KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

BIOCHEMICAL AND HAEMATOLOGICAL PROFILES OF ANAEMIC PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT THE BOLGATANGA REGIONAL HOSPITAL, GHANA.

BENJAMIN AHENKORAH

NOVEMBER, 2015

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY & BIOTECHNOLOGY

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BENJAMIN AHENKORAH (BSc)

NOVEMBER, 2015

DECLARATION

I hereby declare that this work is the report of the research I undertook in partial fulfillment for the award of an MPhil degree and that to the best of my knowledge (except where due acknowledgement has been made in the text), it contains no material which has been accepted for the award of any other degree in this or any other University.

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DEDICATION

THIS WORK IS DEDICATED TO THE LORD JESUS CHRIST FOR HIS GOODNESS, MERCIES AND GUIDANCE IN ALL MY ENDEAVOURS. TO MY PARENTS, FOR THEIR PRAYERFUL SUPPORT AND ENCOURAGEMENT, AND TO MY LOVELY FAMILY FOR THEIR LOVE, CARE AND ATTENTION.

ABSTRACT

Anaemia in pregnancy is a major public health problem associated with maternal morbidity and mortality, especially in developing countries. It is important that there should be a firm diagnosis of anaemia to unravel its possible cause(s) before the prescription of an appropriate therapeutic approach. The use of haemoglobin (HGB) level alone appears insufficient in determining the status of anaemia in pregnancy. It is hypothesised that a combination of biochemical and haematological parameters could enhance the diagnosis. A cross-sectional study was conducted among pregnant women attending their first antenatal visit at the Bolgatanga Regional Hospital Antenatal Clinic. Using a structured questionnaire, the socio-demographic data of the women were obtained. Venous blood was collected for haematological and biochemical analyses. Haematological parameters such as haemoglobin electrophoresis, white blood cells (WBCs), haemoglobin (HGB), haematocrit (HCT), red cell distribution width (RDW), mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV) were determined. The biochemical parameters determined included ferritin, serum iron, total iron binding capacity (TIBC), transferrin saturation (TfS), C-Reactive Protein (CRP), bilirubin, etc. Also, thick blood films were prepared for malaria parasite identification, while early morning stool and midstream urine samples were used for the determination of enteric and urogenital parasites, respectively. It was found that younger age in pregnant women (<30 years) increased the risk of anaemia, with an odds ratio of 1.677(1.081-2.600). The study also found a significant association ($p<0.05$) between parity, gravidity and anaemia in pregnancy. Rural dwelling, and environmental factors such as source of drinking water (borehole, well) and presence of domestic animals also contributed to anaemia in pregnancy, (p-value<0.05). Considering the biochemical parameters, it

was observed that serum iron and transferrin saturation had a significant association with anaemia in pregnancy; p-value<0.05. There was no significant difference (p-value>0.05) with regards to haemoglobinopathies and parasitic infections in the two groups of pregnant women. This study has succeeded in the advocacy for investigating the cause of anaemia before blindly treating patients with haematinics.

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LIST OF ABBREVIATIONS

ANC: Antenatal Clinic

BMS: Biomedical Scientist

CI: Confidence Interval

CRP: C-Reactive Protein

EDTA: Ethylenediaminetetra-acetic acid

GDHS: Ghana Demographic and Health Survey

GSS: Ghana Statistical Services

HbSC: Haemoglobin SC

HbSS: Haemoglobin SS

HGB: Haemoglobin

HCT: Haematocrit

HIV: Human Immunodeficiency Virus

IDA: Iron Deficiency Anaemia

LBW: Low Birth Weight

KHRC: Kintampo Health Research Centre

KNUSTCHRPE: Kwame Nkrumah University of Science and Technology Committee on Human Research, Publications and Ethics.

MCH: Mean Corpuscular Haemoglobin

MCV: Mean Corpuscular Volume

MEIA: Microparticle Enzyme Immunoassay

MOH: Ministry of Health

NHRCIRB: Navrongo Health Research Centre Institutional Review Board

OR: Odds Ratio

PM: Primigravida

MG: Multigravida

RDW: Red Cell Distribution Width

RHB-ANC: Regional Hospital Bolgatanga Antenatal Clinic

RHB: Regional Hospital, Bolgatanga

TDHS: Tanzania Demographic and Health Survey

TIBC: Total Iron Binding Capacity

TfS: Transferrin Saturation

UNICEF: United Nations International Children's Emergency Fund

WBC: White Blood Cell

WHO: World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background to Study

The World Health Organisation defines anaemia in pregnancy as haemoglobin concentration less than 11.0g/dl (WHO, 1992), while the Ministry of Health of Ghana defines it as haemoglobin concentration less than 10.0g/dl (Glover-Amengor *et al.*, 2005). Recent statistics indicate that anaemia affects 41.8% of pregnant women globally, with the highest prevalence in Africa (WHO, 2006). Fifty seven percent of pregnant women in Africa are anaemic, which corresponds to ~17 million affected women, with severe consequence on health, social and economic development (Levin, 1986; Allen, 2000; de Benoist *et al*., 2008).

