absorption. In general, the iron absorbed daily equals the amount needed to compensate its loss, so that the overall iron pool remains stable. This balance is easily breached,

because the capability to absorb iron orally is limited. When iron requirement far exceeds the input, ID and finally IDA, develops. In many cases, these alterations will be secondary to gastrointestinal disease. IDA occurs in 2%-5% of adult males and postmenopausal women in the developed world (Goddard *et al.*, 2000; Sayer and Long, 1993). Iron deficiency anaemia can still occur despite adequate dietary intake. Even though iron absorption increases in response to iron deficiency and in the course of normal pregnancy (Svanberg, 1975; Whittaker *et al.*, 1991)

In addition to digestive disorders, gynaecological diseases, urological diseases and other specific situations, such as intravascular hemolysis can result in ID and IDA. Focusing on digestive diseases, the etiology of ID and IDA of gastrointestinal origin can be divided into two groups: situations with increased loss of iron (the most common in developed countries), and those with decreased iron absorption. In the case of increased loss, the blood loss can occur in the form of visible bleeding (melena, hematemesis, rectal bleeding) or hidden bleeding, which might be more difficult to diagnose. Among the diseases causing the blood loss, benign or malignant gastrointestinal tumors of the colon, stomach, esophagus and small intestine, peptic ulcer and reflux disease, use of nonsteroidal anti-inflammatory drugs (NSAIDs), and inflammatory bowel disease are mostly implicated (Ioannou *et al.*, 2002)

In the second scenario of iron deficiency, (that is reduced iron absorption), causes can be digestive origin, and can be caused by celiac disease, atrophic gastritis, and postsurgical status (gastrectomy, intestinal resection) among others. Celiac disease is very relevant and specific evaluation to exclude it must be performed. In a study on patients referred to a specialized gastroenterological consultation because of ID or IDA, celiac disease was finally the diagnosis in 10% of cases (Corazza *et al.*, 1995) other authors described that at least 2%-3%

of patients with IDA are finally diagnosed as celiac disease (Kepczyk and Kadakia, 1995; McIntyre and Long, 1993).

The prevalence of this disease worldwide is approximately 1%, and it is possible it is being under diagnosed (Catassi and Fasano, 2008)

Microscopic alterations in the duodenal mucosa in non-treated celiac disease patients will lead to them becoming refractory to oral iron treatment. This has also been described in patients with autoimmune atrophic gastritis and gastritis due to *Helicobacter pylori* (*H pylori*) (Barabino, 2002; Hershko *et al.*, 2005; Sarker *et al.*, 2008).

Regarding the role of *H pylori* in the pathogenesis of IDA, a recent meta-analysis indicated that the infection is associated with depleted iron deposits. The mechanism by which *H pylori* induces this alteration is not clear, but it appears to involve gastrointestinal blood loss, diminished iron absorption from the diet, and increased consumption of iron by the bacteria.

Accurate assessment of iron status is difficult and requires a combination of conventional parameters including serum iron, total Iron binding capacity, transferrin and free erythrocyte protoporphyrin. None of these is a very reliable indicator of iron status because of marked variations during iron ingestion and in inflammatory or infectious conditions.

The commonest single reliable biochemical parameter used to measure iron status is serum ferritin, but this has sometimes led to contradictory results (Cardoso *et al.*, 1994)

Ferritin is stable and not affected by recent iron ingestion but is a positive acute phase reactant. The gold standard of assessing iron stores in pregnancy is still

by examination of a stained bone marrow preparation but this is a highly invasive procedure (Letsky, 1995b)

Serum transferrin receptor concentration has been shown not affected by chronic disease or physiological changes in pregnancy and may have a future as a useful new biochemical parameter in assessing iron status (Baynes *et al.*, 1993; Carriaga *et al.*, 1991b).

Table 2.1: Causes of iron deficiency anemia

A. Digestive disorders
i. Increased losses of iron
Cancer/polyp: colon, stomach, esophagus, small bowel
Peptic ulcer, esophagitis
NSAID use
Inflammatory bowel disease: ulcerative colitis, Crohn's disease
Intestinal parasites
Vascular lesions: angiodysplasia, watermelon stomach
Meckel's diverticulum
ii. Reduced iron absorption
Celiac disease
Bacterial overgrowth
Whipple's disease
Lymphangiectasia
Gastrectomy (partial and total) and gastric atrophy
Gut resection or bypass
B. Urological and gynecological disorders
C. Intravascular hemolysis
Prosthetic valves and cardiac myxomas, paroxysmal nocturnal hemoglobinuria, marathon runners, multiple blood donations
D. Deficient iron intake

Source: (Bermejo and García-López, 2009)

2.3.6.4 CLINICAL MANIFESTATIONS OF ID AND IDA

The clinical picture varies greatly from one case to another. Symptoms depend on the speed of onset of anemia, its severity, and the characteristics of the patient. Thus, IDA or ID can be detected in an asymptomatic individual on a screening analysis, or in a person with symptoms that include general weakness, fatigue, irritability, poor concentration, headache, and intolerance to exercise. These symptoms appear even in the figures for ID with normal hemoglobin levels. Patients often show relatively few symptoms spontaneously. Although the impact of ID on the quality of life of the subject is high, they often get used to their symptoms and these are assumed as normal. Some iron-deficient patients, with or without anemia, might have alopecia, atrophy of lingual papillae, or dry mouth due to loss of salivation. Other symptoms, such as weakness or digging fingernails (koilonychia), chlorosis, or the syndromes of Plummer-Vinson or Paterson-Kelly (dysphagia with esophageal membrane and atrophic glossitis) have virtually disappeared. These changes were caused by reduction of iron-containing enzymes in the epithelia and the gastrointestinal tract. Pica, the eating disorder in which there is an irresistible desire to lick or eat non-nutritive and unusual substances, such as soil, chalk, gypsum, ice (pagophagia) or paper, might appear in some cases. Pagophagia is specific to ID (Rector, 1989)

Physical examination might be normal or show pallor of varying degree and systolic murmur in cardiac auscultation.

2.3.6.5 Laboratory diagnosis of iron deficiency and iron deficiency anaemia

There are numerous available laboratory tests to assess iron, but diagnosing IDA is very challenging (Clark, 2009; Logan *et al.*, 2002; Van Vranken, 2010)

Prussian blue staining on bone marrow aspirates is still considered the gold standard for diagnosing iron deficiency (Barron *et al.*, 2001; Ganti *et al.*, 2003;

Thomason and Almiski, 2009), However, it is a highly invasive procedure which is not ideal to perform for each anemic patient at even normal outpatient departments (OPDs). There is therefore a need to find a clinically feasible blood test to assess iron status in patients.

2.3.6.5.1 Diagnosis of IDA using the Classical/conventional iron status parameters

The conventional parameters of assessing of iron status are serum iron, transferrin, total iron-binding capacity (TIBC), transferrin saturation, and ferritin which are widely used in clinical practice. The conventional markers are considerably influenced by acute phase response in inflammatory or infectious conditions, masking the detection of iron deficiency or complicate the clinical interpretation of the test results (Burns *et al.*, 1990b; Cazzola and Beguin, 1992; Harju *et al.*, 1984).

2.3.6.5.2 Ferritin as a marker for ID and IDA diagnosis

The serum ferritin concentration is the most useful and reliable indicator among the classical parameters of assessing iron deficiency (Cook, 2005; Cook *et al.*, 2003; Schrier, 2009).

A low serum ferritin concentration of less than ($<20 \mu\text{g/L}$) confirms iron deficiency. Serum ferritin levels greater than $>100 \mu\text{g/L}$ usually exclude iron deficiency (Munoz *et al.*, 2011).

But ferritin being a positive acute phase reactant (in inflammation), iron deficient patients could have a falsely elevated ferritin levels as high as $100 \mu\text{g/L}$. However, ferritin levels between 20 and $100 \mu\text{g/L}$ are often inconclusive (Guyatt *et al.*, 1992; Nelson *et al.*, 1978).

2.3.6.5.3 Diagnosing iron deficiency anemia with modern indicators of iron status

Since the discovery of ferritin as a useful indicator of iron status, several diffe

rent markers (modern indicators of iron status) have been proposed to clearly differentiate those who are truly iron deficient from those who are non-iron deficient when ferritin levels are inconclusive (that between 20 and 100 µg/L) and also in inflammatory conditions where there is falsely elevated/decreased values in the conventional iron markers. Soluble transferrin receptor, soluble transferrin receptor/log(ferritin) ratio, reticulocyte hemoglobin content, hepcidin, transferrin/log ferritin ratio were identified as some modern indicators of iron status (Clark, 2009)

2.3.6.5.4 Soluble Transferrin Receptor as a reliable marker for the diagnosis of IDA and Anemia of Chronic disease (ACD)

Iron deficiency anemia (IDA) and anemia of chronic disease (ACD) due to inflammation are two common forms of anemia that is confusing in diagnosis. Anaemia of Chronic disease (ACD) is often seen in patients with infections, inflammatory and neoplastic diseases that have persisted for more than 1 or 2 months. ACD is characterised by hypoferraemia in the presence of adequate reticuloendothelial iron stores (Bentley, 1982; Cartwright, 1966; Lee, 1983). Conventional markers of assessing iron status such as serum iron, total iron binding capacity (TIBC) and serum ferritin are poor in distinguishing Anemia of chronic disease (ACD) from Iron deficiency anemia (IDA) as both serum ferritin and transferrin are considerably influenced by acute phase responses in inflammation. Moreover, ACD can coexist with IDA. Therefore, it is extremely difficult to interpret the actual iron status of the patient unless the use of iron indices that is stable in the face of inflammatory or infectious conditions. Soluble transferrin receptor (sTfR), a truncated form of the cellular transferrin receptor, without its transmembrane and cytoplasmic domains, and circulates bound to transferrin (Shih *et al.*, 1990) found on erythroblasts in bone marrow and many other cells has been demonstrated to possess such criteria.

Measurement of sTfR is another marker of iron metabolism that reflects body iron stores and total erythropoiesis (Baynes, 1996). It has been proven to be unaffected by acute phase response unlike ferritin and the other classical iron markers. Thus, it holds the key in diagnosing iron deficiency in the presence of acute and chronic infection or inflammation (PETTERSSON *et al.*, 1994)

The serum level of sTfR in iron deficiency states increases due to upregulated transferrin receptor on the cell surface in response to increased cellular demand. sTfR therefore reflects the tissue iron supply reliably (Baynes, 1996; Worwood, 2002).

Soluble transferrin receptor (sTfR) concentration is not usually affected by inflammation/ infection but rises only in situations where there is an underlying iron deficiency making sTfR levels a better indicator of iron deficiency (ID) when it is associated with inflammation (Angeles Vázquez López *et al.*, 2006; Dimitriou *et al.*, 2000; Hanif *et al.*, 2005; Markovic *et al.*, 2005; PETTERSSON *et al.*, 1994)

There is a growing amount of literature concerning the role of the sTfR/ferritin or sTfR/log ferritin ratio (sTfR-F index) in the evaluation of anemia in patients with chronic inflammatory diseases, especially diagnosing IDA in the setting of chronic inflammation or discriminating iron deficiency in the absence of anemia

(Thomas and Thomas, 2002; Weiss and Goodnough, 2005)

2.3.6.5.5 A new parameter for the diagnosis of iron deficiency anemia: the transferrin/log(ferritin) ratio

Neither the classical nor the modern iron parameters are able to explicitly diagnose Iron deficiency anemia (IDA) in situations where ferritin concentrations are inconclusive (that is ferritin concentrations between 20µg/L and 100 µg/L) (Guyatt *et al.*, 1992; Nelson *et al.*, 1978). Serum ferritin

concentration less than ($<20 \mu\text{g/L}$) confirms iron deficiency anaemia (IDA), whereas serum ferritin levels $>100 \mu\text{g/L}$ usually exclude iron deficiency (Munoz *et al.*, 2011)(Munoz et al, 2011)

Castel et al (2012) combined the classical iron status parameters with ferritin and found that the transferrin (g/L)/log [ferritin ($\mu\text{g/L}$)] ratio [Tf/log(ferr)] is able to completely separate patients with ferritin concentrations between $20\mu\text{g/L}$ and $100 \mu\text{g/L}$ who truly have ID or IDA from those who are non-iron deficient at a Tf/log(ferr) ratio cut-off value of 1.70 (where values less than or equal to ≤ 1.70 are non-iron-deficient; and those with a cut-off value greater than >1.70 are IDA)

2.3.7 FOLATE DEFICIENCY

2.3.7.1 Folic acid and Anaemia in pregnancy

Folate refers to a form of water-soluble B vitamin that are naturally found in foods such as leafy vegetables, egg yolks, liver, some citric fruits and legumes (Kamen, 1997)

Folate deficiency may result from decreased dietary intake, increased demand during pregnancy or haemolytic crisis in malaria infection. Folate deficiency and malaria co-infection greatly predisposes primigravidae women to megaloblastic anaemia observed in a study in West Africa (Fleming *et al.*, 1986)

Similar observation was made in a different study from Nairobi by Mati et al where megaloblastic anaemia was associated with malaria, and prevalence more higher than iron deficiency using bone marrow aspirate and Prussian staining as diagnostic marker (Mati *et al.*, 1971). In the presence of iron deficiency, folate deficiency may be obscured in the peripheral blood film (Letsky, 1995a)

Folate is essential for one-carbon metabolism in amino acid metabolism as well as DNA synthesis, repair and methylation (NCM, 2004; Tamura and Picciano, 2006)

Pregnancy predisposes women to folate deficiency, due to the rapid fetal growth, organ differentiation and high rates of cell division (mitosis) (Scholl and Johnson, 2000; Tamura and Picciano, 2006; WHO, 2004).

For decades, folic acid has been known in obstetrics practice to prevent megaloblastic anemia during pregnancy (Israels and Da Cunha, 1952).

Furthermore, folate deficiency is associated with several negative health outcomes both on gravid mother and the foetus, for example, inadequate maternal folate status has been linked to abruptio placentae, preeclampsia, spontaneous abortion, stillbirth, preterm delivery, low birth weight (Molloy *et al.*, 2008), and serious congenital anomalies of the brain and spine, such as neural tube defects (Hibbard *et al.*, 1965)

2.3.7.2 Folate metabolism and biochemical roles (functions)

Folic acid is a cofactor and cosubstrate for biological methylation and nucleic acid synthesis and also functions as regulatory molecules. It acts as a mobile cofactor in several key enzymatic reactions and carries one-carbon residues. Folates are cosubstrates for the reactions they are involved in (Nijhout *et al.*, 2004)

An additional function of cellular folates is as regulatory molecules that exert allosteric effects on several enzymes in the folate and methionine cycles, such as methylenetetrahydrofolate reductase (MTHFR), glycine-N-methyltransferase, and serine hydroxymethyltransferase (Matthews and Daubner, 1982; Nijhout *et al.*, 2004; Shane, 1995; Wagner, 1995). This regulatory role of folates

is less well understood, but it will be influenced not only by the affinity of the enzymes for folate but also by the steady state concentration of folate in the cell (Nijhout *et al.*, 2006)

Folic acid exists as polyglutamates in green leafy vegetables and other natural sources. These polyglutamates are broken down to monoglutamates by the intestinal enzymes before it can be absorbed in the jejunum. Within the intestinal cells folic acid is first reduced to 7,8 dihydrofolic acid, and then to tetrahydrofolic acid (THFA) by NADPH dependent folate reductase. It is then methylated to N5 methyl THFA and then transported in blood as methyl THFA bound to plasma proteins.

Folates enter mammalian cells as monoglutamates, but are rapidly modified by the addition of 4–8 glutamate residues to form long side chains. Polyglutamation greatly increases the affinity of folates as both substrates of their own enzyme and inhibitors of other enzymes in the folate pathway (Matthews and Daubner, 1982; McGuire and Bertino, 1981)

Polyglutamation also constitutes a mechanism to trap folates within mammalian cells because the long-chain folylpolyglutamates are poorly accepted by the membrane carriers responsible for efflux across the cell membrane (Shane, 1995)

2.3.7.3 FOLIC ACID AND NEURAL TUBE DEFECT

Neural tube defects (NTDs) are congenital malformations that result in foetal mortality, structural and functional disabilities in surviving infants. Approximately 400,000 infants are born each year with spina bifida. There are variable prevalence rates across the globe with higher rates in northern China (Moore *et al.*, 1997) certain parts of England and Wales and Punjab State in India (Verma, 1978)

2.3.7.3.1 Neural tube defects: Pathogenesis and various forms

The formation and closure of the neural tube normally takes place around week 4 of embryonic development and is very critical in the formation of the nervous system. Failure of a portion of the neural tube to close alters significantly the differentiation of central nervous system and the induction of vertebral arches. This can result in a number of developmental malformations along the neuroaxis from the developing brain to the sacrum. These malformations are what we describe as neural tube defects. Forms of neural tube defects are Spina bifida (spina bifida occulta, meningocele, meningocele), anencephaly and encephalocele. In the pathogenesis of spina bifida, the arches of one or more adjacent vertebrae fail to develop with the most commonly affected site being the lumbosacral region. This occurs due to a failure of the mesenchyme to grow in between the neural tube and the surface ectoderm to form the vertebral arches. The severity of this malformation may range from a failure of fusion of the arches in the midline (spina bifida occulta) to defects involving the meninges (meningocele) and spinal cord (meningomyelocele). The location of the defect is frequently indicated by a tuft of hair induced by the underlying abnormality or by an angioma, pigmented nevus or dimple. In the case of anencephaly, it is characterised by the absence of vault of the skull and a greater part of the brain. This malformation is caused by a failure of the cranial part of neural tube to close. The normal forebrain is replaced by an exposed dorsal mass of undifferentiated neural tissue. Most anencephalic infants are still born or die shortly after birth. There are other malformations in which the neural folds not only fail to fuse but also fail to differentiate, invaginate and finally separate from the ectoderm. These include craniorachischisis totalis, rachischisis or myeloschisis and iniencephalus. Encephalocele is a condition in which the brain and meninges herniate through a defect in the calvaria. This defect mostly occurs in the occipital region.

2.3.7.3.2 Development of neural tube in embryogenesis

Gastrulation is a major process in embryonic development and normally occurs by day 16 after fertilization. It is a process in which the intraembryonic mesoderm or the embryonic tissue which differentiate into various organs is formed and the embryo becomes a trilaminar disc namely mesoderm, endoderm and ectoderm. The ectoderm differentiates into the central nervous system, nails, epidermis of skin etc. The notochord develops from the ectoderm and can be seen along the midline of the embryo after one or two days after gastrulation. The notochord develops into neural plate by inducing a wide strip of ectoderm overlying it to get thickened. Some cells at the junction between the neural plate and the rest of ectoderm become specialised to form the primordia of the neural crest. By the 21st day (end of 3rd week), the neural plate gets depressed along the midline forming the neural groove on the neural plate. The neural groove gradually deepens; the two edges of the neural plate come closer and fuse to become the neural tube. With separation of the neural tube from the surface ectoderm, the cells of the neural crest appear as a group of cells lying along the dorsolateral sides of the neural tube. The neural tube initially closes in the middle. The fusion starts around 23rd day and proceeds both cranially and caudally. The anterior neuropore closes by the 25th day and the posterior neuropore by the 27th day. The closure of neural tube is completed by the end of 4th week (Bachiller *et al.*, 2000; Nieuwkoop, 1999)

2.3.7.3.3 Mechanism of neural tube closure

Three main stages are involved in formation and closure of the neural tube namely, formation of the neural groove, elevation of the neural walls and neural fold fusion.

Studies in animals have shown that notochord induces the transformation of overlying ectodermal cells to form the neural plate (Bachiller *et al.*, 2000; Nieuwkoop, 1999). As neurulation progresses, cells in the superficial

neuroectoderm undergo an apical constriction thereby shaping the neural groove (Davidson and Keller, 1999).

The elevation of the neural walls is brought about by several mechanisms. These include:

- I. Convergent extension, which has two components: extension in the longitudinal direction and narrowing in the transverse direction at the same time. The cells within the neural plate increase in number, migrate towards the midline and undergo certain morphological changes that lead to the neural groove deepening to form the neural tube (Jacobson and Gordon, 1976). Jacobson in 1978 observed that anteroposterior elongation of the neural plate during convergent extension results in a transverse buckling that promotes the elevation of neural folds (Jacobson, 1978)
- II. Contraction of the apical microfilament bundles, which are arranged like a purse string around the cellular apices, is a crucial component of neural fold elevation. As a result of this apical constriction, all the neural plate cells become wedge shaped and as a whole, the neural plate curls up (Ferreira and Hilfer, 1993; Hildebrand and Soriano, 1999; Nagele *et al.*, 1987; Schoenwolf *et al.*, 1988; Smith and Schoenwolf, 1997; Van Straaten *et al.*, 1996) Apical constriction can result in anteroposterior shortening of the neuroepithelium and convergent extension may compensate for this (Jacobson and Gordon, 1976; Schoenwolf and Alvarez, 1989). Elongation of the neural plate shapes the depression into a groove (Jacobson and Gordon, 1976)
- III. Formation of medial hinge point (MHP): The medial neural plate cells decrease in height and become markedly wedge shaped under the influence of the underlying notochord (Placzek *et al.*, 2000; Smith and Schoenwolf, 1989; van Straaten *et al.*, 1988). MHP facilitates the bending of

neural plate in its midline and plays a major role in generating the normal cross-sectional morphology of the neural tube (Smith and Schoenwolf, 1989)

IV. Expansion of the underlying mesoderm also facilitates the elevation of neural walls (Hildebrand and Soriano, 1999; Van Straaten *et al.*, 1996).

2.3.7.3.4 Reopening of the neural tube

Neural tube defects arise when the neural tube fails to close properly. The second proposed model for neural tube formation is the reopening of the neural tube. (Campbell and Sohal, 1990)

2.3.7.4 Epidemiological correlation between folate and neural tube defects (NTDs)

The possible relation between folic acid deficiency and NTDs in humans was first reported by Hibbard (1964). He observed that there was a higher incidence of abnormal folate metabolism in women who had pregnancies associated with foetal malformations.

Smithells and co-workers in 1976 observed that maternal red blood cell folate levels were significantly lower when the foetus had NTDs in comparison to when the foetus did not have NTDs (Smithells *et al.*, 1976). This led to periconceptional folic acid supplementation for pregnant women in an attempt to prevent neural tube defects (Smithells *et al.*, 1980)

Lawrence and co-workers in 1981 reported findings from a randomised control trial that revealed that 4mg periconceptional supplementation of folic acid is able to reduce the risk of recurrence of NTDs (Lawrence *et al.*, 1981). Similar findings in non-randomised trial showed that administration of multivitamins containing

360 micrograms of folic acid in the immediate pre-pregnancy period to women who have had NTDs in their previous pregnancies saw 86% reduction in risk of developing NTDs (Smithells *et al.*, 1980)

Another study by the British Medical Research Council (MRC) in a large multicentre randomised control trial to assess the effect of folic acid supplementation to women with history of NTDs revealed similar findings of reduced risk of NTDs recurrence (Wald and Sneddon, 1991). A community based study to assess the protective effect of periconceptional folic acid supplementation (multivitamin containing 800 microgram of folic acid) in preventing the first occurrence of NTDs was conducted (Czeizel and Dudas, 1992) and the preventive effect was demonstrated even lower doses of 400 microgram (Berry *et al.*, 1999).

2.3.7.5 Genetic defects in folate metabolism, the gateway to the formation of Neural tube defects NTDs

Supplementation of folic acid reduces the incidence of neural tube defects by overriding genetic defects in folate metabolism. Key defects folic acid metabolism that lead to NTDs formation include ;

2.3.7.5.1 Methionine synthase gene defects

The common polymorphism in the methionine synthase gene is A2756G substitution, which leads to a change of aspartic acid to glycine (D919G (van der Put *et al.*, 1997). This substitution could lead to an improper cofactor oxidation level, which can decrease methionine synthase activity and increase cellular homocysteine level (Chen *et al.*, 1997). Methionine synthase along with cofactor Vit B 12 catalyses the transfer of methyl group from N5methyl THFA to homocysteine resulting in formation of methionine. Decreased levels of methionine result in decreased levels of methionine synthase (SAM). The low enzymatic activity also leads to increased levels of homocysteine. The deficiency of SAM could affect the cells' ability to methylate important compounds

such as DNA, lipids, proteins and myelin, thereby impairing cell function, which could result in defective neurulation and causes the formation of NTDs (Harmon *et al.*, 1999)

2.3.7.5.2 Methionine synthase reductase (MTRR) gene defects

MTRR regulates methionine synthase activity by reductive methylation. Defective MTRR reduces the functional ability of methionine synthase and thereby decrease methylation of homocysteine to methionine (Leclerc *et al.*, 1998).

2.3.7.5.3 Methylene tetrahydrofolate dehydrogenase (MTHFD) gene defects

MTHFD gene defects results in a decreased activity of MTHFD enzyme that has three critical roles to play: First, MTHFD enzyme is an NADP dependent methylene tetrahydrofolate dehydrogenase, ATP dependent formyl tetrahydrofolate synthase and methylene tetrahydrofolate cyclohydrolase. Hol and co-workers identified patients with familiar history of NTDs have G878A substitution on mutational analysis of MTHFD gene (Hol *et al.*, 1998).

2.3.7.5.4 Cystathionine beta synthase (CBS) gene defects

CBS catalyses the irreversible synthesis of cystathionine from homocysteine and serine. Defect in CBS gene affects this process leading to an increased cellular homocysteine level. These mutations lead to reduced enzymatic activity (Kraus *et al.*, 1999)

2.3.8 Vitamin B12 deficiency

Vitamin B12 deficiency in pregnancy has also been described in many parts of the world such as India (Baker *et al.*, 1962). This problem has a multifaceted aetiology both economic reasons and cultural beliefs of food taboos; the diet of pregnant women in many developing countries is mostly vegetarian in nature. Serum vitamin B12 levels decrease markedly in pregnancy leading to vitamin B12 deficiency but not necessarily causing Vitamin B12 anaemia.

(Cooper, 1973; Pinto *et al.*, 1973). But Letsky argued that stores of vitamin B12 are sufficient enough to meet the extra and increasing demands during pregnancy (Letsky, 1995b). Apart from megaloblastic anaemia, vitamin B12 deficiency has been found to be associated with infertility (Sanfilippo and Liu, 1991). Results from a study in Zimbabwe by Savage *et al.* suggested vitamin B12 deficiency may be far more common than expected (Savage *et al.*, 1994) and was proposed that measurement of methylmalonic acid and homocysteine in serum be used as a more sensitive indicator of vitamin B12 deficiency (Green, 1995; Savage *et al.*, 1994). Re-examination of the possible role of vitamin B12 deficiency using these new parameters would be worthwhile.

2.3.9 Parasitic disease

2.3.9.1 Malaria and anemia in pregnancy

Malaria co-infection in pregnancy causes haemolytic anaemia and increase the risk of folate deficiency. An increase in parasitaemia levels in pregnancy is more pronounced in primiparous women (Brabin, 1983; Gilles *et al.*, 1969; McGregor, 1984; Steketee *et al.*, 1988).

In several parts of Africa, malaria plays critical role in the aetiology of anaemia (Gilles *et al.*, 1969; Jackson *et al.*, 1991; Shulman *et al.*, 1996). Policies that reduces malaria transmission has shown positive improvement in the levels of mean haemoglobin (Draper, 1960; Fleming *et al.*, 1986; Gilles *et al.*, 1969; NIN, 1974; Scofield *et al.*, 1964)

Pregnant women are highly susceptible to malaria due to the ability of the parasites to sequestrate into the placenta and avoiding clearance by the reticuloendothelia system especially the spleen. The parasites cause damage to the placental tissue which triggers inflammatory responses, causing the thickening of the placental wall. Thickened placental wall impedes the flow of

blood carrying nutrients, oxygen and other biomolecules from the mother to the foetus, hence the low birth weight infants (Fried and Duffy, 1996).

2.3.9.2 Hookworm Infestation as an aetiological cause of anaemia in pregnancy

Hookworm infection with *Necator americanus* or *Ankylostoma duodenale* is common in many tropical areas. The development of iron deficiency anaemia as a result of hookworm infection depends on the content and bioavailability of iron in the diet, the size of the body's iron stores and the severity and duration of the infection (Crompton and Whitehead, 1993; Fleming, 1981; Gilles *et al.*, 1964; Miller, 1979). They infect and cause inflammatory lesions in the terminal ileum preventing Vitamin B12 absorption.

Although laboratory diagnosis of hookworm infection is simple, prevalence rates for pregnant women are rarely published. *Trichuris trichuria*, *Schistosoma haematobium* and *Schistosoma mansoni* may also be implicated but rarely causes severe anaemia.

2.3.9.3 Inflammation and anaemia

Acute and chronic inflammations are leading frontiers in the aetiology of anaemia which must be carefully studied (Rosenthal, 1997; Willoughby, 1977; Yip and Dallman, 1988). Infection with tuberculosis, brucellosis and amoebiasis is associated with anaemia. Mild transient infections, such as urinary tract infections, which are common in pregnancy, have been associated with haematological abnormality. The main form of anaemia associated with inflammatory conditions is normocytic normochromic anaemia due to the inhibition of erythropoiesis and failure of incorporation of iron into erythroblasts (Gaspar *et al.*, 1993).

Inflammatory conditions may be a source of error in diagnosing iron deficiency anaemia, as for example, serum ferritin, which is most commonly used to define iron deficiency, is raised in inflammation.

2.3.9.4 Pathogenesis of HIV Infection in anaemia in pregnancy

The higher seropositivity in antenatal clinics (between 6% and 30%) in some developing countries, has given a cause for concern. Infection with HIV has been included in the differential diagnosis of anaemia in pregnancy. A lot of haematological disturbances are associated with HIV infection (Costello, 1997; Davis and Zauli, 1995; Hoxie, 1995; Scadden *et al.*, 1989; Zon *et al.*, 1987; Zon and Groopman, 1988)

Although it is primarily characterized by a specific deficit in CD4T lymphocytes, other cytopenias such as neutropaenia, thrombocytopaenia and anaemia do occur. Anaemia has been reported in 70% to 92% of homosexual male patients with HIV infection and concurrent opportunistic infection (Hoxie, 1995; Mir *et al.*, 1989; Scadden *et al.*, 1989; Zon *et al.*, 1987). Abnormal haematopoiesis in HIV infection may be further exacerbated by antiviral agents, such as zidovudine or azidothymidine (Richman *et al.*, 1987)

Whereas some authors have suggested that anaemia occurs particularly in the later stages of HIV infection, others report that it may present the first opportunity to diagnose HIV infection (Fleming, 1989b; Street and Miliken, 1993)

In general the incidence of anaemia increases in frequency with progressive immunological deterioration and advancement of disease but isolated cytopenia may be the presenting manifestation of HIV infection unassociated with immune deterioration (Scadden *et al.*, 1989)

Only a few studies originate from developing countries where prevalence of asymptomatic HIV infection and anaemia in both children and pregnant women is high (Adewuyi and Chitsike, 1994; Bobat *et al.*, 1990; Fleming, 1989b). Adewuyi and Chitsike (1994) reported anaemia in 84% of 46 children with AIDS.

Fleming (1989a) described a case of severe anaemia and AIDS from Zambia and suggested that HIV infection should be included in the differential diagnosis of anaemia in pregnancy

The incidence and severity of the cytopoenia generally correlate to the stage of the disease with anaemia being the most commonly encountered haematologic abnormality and a significant predictor of progression to AIDS or death (Odunukwe *et al.*, 2005; Volberding, 2002). An obvious cause of anaemia in patients with HIV infection is blood loss which may be associated with conditions such as neoplastic disease e.g. Kaposi sarcoma in the gastrointestinal tract or gastrointestinal lesions that accompany opportunistic cytomegalovirus infection. Other than blood loss, the pathophysiology of HIV-associated anaemia may involve three basic mechanisms: namely decreased red blood cell (RBC) production, increased RBC destruction and ineffective RBC production.

2.3.9.4.1 Decreased RBC production

This may be a consequence of infiltration of the bone marrow by tumor (Sipsas *et al.*, 1999) or infection (Hambleton, 1996), use of myelosuppressive drugs, HIV infection, a decreased production of erythropoietin, or refractive response to erythropoietin (Spivak *et al.*, 1989) or hypogonadism (Dobs, 1998).

2.3.9.4.2 Increased RBC destruction (haemolysis)

Increased or premature RBC destruction in the spleen or the circulatory system may occur in patients with HIV infection. Haemolytic anaemia may result from RBC auto-antibodies or disseminated intravascular coagulation (Coyle, 1997). Haemolysis may also develop as a consequence of the use of various medications (Soriano *et al.*, 2002).

2.3.9.4.3 Ineffective RBC production

Anaemia may result from nutritional deficiencies – most commonly deficiencies in iron, folic acid or vitamin B12. In patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology. Vitamin B12 deficiency may result from malabsorption in the ileum or from gastric pathology caused by an array of infections or other conditions that affect the gastric mucosa in HIV-infected patients (Harriman *et al.*, 1989). Some risk factors currently associated with anaemia in HIV infection are: history of clinical AIDS, CD4 cell count <200 cells mm⁻³, plasma viral load, sex (women), race (black), zidovudine use, increasing age, lower body mass index, history of bacterial pneumonia, oral candidiasis and history of fever (Levine *et al.*, 2001).

2.3.9.5 Haemoglobinopathies and G6PD deficiency in Anaemia

Sickle cell disease (SCD) has a prevalence rate of about (1-2%) among infants in sub-Saharan Africa which normally results in sickle cell anaemia due to hemolytic crisis. Other hemoglobinopathies such as haemoglobin SC (HbSC) and β -thalassaemia are also prevalent which predisposes individuals to the development of anaemia. Majority of these patients are able to reach reproductive age and are able to conceive due to the improvement in health care delivery (Rust and Perry, 1995). Normocytic normochromic anaemia with haemoglobin values (6 to 9 g/dL) are the commonest form seen among these individuals (Letsky, 1995a)

Sickling crises may be precipitated by hypoxia, acidosis or dehydration and may be life threatening (Fleming, 1989b). Outcome for both mother and baby will improve with intensive antenatal supervision and aggressive treatment including blood transfusion after crises resulting in severe anaemia. Sickle cell trait is not normally associated with anaemia, although crises can occur in exceptional circumstances and in the postpartum period (Letsky, 1995b; Pastorek and Seiler, 1985)

The patterns of red cell changes in α -thalassaemia have been well described in a study from Vanuatu (Williams *et al.*, 1996). A mild hypochromic microcytic anaemia is seen in both the homozygous state for α^+ thalassaemia ($\sim\alpha/-\alpha$) and the heterozygous state for α^0 thalassaemia. This must not be confused with iron deficiency anaemia (Bowden *et al.*, 1985)

G6PD deficiency, the commonest red blood cell enzymopathy can result in a shortened red cell lifespan and haemolytic anaemia which may be severe. Administration of certain drugs such as sulfadoxine pyrimethamine (S.P) and other sulfur containing drugs as antimalarial prophylaxis during pregnancy further predisposes individuals with G6PD deficiency to haemolytic crisis. Haemolysis induced by infection is also a major contributing factor in G6PD deficient pregnant women (Bowden *et al.*, 1985)

2.3.10 Miscellaneous causes of anaemia in pregnancy

The less common aetiological agents of anaemia exist in both industrialized and developing countries but can be detected in the developed world than the developing countries due to advance technologies. This point is vindicated by a study by Bates and co-workers in Ghana where splenic lymphoma with villous lymphocytes was described in association with anaemia (Bates *et al.*, 1992). There are several of such conditions that are often wrongly classified as hyperreactive malarial splenomegaly (tropical splenomegaly syndrome) (Bates, 1991; Hamilton *et al.*, 1966; Marsden *et al.*, 1965).

2.4 SPONTANEOUS ABORTION, AN OBSTETRICS COMPLICATION

Spontaneous abortion is the termination of pregnancy (expulsion of foetus) before 20 completed gestational weeks from the last menstrual period, or less than 500 g fetal weight (WHO, 1977)

The rate of spontaneous abortion among North American women is about 15%– 20% and prevalence varies in different age categories, 15% in women younger than 25 years and increases to 35% in women older than 38 years (Tallia *et al.*, 2001). Wilcox *et al.* conducted a prospective study of 221 women who were trying to conceive. The rate of spontaneous abortion was 12% among clinically diagnosed pregnancies. Another of his study also demonstrated a 12% rate of spontaneous abortion among clinically diagnosed pregnancies (Wilcox *et al.*, 1988).

2.4.1 Types of Spontaneous abortion

There are two types of spontaneous abortion: sporadic and recurrent. Classically, recurrent pregnancy loss is defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation. Although chromosomal abnormalities are the cause of about 50% of all spontaneous abortions, the remaining 50% of the causes needs to be researched into and identified (Cramer and Wise, 2000)

Stirrat identified recurrent abortion affects about 1% of couples (Stirrat, 1990) and its highly frustrating for both patients and physicians. Recurrent spontaneous abortion occurs even if the fetus has a normal chromosome complement (Stirrat, 1990; Sullivan *et al.*, 2004)

Unlike recurrent abortion, sporadic abortions usually occur due to random fetal chromosomal abnormalities. At least 25% or as many as 50% of all women will experience one or more in their reproductive age (Greenwold and Jauniaux, 2002; Stephenson *et al.*, 2002; Stirrat, 1990), with the risk increasing with increasing maternal age (Regan and Rai, 2000)

2.4.2 Etiology of spontaneous abortion

The etiology of recurrent pregnancy loss includes chromosomal abnormalities, uterine anatomic anomalies, immunologic disorders such as antiphospholipid antibody syndrome, clotting disorders such as hyperhomocystinemia, infe

ctious diseases, endocrinopathies and sperm DNA fragmentation (Cramer and Wise, 2000)

The human placenta is hemochorial and successful pregnancy requires the development of an adequate uteroplacental circulation. Many studies have demonstrated that the maternal arterial circulation is established in the placenta by 10–12 weeks of gestation (Hustin and Schaaps, 1987; Jaffe and Woods, 1993; Jauniaux *et al.*, 2000; Kliman, 2000b). Defective placentatal development is implicated in the pathophysiology of early pregnancy loss (Graham *et al.*, 2000).