Studies in Africa have shown a high prevalence of anaemia in pregnancy, ranging from 41-83% in different settings (Meda *et al*., 1999; Antelman *et al*., 2000; TDHS, 2005; Uneke *et al*., 2007; Kidanto *et al*., 2009; Haggaz *et al*., 2010). There is however, significant variation in prevalence of anaemia, both within and between countries, necessitating a need for local data to help inform the formulation of effective preventive programmes.

Anaemia remains a major public health problem in Ghana and more effort is needed to combat it. Two national representative survey documents on the prevalence of anaemia among children under five and pregnant women showed that 84% of children under five, 71% of school-age children, 65% of pregnant women and 59% of lactating mothers were anaemic (MOH and UNICEF, 1998). Prevalence of anaemia was above

the 40% threshold, defined by WHO, representing a severe public health problem (Barbin *et al.,* 2001).

Among children under five, the prevalence of severe anaemia (haemoglobin <7.0 g/dL) was also very high, at 10%. For all population groups (children under five, school-age children, pregnant and lactating women), the prevalence of anaemia was higher in rural areas than in urban areas. Among children under five, the prevalence of anaemia was high in all regions (MOH and UNICEF, 1998).

In 2003, according to the Ghana Demograpic and Health Survey (GDHS) carried out by the Ghana Statistical Service and Ghana Health Service, the prevalence of anaemia among children aged 6-59 months was 76% and that of severe anaemia was 6%. Prevalence of anaemia was significantly higher in rural areas (80%) than in urban areas (68%). By region, there were substantial disparities, ranging from a prevalence of 61% in Greater Accra region to 83% in Northern region (GSS, 2004). Overall, the prevalence of anaemia was higher in rural areas than in urban areas and disparities across regions were marked, ranging from 34% in Brong Ahafo region to 51% in Upper East region (GSS, 2004).

Anaemia in pregnancy is associated with negative consequences for both the woman and neonate. Foetal anaemia, low birth weight (LBW), preterm birth, low appearance, pulse, grimace, activity and respiratory (APGAR) score, intrauterine growth restriction, and perinatal mortality have been associated with anaemia (Scholl & Hediger 1994; Msolla & Kinabo, 1997; Allen, 2000; Lone *et al*., 2004; Adam *et al*., 2007; Kidanto *et al*., 2009; Haggaz *et al*., 2010). In the women themselves it may cause low physical activity and increased risk of maternal morbidity and mortality,

especially in those with severe anaemia (Scholl & Hediger, 1994; Allen, 2000; de Benoist *et al*., 2008).

The cause of anaemia in pregnancy is multi-factorial. Low caloric intake, leading to deficiencies in iron, folate, vitamin B_{12} and vitamin A, as well as intestinal parasitic infections, malaria, haemoglobinopathies and HIV have all been shown to be the main causes of anaemia among pregnant African women (Msolla & Kinabo, 1997; Verhoeff *et al*., 1999a; Allen, 2000; Antelman *et al*., 2000; Uneke *et al*., 2007).

The adverse socio-economic and health impacts of anaemia in pregnancy have made anaemia prevention and control a top priority in many countries. To formulate and to implement policies aimed at preventing and reducing these adverse outcomes requires adequate knowledge of the distribution and predisposing factors for the disorder at the sub-population level. Unfortunately this knowledge is often unavailable or is inadequate in most developing countries (Ayoya *et al.*, 2006). This cross-sectional study was designed to determine the biochemical and haematological profiles of anaemic pregnant women attending Antenatal Clinic at the Bolgatanga Regional Hospital. The study involved both rural and urban pregnant women attending the Bolgatanga Regional Hospital. The findings from this study will provide the basis for local micronutrient fortification and supplementation programmes, promote the formulation and implementation of effective anaemia control and preventive measures, as well as identifying future research needs.

1.2 Aim of the Study

To determine the biochemical and haematological profiles of anaemic pregnant women attending Antenatal Clinic at the Bolgatanga Regional Hospital.

1.3 Specific Objectives:

1. To use a questionnaire to find out the economic and socio-cultural background of pregnant women in order to determine some factors responsible for the nutritional status.

2. To assess the haematological profiles and the haemoglobin genotype of pregnant women attending Antenatal Clinic at the Bolgatanga Regional Hospital.

3. To determine the possible parasitic agents associated with the low haemoglobin levels.

4. To determine the biochemical iron indicators (ferritin, serum iron, TfS and TIBC), bilirubin (direct and total) and C-Reactive Protein levels of pregnant women.

1.4 Study Hypothesis

H_1- Anaemia causes significant changes in biochemical and haematological parameters in pregnant women.

1.5 Justification of Study

The anaemic condition in Ghana can be attributed to iron deficiency and parasitic infections such as malaria, hookworm and schistosomiasis. Anaemic pregnant women exhibit low physical activity and increased risk of maternal morbidity and mortality, especially in those with severe anaemia (Scholl & Hediger, 1994; Allen, 2000; de Benoist *et al*., 2008). Anaemia in pregnancy is also associated with negative consequence for the neonate.