Sacks *et al.* demonstrated that abnormal placentation leads to placental oxidative stress and syncytiotrophoblast dysfunction (the outer cellular portion of the developing embryo that forms the placenta), and it has been linked as a cause of early abortion. Pregnancy has been characterized as an inflammatory state with the leukocytes showing changes similar to those seen in sepsis (Sacks *et al.*, 1998). Early pregnancy is characterized by a rise in the peripheral polymorphonuclear leukocyte counts. Increased generation of superoxide radicals from activated polymorphonuclear leukocytes has been demonstrated during early pregnancy (Fait *et al.*, 2005). Increased generation of reactive oxygen species was demonstrated in leukocytes by significantly higher levels of granulocyte spontaneous chemiluminescence in the recurrent abortion patients compared to a control group of healthy women (Safronova *et al.*, 2003).

A disruption of the balance between the prooxidant and antioxidant factors may occur in patients with recurrent abortions. An increase in the scavenging of oxygen radicals by antioxidants results in a decrease or depletion of cellular antioxidant levels.

Decreased concentrations of plasma ascorbic acid, α -tocopherol, total thiols, and erythrocyte reduced glutathione (GSH) in patients with unexplained

recurrent pregnancy loss or in patients with autoimmune or luteal phase insufficiency has been described (Vural *et al.*, 2000). However a study by Nicotra *et al* demonstrated no significant differences in plasma levels of triglycerides, cholesterol, cholesterol esters, phospholipids, lipoperoxides, vitamin E, and erythrocyte glutathione peroxidase activity in women with recurrent abortion compared with controls (Nicotra *et al.*, 1994)

Jauniaux *et al* suggested that early pregnancy loss may also result from premature oxygenation of the early embryonic environment (Jauniaux *et al.*, 2000).

2.4.3 G6PD Deficiency, oxidative stress and Spontaneous abortion

Oxidative stress as a result of Glucose-6-Phosphate Dehydrogenase enzyme deficiency and loss of other antioxidant defenses have been implicated and shown to be associated with recurrent pregnancy loss (Simsek *et al.*, 1998)

Oxygen is useful for sustaining life in cells, it undergoes extensive metabolism that can result in the production of toxic derivatives in the electron transport chain in the mitochondria during the generation of ATP. The end products of oxygen metabolism may include molecules in an activated electronic state with unpaired electrons that are highly reactive with molecules found in biological systems. These activated derivatives are referred to as reactive oxygen species (ROS) (Halliwell and Gutteridge, 1988). ROS extensively damage cellular organelles including the mitochondria, nuclear and mitochondrial DNA, and cell membrane, leading to failure to repair and subsequently, cell death (Kowaltowski and Vercesi, 1999; Pierce *et al.*, 1991; Ronnenberg *et al.*, 2002). To prevent ROS-induced damage, cells have developed its own antioxidant systems. As a result, there is a delicate balance between ROS production and antioxidant activity that maintains a physiologic balance leading to cellular homeostasis. When this fine balance is breached by an excess ROS production,

oxidative stress results leading to cell damage and cell dysfunction (Agarwal and

Allamaneni, 2004; Agarwal *et al.*, 2005)

Markers of ROS-induced membrane damage such as lipid peroxidation products, has been found in higher levels immediately before abortion implicating oxidative stress in spontaneous abortion (Sane *et al.*, 1991)



Chapter 3**MATERIALS AND METHODS****3.1 STUDY SITE**

This cross sectional study was carried out at the Tamale Teaching Hospital (TTH), Tamale in the Northern region of Ghana from March 2014 to October 2015.

3.2 STUDY SUBJECTS AND ELIGIBILITY CRITERIA

A total of 248 pregnant women between 16 and 42 years of age, who visited the TTH for antenatal care were eligible for recruitment into this study. The pregnant women were classified into three subgroups according to the stage of pregnancy (gestational age), thus, first, second and third trimester. The gestational age was estimated from an interview based on the date of last normal menstruation period (LNMP), measurement of fundal height (symphysiofundal height) and results of their first trimester ultrasound scan.

Pregnant women with known medical conditions such renal disorders, hypertension, diabetes, diagnosed complicated pregnancies were excluded from this study. Informed consent was obtained from all the study participants.

Ethical approval for the study was obtained from the Tamale Teaching Hospital (TTH) and the Committee on Human Research, Publication and Ethics (CHRPE/KNUST-SMS/KATH) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.3 SAMPLE SIZE DETERMINATION

Materials and Methods

The target group of this study was pregnant women between ages 16 and 42 who visited TTH for antenatal care in the Tamale metropolis. The minimum sample size for the study was calculated to be 275 pregnant women, using 76.6% prevalence rate of anaemia among pregnant women per the study of Bondevik and co-workers (Bondevik et al, 2001), with an expected difference of 5% between the sample and the general population and a type I error (α) of 0.05

$$n = \frac{z^2(1-p)p}{d^2}$$

Where n = minimum sample size; Z = standard normal variance = 1.96 to obtain 95% confidence interval, (β = 5%) and a type 1 error probability of 5%; d = Absolute standard error = 0.05; p = prevalence = 76.6%.

Using the above formula, the calculated sample size was approximately 275. Three hundred (300) participants were therefore recruited initially for the study

Out of the 300 pregnant women who were recruited into this study, 52 of them had incomplete data during the data entry process and were excluded, leaving 248 participants with complete data set (from obstetrics history to laboratory data).

3.4 DATA COLLECTION

3.4.1 Questionnaire and obstetrics history retrieval from ANC folder

Questionnaires were administered to the study participants to obtain information about their socio-demographic characteristics such as age, occupation, educational level, religion, hometown/region, tribe etc. The questionnaire also captured information such as the last menstrual period (LMP), haematinic intake, appetite for food, history of regular antenatal visit, alcohol intake and past medical history.

Materials and Methods

Obstetrics history such as gravidae , parity, history of abortion (spontaneous or induced) was retrieved from the antenatal record booklet.

3.4.2 SAMPLE COLLECTION AND PREPARATION

Two separate volumes of 6 ml of venous blood each was collected from the antecubital vein of each patient under aseptic conditions. A volume of 4 ml of the blood sample was dispensed into vacutainer plain-gel tube and allowed to clot. The sample was then centrifuged at 500 RPM for 10 minutes and the serum separated and aliquoted into cryotubes and stored at -80°C. The serum was used for biochemical analysis.

The other 8 ml of blood sample was dispensed into an ethylene diamine tetraacetic acid (EDTA) anticoagulated tube (2ml and 6ml respectively). The 2ml of blood in the EDTA tube was used for other haematological analysis and the 6ml for G6PD Methaemoglobin reduction test.

3.5 LABORATORY ASSAY

3.5.1 HAEMATOLOGICAL ANALYSIS

The EDTA anticoagulated blood was analyzed using a fully automated blood cell counter; Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) for the determination of haemoglobin concentration, haematocrit (HCT); red blood cell counts (RBC), mean corpuscular volume (MCV), mean haemoglobin concentration (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW) and other haematological parameters.

The Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) is an automated, multi-parameter haematology analyzer which is designed to classify formed elements of EDTA-anticoagulated blood:

3.5.1.1 Sample Analysis Cycle Overview

Aspiration – the Sysmex XE -2100 aspirates approximately 30 μL of whole blood for the analysis.

WBC Measurement Process

A dilution is delivered to the WBC chamber where it is mixed with 1.0 mL of WBC lysing reagent. The WBC count is obtained by impedance as they pass through the aperture of a transducer.

RBC/PLT Measurement

An electrical impedance method is used to obtain red blood cell and platelets data by generation of pulses. If the pulse produced is above the platelet lower threshold, the pulse is counted as a platelet. On the other hand, if the pulse is greater than the lower threshold, the pulse is counted as Red blood cells.

3.5.1.2 RBC Count

The RBC count is determined directly and absolute numbers determined.

3.5.1.3 Mean Cell Volume (MCV)

The Mean Cell Volume (MCV) is the average volume of all the RBCs and it is derived from the RBC size-distribution data. MCV is reported in femtoliters (fL).

3.5.1.4 Haematocrit (HCT)

Haematocrit is the ratio of RBCs to plasma. The HCT is calculated from the RBC count and the MCV

3.5.1.5 Mean Cell Haemoglobin (MCH)

The Mean Cell Haemoglobin (MCH) is the average amount of haemoglobin contained in the RBC. The MCH is calculated as haemoglobin concentration divided by Red blood cell count.

3.5.1.6 Mean Cell Haemoglobin Concentration (MCHC)

The Mean Cell Haemoglobin Concentration (MCHC) is the ratio of hemoglobin to the pack cell volume (PCV) or hematocrit. MCHC is calculated from hemoglobin concentration divided by PCV

3.5.1.7 PLT Count

The platelet count is obtained from the platelet histogram after the data have been analyzed by the platelet algorithm.

3.5.1.8 Haemoglobin Measurement

Haemoglobin measurement is obtained by a modified methaemoglobin method. A portion of the lysed, diluted sample from the WBC mixing chamber is used for HGB measurement. A low-energy light-emitting diode (LED) is used as the light source and shines through the HGB flow cell at 540 nm absorbance filter membrane. The haemoglobin concentration is directly proportional to the absorbance of the sample

3.5.2 PREPARATION OF BLOOD FILM FOR MALARIA PARASITES

Microscopy is the gold standard by which the various parasitic stages of plasmodium in blood films were identified. Thick blood film was prepared by placing a small drop of blood collected in the EDTA tube by the use of a small plastic bulb pipette. The drop of blood was then dispensed at the centre of a sterilized, grease free glass slide. Without delay, the drop of blood was spread

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to make the thick smear covering evenly an area about 15 × 15 mm. The film was prepared in such a way that it was possible to see through but cannot read writings or prints through. It was then labeled with laboratory number using a lead pencil. The film was then allowed to air dry by placing in a horizontal position and a safe place. After the slide was air-dried, it was then stained with (10%) Giemsa stain (BDH Laboratory Supplies, Poole BH15 ITD, England) for a period of 10 minutes. The slides were then placed in a shallow tray supported on two rods, in a staining rack for immersion in a staining trough. The diluted stain was then poured into the staining trough and the slides were then stained for 10 minutes using a 10% Giemsa solution. The slides were then washed with distilled water and left to dry at room temperature. When the thick film was completely dried, a drop of immersion oil was applied to an area of the film which was well stained around the edges and oil spread to cover an area 10 mm in diameter. The slide was then mounted on a light microscope to examine for malaria parasites and malaria pigment using 100 × objectives. At least 100 high power microscope fields were examined for malaria parasites. Approximate numbers of parasites (trophozoites, schizonts, and gametocytes) were reported as present and quantified using the following plus sign scheme where the number of pluses correlates with the severity of the infection (Cheesbrough, 2006).

Parasites

1-10 per 100 high power fields +

11-100 per 100 high power fields ++

1-10 in every high power field +++

More than 10 in every high power field. ++++ (Cheesbrough, 2006)

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The thin blood film was prepared by placing a drop of blood on one end of a clean, grease free dry slide for blood film comment (Red Cell morphology) and malaria parasite species identification. Using a clean smooth edged spreader, the spreader was drawn backwards to touch the drop of blood and allow the blood to extend along the edge of the spreader. The spreader was held at an angle of about 30° to make a film of about 40–50 mm in length equivalent to two thirds of the slide. The thin blood film was air-dried by waving the slide back and forth protecting it from dust and insects. The film was then fixed in absolute alcohol by dipping for few seconds. The alcohol-fixed blood smear was then stained with Leishman stain for 10 minutes. The stain was then washed off with running tap water; back of the slide wiped and allowed to stand in a draining rack for the smear to dry. It was then observed under light microscope using 100× objective lens for blood cell morphology and malaria parasites (Cheesbrough, 2006)

3.5.3 Methaemoglobin reduction test for G6PD Screening

3.5.3.1 Principle of test

Haemoglobin is oxidized to methaemoglobin by an oxidizing reagent, sodium nitrite. A redox reagent, methylene blue acts to catalyse and set in action the pentose phosphate pathway causing an enzyme to reverse the methaemoglobin back to haemoglobin in red blood cells with no G6PD defect. In G6PD deficient red blood cells, there is no reverse conversion of methaemoglobin to haemoglobin.

6 ml of EDTA blood samples were divided into 3 different glass test tubes and labeled Test, Negative and Positive control. In each of the 3 test tubes, 2 ml of blood was added. 0.1 ml of freshly prepared sodium nitrite, 0.1ml Methylene blue reagents were added into the tube labeled as Test and 0.1 ml of sodium nitrate into the test tube marked as Positive control. 2mls of blood was added

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to the test tube labeled as Negative control without any reagent. The 3 tubes were corked with cotton wool with gentle mixing. All the three tubes (Test, Normal and Positive control) were incubated in a waterbath at a temperature of 35 to 37 °C for 1 hour 30 minutes as described in Monica Cheesbrough, District Laboratory Practice in Tropical Countries. The final test was read by qualitatively observing and comparing the colour change of the solution in each tube. The test was interpreted as follows; colour of test solution is similar to the red colour of the "Negative tube" indicates normal G6PD activity. Colour of test solution is similar to the brown colour of the "Positive control tube" is suggestive of reduced G6PD activity and G6PD deficiency in homozygote individuals (Cheesbrough, 2006).

3.5.4 Sickle Cell slide test for Sickling status

3.5.4.1 Test principle

Two drops of fresh blood is mixed on a clean slide with a reducing agent such as sodium metabisulphite and covered with a cover glass. It is then incubated at room temperature for about 30 minutes to an hour making sure the mixture does not dry up. The sodium metabisulphite reduces the oxygen tension of the red blood cells and cells containing haemoglobin SC (HbS) sickle.

Negative and positive controls were set up by delivering a drop of blood from a person that does not have a sickle cell disorder and a person with a known sickle cell trait respectively. Equal volumes of sodium metabisulphite was added, mixed and incubated. It was then examined microscopically after 15 minutes using the 40x objective lens for fine details.

3.5.5 EXAMINATION OF STOOL FOR PARASITES

Stool samples were collected, examined for intestinal parasites using accepted techniques. 6mls of normal saline was added to a small portion of stool and emulsified. A drop of the mixture was then transferred on a glass slide and covered with a cover slip to be examined under the microscope for parasitic eggs or trophozoites. No ova, protozoa or larvae seen on examination (Cheesbrough, 2006).

3.5.6 BIOCHEMICAL ANALYSIS

The serum was analyzed for the determination of iron, transferrin, total Iron Binding Capacity (TIBC), transferrin receptor, Ferritin, Vitamin B12 and Folate.

Serum iron concentration, Transferrin, TIBC were measured by colorimetric and immunoturbidimetric methods respectively, using Elitech Clinical Systems (France) and Fortress Diagnostics (UK) assay kits respectively and The Vitalab Selectra E Clinical Chemistry analyser (Vital Scientific, The Netherlands) to perform the analysis.

3.5.6.1 Serum Iron measurement

Serum Iron was determined using Ferrozine without deproteinization method and by the use of The Vitalab Selectra E Clinical Chemistry autoanalyser (Vital Scientific, The Netherlands) to perform the analysis.

Reagents, calibrators (ELICAL 2) and controls (ELITROL I and II) were obtained from Elitech Diagnostics (Sees, France).

Chemical reactions occurred between serum Iron, Chomazurol B and cetyltrimethyl ammonium bromide to form a coloured compound. The

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intensity of the colour is proportional to the iron concentration which was measured by the autoanalyser.

Serum iron is considerably influenced by acute phase response in inflammatory or infectious conditions and also has diurnal variability, masking the detection of iron deficiency (Burns *et al.*, 1990b; Cazzola and Beguin, 1992; Harju *et al.*, 1984).

3.5.6.2 Transferrin measurement

Transferrin concentration was assayed using the immunological agglutination principle (immunoturbidimetric assay) described in the reagents insert from Fortress diagnostics (UK) catalogue number BXC0741A and using The Vitalab Selectra E Clinical Chemistry analyser (Vital Scientific, The Netherlands) to perform the analysis

3.5.6.2.1 Test principle

This is Immunoturbidimetric assay

The autoanalyser uses 2UI of serum sample and add 250UI of reagents buffer (R1) containing (phosphate buffered saline, polyethylene glycol, sodium azide).

50UI of anti-transferrin antibody reagent is added to the mixture, which then reacts with the antigen in the sample to form an antibody/ antigen complex. This is measured at an absorbance of 340nm after 5minutes of incubation.

3.5.6.3 Total Iron Binding capacity (TIBC) measurement

TIBC was assayed using the Colorimetric method described in the reagents insert from Fortress diagnostics (UK) catalogue number BXC0233. Analysis was performed using The Vitalab Selectra E Clinical Chemistry analyser (Vital Scientific, The Netherlands).

3.5.6.3.1 Test Principle

This is Colorimetric method

Excess iron is added to serum in order to saturate serum transferrin.

Basic magnesium carbonate precipitates the unbound iron. The level of iron in the supernatant is determined once the solution is centrifuged at 3000 RPM for 10 minutes.

3.5.6.3.2 Assay procedure

3.5.6.3.2.1 SAMPLE PREPARATION FOR THE AUTOANALYSER

The sample was manually prepared before being introduced to the autoanalyser for analysis

0.5 ml of serum was added to 1.0 ml of Iron solution.

It was then mixed and allowed to stand for 5-30 minutes at 20-25 °C. About 180mg equivalent to one spatula full of basic magnesium carbonate was added to the mixture, mixed and allowed to stand for 30 minutes to 1 hour at 20-25 °C with frequent mixing. The mixture was then centrifuged for 10 minutes at 3000 RPM and supernatant removed for analysis.

The supernatant was then analysed with The Vitalab Selectra E Clinical Chemistry analyser (Vital Scientific, The Netherlands) using Iron reagent.

Transferrin Saturation (TSAT), Transferrin/log(Transferrin) ratio, Transferrin receptor log(Transferrin) ratio (TRF -F index) were determined using an established formulars.

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From determined formulars provided in the assay kit and other scientific publications, TSAT%, Transferrin /log (Ferritin) ratio, TfR/log (Ferr) were calculated for each of the specimens using the relationship:

$$\text{TSAT (\%)} = \frac{\text{Serum iron } (\mu\text{mol/L}) \times 100}{\text{TIBC } (\mu\text{mol/L})}$$

Transferrin /log (Ferritin) ratio (Castel et al., 2012) shall be calculated as;

$$\text{Tf/log (Ferr)} = \frac{\text{Transferrin (g/L)}}{\text{Log (Ferritin) } (\mu\text{g/L})}$$

Transferrin receptor/ log (Ferritin) ratio (Punnonen et al., 1997) calculated as;

$$\text{TRf-F index} = \frac{\text{Transferrin receptor } (\mu\text{g/L})}{\text{Log (Ferritin) } (\mu\text{g/L})}$$

3.5.7 ELISA ASSAYS

A solid phase sandwich enzyme-linked immunosorbent assay (an immunoenzymometric sequential assay) was used to determine Serum Folic acid, Serum Ferritin; Serum Vitamin B12 levels using ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) at the Noguchi Memorial and Medical Research Centre, Department of Immunology, University of Ghana, Legon-Accra.

3.5.7.1 FERRITIN MEASUREMENT

Ferritin was determined using commercially prepared Ferritin Quantitative test kit based on a solid phase enzyme-linked immune sorbent assay from

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Elabscience Biotechnology (Wuhan, Hubei Province, China). The assay system utilizes one anti-Ferritin antibody for solid phase (micro titre wells) immobilization and another mouse monoclonal anti-Ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample was allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate was then added, resulting in the sandwich formation of Ferritin between solid phase and conjugated antibodies. After second wash step a solution of chromogen-substrate was added and incubated for 15 minutes, resulted in the development of a blue color. The colour development was stopped with the addition of stop solution, and the colour was changed to yellow and measured spectrophotometrically at 450nm using ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). The concentration of Ferritin is directly proportional to the colour intensity (optical density, OD) of the test sample, which was calculated by comparing the OD of the samples (test) to the standard curve.

3.5.7.2 HUMAN SOLUBLE TRANSFERRIN RECEPTOR (sTfR)/CD71 MEASUREMENT

Soluble Transferrin Receptor (sTfR) was determined using Sandwich-ELISA method. The commercially available micro ELISA plate was obtained from Elabscience Biotechnology (Wuhan, Hubei Province, China), and was pre-coated with an antibody specific to TfR/CD71. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then biotinylated detection antibody specific for TfR/CD71 and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain TfR/CD71, biotinylated detection antibody and Avidin-HRP conjugate

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appeared blue in color. The enzyme substrate reaction was terminated by the addition of sulphuric acid solution and the colour turned yellow. The optical density (OD) was measured spectrophotometrically using ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) at a wavelength of 450nm±2nm. The OD value is proportional to the concentration of TfR/CD71, calculated by comparing the OD of the samples (test) to the standard curve.

3.5.7.3 FOLIC ACID (FA) ESTIMATION

Folic acid (FA/VB9) concentration was assayed using Competitive-ELISA technique. A commercially available microtitre plate was obtained from Elabscience Biotechnology (Wuhan, Hubei Province, China), already pre-coated with FA/VB9. During the reaction, FA/VB9 in the sample or standard competed with a fixed amount of FA/VB9 on a solid phase supporter for sites on the Biotinylated Detection Ab specific to FA/VB9. Excess conjugate and unbound sample or standard were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) were added to each microplate well and incubated. Then a substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm±2nm using ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). The concentration of FA/VB9 in the sample was then determined by comparing the OD of the samples to the standard curve.

3.5.7.4 VITAMIN B12 (VB12) MEASUREMENT

Vitamin B12 (VB12) concentration was determined using a Competitive-ELISA technique.

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The commercially available micro ELISA plate was obtained from Elabscience Biotechnology (Wuhan, Hubei Province, China), and was pre-coated with VB12. During the reaction, VB12 in the sample or in the standard competed with a fixed amount of VB12 on a solid phase supporter for sites on the Biotinylated Detection Ab specific to VB12. Excess conjugate and unbound sample or standard were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) were added to each microplate well and incubated. Then a substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of $450\text{nm} \pm 2\text{nm}$ using ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). The OD value is proportional to the concentration of VB12, calculated by comparing the OD of the samples (test) to the standard curve.

3.5.8 SEROLOGICAL DIAGNOSIS OF HIV, HBsAg and HCV

HIV, HBsAg, HCV were diagnosed using both the Rapid test kit HIV 1/2 and Oraquick Rapid HIV1/2 Antibody Test (Orasure Technologies, Inc, USA), HBsAg HCV rapid chromatographic test kits respectively. Double lines appearing at both test and control columns of the test kit indicates positive test, while a single line appearing at the control column shows negative test.

3.6 RATIONALE FOR THE VARIOUS ASSAYS

- Full blood count was determined to enable estimation of the prevalence of anaemia in the study population by the use of the Red blood cell indices (Haemoglobin concentration (Hb), Haematocrit (HCT)).
- Full blood count (MCV, MCH, MCHC) were used in conjunction with the thin film comment to classify the anaemia into the various types i.e. microcytic hypochromic anaemia, Normocytic normochromic anaemia or Macrocytic anaemia.

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- Some of the possible cause of Microcytic hypochromic anaemia (Iron deficiency anaemia (IDA), anaemia of chronic (ACD), were establish using Serum Iron, TIBC, Serum Transferrin receptor, Serum Ferritin, Transferrin/log (Ferritin) ratio, TRf-F index, Hb electrophoresis, Full blood count, and film comments in the thin film preparation.
- Transferrin/ Log (Ferritin) ratio was used in the diagnosis of IDA when classical markers of iron status (serum Iron, TIBC, Transferrin, TSAT% and Ferritin) give inconclusive results.
- TRf/log (Ferritin) index enabled the diagnosis and differentiation of IDA and anaemia of chronic disease (ACD)
- Possible cause of normocytic normochromic anaemia were established using G6PD deficiency test, sickling test, Hb electrophoresis for sickle cell disease (i.e. congenital cause of haemolytic anaemia), thick film for malaria, stool examination for parasitic infection as well as HIV, HBsAg, HCV test (infectious cause of haemolytic anaemia).
- The various cause of Macrocytic anaemia (i.e. megaloblastic anaemia) was determined using serum Vitamin B12, serum Folate.

3.7 STATISTICAL ANALYSIS

The data was analysed by the use of sigma plot for windows version 11.0 systat, Inc. Germany, Microsoft Excel and GraphPad Prizm version 5.0, San Diego California, USA (GraphPad software, San Diego California USA, www.graphpad.com). Continuous data are expressed as mean $\pm$ SEM whilst categorical data are expressed as proportions. In all the statistical analysis, a value of $p < 0.05$ was considered significant. Unpaired t-test was used to compare the means.

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Association between variables was determined with linear and logistic regression analysis. The differences between means of more than two groups were tested by performing one-way ANOVA

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Chapter 4

RESULTS

4.1 BIOCHEMICAL AND HEMATOLOGICAL PROFILE OF STUDIED POPULATION

Two hundred and forty eight (248) pregnant women were recruited for this study with the mean age of the participants being 29.07 ± 5.262 . The mean $\pm$ SD for Serum Iron concentration, Total Iron Binding Capacity (TIBC), Vitamin B12, Folic acid concentration, soluble Transferrin receptor (sTRF), Serum ferritin, Transferrin (Tf), Transferrin Saturation (TSAT), TRf/log(F) and Tf/log(F) concentration were

16.37 ± 8.581 , 64.38 ± 20.08 , 30627 ± 15211 , 835.6 ± 437.4 , 130.6 ± 51.17 , 49.62 ± 38.05 , 2.818 ± 0.9034 , 29.93 ± 20.3 , 106.1 ± 266.4 and 2.233 ± 4.884 respectively as shown in table 4.1, whereas the mean $\pm$ SD for RBC, hemoglobin, hematocrit, MCV and MCH were 4.285 ± 0.6177 , 11.67 ± 1.773 , 33.68 ± 4.528 , 79.22 ± 8.165 , 28.15 ± 3.117 respectively as shown in table 4.1

The data was stratified into three (3) major categories; hematinic intake, anaemia and obstetrics history of spontaneous abortion

On hematinic Intake, the study participants (pregnant women) were further classified as non-hematinic (hematinic naïve) and hematinic

The group stratified as non-haematinic (hematinic naïve) are pregnant women in their early stage of pregnancy (early gestation period up to 13 weeks after the last menstrual period (LMP) where the foetus weighs approximately 13g and it is up to 8cm long (Oke et al, 2011), first antenatal visit in the current pregnancy and have not yet taken any hematinic, vitamin or mineral supplements.

They have also had several antenatal visits in that current pregnancy and are taken hematinic, vitamin or mineral supplements from the antenatal clinic

4.1.1 Biochemical and hematological variations based on Intake of hematinic

When the study participants were categorized based on intake of haematinics, the mean Vitamin B12 and hematocrit were significantly higher among hematinic naïve participants (33222 ± 16126 ; $p=0.0189$ and 34.33 ± 5.197 ; $p=0.0488$ for vitamin B12 and hematocrit respectively) compared to their counterparts on hematinic. Folic acid concentration was lower in the no hematinic than the hematinic group and levels were significant (954.7 ± 394.0 ; $p < 0.0001$) as shown in table s 4.1. The other parameters were not significantly different among the groups.



Results

Table 4.1 shows biochemical and hematological profile of study participants stratified by hematinic intake

Variable P Value	Total	No Hematinic	Hematinic	
Age		29.19 ±4.878		0.7583
Biochemical Profile				
Serum Iron Concentration	16.37±8.581	15.68 ±7.203	16.89 ±9.487	0.2737
TIBC	64.38±20.08	62.67 ±18.77	65.68 ±21.00	0.2442
Vitamin B12 (pmol/l)	30627±15211	33222 ±16126	28657 ±14221	0.0189
Folic Acid (nmol/L)	835.6±437.4	678.7 ± 443.9	954.7 ±394.0	< 0.0001
Soluble Transferrin Receptor (sTRf)	130.6±51.17	127.5 ±51.94	132.9 ±50.65	0.4104
Serum Ferritin	49.62±38.05	51.30 ±39.81	48.34 ± 36.75	0.5447
Transferrin (Tf) (g/L)	2.818±0.9034	2.744 ±0.8454	2.874 ±0.9441	0.262
Transferrin Saturation (TSAT %)	29.93±20.3	29.47 ±18.91	30.28 ±21.36	0.7569
sTRf/log(F)	106.1±266.4	89.44 ±53.00	118.8 ±350.4	0.3919
Tf/log(F)	2.233±4.884	1.876 ±1.013	2.503 ± 6.414	0.318
Haematological profile				
WBC	6.665±1.833	6.576 ±1.803	6.733 ±1.860	0.5054
RBC	4.285±0.6177	4.326 ±0.6891	4.254 ±0.5581	0.3621
Hb	11.67±1.773	11.88 ±1.927	11.50 ±1.634	0.0972
HCT	33.68±4.528	34.33 ±5.197	33.18 ±3.893	0.0488
MCV	79.22±8.165	80.01 ±8.540	78.61 ± 7.845	0.182
MCH	28.15±3.117	28.38 ±3.053	27.97 ±3.164	0.3032
MCHC	35.55±2.029	35.53 ±2.080	35.57 ±1.997	0.8786
PLT	237.3±88.69	246.0 ±72.61	230.7 ±98.91	0.1783
RDW-SD	48.04±40.2	44.84 ±33.54	50.48 ±44.60	0.2754
RDW-CV	14.33±2.748	14.50 ±2.415	14.19 ±2.978	0.3896

(n=248)		(n=141)
29.07 ± 5.262	(n=107)	28.98 ± 5.550

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Continuous data are presented as Mean $\pm$ SD and compared using unpaired t-test, Total iron binding capacity, TSAT: Transferrin saturation, sTfR/log(F): ratio of soluble Transferrin receptor concentration and logarithm of ferritin concentration Tf/log(F): ratio of Transferrin concentration and logarithm of ferritin concentration. WBC: White blood cells, Hb: haemoglobin concentration, HCT: haematocrit, RBC: red blood cell counts, MCV: mean corpuscular volume, MCH: mean haemoglobin concentration, MCHC: mean cell haemoglobin concentration, RDW: red cell distribution width

4.1.2 Prevalence of anaemia in studied population

The prevalence of anaemia among the study participants was 33.87% when haemoglobin concentration was used as the criteria for determining anaemia, the prevalence was however 43.55%, when hematocrit was used. The prevalence of microcytic hypochromic anaemia and normocytic normochromic anaemia were 12.10% and 14.92% respectively. None of the pregnant women (0%) had macrocytic hypochromic anaemia. When the studied population was stratified based on intake of hematinics, the prevalence of anaemia was significantly higher in those who took hematinics (39.72%; $p=0.0256$ -using haemoglobin and 48.94%; $p=0.0495$ -using hematocrit) than in those who were haematinic-naïve (26.17% and 36.45% using haemoglobin and hematocrit respectively). The prevalence of microcytic hypochromic anaemia was also significantly higher in those who took haematinics (16.31%; $p=0.0194$) than in those who were not on haematinics (6.54%) as shown in figure 4.1.



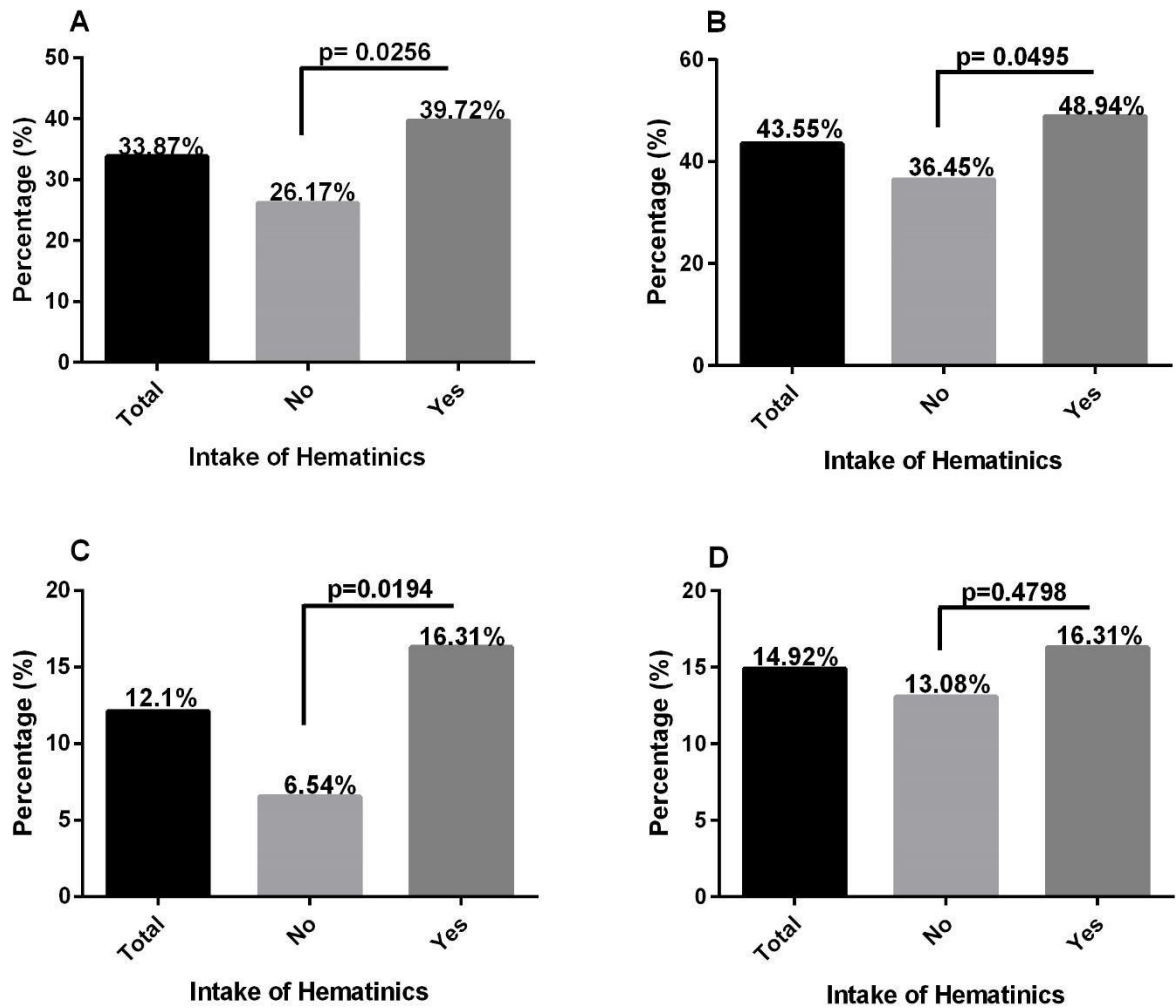


Figure 4.1: The prevalence of Anaemia using hemoglobin concentration (A), Anaemia using hematocrit (B), microcytic hypochromic anaemia (C) and normocytic normochromic anaemia (D) among the studied participants classified intake of hematinics

4.1.3 Prevalence of iron deficiency and iron deficiency anaemia

The prevalence of Iron deficiency and iron deficiency anaemia ranged from 16.94% to 77.42% and 7.26% to 27.82% respectively using various iron markers and hemoglobin concentration as criteria. Using the classical (conventional) iron markers (that is serum iron, Ferritin, Transferrin, TSAT, TIBC), Iron deficiency prevalence were 20.16%, 20.98%, 16.94%, 23.78% and 31.05% respectively. When the two modern indicators of Iron status were used (that is Transferrin/log Ferritin ratio and Transferrin receptor), prevalence of Iron deficiency were 50% and 77.42% respectively. Iron deficiency anaemia using the conventional Iron markers were 7.26%, 4.44%, 7.66%, 9.27% and 11.69% using serum iron, Ferritin, Transferrin, TSAT and TIBC as markers respectively. The modern indicators of iron status gave Iron deficiency anaemia prevalence as 16.13% and 27.87% respectively for Transferrin/log Ferritin ratio and Transferrin receptor. For most of the iron markers, there were no significant differences in iron deficiency and iron deficiency anaemia between those who were on haematinics and those who were not. However, the prevalence of iron deficiency anaemia was higher among those who were on haematinics (33.33%; $p=0.0262$) than those who were not (20.56%) when the transferrin receptor concentration was used with hemoglobin as a criterion for iron deficiency as shown in table 4.2 below.

Table 4.2: Prevalence of iron deficiency and iron deficiency anaemia using various markers of assessing iron status

Variable	Total (n=248)	No hematinic (n=107)	Hematinic (n=141)	P Value
Iron Deficiency Markers				
<i>Serum Iron</i>	50(20.16)	23(21.50)	27(19.15)	0.6483
<i>Ferritin</i>	52(20.98)	23(21.50)	29(20.57)	0.8589
<i>Transferrin</i>	42(16.94)	20(18.69)	22(15.60)	0.5207
<i>TSAT</i>	59(23.79)	26(24.30)	33(23.40)	0.8698
<i>TIBC</i>	77(31.05)	32(29.91)	45(31.92)	0.7349
<i>Tf/log ferr</i>	124(50.00)	50(46.73)	74(52.48)	0.3695
<i>Transferrin receptor</i>	192(77.42)	82(76.64)	110(78.0)	0.797
IDA Markers				
<i>Serum Iron</i>	18(7.26)	8(7.48)	10(7.09)	0.908
<i>Ferritin</i>	11(4.44)	3(2.80)	8(5.67)	0.2769
<i>Transferrin</i>	19(7.66)	6(5.61)	13(9.22)	0.2895
<i>TSAT</i>	23(9.27)	9(8.41)	14(9.93)	0.6832
<i>TIBC</i>	29(11.69)	10(9.35)	19(13.48)	0.3162
<i>Tf/log ferr</i>	40(16.13)	12(11.21)	28(19.86)	0.7434
<i>Transferrin receptor</i>	69(27.82)	22(20.56)	47(33.33)	0.0262

Categorical data are presented as proportion and compared using chi-square.