This study therefore sought to determine the possible causes of anaemia in pregnancy, by evaluating the biochemical and haematological profiles of pregnant women attending Antenatal Clinic at Regional Hospital, Bolgatanga (RHB) and also provide possible treatment and preventive guidelines.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction: Anaemia

Anaemia refers to a condition in which there is a decrease in the oxygen carrying capacity of blood, as a result of a reduction in the number of normal circulating red blood cells or to a lower haemoglobin concentration or haematocrit than that which is normal for a person's age, sex and geographical location (Gupta and Kalia, 2004). Due to the existence of differences in haemoglobin distribution amongst healthy populations in different geographical areas of the world, many countries have established their own haemoglobin thresholds to define anaemia in pregnancy. In Ghana for example, the Ministry of Health's haemoglobin threshold for anaemia at all stages of pregnancy is 10.0g/dl (Glover-Amengor *et al.*, 2005; Baiden *et al.*, 2006).

To ensure that research data from different parts of the world can be compared and interpreted uniformly, the World Health Organisation has recommended the use of a haemoglobin concentration threshold of 11.0g/dL to define anaemia in pregnancy throughout the world (de Benoist *et al.*, 2008; Dim and Onah, 2007; WHO Global Database on Anaemia, 2008).

2.2 Distribution and public health significance of anaemia

Anaemia in pregnancy is an important public health problem in both developing and developed countries. In 2008, the WHO estimated that 41.8% (56.4 million women) of pregnant women in the world and 55.8% of pregnant women (19.3 million women) in Africa were anaemic. Most of the pregnant women with anaemia live in Africa and Asia (WHO global database on anaemia, 2008). Between 35% and 75% of all pregnant women in developing countries are anaemic, by WHO standards, compared

to less than 18% anaemia prevalence in industrialized countries (WHO, 1992; Ayoya *et al*., 2006). The exact prevalence of anaemia in most sub-populations in most developing countries is unknown. The national estimates are often an underestimation of the magnitude of the situation at the community level. In India for example, WHO estimates that 49.7% of all pregnant women are anaemic (WHO global database on anaemia, 2008). Agarwal *et al*. (2006), in a survey involving 1148 pregnant women in seven Indian states, found an overall anaemia prevalence of 84%. In five out of the seven states, the prevalence ranged between 91 and 97%. It was also reported by Awan *et al*. (2004) that the prevalence of anaemia in pregnancy in Railway Colony, Multan, Pakistan was 96%, compared to the WHO estimate of 30.1% for Pakistan.

The prevalence of anaemia in pregnancy in Ghana is higher in rural areas (70.8%) and in Northern Ghana (74.1%), when compared to that of urban areas (61.5%) and Southern Ghana, respectively (de Benoist *et al*., 2008). In Ghana, India and many other developing countries, the sub-national prevalence of anaemia in pregnancy remains unknown in most communities. Anaemia prevalences of 34%, 70% and 57% were reported by Engmann *et al.* (2008), Baiden *et al*. (2006) and Glover-Amengor *et al*. (2005) in Accra, Navrongo and the Sekyere West Districts of Ghana, respectively. The latter two studies used the Ministry of Health's threshold of 10.0g/dl to define anaemia in pregnancy. In the Sekyere West District, only 2 out of 205 pregnant women (all in the third trimester of pregnancy) had haemoglobin levels greater than or equal to 11.0g/dL. The prevalence of anaemia in some selected countries is shown in Table 2.1.

Table 2.1: Prevalence of Anaemia in Selected Countries

Country	Prevalence		Public Health Significance*
	%	95% CI	
Papua New Guinea	55.2	24.2-82.6	Severe
Ghana	64.9	58.0-71.2	Severe
India	49.7	47.9-51.5	Severe
Nigeria	66.7	59-73.6	Severe
USA	5.7	3.6-8.9	Mild
UK	15.5	3.8-44.7	Moderate

* 0.00-4.99% = no significance, 5.00-19.99% = mild, 20.00-39.99%= moderate
≥40.00% = severe. Anaemia is a mild public health problem in developed countries (USA and UK) but a severe problem in developing countries (the rest of the countries in the table)

Compiled from WHO global database on anaemia; Available at www.who.int/vmnis

2.3 CLINICAL FEATURES OF ANAEMIA IN PREGNANCY

Pregnant women with anaemia may not have any symptom as the body's organ systems get adjusted to the reduction in haemoglobin mass however, they may present with vague complaints of ill health, fatigue, loss of appetite, digestive upset, and dyspnoea, especially on exertion, lethargy, headache, dizziness and palpitation. Clinical examination may reveal pallor of the mucous membranes and conjunctivae, tachycardia, systolic flow murmurs, pale nail beds, and occasionally koilonychias (brittle spoon-shaped nails) (Cheesbrough, 2004).

2.4 CAUSES OF ANAEMIA IN PREGNANCY

Anaemia in pregnancy in most developing countries is often multi-faceted. The causes may be physiological or pathological. Physiological anaemia in pregnancy results from haemodilution, caused by the expansion of maternal plasma volume (by 40-50%) in excess of that of red cell mass (20-25%) (McCarthy & Hunter, 2003; Awan *et al*., 2004).