4.1.4 Biochemical and hematological variations in anaemia

When the study population was stratified based on anaemia using haemoglobin, the variations in the biochemical parameters and some haematological parameters between those with anaemia and those without anaemia were not significantly different, even though most of them were elevated in those with anaemia compared to those without anaemia (Tables 4 and 5). RBCs, haematocrit, MCH and MCHC were however significantly ($p < 0.0001$, $p < 0.0001$, $p = 0.001$ and $p < 0.0001$ for RBCs, haematocrit, MCH and MCHC respectively) higher in pregnant women without (4.521±0.5425, 35.74±3.82, 28.61±2.54, 36.02±1.818 for RBCs, hematocrit, MCH and MCHC respectively) anaemia than those with (3.823±0.4817, 29.64±2.718, 27.24±3.871, 34.64±2.114 for RBCs, hematocrit, MCH and MCHC respectively) anemia as shown in Table

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Table 4.3: Biochemical profile stratified by presence or absence of anaemia

Variable	No Anaemia (n=164)	Anaemia (n=84)	P Value
Age (years)	29.4±5.392	28.42±4.963	0.163
Biochemical Profile			
Iron (μmol/L)	16.29±8.255	16.53±9.235	0.8324
TIBC (μmol/L)	64.3±19.57	64.53±21.17	0.9314
Vit B12 pmol/L	30475±15226	30923±15267	0.827
FA (nmol/L)	853±452.1	801.6±407.6	0.3821
sTRF/CD71) ng/mL,	128.4±52.41	134.9±48.69	0.3401
Serum Ferritin ng/mL,	47.56±36.94	53.64±40.06	0.2341
Transferrin (g/L)	2.822±0.8689	2.811±0.9726	0.9275
Transferrin Saturation (TSAT %)	29.85±20.37	30.08±20.28	0.9353
sTRf/logFer (ug/L)	115.9±326.4	87±38.79	0.4199
Transferrin/log(Ferr) g/L, ug/L	2.447±5.973	1.815±0.8182	0.3361
Haematological Profile			
RBC (x106/μL)	4.521±0.5425	3.823±0.4817	< 0.0001
HCT (%)	35.74±3.82	29.64±2.718	< 0.0001
MCV (fL)	79.6±7.602	78.48±9.169	0.3101
MCH (pg)	28.61±2.54	27.24±3.871	0.001
MCHC (g/dL)	36.02±1.818	34.64±2.114	< 0.0001
PLT (x103/μL)	241.2±80.05	229.7±103.6	0.3351

Continuous data are presented as Mean ± SD and compared using unpaired t-test

TIBC: Total Iron Binding Capacity, VitB12: Vitamin B12, FA: Folic acid, sTRF/CD71:

Soluble Transferrin Receptor, sTRf/log Fer: Soluble Transferrin Receptor over logarithm Ferritin ratio

Anaemia: hemoglobin<11g/dl or hematocrit<33% ((WHO, 1992)

KNUST

4.1.5 Routine laboratory investigations and obstetric profile of study participants stratified by anaemia

The study shows that, 3.63%, 12.90%, 17.34%, 12.90%, 20.97% and 2.02% of the studied population had hookworm infection, HIV, hepatitis C, hepatitis B, partial G6PD defect and full G6PD defect respectively. 14.11% of the studied population was sickling positive while 25.81% had malaria. More than half (56.85%) of the population were on haematinics with most of them attending antenatal care regularly. About 66% of the studied population had had spontaneous abortion in their previous pregnancies. The prevalence of HIV infection was higher in those with anaemia (19.04%; $p=0.0389$) than those without anaemia (9.76%). The proportion of pregnant women who were on haematinics was significantly higher in those with anaemia (66.67%; $p=0.0256$) than those without anaemia (51.83%). Among those who had appetite for food, more were anaemic (69.05%; $p=0.0198$) compared to those without anaemia (53.66%) as shown in table 4.4 below.

KNUST

Table 4.4 shows the routine Laboratory investigations and obstetric profile stratified by anaemia

Variable	Total	No Anaemia (n=164)	Anaemia (n=84)	P Value
Hookworm in Stool				
Yes	9(3.63)	5(3.05)	4(4.76)	0.4948
HIV (1 & 2)				
Reactive	32(12.90)	16(9.76)	16(19.04)	0.0389
HBsAg				
Reactive	32(12.90)	21(12.81)	11(13.1)	0.9485
HCV				
Reactive	43(17.34)	31(18.90)	12(14.29)	0.3634
G6PD				
Partial Defect	52(20.97)	33(20.12)	19(22.62)	0.6475
Full Defect	5(2.02)	3(1.83)	2(2.38)	0.7699
Sickling				
Positive	35(14.11)	26(15.85)	9(10.71)	0.2712
Malaria Parasites				
Present	64(25.81)	44(26.83)	20(23.81)	0.607

Parity				
Null	60(24.19)	38(23.17)	22(26.19)	0.5992
Uni	100(40.32)	71(43.29)	29(34.52)	0.1828
Multi	88(35.48)	55(33.54)	33(39.29)	0.3705
Gravida				
I	14(5.65)	7(4.27)	7(8.33)	0.1893
II	45(18.15)	32(19.51)	13(15.48)	0.4351
Multigravida	189(76.21)	125(76.22)	64(76.19)	0.9959
Spontaneous Abortion				
Yes	163(65.73)	110(67.07)	53(63.10)	0.898
Induced Abortion				
Yes	33(13.31)	25(15.24)	8(9.52)	0.2094
Regular Antenatal Attendance				
Yes	184(74.19)	122(74.39)	62(73.81)	0.9212
Hematinic Intake				
Yes	141(56.85)	85(51.83)	56(66.67)	0.0256
Appetite for Food				
Yes	146(58.87)	88(53.66)	58(69.05)	0.0198

Categorical data are presented as proportion and compared using chi-square

4.2 BIOCHEMICAL AND HEMATOLOGICAL VARIATIONS IN GESTATION PERIODS

There were no marked variations in almost all the biochemical and hematological profiles when the studied population was stratified based on gestation period. There were however significant variations in the folic acid concentrations and RDW-CV among the three gestation periods ($p < 0.0001$). The folic acid levels of women in their first trimester (503.2 ± 339.7) was lower as compared to those in their second trimester (986.1 ± 407.3) as well as those in their third trimester (1017 ± 362) as shown in table 4.5 and 4.6.

Table 4.5 shows the biochemical characteristics of study participants stratified by Gestation period

Variable	1st Trimester (n=83)	2nd Trimester (n=76)	3rd Trimester (n=89)	F value	P value
Age	29.8±4.355	29.46±6.281	28.06±4.973	F(2,245) =2.686	0.0702
Iron	15.98±8.315	15.56±8.544	17.42±8.844	F(2,245) =1.099	0.3348
TIBC	64.02±19.54	67.29±20.16	62.23±20.44	F(2,245) =1.324	0.268
vit B12 pmol/l	33910±17167	28601±13500	29295±14281	F(2,245) =2.997	0.0518
FA (nmol/L)	503.2±339.7	986.1±407.3*	1017±362†	F(2,245) = 50.65	< 0.0001
TRF/CD71) ng/mL, ug/L	130.5±49.5	126.7±54.97	134±49.67	F(2,245) = 0.4132	0.6620
Serum Ferritin ng/mL, ug/L	52.67±42.45	47.38±28.27	48.69±41.1	F(2,245) = 0.4236	0.6551
Transferrin(g/L)	2.796±0.8923	2.942±0.9135	2.733±0.9034	F(2,245) = 1.143	0.3206
Transferrin Saturation (TSAT %)	29.08±18	27.35±20.43	32.89±21.99	F(2,245) = 1.632	0.1976
TRf/logFer (ug/L)	92.14±54.97	86.89±44.78	135.5±439.7	F(2,245) = 0.8538	0.4270
Transferrin/log(Ferr) g/L,ug/L	1.98±1.277	2.006±1.112	2.662±8.006	F(2,245) = 0.5347	0.5865

Continuous data are presented as Mean ± SD and compared using anova

* comparing folic acid concentrations in 1st and 2nd Trimesters of gestation and the difference is significant

† comparing folic acid concentrations in 1st and 3rd Trimesters of gestation and the difference is significant

Table 4.6 shows the haematological characteristics of pregnant women by Gestation period

Variable	1st Trimester (n=83)	2nd Trimester (n=76)	3rd Trimester (n=89)	P Value	Variable
<i>WBC</i>	6.714±1.872	6.781±1.774	6.52±1.858	F(2,245) = 0.4603	0.6316
<i>RBC</i>	4.276±0.7041	4.377±0.5793	4.214±0.5573	F(2,245) = 1.444	0.2379
<i>Hb</i>	11.71±2.133	11.77±1.423	11.54±1.682	F(2,245) = 0.3792	0.6848
<i>HCT</i>	33.8±5.588	33.87±3.618	33.39±4.143	F(2,245) = 0.2749	0.7599
<i>MCV</i>	79.71±8.123	78.18±9.146	79.64±7.28	F(2,245) = 0.8808	0.4158
<i>MCH</i>	28.3±3.006	27.83±3.304	28.28±3.077	F(2,245) = 0.5692	0.5667
<i>MCHC</i>	35.54±2.128	35.64±2.121	35.49±1.868	F(2,245) = 0.1238	0.8836
<i>PLT</i>	239.6±74.03	242.4±83.88	230.7±104.4	F(2,245) = 0.3969	0.6728
<i>RDW-SD</i>	46.23±37.98	46.71±34.51	50.85±46.48	F(2,245) = 0.3406	0.7117

RDW-CV	14.57±2.645	14.75±3.094	13.74±2.441#	F(2,245) = 3.322	0.0377
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Continuous data are presented as Mean ± SD and compared using anova



4.2.1 Prevalence of abnormalities in routine ANC laboratory investigations and obstetric profile stratified by gestation period

The prevalence of malaria significantly ($p=0.0441$) declined from the 1st trimester (32.53%) to 2nd trimester (26.31%) and then to 3rd trimester (19.10%). However the distribution of pregnant women who attended antenatal care regularly as well as those who were on haematinics, increased significantly ($p< 0.0001$ and $p< 0.0001$ for regular antenatal attendance and intake of haematinics respectively) from 1st trimester (54.22%- regular antenatal attendance and 18.07% for intake of hematinics) to 2nd trimester (72.37%- regular antenatal attendance and 59.21% for intake of hematinics) to 3rd trimester (94.38%- regular antenatal attendance and 91.01% for intake of hematinics) as shown in table 4.7.

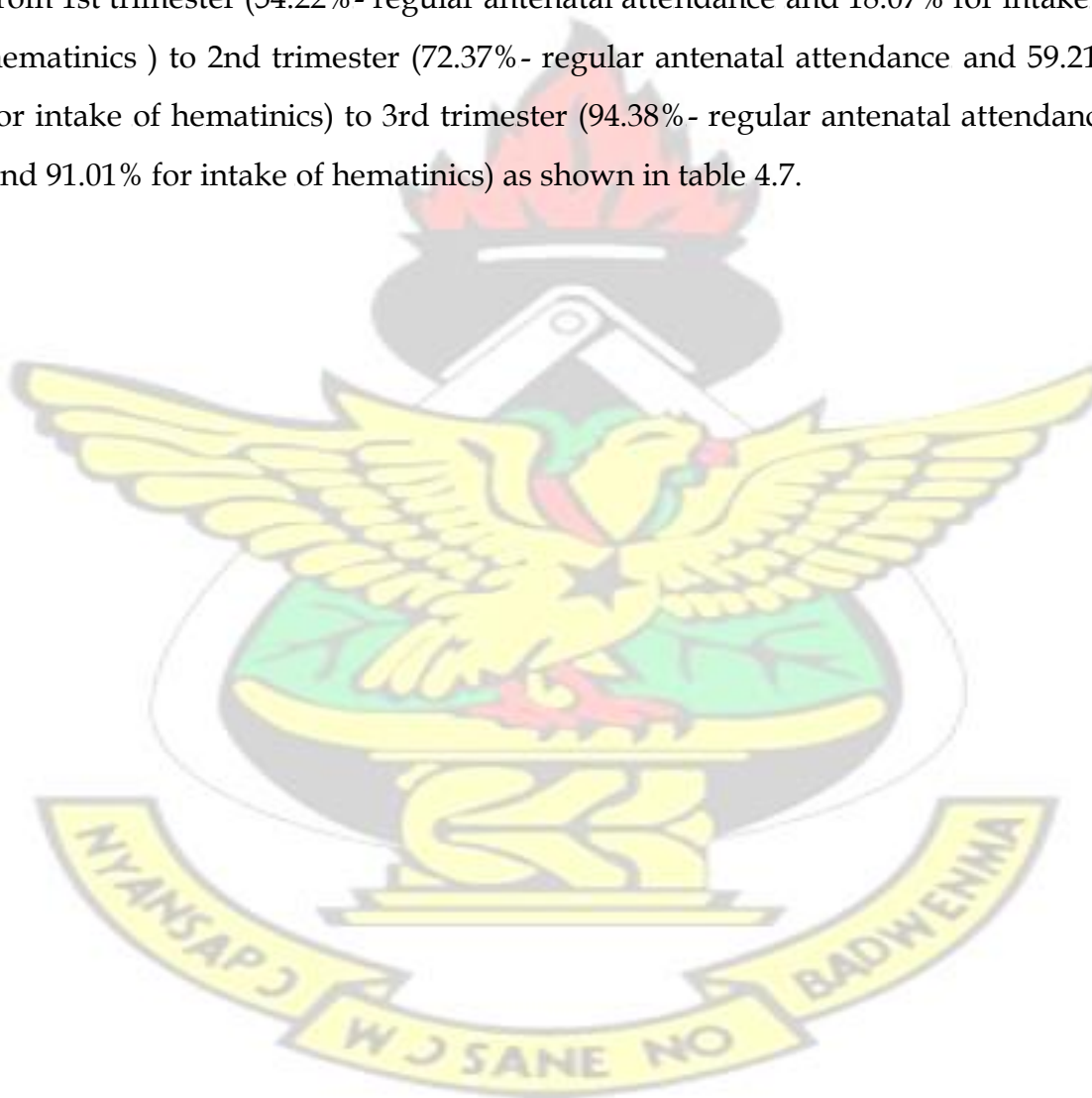


Table 4.7 shows Routine ANC and obstetric characteristics stratified by gestation period

Variable	1st Trimester (n=83)	2nd Trimester (n=76)	3rd Trimester (n=89)	P Value
Hookworm in Stool				
Yes	3(3.61)	4(5.26)	2(2.25)	0.6194
HIV (1 & 2)				
Reactive	14(16.87)	9(11.84)	9(10.11)	0.1889
HBsAg				
Reactive	11(13.25)	8(10.53)	13(14.61)	0.7803
HCV				
Reactive	17(20.48)	17(22.37)	9(10.11)	0.0685
G6PD				
Partial Defect	12(14.46)	21(27.63)	19(21.35)	0.2819
Full Defect	1(1.20)	2(2.63)	2(2.25)	0.6333
Sickling				
Positive	9(10.84)	11(14.47)	15(16.85)	0.2589
Malaria Parasites				
Present	27(32.53)	20(26.31)	17(19.10)	0.0441
Regular Antenatal Attendance				
Yes	45(54.22)	55(72.37)	84(94.38)	< 0.0001
Hematinic Intake				
Yes	15(18.07)	45(59.21)	81(91.01)	< 0.0001

Appetite for Food

Yes

44(53.01)

46(60.53)

56(62.92)

0.1887

Categorical data are presented as proportion and compared using chi-square for trend

75



4.2.2 Figure 4.2 shows the prevalence of anaemia in various gestation periods

There were no significant variations in the prevalence of various types of anaemia diagnosed using hemoglobin and hematocrit as markers. The prevalence of microcytic hypochromic and normocytic normochromic anaemia at varying gestation periods also showed no significant difference. However, anaemia prevalence was lowest in second trimester; but trend was not significant. (Figure 4.2)

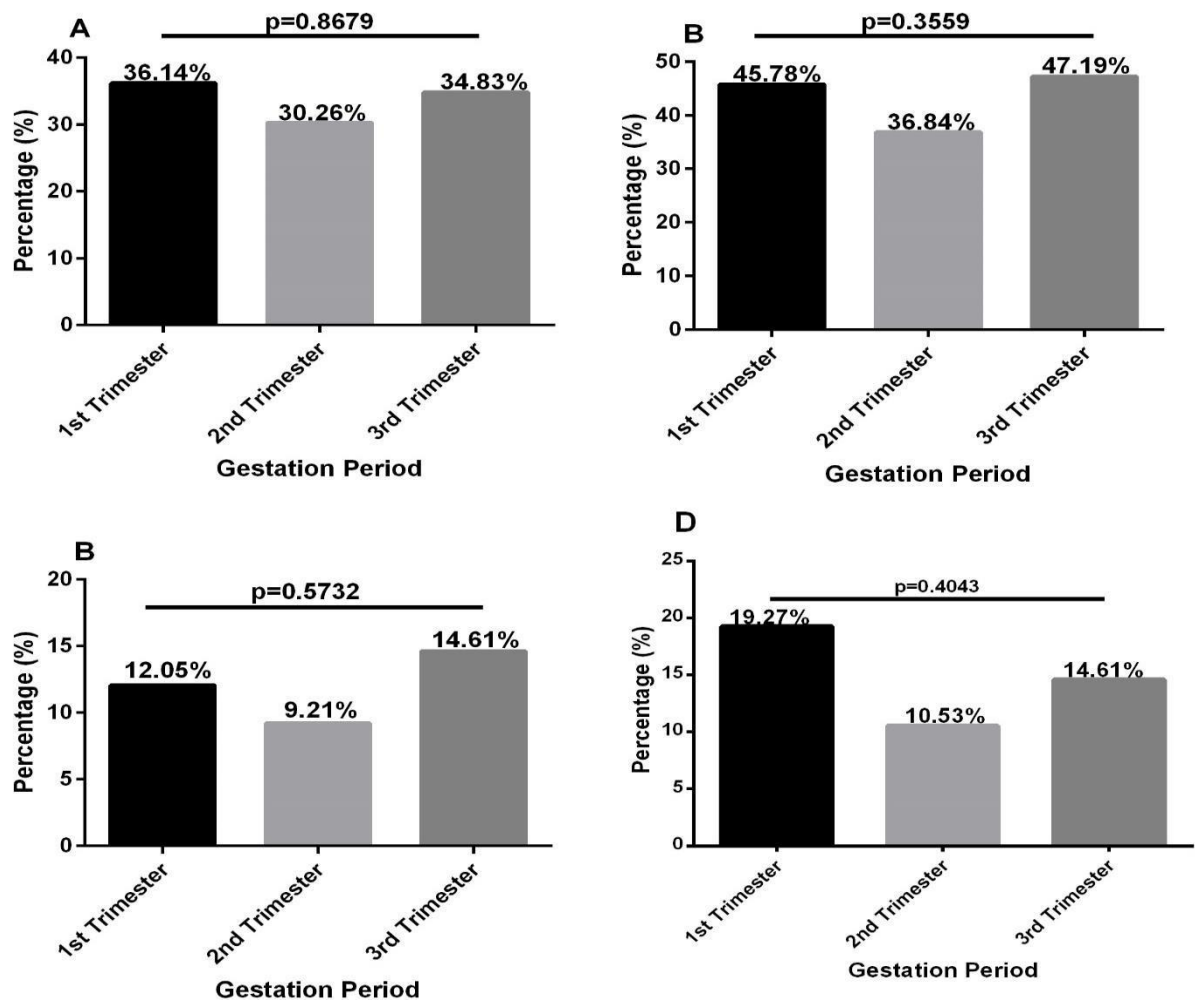


Figure 4.2 shows the prevalence of anaemia using hemoglobin concentration as a marker (A), anaemia using hematocrit (B), prevalence of microcytic hypochromic

anaemia (C) and normocytic normochromic anaemia (D) among the studied participants at different gestation periods

4.2.3 Prevalence of iron deficiency and iron deficiency anaemia at different gestation periods

The prevalence of iron deficiency ranged from 21.69% to 79.52%, 11.84% to 72.37% and 13.48% to 79.78% for pregnant women in their 1st, 2nd and 3rd trimesters respectively, using various iron markers as criteria for iron deficiency classification. (Table 10).