Of the pathological causes of anaemia in pregnancy, iron deficiency resulting from inadequate intake, increased physiological demand, malabsorption or chronic blood loss is perhaps the most important. It accounts for at least 50% of all anaemias in pregnancy in developing countries (WHO, 1992; Allen, 2000). Other causes of anaemia in pregnancy include acute and chronic infection (malaria, hookworm, *Schistosoma haematobium* and recently, HIV); deficiency of vitamins (vitamin B12, folic acid and vitamin A) and haemoglobinopathies, like the thalassaemia and sickle cell disease (Fleming, 1989a; Meda *et al*., 1999; Ayoya *et al*., 2006).

Nulliparity and grand multi-parity, low socio-economic status, young age, illiteracy, low birth spacing and cultural factors such as taboos prohibiting pregnant women from consuming meat or egg-based foods also predispose pregnant women in developing countries to anaemia (Awan *et al*., 2004; Dim & Onah, 2007; Munasinghe and van den Broek, 2007). These predisposing factors are not universal in their significance. Their significance varies from one antenatal population to another. Within the same population, not all of them might even be significant.

2.5 CLASSIFICATION OF ANAEMIA

Anaemia may be classified based on a number of criteria, including the pathologic mechanisms causing the anaemia and the morphological appearance of the red cells during anaemia. The advantages of the morphological system of classification are that:

- The size of the red cells and the haemoglobin content of the red cells usually suggest the nature of the underlying disorder causing the anaemia.
- Red cell indices based on automated haematology analysers may also suggest the nature of the underlying defect before anaemia occurs. They may also identify disorders such as thalassaemia traits in which anaemia may not occur (Cheesbrough, 2004).

Based on red cell size, anaemia may be described as microcytic (red cells smaller than normal or MCV<80fL), normocytic (normal size or 79<MCV<95fL) and macrocytic (MCV> 95fL). Based on the red cell haemoglobin content, anaemia may be described as hypochromic (paler red cells with MCH<27pg) or normochromic (normal red cells with MCV>26pg) (Hoffbrand *et al*., 2001; Cheesbrough, 2004). A combination of descriptive information on red cell size and haemoglobin content is often used to give the morphologic picture of red cells in anaemia. Figure 2.1 shows the commonest morphological features of red cells in anaemia and the conditions associated with these features.

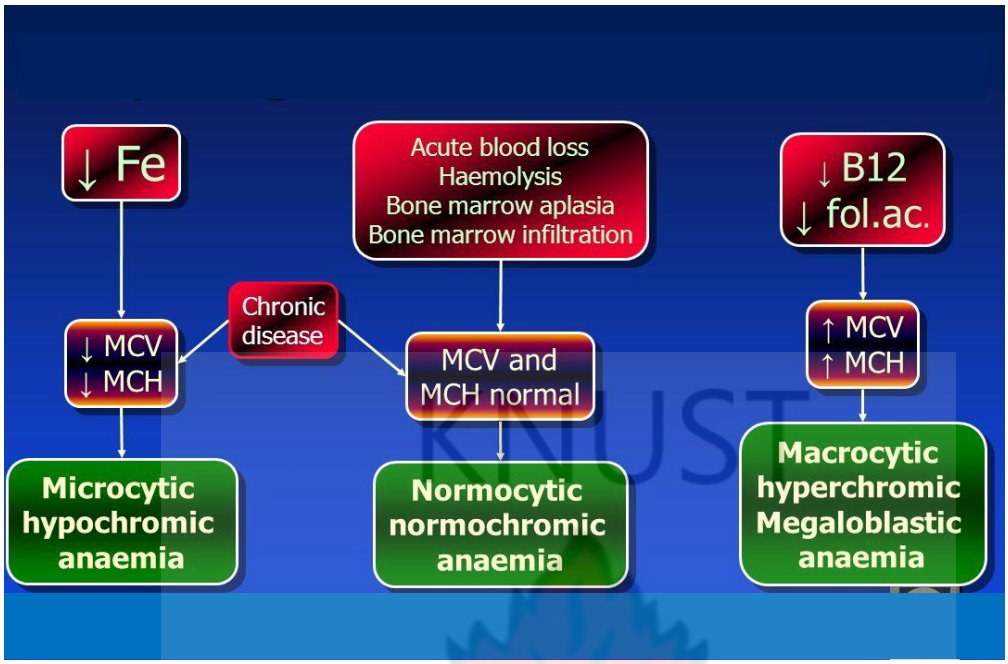

Figure 2.1 Morphological Classification of Anaemia

(Courtesy:*www.tuo.agh.edu.pl*)

2.6. Haemoglobin structure: An overview

Knowing that disorders of haem metabolism or globin synthesis can lead to microcytic anaemia, an appreciation of haemoglobin structure and how it changes over the first few months after birth is important. Haemoglobin is a 68000 Dalton iron-containing metallo-protein found in the red blood cells of vertebrates (Sittivilai, 2004). Haemoglobin is produced by a multi-step process involving several enzymes in mitochondria and the cytosol. It consists of an iron-containing protoporphyrin ring (haem) associated with each of four polypeptide globin chains. The polypeptides exist as a pair of similar globin chains (Haen, 1995).

Except for the first few weeks after conception, the dominant haemoglobin is foetal haemoglobin (Hb F: α2γ2), composed of the haem associated with a pair each of alpha and gamma-globin chains. As pregnancy progresses, the foetus transitions to an adult haemoglobin pattern by gradually decreasing the amount of Hb F and increasing the amounts of haemoglobin A (α2ß2) and haemoglobin A2 (α2δ2). Between 6 and 10 months after birth, most children have a distribution of haemoglobin types similar to that of adults. Thus, disorders of beta-globin genes, such as beta-thalassaemia or sickle cell disease, may not become apparent until partway through the first postnatal year when normal beta-globin chain synthesis is supposed to begin (Richardson, 2007). The structure of normal adult human haemoglobin is shown in Figure 2.2.

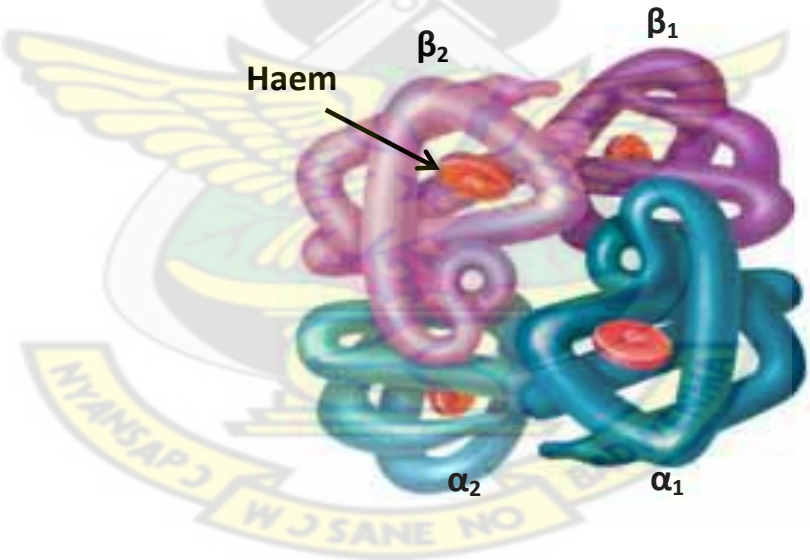

Figure 2.2: The haemoglobin molecule: note four haem molecules tucked inside globin chains. (Adapted from Ciela Betty, 2007)

2.6.1. IRON DEFICIENCY IN PREGNANCY

Iron deficiency and iron deficiency anaemia are the most prevalent nutritional deficiency problems during pregnancy. They are more common in pregnant women

than in any other population group. Due to the fact that iron deficiency anaemia is the leading cause of anaemia in most developing countries, anaemia and iron deficiency anaemia are often used interchangeably and the prevalence of anaemia taken to be equal to that of iron deficiency (Tolentino and Friedman, 2007; Okwu and Ukoha, 2008). It has increasingly been observed in many tropical countries that the proportion of anaemic pregnant women is usually higher than the proportion of pregnant women with depleted iron stores. (Mockenhaupt *et al.*, 2000; Engmann *et al.*, 2008)

Anaemia is often a late manifestation of iron deficiency. The sequence of events leading to the development of iron deficiency anaemia usually occurs in three stages as follows:

1. When demand for iron or blood loss exceeds intestinal iron absorption, a negative iron balance ensues. Iron is mobilized from stores; storage iron decreases; plasma ferritin decreases; iron absorption increases, and plasma iron-binding capacity increases. This stage is known as *iron depletion.*

2. After iron stores are depleted, the plasma iron concentration falls, saturation of transferrin falls below 15%, and the percentage of sideroblasts decreases in the marrow. As a result of lack of iron for haem synthesis, red cell protoporphyrin increases. This second stage is *iron deficient erythropoiesis;* anaemia may not yet be present.

3. The third stage is *iron deficiency anaemia;* in addition to the above abnormalities, anaemia is detectable. The anaemia is at first normochromic and normocytic but gradually becomes microcytic, and finally microcytic and hypochromic (Tarek-Elghetany and Banki, 2006).

Deficiency of iron has been reported to adversely affect pregnant women. Pre-term delivery, low birth weight, suppression of maternal immune response, work incapacitation and diminished mental capacity of children born to iron deficient mothers are some of the consequences of iron deficiency anaemia (UNICEF, 2001)

2.6.2. Iron requirements during pregnancy

Due to the cessation of menses, iron requirements during the first trimester of pregnancy are usually low until gestational week 16 when maternal red cell mass and plasma volume expansion increase the demand for iron. The need for iron increases almost linearly until term. Although red cell mass expansion ceases in the last 5–10 weeks of pregnancy, demand for iron is still high due to increase in erythropoiesis in the developing foetus as well as to an increased transfer of iron to the placenta (Allen, 2000). Each day, a pregnant woman requires 1.5-3.0mg of iron. About 0.5-1.0mg of the daily iron requirement is used to replace iron lost through urine, sweat and faeces. The remaining 1-2mg of iron is used for expansion of red cell mass as well as for foetal and placental growth (Hoffbrand & Pettit, 1993). The total amount of iron required for an average pregnancy is about 1000mg. Of this, 350mg is transferred to the foetus and placenta, 250mg is lost in blood at delivery, and 240mg is basal losses (van den Broek, 2003). The additional 450mg of iron required for the expansion of maternal red blood cell mass contributes to depletion of iron stores during gestation. The increased demand for iron is partially offset by cessation of menstruation. This compensation is however, not enough to meet the demands for iron during pregnancy, hence supplementation may be required (WHO, 1992).