IDA also ranged from 3.61 to 30.12, 2.63 to 25 and 3.37 to 28.09 for pregnant women in their 1st, 2nd and 3rd trimesters respectively, using various iron markers and hemoglobin concentration as criteria for classification of IDA. (Table 4.8)



Table 4.8: Prevalence of iron deficiency and iron deficiency anaemia stratified by gestation period

Variable	1st Trimester (n=83)	2nd Trimester (n=76)	3rd Trimester (n=89)	P Value
Iron Deficiency Markers				
Serum Iron ($\mu\text{mol/L}$)	18(21.69)	20(26.32)	12(13.48)	0.1704
Ferritin(ng/mL)	17(20.48)	18(23.68)	17(19.10)	0.8136
Transferrin(g/L)	17(20.48)	9(11.84)	16(17.98)	0.6818
TSAT (%)	21(25.30)	22(28.95)	16(17.98)	0.2495
TIBC ($\mu\text{mol/L}$)	26(31.33)	28(36.84)	23(25.84)	0.4227
Tf/log ferr(g/L,ug/L)	41(49.40)	43(56.58)	40(44.94)	0.5417
Transferrin receptor(ng/mL)	66(79.52)	55(72.37)	71(79.78)	0.9484
IDA Markers				
Serum Iron ($\mu\text{mol/L}$)	8(9.64)	7(9.21)	3(3.37)	0.11
Ferritin(ng/mL)	3(3.61)	4(5.26)	4(4.49)	0.7857
Transferrin(g/L)	8(9.64)	2(2.63)	9(10.11)	0.8769
TSAT (%)	10(12.05)	7(9.21)	6(6.74)	0.2308
TIBC($\mu\text{mol/L}$)	10(12.05)	10(13.16)	9(10.11)	0.6863
Tf/log ferr(g/L,ug/L)	13(15.66)	14(18.42)	13(14.61)	0.8409
Transferrin Receptor(ng/mL)	25(30.12)	19(25)	25(28.09)	0.7763

Categorical data are presented as proportion and compared using chi-square for trend

4.3 BIOCHEMICAL AND HEMATOLOGICAL VARIATIONS IN SPONTANEOUS ABORTION

The prevalence of spontaneous abortion among the study participants was 66%. When the study was stratified based on obstetric history of spontaneous abortion to identify the biochemical and haematological variations in pregnant women with a history of spontaneous abortion and those without spontaneous abortion, there were no significant differences in most of the biochemical and haematological parameters between those with a history of spontaneous abortion and those without spontaneous abortion as shown in tables 11 and 12. However, there was a marked difference in the level of folic acid, with those with a history of spontaneous abortion (1074 ± 301.7 ; $p = < 0.0001$) having significantly higher levels than those without a history of spontaneous abortion (378.5 ± 255.5) as shown in table 4.9.

Table 4.9 Biochemical and Hematological profile stratified by obstetric history of spontaneous abortion

Variable	No Spontaneous Abortion (n=85)	Spontaneous Abortion (n=163)	P Value
Age	29.24 $\pm$ 4.587	28.98 $\pm$ 5.593	0.7193
Biochemical Profile			
Iron	15.7 $\pm$ 6.896	16.72 $\pm$ 9.342	0.377
TIBC	61.19 $\pm$ 19.16	66.04 $\pm$ 20.41	0.071
Vit B12 pmol/l	32689 $\pm$ 16043	29552 $\pm$ 14694	0.1234
FA (nmol/L)	378.5 $\pm$ 255.5	1074 $\pm$ 301.7	< 0.0001
TRF/CD71) ng/mL	134.6 $\pm$ 48.2	128.5 $\pm$ 52.68	0.3731
Serum Ferritin ng/mL	49.81 $\pm$ 41.89	49.52 $\pm$ 36.02	0.9543
Transferrin(g/L)	2.672 $\pm$ 0.8729	2.894 $\pm$ 0.9122	0.0651
Transferrin Saturation (TSAT %)	30.59 $\pm$ 19.19	29.58 $\pm$ 20.9	0.7128
TRf/logFer (ug/L)	139.9 $\pm$ 449.8	88.47 $\pm$ 49.42	0.1492
Transferrin/log(Ferr) g/L,ug/L	2.735 $\pm$ 8.208	1.97 $\pm$ 1.113	0.2425
Haematological Profile			
WBC	6.622 $\pm$ 1.903	6.687 $\pm$ 1.801	0.7911
RBC	4.243 $\pm$ 0.5879	4.306 $\pm$ 0.6334	0.4451

Prevalence

Hb	11.41±1.559	11.8±1.865	0.1017
HCT	32.94±3.78	34.06±4.839	0.0629
MCV	78.41±8.053	79.64±8.215	0.2618
MCH	27.86±3.056	28.3±3.147	0.3009
MCHC	35.56±2.191	35.55±1.946	0.9647
PLT	235.6±76.55	238.1±94.61	0.8331
RDW-SD	45.36±34.17	49.45±43.07	0.4488
RDW-CV	14.41±2.677	14.28±2.791	0.7325

Continuous data are presented as Mean $\pm$ SD and compared using unpaired t test

4.3.1 of abnormalities in routine ANC laboratory investigations and obstetric profile stratified by obstetric history of spontaneous abortion

There were no significant differences in distribution of abnormalities in most of the routine ANC laboratory investigations between pregnant women with a history of spontaneous abortion and those without. However the proportion of those with a history of spontaneous abortion (25.77%; 0.0101) who had a partial defect in G6PD was significantly higher than among those without a history of spontaneous abortion (11.76%) as shown in table 4.13. Also the proportion of women who had regular antenatal attendance was significantly higher among those with a history of abortion (78.53%; 0.0308) than in those without (65.88%) as shown in table 4.10.

Table 4.10: Routine ANC Laboratory investigations and obstetric profile stratified by obstetric history of spontaneous abortion

Variable	No Spontaneous Abortion (n=85)	Spontaneous Abortion (n=163)	P Value
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Hookworm in Stool			
Yes	3(3.53)	6(3.68)	0.9517
HIV (1 & 2)			
Reactive	10(11.76)	22(13.50)	0.6993
HBsAg			
Reactive	12(14.13)	20(12.30)	0.6804
HCV			
Reactive	13(15.29)	30(18.40)	0.5391
G6PD			
No Defect			
Partial defect	10(11.76)	42(25.77)	0.0101
Full defect	3(3.53)	2(1.23)	0.2208
Sickling			
Positive	9(10.59)	26(15.95)	0.2496
Malaria Parasites			
Present	21(24.71)	43(26.38)	0.7749
Regular Antenatal Attendance			
Yes	56(65.88)	128(78.53)	0.0308
Appetite For Food			
Yes	44(51.76)	102(62.58)	0.1005

Categorical data are presented as proportion and compared using chi-square

Prevalence

4.3.2 of anaemia in pregnant women with obstetrics history of spontaneous abortion

The prevalence of anaemia using both hemoglobin concentration and hematocrit, as well as the prevalence of microcytic hypochromic anaemia were higher among those without a history of spontaneous abortion. These differences were however not significant. The prevalence of normocytic normochromic anaemia was significantly higher among pregnant women without (21.18%; $p=0.0458$) a history of spontaneous abortion than in those with (11.66%) a history of spontaneous abortion as shown in figure 4.3

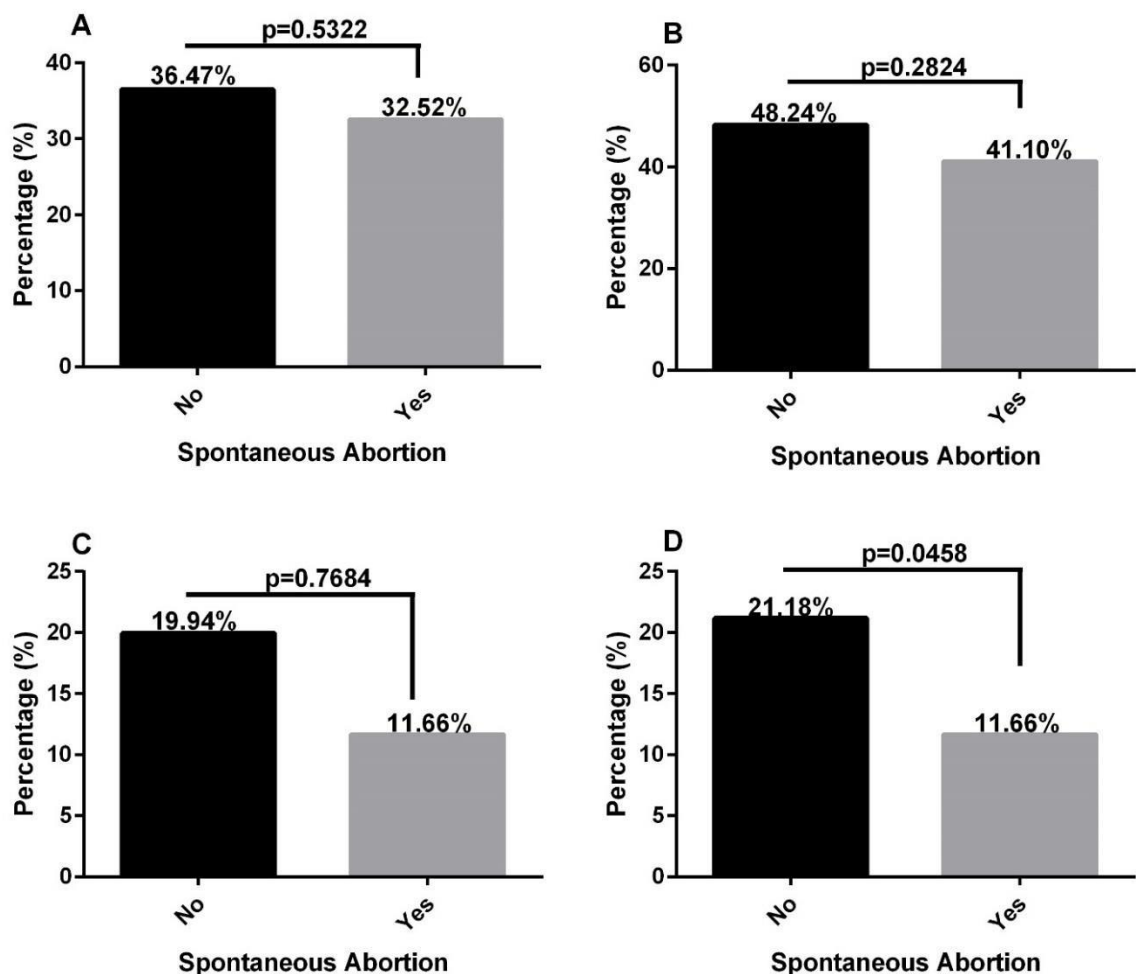


Figure 4.3: The distribution of Anaemia using hemoglobin concentration (A), Anaemia using hematocrit (B), microcytic hypochromic anaemia (C)

and normocytic normochromic anaemia (D) among the studied participants classified by obstetric history of spontaneous abortion.

4.3.3 of iron deficiency and iron deficiency anaemia stratified by obstetric history of spontaneous abortion

There were no significant differences in the prevalence of iron deficiency and iron deficiency anaemia using most of the various iron markers as criteria. There was however a higher prevalence of iron deficiency and Iron deficiency anaemia among women without (23.53%; $p=0.0456$ for iron deficiency, 12.94%; 0.0240 for IDA) a history of spontaneous abortion as compared to those with (13.50% for Iron deficiency and 4.91% for IDA) a history of spontaneous abortion, when transferrin concentrations were used as a marker for iron deficiency as shown in table 4.11

Table 4.11: Prevalence of iron deficiency and IDA stratified by obstetric history of spontaneous abortion

Variable	No Spontaneous Abortion (n=85)	Spontaneous Abortion (n=163)	P Value
Iron Deficiency Markers			
Serum Iron	17(20.00)	33(20.25)	0.9635
Ferritin	19(22.35)	33(20.25)	0.6988
Transferrin	20(23.53)	22(13.50)	0.0456
TSAT	20(23.53)	39(23.93)	0.9444
TIBC	23(27.06)	54(33.13)	0.3268
Tf/log ferr	39(45.88)	85(52.15)	0.349
Transferrin receptor	70(82.35)	122(74.85)	0.1796
IDA Markers			
Serum Iron	7(8.24)	11(6.75)	0.6684
Ferritin	5(5.88)	6(3.68)	0.4242
Transferrin	11(12.94)	8(4.91)	0.0240
TSAT	9(10.59)	14(8.59)	0.6064
TIBC	8(9.41)	21(12.88)	0.4194
Tf/log ferr	12(14.12)	28(17.17)	0.534
Transferrin receptor	26(30.59)	43(26.38)	0.4828

Categorical data are presented as proportion and compared using chi-square

Prevalence

4.4 DETERMINANTS OF ANAEMIA IN PREGNANCY

The effect of various abnormalities and obstetric variables on anaemia is shown in tables 4.15, 4.16, 4.17 and 4.18. Low ferritin concentrations (OR= 2.291; 95%CI=1.102-4.763; $p=0.026$), low transferrin levels (OR=0.480; 95%CI=0.238-0.967; $p=0.04$), non-intake of hematinics (OR=1.859; 95%CI=1.075-3.213; $p=0.026$), as well as absence of appetite for food (OR=1.927; 95%CI=1.106-3.356; $p=0.021$) were the variables that significantly increase the risk of anaemia from the univariate analysis. (Tables 4.12 and 4.13)

After adjusting for confounding variables, the risk factors for anaemia are low ferritin concentration (aOR=0.664; 95%CI=0.253 -1.741; $p=0.405$), advance gestation; 2nd trimester (aOR=2.459; 95%CI=1.083 -5.583; $p=0.031$) and 3rd trimester (aOR=2.688 95%CI=1.118-6.463; $p=0.027$), non-intake of hematinics (OR=1.856; 95%CI=1.052-3.277; $p=0.033$) and absence of appetite for food (OR=1.86; 95%CI=1.044-3.312; $p=0.035$). (Tables 4.12 and 4.13)

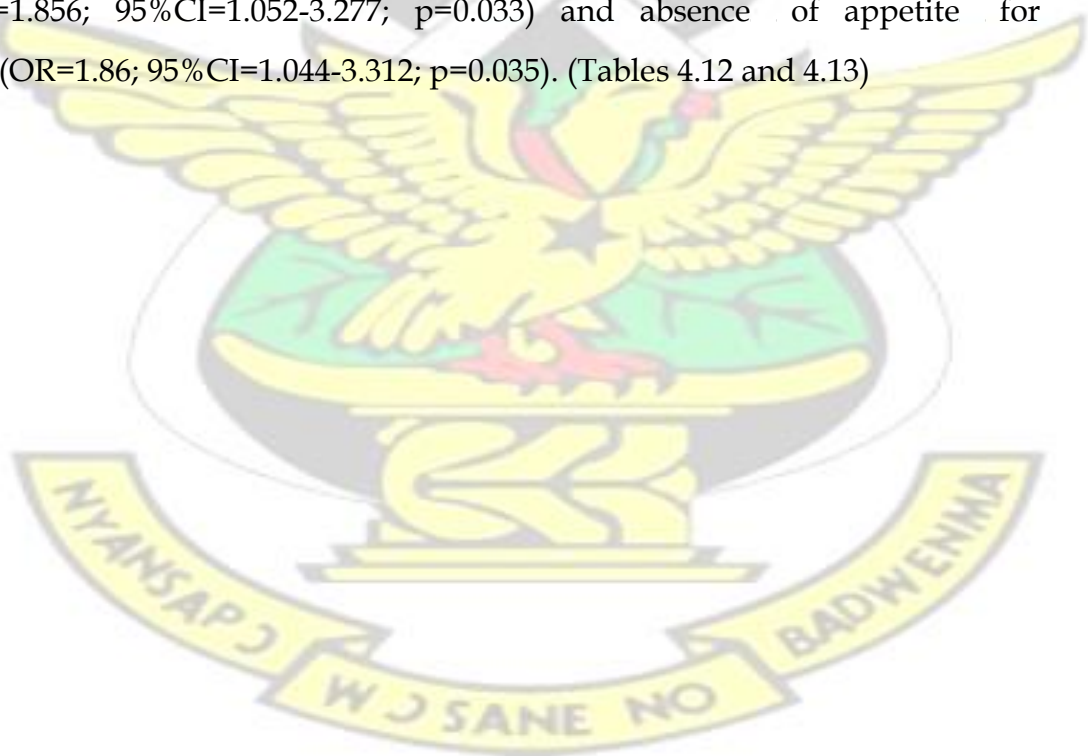


Table 4.12 : Effect of abnormal routine ANC laboratory investigations on anaemia

Variable	OR	P Value	aOR	P Value
Hookworm In				
Stool				
No				
Yes	0.629 (0.164-2.407)	0.498	0.683(0.1612.905)	0.606
HIV (1&2)				
Non-Reactive				
Reactive	0.532(0.2511.127)	0.099	0.573(0.2541.293)	0.18
HbsAg				
Non-Reactive				
Reactive	0.975(0.4462.130)	0.949	1.013(0.4432.315)	0.975
HCV				
Non-Reactive				
Reactive	1.398(0.6772.889)	0.365	1.335(0.6242.856)	0.456
G6PD				
No Defect				
Partial Defect	0.855(0.4511.621)	0.631	0.86(0.439-1.686)	0.661
Full Defect	0.738(0.120-4.531)	0.743	1.476(0.227-9.613)	0.684
Sickling				
Negative				
Positive	1.570(0.7003.524)	0.274	1.69(0.722-3.954)	0.227
Malaria Parasites				
Absent				
Present	1.173(0.6382.158)	0.607	1.209(0.6332.309)	0.566