2.6.3. Iron absorption and transport

Iron is absorbed in the duodenum and upper jejunum. Absorption of iron is determined by the type of iron molecule and by what other substances are ingested. Iron absorption is best when food contains haem iron as in meat. Dietary non-haem iron must be reduced to the ferrous state and released from food binders by gastric secretions. Non-haem iron absorption is reduced by other food items (e.g., vegetable fibre, phytates and polyphenols; tea tannates, phosphoproteins; and bran) and antibiotics like tetracycline (Lichtin, 2008a). Ascorbic acid is the only common food component known to increase non-haem iron absorption by reducing ferric iron to the more soluble and absorbable ferrous form (WHO, 1992).

Iron from intestinal mucosal cells is transferred to transferrin, an iron-transport protein synthesized in the liver. Transferrin can transport iron from intestinal luminal cells and macrophages to specific receptors on erythroblasts, placental cells, and hepatocytes. For haem synthesis, transferrin transports iron to the erythroblast mitochondria, which insert the iron into protoporphyrin to form haem. Transferrin (plasma half-life, 8 days) is then extruded for reutilization. Synthesis of transferrin increases with iron deficiency but decreases with any type of chronic disease (Andrews, 2005).

2.6.4. Causes of iron deficiency in pregnancy

Iron deficiency in pregnancy may result from nutritional deficiency, increased iron requirements in pregnancy, malabsorption or chronic blood loss. Nutritional iron inadequacy is a major cause of iron deficiency among pregnant women in developing countries, where plant-based food containing inadequate or less absorbable iron is widely consumed (Munasinghe & van den Broek, 2007). The increased demand for

iron to meet maternal red cell mass expansion, foetal and placental growth leads to further depletion of maternal iron stores, especially among those who enter pregnancy with partially depleted iron stores as is often the case in developing countries (WHO 1992).

Malabsorption secondary to gastrectomy, gluten-induced gastroenteropathy or atrophic gastritis, gastrointestinal bleeding (may be due to peptic ulcer, hookworm infection, oesophageal varices, carcinoma or ulcerative colitis) and rarely haematuria, haemoglobinuria are often associated with iron deficiency, hence predispose the pregnant woman to anaemia (Rockey and Cello, 1993; Lichtin, 2008a).

2.6.5. Diagnostic features of iron deficiency anaemia

In addition to the usual manifestations of anaemia (section 2.3), some uncommon symptoms occur in severe iron deficiency. Patients may have pica, an abnormal craving to eat unusual substances (e.g., ice, dirt, paint). Other findings include a painless glossitis, cheilosis, concave nails (koilonychias), and, rarely, dysphagia caused by a postcricoid oesophageal web (Plummer-Vinson or Paterson-Kelly syndrome) (Mehta and Hoffbrand, 2005; Wikipedia, 2012).

The gold standard for the laboratory diagnosis of iron deficiency is still the examination of a bone marrow biopsy suitably stained by the Perl's method. Due to its invasiveness, bone marrow examination is not a routine procedure (Munasinghe & van den Broek, 2007). In iron deficiency anaemia, all red blood cell indices are usually reduced, in proportion to the severity of the iron deficiency. The red cell distribution width (RDW) is usually greater than 15%; the peripheral blood film

shows pencil cells and reduced red cell and reticulocyte counts. Platelet count is usually elevated (Haen, 1995; Cheesbrough, 2004).

Although serum ferritin concentration of less than 12μg/L is usually used as an indicator of iron deficiency, serum ferritin cut-off of 30μg/L was recommended by van den Broek & Letsky (2000), as being the best indicator of deficient iron stores in tropical countries (higher sensitivity and specificity). This high ferritin threshold, they claim, adjusts for the rise in ferritin associated with the widespread inflammatory processes found in pregnant women in developing countries (Munasinghe & van den Broek, 2007). Other indicators of iron deficiency are a low serum iron level, an elevated serum transferrin and transferrin receptor (TfR) and a high total iron binding capacity (TIBC). The free erythrocyte protoporphyrin (FEP) and zinc erythrocyte protoporphyrin (ZEP) are usually elevated, reflecting impaired incorporation of iron into protoporphyrin to form haem (Brittenham, 2005).