Table 4.13: Effect of biochemical profile on anaemia

Variable	OR	P Value	aOR	P Value
Serum Iron Concentration				
Low	0.868(0.453-1.665)	0.671	0.958(0.372-2.469)	0.929
Normal				
High	0.488(0.096-2.489)	0.388	0.579(0.107-3.121)	0.525
TIBC				
Low	0.494(0.235-1.038)	0.063	1.293(0.149-11.197)	0.815
Normal				
High	0.662(0.365-1.200)	0.174	0.664(0.253-1.741)	0.405
Ferritin				
Low	2.291(1.102-4.763)	0.026	2.137(1.004-4.546)	0.049
Normal				
High	1.434(0.526-3.913)	0.481	1.393(0.486-3.986)	0.537
Transferrin				
Low	0.480(0.238-0.967)	0.04	0.511(0.248-1.053)	0.069
Normal				
High	0.566(0.293-1.093)	0.09	0.557(0.280-1.111)	0.097
TSAT				
<15	0.746(0.407-1.367)	0.343	0.656(0.280-1.538)	0.332
≥15				
Tr/Log Fer				
≤1.7				
>1.7	1.155(0.682-1.955)	0.592	0.879(0.406-1.904)	0.743

4.14 Effect of Obstetric profile on anaemia

Variable	OR	P Value	aOR	P Value
Gestation Period				
1st Trimester				
2nd Trimester	1.304(0.672-2.532)	0.432	2.459(1.083-5.583)	0.031
3rd Trimester	1.059(0.567-1.979)	0.857	2.688(1.118-6.463)	0.027
Parity				
Null				
Uni	1.417(0.718-2.798)	0.315	1.248(0.606-2.572)	0.548

Results

<i>Multi</i>	0.965(0.489-1.904)	0.918	0.96(0.471-1.956)	0.91
Gravida				
≤2				
>2	1.002(0.540-1.857)	0.996	1.35(0.694-2.625)	0.377
Spontaneous Abortion				
No				
Yes	1.191(0.687-2.065)	0.532	1.674(0.895-3.130)	0.107
Induced Abortion				
No				
Yes	1.709(0.735-3.973)	0.213	1.25(0.511-3.057)	0.625
Regular Antenatal Attendance				
Yes				
No	0.970(0.533-1.767)	0.921	0.599(0.296-1.210)	0.153
Hematinic Intake				
Yes				
No	1.859(1.075-3.213)	0.026	1.856(1.052-3.277)	0.033
Appetite For Food				
Yes				
No	1.927(1.106-3.356)	0.021	1.86(1.044-3.312)	0.035

4.5 DETERMINANTS OF SPONTANEOUS ABORTION

The impact of various conditions and obstetric variables on the occurrence of spontaneous abortion is shown in tables 4.18, 4.19, 4.20 and 4.21. A partial defect in G6PD (OR= 0.394; 95%CI=0.186-0.832; $p=0.015$), multi-parity (OR= 2.189; 95%CI=1.076-4.453; $p=0.031$) and irregular attendance at ANC (OR= 1.894; 95%CI=1.056-3.395; $p=0.032$) were variables that significantly increases the risk of spontaneous abortion. (Tables 4.13 and 4.14)

After adjusting for confounding variables, the risk factors for spontaneous abortion are partial defect in G6PD (OR= 0.447; 95%CI=0.208-0.961; $p=0.039$), multi-parity (OR= 2.724; 95%CI=1.277-5.809; $p=0.01$) and irregular attendance at ANC (OR= 2.036; 95%CI=1.093-3.793; $p=0.025$). (Tables 4.15 and 4.18)



Table : Effe

4.15 ct of abnormal routine ANC laboratory investigations on occurrence of spontaneous abortion

Variable	OR	P Value	aOR	P Value
Hookworm In Stool				
No				
Yes	0.957(0.233-3.927)	0.952	1.219(0.282-5.270)	0.791
HIV(1&2)				
Non-Reactive				
Reactive	0.855(0.385-1.899)	0.7	0.852(0.359-2.018)	0.715
HbsAg				
Non-Reactive				
Reactive	1.175(0.545-2.536)	0.681	1.241(0.556-2.773)	0.598
HCV				
Non-Reactive				
Reactive	0.8(0.393-1.630)	0.54	0.707(0.336-1.488)	0.361
G6PD				
No Defect				
Partial Defect	0.394(0.186-0.832)	0.015	0.447(0.208-0.961)	0.039
Full Defect	2.479(0.405-15.194)	0.326	3(0.467-19.274)	0.247
Sickling				
Negative				
Positive	0.624(0.278-1.400)	0.253	0.582(0.252-1.346)	0.206
Malaria Parasites				
Absent				
Present	0.916(0.501-1.674)	0.775	0.812(0.430-1.532)	0.52

Table : Effe

4.16 ct of biochemical profile on occurrence of spontaneous abortion				
Variable	OR	P Value	aOR	P Value
Serum Iron Concentration				
<i>Low</i>	0.939(0.488-1.810)	0.791	0.791(0.399-1.568)	0.502
<i>Normal</i>				
<i>High</i>	0(0.00-0.00)	0.000	0(0-0)	1.000
TIBC				
<i>Low</i>	1.82(0.875-3.785)	0.109	1.849(0.855-3.999)	0.119
<i>Normal</i>				
<i>High</i>	0.862(0.469-1.581)	0.630	0.784(0.416-1.480)	0.453
Ferritin				
<i>Low</i>	1.171(0.614-2.235)	0.631	1.168(0.591-2.307)	0.655
<i>Normal</i>				
<i>High</i>	1.356(0.525-3.501)	0.529	1.521(0.560-4.131)	0.411
Transferrin				
<i>Low</i>	1.854(0.928-3.704)	0.080	1.931(0.931-4.005)	0.077
<i>Normal</i>				
<i>High</i>	0.772(0.383-1.554)	0.468	0.668(0.318-1.403)	0.287
TSAT				
<15	0.978(0.528-1.813)	0.944	0.816(0.427-1.558)	0.538
≥15				
Tr/Log Fer				
≤1.7				
>1.7	0.778(0.460-1.316)	0.349	0.77(0.444-1.335)	0.352

4.17 ct of hematological profile on occurrence of spontaneous abortion

Variable	OR	P Value	aOR	P Value
WBC				
<i>Low</i>	1.214(0.544-2.706)	0.636	1.126(0.484-2.619)	0.783

Table : Effe

<i>Normal</i>				
<i>High</i>	1.986(0.274-14.390)	0.497	1.428(0.180-11.364)	0.736
RBC				
<i>Low</i>	1.146(0.629-2.090)	0.656	1.031(0.548-1.941)	0.924
<i>Normal</i>				
<i>High</i>	0.597(0.208-1.715)	0.338	0.698(0.234-2.084)	0.519
Haemoglobin				
<11.0	1.191(0.687-2.065)	0.532	1.177(0.662-2.095)	0.579
≥11.0				
HCT				
<33	1.335(0.788-2.263)	0.283	1.322(0.762-2.293)	0.320
≥33				
MCV				
<i>Low</i>	1.161(0.683-1.974)	0.581	1.456(0.823-2.576)	0.197
<i>Normal</i>				
<i>High</i>	1.382(0.222-8.595)	0.729	1.002(0.150-6.697)	0.999
MCH				
<i>Low</i>	1.165(0.656-2.069)	0.602	1.352(0.734-2.492)	0.334
<i>Normal</i>				
<i>High</i>	0.25(0.055-1.132)	0.072	0.213(0.045-1.014)	0.052
PLT				
<i>Low</i>	1.126(0.508-2.493)	0.77	1.111(0.485-2.546)	0.804
<i>Normal</i>				
<i>High</i>	0.972(0.174-5.435)	0.974	1.253(0.213-7.375)	0.803

Table 4.18 : Effect of obstetric history on occurrence of spontaneous abortion

			<i>Result</i>	
Variable	OR	P Value	aOR	P Value
Gestation				
1st Trimester		0.000		0.000
2nd Trimester	0.089(0.042-0.190)		0.087(0.038-0.196)	
3rd Trimester	0.08(0.038-0.168)	0.000	0.066(0.028-0.155)	0.000
Parity				
Null		0.652		0.406
Uni	1.179(0.577-2.408)		1.373(0.651-2.895)	
Multi	2.189(1.076-4.453)	0.031	2.724(1.277-5.809)	0.010
Gravida				
≤ 2		0.000		0.000
>2	0.056(0.026-0.118)		0.001(0.000-0.012)	
Induced Abortion				
No		0.149		0.723
Yes	1.726(0.822-3.626)		1.161(0.510-2.640)	
Regular Antenatal Attendance				
Yes				
No	1.894(1.056-3.395)	0.032	2.036(1.093-3.793)	0.025
Hematinic Intake				
Yes		0.000		0.000
No	3.615(2.086-6.266)		4.546(2.385-8.667)	

Appetite For Food

Yes		0.101		0.135
No	1.558(0.916-2.649)		1.529(0.876-2.668)	

Chapter 5 **DISCUSSION**

This study aimed at determining the hemato-biochemical indices and their correlation with obstetrics profile and complications among pregnant women in the Northern region of Ghana and to determine the prevalence, types and risk factors predisposing pregnant women to the development of anemia and spontaneous abortion

5.1 HEMATO-BIOCHEMICAL VARIATIONS

The importance of folic acid during pregnancy cannot be over emphasized; several studies have demonstrated the importance of folic acid to the developing fetus. Rapid mitosis occurring in the developing foetus depends on adequate supply of folates for tissue growth. Folate homeostasis is sometimes breached in the early periods of gestation mostly during placental formation, gastrulation or organogenesis of pregnancy (Hibbard, 1975).

Maternal folate deficiency may affect foetal growth and development, which is often, presented as small for gestation age (SGA) and underweight babies (LBW). Folic acid deficient women also have a higher incidence of spontaneous abortion, abruptio placentae and foetal malformation (Hibbard, 1975).

Folic acid levels as seen in this study were higher among pregnant women on hematinics as expected since these hematinics contain folic acid as well as other micronutrients. Consequently, the intake of hematinics leads to higher serum folic acid levels than in the hematinic-naïve pregnant women. This study also saw an increasing trend in the number of regular antenatal attendance as well as number of pregnant women on hematinics as the gestation increased with

folic acid levels increasing with advancing gestation in this current study. Similarly, the higher levels of folic acid in the second and third trimester could be as a result of the fact that most of the pregnant women in this category attended antenatal care regularly and severally and on each visit, were given hematinics and folic acid supplements.

5.1.1 Folic acid levels and occurrence of spontaneous abortion

5.1.1.1 Prevalence of spontaneous abortion

The prevalence of spontaneous abortion among pregnant women in this study was 65.72%. Tallia *et al.* (2001) in a similar study in Northern America found the rate of spontaneous abortion in North American women to be between 15%–20% for women among women younger than 25 years and 35% in women older than 38 years. Also, a prospective study conducted among clinically diagnosed pregnancies established a 12% prevalence of spontaneous abortion (Wilcox *et al.*, 1988) which are all lower than the prevalence rate obtained in this study.

In a study by Jibril *et al* in Nigeria on women admitted to the gynaecological ward, the prevalence of spontaneous abortion among the pregnant women was 53.2% (Jibril *et al.*, 2014). (Meseret *et al.*, 2015) in Northwest Ethiopia found 10.9% prevalence rate of spontaneous abortion among rural women in Gonji district.

5.1.1.2 Folic acid levels and obstetrics history of spontaneous abortion

This study showed a higher level of folic acid concentrations among women with obstetrics history of spontaneous abortion as shown in table 4.11. This brings to the fore the impact of high folate levels on the safety of pregnancies. Data from one large randomized trials on folic acid supplementation was analysed and the authors reported that a periconceptional use of folic acid was

associated with a significant increase in the risk of spontaneous abortion (Hook and Czeizel, 1997). Published reports on the relation between folic acid supplementation during pregnancy and risk for miscarriage provide conflicting reports. The finding in this study and that of Hook and Czeizel (1997) are in agreement with those of Windham *et al.* (2000). These findings are however in contradiction to those of Gindler *et al.* (2001) and Wald and Hackshaw (2001) who observed no association between folic acid levels and the occurrence of spontaneous abortion. Studies by Wouters *et al.* (1993) and Nelen *et al.* (2000) found an association between low levels of folic acid and the occurrence of spontaneous abortion. Results from this study also indicates the higher number of women with obstetrics history of spontaneous abortion were those who attended antenatal care regularly and were given hematinic and folic acid supplements on each visit. This could explain the higher folic acid levels found in their blood as a result of increased consumption from antenatal care clinics.

Despite a well-documented benefit of folic acid consumption, a study in 1997 examined pregnancy outcomes among women who participated in randomised controlled trials to prevent NTDs (Czeizel and Dudas, 1992; Wald and Sneddon, 1991). Findings in that study indicated 16% of women who had taken a multivitamin containing 800g of folic acid developed spontaneous abortion (miscarriage) (Hook and Czeizel, 1997). Serum folate concentrations above 45 nmol/L are often considered highly elevated (abnormal levels) (Wald and Sneddon, 1991). The average serum folic acid concentration among pregnant women with obstetrics history of spontaneous abortion in our study was 1074 nmol/L which is highly unacceptable compared to the normal serum folic acid levels. This phenomenon of increased serum folic acid concentration and occurrence of spontaneous abortion could be explained that folate extends the viability of fetuses that might be lost in early gestation to be recognized as miscarriages (abortion), thereby increasing the gestation age of the non viable fetus to an age where its expulsion becomes noticeable. MRC in their own analysis in the control trial, indicated that only women who became pregnant

showed a smaller increase (6%) of spontaneous abortion, but it was not statistically significant (Wald and Hackshaw, 1997). Windham and co-workers (Windham *et al.*, 2000) reported a non-significant increased occurrence of spontaneous abortion among women interviewed during the first trimester who reported having taken multivitamins or folic acid during the prenatal period.

5.2 ANAEMIA IN PREGNANCY

5.2.1 Prevalence of anaemia

Pregnant women are mostly vulnerable to developing anaemia due to an increased demand for iron and other macro and micronutrients as a result as an increased physiological demand associated with pregnancy. Iron deficiency and iron deficiency anaemia in pregnancy poses serious threat to both the mother and the developing foetus which include increased risks of maternal mortality and morbidity, premature delivery, and small for gestation age (SGA) or low birth weight (Malee, 2008). In this current study, the prevalence of anemia was found to be 33.87% and 43.55% when the hemoglobin concentration and the hematocrit were used respectively in the classification of anaemia. The prevalence of anaemia in this study as determined by the hemoglobin concentration falls in the moderate public health significance category (WHO, 2001b) and is comparable to several other studies from Ghana and other parts of Africa. Engmann *et al.* (2008) reported 34% among pregnant women in Ghanaian urban communities, 32% in similar work in Ghana by Stephens *et al.* (2014), as well as the 33% from Zambia and the 34% from South Africa is reported in a study by Brabin *et al.* (2001). It was however lower than the 44% reported by Obirikorang *et al.* (2015) in their study among a similar Ghanaian population and 76.6% reported by Bondevik *et al.* (2000) among Nepalese pregnant women but higher than the 23.2% found by Buseri *et al.* (2008) among Nigerian pregnant women. The differences in prevalence rates among this study and the others could be due to the differences in sample sizes, cut-off points used in defining anaemia, differences in demography and geographical location, effect of social stratification that defines the severity or

otherwise in worm infestation, malaria infection, parity and gravidae rate, dietary pattern and socioeconomic status in different locations.

5.2.2 Anaemia and hematinic intake

It was also revealed in this study that the prevalence of anemia was higher in pregnant women on hematinics (pregnant women in this category were in the third trimester) than their counterparts who were hematinic naïve (pregnant women classified as hematinic naïve were in the first trimester). This is contrary to findings by (AL- Shawi *et al.*, 2012), who found a higher prevalence of anemia among hematinic naïve pregnant women. Several studies have reported a positive association between anemia and the age of pregnancy, therefore, as gestation increases, the prevalence of anemia increases among pregnant women (MacDonald *et al.*, 2007). Most of the hematinic naïve pregnant women were in the first trimester of the pregnancy, with the highest number of pregnant women on hematinic being in the third trimester of pregnancy as revealed in this study. A higher prevalence of anemia among pregnant women on hematinic may be due to the fact that they were in their late stage of the pregnancy and therefore have a higher physiological burden that comes with pregnancy. Further as pregnancy ages, the foetal demand for maternal micronutrients particularly iron, increases and hence a further increase of the burden on the mother leading to anemia thus the administration of hematinic. However, there is an alternative explanation from a study by Hemminki and Rimpelä (1991) that suggest that, women close to iron depletion at the time of fertilization and conception, there is the development of iron deficiency in early gestational age and therefore, iron or hematinic supplementation would not be able to prevent or correct the iron depletion. Similar findings have been described by Allen (2000), who indicated that iron supplementation will often fail to prevent iron deficiency and iron deficiency anemia in women who became pregnant with already iron depleted stores. This offers a better explanation to the observation seen in this current study.

Drug side effects mostly constipation and darker stool seen on iron consumption and non-compliance may also explain this finding (Allen, 2000; Müngen, 2003). Similarly, pregnant women with no obstetric history of spontaneous abortion had a higher prevalence of normocytic normochromic anaemia than those with a history of abortion. The reason for this finding is not clear from this study and may warrant further investigations.

5.2.3 Chronic infections and anaemia

This study also highlights the impact of chronic diseases such as HIV infection on anaemia in pregnancy. The role of other chronic infections has been discussed, but not extensively studied in pregnant women. In the last decade, HIV infection has become more prevalent and it must now be considered a possible aetiological factor (Buseri *et al.*, 2008; van den Broek and Letsky, 2000). This study found an overall HIV prevalence of 12.9% with a higher number of them being anaemic. With reported incidence of HIV seropositivity in antenatal clinics of between 6% and 30% in some developing countries, it is prudent infection with HIV be included in the differential diagnosis of anaemia in pregnancy. A lot of haematological derangements associated with HIV infection has been reported in several studies (Costello, 1997; Davis and Zauli, 1995; Hoxie, 1995; Scadden *et al.*, 1989; Zon *et al.*, 1987; Zon and Groopman, 1988). Impaired haematopoiesis occurs in HIV infection and these effects are further exaggerated by the use of antiviral agents, such as zidovudine or azidothymidine (Richman *et al.*, 1987). In general the incidence of anaemia increases as the disease advances (Scadden *et al.*, 1989). An obvious cause of anaemia in patients with HIV infection is blood loss which may be associated with conditions such as neoplastic disease e.g. Kaposi sarcoma in the gastrointestinal tract or gastrointestinal lesions that accompany opportunistic cytomegalovirus infection. Other than blood loss, the pathophysiology of HIV-associated anaemia may involve decreased red blood cell (RBC) production, increased RBC destruction and ineffective RBC production.

In this study the most prevalent morphological form of anaemia is normocytic normochromic anaemia. This is consistent with the type of anemia seen in infections and inflammatory conditions. Gaspar and co-workers reported similar finding that anaemia associated with inflammatory or infectious conditions is normocytic normochromic anaemia and explained that it could be due to inhibition of erythropoiesis and failure of incorporation of iron into erythroblasts by the infectious agent (Gaspar *et al.*, 1993)

5.2.4 Iron deficiency and iron deficiency anemia

Iron deficiency (ID) is the most common nutritional deficiency worldwide (Denic and Agarwal, 2007). It arises when iron consumption is far less than the body requirement or when there is impaired absorption from the gastrointestinal tract (GIT) in certain disease conditions. Iron deficiency worsens in high metabolic states that require extra iron supply such as pregnancy which has the tendency to deplete iron stores (Clark, 2009). The high physiological and metabolic demand for iron imposed by growing hemochorial placenta, rapidly developing foetus and maternal tissue predisposes pregnant women to the development of iron deficiency (Umbreit, 2005).