2.6.6 The thalassaemia syndromes

The thalassaemias are a heterogeneous group of autosomal recessive disorders of haemoglobin synthesis, all of which result from a reduced rate of production of one or more of the globin chains. As a group, the thalassaemias constitute one of the most common single gene disorders in the world – with estimates of gene frequencies ranging from 2.5% to 15% in some areas of the tropics (Xu *et al.*, 2004). The thalassaemias are widely distributed around the world however, some forms of the disorder occur in higher frequencies in certain geographical locations. Beta-thalassaemia is more common amongst people with Mediterranean ancestry, especially those from Northern Italy and Greece, where 5-10% of the population carry the heterozygous trait (Xu *et al.*, 2004). Beta-thalassaemia is also found in few

locations in Africa. Alpha-thalassaemia occurs predominantly in Asia (especially in China, Philippines and Thailand) and throughout Africa (Modell & Darlisson, 2008). The thalassaemias follow a pattern of distribution along the tropics where malaria is or was highly prevalent. It has been suggested by Luzzi *et al.* (1990) that the thalassaemias afford their carriers some protection against death from *Plasmodium falciparum* infection by depriving the parasites of nutrients normally derived from digestion of haemoglobin. Other researchers have suggested that, parasitized thalassaemic cells bind more antibody than normal control red cells thus, facilitating the clearance of malaria parasites from the blood (Teo & Wong 1985; Luzzi *et al.*, 1990). The distribution of thalassaemias in Ghanaian communities is largely unknown. Mockenhaupt *et al*. (2000) reported that alpha-thalassaemia and beta-thalassaemia respectively affected as much as 33% and 1% of antenatal populations in a Ghanaian community. The same study demonstrated that alpha- thalassaemia trait protects from malaria. Mockenhaupt *et al.* (2004), also reported the prevalence of α^{+} in children in Tamale, Ghana, and its surrounding areas to be 31.8%. The protective effect of thalassaemia trait against severe malaria was also demonstrated. The protection however, did wane with increasing age.

2.6.7 Beta-thalassaemia syndrome

Two gene clusters, one on each of chromosome 11, control beta-like globin chain synthesis. Gene mutation may result in defects in one or both genes. These mutations result in ß-globin chain production ranging from nearly normal levels to virtually absent levels. A defect in one gene results in beta-thalassaemia carrier trait. Carriers of ß-thalassaemia are usually symptom-free, except during periods of stress such as pregnancy, when they may become severely anaemic. Splenomegaly is rare.

Haemoglobin values are in the 9–11g/dL range (Chui *et al.*, 2006). The red cells show hypochromasia and microcytosis with characteristically reduced red cell indices. The reticulocyte count is often normal. The bone marrow shows moderate erythroid hyperplasia. The characteristic finding is an elevated HbA_2 level in the 4–6% range (Chui *et al.*, 2006). There is a slight elevation of HbF in the 1–3% range in about 50% of cases. Serum ferritin, TIBC, FEP and TfS are usually normal in the absence of concurrent iron deficiency (Wonke *et al.*, 2007). If both genes are defective, ß-thalassaemia major or Cooley's anaemia arises; no beta globin chains will be produced 3-6 months after birth when the normal switch from foetal to adult haemoglobin is expected. The patient presents with hepatosplenomegaly, poor growth, jaundice due to chronic haemolysis and if untreated, frontal bossing and maxillary hyperplasia (due to bone marrow hyperplasia in response to anaemia). Without chronic blood transfusion and iron chelation therapy, the condition is incompatible with life. Allogeneic stem cell transplantation is curative but the need for lifelong immunosuppression has limited its widespread application (Bennett *et al.*, 2005).

Anaemia is usually severe (Hb<7.0g/dl) with reticulocytosis, basophilic stippling, target cells and nucleated red cells on blood smear (Olivieri, 1999). Haemoglobin electrophoresis reveals that Hb A and Hb F constitute 0-10% and 90-100% of total haemoglobin respectively. Hb A_2 is usually elevated. Serum bilirubin is also usually elevated with absent haptoglobin levels in serum as well. Confirmation of diagnosis of beta-thalassaemia requires electrophoresis at acid pH, high performance liquid chromatography, analysis of globin chain synthesis and most recently molecular techniques. In thalassaemia intermedia, the amount of ß-globin chains produced is

between that of thalassaemia trait and thalassaemia major. The patients require periodic blood transfusions (Olivieri, 1999).

2.6.8 Alpha- thalassaemia syndrome

The fact that both adult (HbA) and foetal (HbF) haemoglobins have α-globin chains, genetic disorders of α-chain synthesis may result in defective foetal and adult haemoglobin production. Four genes, a pair on each of chromosome 16, control alpha globin chain synthesis. Deletions and occasionally mutations in these genes result in stop codons, thus, an alpha globin gene either codes for alpha globin production ("is on/active") or does not ("is off/inactive"). In the normal human being, all four globin genes are functional (Weatherall, 2005).

Deletion or inactivation of one globin gene results in a person being a 'silent' carrier of alpha thalassaemia (a-/aa). Two gene deletions either on the same chromosome (cis: aa/--) or on different chromosomes (trans: a-/a-) result in alpha thalassaemia carrier state. Affected persons have mild microcytic hypochromic anaemia or are non-anaemic.