There are several laboratory assays to diagnose iron deficiency but no single biomarker for assessing iron deficiency has been confirmed (Raza *et al.*, 2011). A combination of several methods or markers enables a better understanding of iron status of a patient (ALPER *et al.*, 2000). The prevalence of iron deficiency and iron deficiency anaemia as revealed in this study ranged from 16.94% to 77.42% and 7.26% to 27.82% respectively using various iron markers as criteria. The findings of the study of Obirikorang *et al.* (2015) falls within the range established in this study.

5.2.4.1 Ferritin as a marker for the diagnoses of Iron deficiency (ID) and iron deficiency anaemia (IDA)

Serum ferritin has emerged as a single best marker for the diagnosis of iron deficiency and iron deficiency anemia (Cook, 2005; Cook *et al.*, 2003; Schrier, 2009) comparable to the gold standard of bone marrow aspirate which is a highly invasive procedure (Barron *et al.*, 2001; Ganti *et al.*, 2003; Thomason and Almiski, 2009). The prevalence of iron deficiency and iron deficiency anemia in this current study as determined by ferritin levels were 16.94% and 4.44% respectively. These figures are comparable to those by Engmann *et al.* (2008) who reported 16% and 7.5% iron deficiency and iron deficiency anaemia among pregnant women in Ghana (Engmann *et al.*, 2008). Obirikorang *et al.* (2015) conducted a similar study in Ghana on the same target group and found (21.5%) iron deficiency and (10.4%) iron deficiency anaemia, 28.5% among pregnant women in Southern Iran by Karimi *et al.* (2002) which were higher than the prevalence rate obtained in our study. The prevalence rate in this study is also lower than the range reported in other developing countries (25-35%) (Isah *et al.*, 1985; Lewis and Rowe, 1986; Mashako *et al.*, 1991; Sifakis and Pharmakides, 2000) and even much lower than the prevalence found in the industrialized countries (5-8%) (Sifakis and Pharmakides, 2000).

5.2.4.2 Iron deficiency and iron deficiency anaemia as diagnosed by the modern indicators of iron status

Ferritin is an acute phase reactant whose concentration increases in inflammatory conditions (Burns *et al.*, 1990a; Cazzola and Beguin, 1992; Harju *et al.*, 1984) and this variation can mask the diagnosis of iron deficiency since normal ferritin levels or ferritin as high as 100 µg/L can occur in iron deficient patients (Beard, 1994). Serum ferritin concentration less than 20 µg/L confirms

iron deficiency anaemia (IDA), whereas serum ferritin levels $>100 \mu\text{g/L}$ usually rule out iron deficiency (Munoz *et al.*, 2011)

In inconclusive situations (when ferritin concentrations are between $20\mu\text{g/L}$ and $100 \mu\text{g/L}$) (Guyatt *et al.*, 1992; Nelson *et al.*, 1978), it is difficult to determine individuals who are truly iron deficient from non-iron deficient patients.

To cater for these lapses, two modern indicators of assessing iron status were explored; the transferrin/log ferritin ratio and transferrin receptor. These have been shown to be better markers for the diagnosis of iron deficiency in other studies (Angeles Vázquez López *et al.*, 2006; Dimitriou *et al.*, 2000; Hanif *et al.*, 2005; Markovic *et al.*, 2005; PETTERSSON *et al.*, 1994)

5.2.4.2.1 Iron deficiency (ID) and iron deficiency anaemia (IDA) as diagnosed using Transferrin/log Ferritin as an index

The prevalence of ID and IDA as revealed from this study using transferrin: log ferritin as marker were 50% and 16.13% for ID and IDA respectively. Castel *et al.* (2012), conducted a study using the transferrin/log ferritin as an index for the assessment of iron deficiency and iron deficiency anaemia and found 29% prevalence rate of IDA which is higher than the figure obtained in this study.

5.2.4.2.2 ID and IDA using transferrin receptor as a marker

When transferrin receptor was used as an index for the assessment of iron status, 77.42% and 27.8% prevalence was obtained for ID and IDA respectively.

Soluble transferrin receptor (sTfR) concentration has shown much promise in the diagnoses of iron deficiency since it is not affected by inflammation/ infection. Thus, sTfR levels are a better indicator of iron deficiency (ID) (Angeles Vázquez López *et al.*, 2006; Dimitriou *et al.*, 2000; Hanif *et al.*, 2005; Markovic *et*

al., 2005; PETTERSSON *et al.*, 1994) and it is capable of detecting even mild iron deficiency as well (Skikne *et al.*, 1990)

A study by Olivares *et al.* (2000) found 31.3% and 12.8% prevalence of iron deficiency and iron deficiency anaemia respectively using transferrin receptor as an index for the diagnosis of iron deficiency which is lower than the prevalence rate obtained in this study. However, the prevalence of ID and IDA in this study is comparable to that obtained in a study in India by Khosla *et al.* who found 80% iron deficiency anaemia (Khosla *et al.*, 2002) and 44 to 61% by van den Broek and Letsky (2000) who also used a similar marker for the diagnosis of iron deficiency. There are marked variations between figures obtained using the modern indicators of assessing iron status compared to using ferritin alone or a combination of the conventional/classical markers of assessing iron deficiency and iron deficiency anemia. These variations could be due to an underlying inflammatory conditions, diurnal variations, ge stational age, haemodilution that affect levels of the conventional markers of iron status thereby, masking the diagnosis of iron deficiency. The modern indicators of iron status especially transferrin receptor are not influenced by these factors (Carriaga *et al.*, 1991a)

5.2.5 Determinants of anaemia

5.2.5.1 Low ferritin as a predictive indicator of anaemia

This study highlights the impact of low levels of ferritin on the occurrence of anaemia among pregnant women. The effect of ferritin on the incidence of anaemia has been demonstrated in several studies from parts of the world. In this study ferritin was a strong predictor of anaemia even after controlling for confounding factors. In a similar study on anaemia in Nepalese pregnant women, (Bondevik *et al.*, 2000) found low serum ferritin being significantly associated with increased risk of severe anaemia; the odds (95% CI) were 3.21 (1.56, 6.60) and after adjustment for potential confounders, the odds were 2.05 (0.87, 4.85) (95% CI). Transfer of iron to the developing fetus is enhanced by an

increase in maternal iron absorption during pregnancy and this is regulated by the placenta in the foeto-maternal circulatory system. Serum ferritin usually falls markedly between 12 and 25 weeks of gestation, as a result of increased utilization to increase the maternal red cell mass due to the obvious hemodilutional effect. Iron transfer to the fetus occurs after week 30 of gestation, which is the same time there is rapid increase in maternal iron absorption. Free iron binds to ferritin in placental cells and then transferred to apotransferrin, which enters from the fetal side of the placenta and exits as holotransferrin into the fetal circulation (Harris, 1992; Starreveld *et al.*, 1995). Hence, low levels of ferritin predisposes a pregnant mother to developing anaemia during pregnancy

5.2.5.2 Effect of 2nd and 3rd trimester gestation and occurrence of anaemia

This study also shows an association between gestation and the occurrence of anemia. The findings revealed that pregnant women in the second and third trimesters were more likely to become anemic than those in the first trimester of pregnancy. This finding is in consonance with the study of Bodeau-Livinec *et al.* (2011), who studied the prevalence of anaemia throughout pregnancy in the Beninois hospitals. In that study the prevalence of anaemia was (64%) during the second (2nd) and third (3rd) trimesters of gestation. A similar trend was observed by Karaoglu *et al.* (2010) in a study of nutritional anaemia among pregnant women in Turkey. This trend of anemia in the 2nd and 3rd trimesters of gestation could be as a result of increased plasma volume expansion in the late second trimester and early third trimester that leads to a decrease in haemoglobin concentration (Xiong *et al.*, 2003) and increased red blood cells (Chesley, 1972; Hytten and Paintin, 1963). In advance stage of pregnancy (late gestation, second and third trimesters)(13 to 26 weeks second trimester), there is rapid foetal growth and by the end of the second trimester, the foetus weighs approximately 70g and is 30cm long while the foetal organs have begun

to mature (Oke *et al.*, 2011). During the third trimester (26 to 40 weeks), the fetal organs have undergone complete maturation (Lawson and Starrant, 1988) and there is rapid foetal growth and development which requires increased micro and macro nutrients, other growth signals from maternal circulation to meet this high demands of the fetus, hence the incidence of anemia seen.

5.2.6 Folate and Vitamin B12 deficiency

It is interesting to note that none of the pregnant women in this study had folate or Vitamin B12 deficiency and thus no macrocytic anemia among the pregnant women. This finding is consistent with the exceedingly higher serum folic acid and Vitamin B12 concentrations recorded in our data. This finding is contrary to the findings of (Karaoglu *et al.*, 2010) who identified 34.5% and 71.7% Vitamin B12 and folate deficiencies respectively and several other studies that have found higher prevalence of the deficiency of these micronutrients. This trend observed could be attributed to the antenatal care policy of the Ghana Health Service in providing folic acid and Vitamin B12 supplementation during every antenatal visit.

5.3 DETERMINANTS OF SPONTANEOUS ABORTION

5.3.1 G6PD deficiency and occurrence of spontaneous abortion

How does it feel for a woman to consistently lose pregnancy in our African traditional setting where miscarriages are often associated with witchcraft and sorceries? There is profound emotional and psychological trauma associated with spontaneous abortion. Women experience moderate to severe grief and have feelings of guilt or anxiety accompanying abortions (Adolfsson *et al.*, 2004). The families find it difficult to cope with the situation, and these women may require psychotherapeutic support at most times.

This study has demonstrated that a partial G6PD deficiency as a risk factor for the occurrence of spontaneous abortion after adjusting for confounding

factors. G6PD is essential for protection against oxidative stress in erythrocytes. Its full defect or partial deficiency therefore causes increased susceptibility of erythrocytes to H_2O_2 and other reactive oxygen species that can lead to hemolytic anaemia and spontaneous abortion (Toncheva and Tzoneva, 1985). Oxidative stress as a result of Glucose-6-Phosphate Dehydrogenase enzyme deficiency and loss of other antioxidant defenses have been implicated and has been shown by the study of Simsek and co-workers to be associated with recurrent pregnancy loss (Simsek *et al.*, 1998). In G6PD deficient pregnant women, the G6PD activity is reduced, and this increases hydrogen peroxide (H_2O_2) level and thus induces the formation of hydroxyl radical ($\cdot OH$). Hydroxyl radicals are highly reactive and may cause damage to important components that maintain cell integrity and survival in the placenta particularly the syncytiotrophoblast (Burton *et al.*, 2003; Kliman, 2000a). The syncytiotrophoblast synthesizes human chorionic gonadotropin (hCG) required for the development of the placenta. In addition, syncytiotrophoblast is located at the surface of the villi, where it is susceptible to the attack of ROS (Burton *et al.*, 2003; Kliman, 2000b). If the capacity of oxidant is more than that of antioxidant, such as in G6PD full deficiency or partial deficiency, syncytiotrophoblast or trophoblast degenerates, leading to abnormal placentation and may result in spontaneous abortion (Jauniaux *et al.*, 2000; Kliman, 1994). The same can be said about women with a partial defect, thus the essence of the findings in this current study.

Several other studies have identified glutathione and glutathione peroxidase as potent antioxidants that neutralize free radicals and lipid peroxides to maintain intracellular homeostasis acting as a scavenger of ROS and also maintains the functional sulphhydryl groups. In a large case-control study, gene polymorphisms of glutathione S-transferase class 1 (GSTM1) were studied. Increased risk of recurrent pregnancy loss was found to be associated with the GSTM1 genotype null polymorphism in patients with recurrent pregnancy loss (Miller *et al.*, 2000; Sata *et al.*, 2003). Animal studies have

demonstrated the protective effects of glutathione peroxidase against oxidative damage in neurogenic tissue development in fetuses (McLean *et al.*, 2005). The sulfhydryl group of glutathione requires NADPH as a cofactor which is obtained as one of the metabolites in the Pentose Phosphate Pathway (PPP). The rate limiting step of this critical pathway is catalyzed by Glucose 6 phosphate dehydrogenase enzyme (G6PD). Deficiency of this enzyme could result in an imbalance between ROS and the glutathione antioxidant system leading to the generation of oxidative stress. Burton *et al.* identified placental oxidative stress as a main contributing factor that impairs placental development and degeneration of syncytiotrophoblast in early pregnancy that may lead to complications such as recurrent abortions, preeclampsia, and congenital anomalies (Burton *et al.*, 2003) by inducing apoptosis leading to derangements in placental invasion.

5.3.2 Multiparity and the risk of spontaneous abortion

Also, multiparity was found to be a risk factor for the occurrence of spontaneous abortion. This suggests that the probability of spontaneous abortion in multiparous women is higher than in primiparous or nulliparous women.

Similar findings have been reported in other studies (Everett, 1997; Jibril *et al.*, 2014; Pollack *et al.*, 2010). The mechanism of multiple births pre-disposing a woman to spontaneous abortion could be explained with possible endometrial weakening during repeated pregnancies, which impedes successful implantations during subsequent births (RASMUSSEN *et al.*, 2000). Risk is also higher in advanced age and increased parity (Camargo *et al.*, 2011). There is also evidence of a strong relation between age and the occurrence of spontaneous abortion (Andersen *et al.*, 2002; Everett, 1997). It is therefore valid to also indicate that the impact of parity on the occurrence of spontaneous abortion could be attributed to age.

5.3.3 Antenatal care and the occurrence of spontaneous abortion

In discussing spontaneous abortion, it is essential to scrutinise the part played by antenatal care, as being paramount. Various studies have illustrated some unquestionable benefits of antenatal care (Carroli *et al.*, 2001; Künzel and Misselwitz, 2003; Villar and Bergsjg, 1997) and hence the need for regular antenatal care. This study highlights the impact of non-attendance to antenatal was found to impact on the occurrence of spontaneous abortion. Non-attendance of antenatal care was found to be a risk factor for the occurrence of spontaneous abortion in this study which is in consonance with several other studies. Antenatal care is one of the “four pillars” of safe motherhood, as formulated by the Maternal Health and Safe Motherhood Program, Division of Family Health, of the World Health Organization (WHO) (WHO, 1996). Among regular checks on the foetal health status, several interventions for some known causes of spontaneous abortion such as anemia and malaria (McGready *et al.*, 2012), infections (Parazzini *et al.*, 1997), diabetes mellitus (Brydon *et al.*, 2000) among others are provided during antenatal. It can therefore be said pregnant women who do not attend antenatal care or do so irregularly face the risk of spontaneous abortions since the possible causes may not be detected and attended to.

Chapter 6

CONCLUSION

This study highlights the impact of folic acid, Vitamin B12 and other hematinic supplementation in obstetrics practice in preventing nutritional causes of anaemia, neural tube defects and other pregnancy related complications. However, the serum folate levels found among the pregnant women in this study was abnormally higher (supraphysiologic) and levels were associated with recurrent spontaneous abortion among the study group.

This brought to the fore the impact of high folic acid levels on the safety of pregnancies. In Ghana, the common practice adopted by the various antenatal clinics/hospitals is a routine folic acid, Vitamin B12 and iron supplements in each monthly or weekly antenatal visit irrespective of levels in the blood or age of gestation. Formation and closure of neural tube occurs at the 4th week of gestation; hence continues supply of folic acid at advance gestation in pregnancy to prevent neural tube defects has no physiologic effect.

This study also highlights the prevalence of iron deficiency and iron deficiency anaemia using both the conventional or classical parameters and new diagnostic tools (transferrin/log ferritin and transferrin receptor (sTfR) in assessing iron status.

The study showed lower prevalence of iron deficiency and iron deficiency anaemia using the conventional markers as compared to the modern indicators which gave higher prevalence rate of ID and IDA which seem to suggest that iron deficiency and iron deficiency anaemia are being underdiagnosed using the classical or conventional parameters of assessing iron status. It also indicates the usefulness of the new diagnostic tools (transferrin/log ferritin and transferrin receptor (sTfR) in identifying iron deficiency and iron deficiency anaemia with high sensitivity and specificity.

Results from this study indicate that anaemia and iron deficiency is still a major challenge among pregnant women in Ghana.

The prevalence of anaemia in this study as shown by the modern indicators of iron status is very alarming and a severe public health concern per the definition by the World Health Organization. Furthermore, this study indicates that hematinic and other iron supplementation do not prevent iron deficiency occurrence among most pregnant women in the advanced stage of gestation.

HIV, Hepatitis B viral and other infections are highly prevalent among pregnant women in Ghana as it is highly associated with anaemia as seen in this study.

There is profound emotional and psychological trauma associated with recurrent spontaneous abortion as women and family finds it difficult to cope with the loss. This study revealed a higher prevalence of spontaneous abortion among pregnant women in Ghana, figures extremely higher than that found in the developed countries. This was found to be associated with G6PD partial defect.

This study also highlighted the risk of multi-parity and default in antenatal clinic attendance in the spontaneous loss of pregnancy. Women who have had multiple child birth had increased risk of spontaneous abortion due to a possible endometrial weakening associated with repeated pregnancies. Antenatal care and visit during pregnancy cannot be overemphasized as it is one of the “four pillars” of safe motherhood. Pregnant women who did not attend antenatal clinic regularly had a higher risk of occurrence of spontaneous abortion as revealed in this study.

Recommendations for policy review and further work

This call for further research and rigorous evaluation of the existing antenatal care program that offers folic acid and Vitamin B12 in each month or week of antenatal visit even in advance stages of pregnancy, regardless of serum levels of these micronutrients. This is to prevent these abnormally higher serum levels and the occurrence of spontaneous abortion as seen in this study.

Proper dietary intake should be the utmost aim as it may be sufficient to prevent folate or Vitamin B12 deficiency, one of the key goals of haematinic and multivitamin supplementation in obstetrics practice.

The modern indicators for assessing iron status should be gradually introduced in routine clinical practice so it can augment or gradually replace the conventional parameters of iron status that is; serum iron, transferrin, TIBC, TSAT% and ferritin alone in clinical laboratories. They would be very useful in clinical practice as it will replace bone marrow examination that is currently considered the gold standard in assessing iron status which is regarded as highly invasive procedure.

Again, a more thorough investigations which should include modern markers of iron status rather than the routine obstetrical practice of determining haemoglobin, haematocrit, mean cell volume, serum iron, TIBC, TSAT%, ferritin which are unreliable as early predictors of iron deficiency and anaemia in pregnancy and are also influence by inflammatory or infectious conditions. A thorough assessment of this approach should therefore be considered.

Antenatal screening for G6PD status among pregnant women should be of high priority in obstetrics practice in Ghana. Pregnant women found to have

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partial or full defect of this enzyme must be monitored closely during their gestation period to prevent the spontaneous loss of fetus.

Antenatal care and visit during pregnancy should be advocated by all stakeholders in Maternal and Child Health as it is one of the “four pillars” of safe motherhood. Pregnant women who did not attend antenatal clinic regularly has a higher risk of occurrence of spontaneous abortion and other pregnancy related complications that pose greater threat to the health of both mother and the unborn child.



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