Deletion of three globin genes (a-/--) results in haemoglobin H ($ß_4$) disease. Persons with this disorder have variable degrees of microcytic hypochromic anaemia (Hb:7-11 g/dL) and hepatosplenomegaly but it is unusual to find severe thalassaemic bone changes or growth retardation (Weatherall, 2005).

Patients usually survive into adult life, although the course may be interspersed with severe episodes of haemolysis associated with infection or worsening of the anaemia due to progressive hypersplenism (Weatherall, 2005 and Tarek-Elghetany and Banki, 2006). Deletion of all four globin genes on the other hand, results in complete failure

of foetal and adult haemoglobin synthesis due to absence of alpha globin chains. Intrauterine death usually occurs and the condition is referred to as haemoglobin Bart (γ_4) or hydrops foetalis (Johnston, 2005). The genetic defects, clinical features and the pattern of haemoglobin electrophoresis in the alpha-thalassaemia syndromes are summarised in table 2.3.

Table 2.3 The Alpha Thalassaemia Syndromes

Syndrome	Defective genes	Genotype	Clinical features	Newborn	After first year
Hydrops foetalis	4	—/—	Foetal or neonatal death with severe anaemia	Hb Bart's > 80% Hb H, Hb Portland	
Hb H disease	3	—/-α (—/ααᶜˢ*)	Chronic haemolytic anaemia	Hb Bart's 20–40% (Hb CS*)	Hb H 5–30% (Hb CS 2–3%)
Thalassaemia minor	2	—/αα -α/-α αᵀα/-α	Asymptomatic, mild anaemia, thalassaemic indices	Hb Bart's 5–10%	None
Silent carrier	1	-α/αα (αα/ααᶜˢ*)	No clinical or haematological abnormality	Hb Bart's ± 1–2% (Hb CS*)	None (Hb CS* 1%)

* CS = constant spring

Source: Tarek-Elghetany and Banki, 2006

2.6.9 Laboratory diagnosis of alpha-thalassaemia

Alpha-thalassaemia trait is diagnosed most commonly when evaluation of a microcytic anaemia shows no evidence of iron deficiency or of beta-thalassaemia trait. The haemoglobin electrophoresis pattern in patients having alpha thalassaemia trait is normal. DNA analysis is the means of achieving diagnostic certainty. Hb H

disease can be diagnosed by demonstrating the fast-migrating Hb H or Bart's fractions on Hb electrophoresis. Recombinant DNA approaches of gene analysis (particularly the PCR) have become the standard for prenatal diagnosis and genetic counselling (Lafferty, 1998).

2.7.0 Anaemia of chronic disease

Any inflammatory state, acute or chronic, can produce either normocytic or microcytic anaemia, sometimes called anaemia of chronic disease or anaemia of inflammation. Cytokines such as interleukins-1 and 6; tumour necrosis factor-alpha (TNF-α); and interferon-alpha, -beta, and –gamma (Interferon-α,–β and –γ) are produced in inflammatory states, including cancer, acute or chronic infection, and autoimmune disorders (e.g., inflammatory bowel disease, connective tissue disease) (Weiss, 2002). These cytokines alter iron homeostasis, promote accumulation of iron in storage sites (macrophages in the marrow and reticuloendothelial system) and inhibit bone marrow erythroblast proliferation and differentiation (Weiss, 2002). Hepcidin, released by hepatocytes in response to inflammation, also inhibits iron release from macrophages. The result can be either microcytic or normocytic anaemia (Lichtin, 2008b). The cause of inflammation is usually clinically apparent in people who have this type of anaemia. When there are no clinical signs of inflammation but laboratory findings suggest anaemia of inflammation, checking for serologic markers of inflammation such as C-reactive protein (CRP) may be helpful (Weiss and Goodnough, 2005).

If anaemia of chronic disease is suspected, serum iron, transferrin, transferrin receptor, and serum ferritin should be measured. Haemoglobin concentration is

usually greater than 8g/dL, unless an additional mechanism contributes to the anaemia (Weiss, 2002). Elevated ferritin level, elevated erythrocyte sedimentation rate, and increased C-reactive protein concentrations, but decreased serum iron concentrations and transferrin saturation are usually associated with anaemia of chronic disease (Wians *et al.*, 2000).

Blood transfusions are rarely indicated because the anaemia is usually mild. Treatment is usually targeted at the cause of the inflammation. Recombinant human erythropoietin may be administered to induce erythropoiesis (Weiss and Goodnough, 2005).

2.7.1 Adverse effects of anaemia in pregnancy

Anaemia in pregnancy is associated with unfavourable outcome for both the mother and foetus. Pre-term delivery, low birth weight, increased risk of maternal and foetal mortality (Allen, 2000) neonatal growth stunting, neurological dysfunction, and poor cognitive development in infants (especially the pre-term ones) are some of the adverse effects of anaemia in pregnancy (Fleming, 1989b; El Guindi *et al.*, 2004; de Benoist *et al.*, 2008). About 20% of all maternal deaths in some developing countries can be attributed to anaemia in pregnancy. Anaemia causes maternal mortality by increasing the risk of maternal death, through haemorrhage at delivery, by increasing maternal susceptibility to infection and in severe cases, by causing cardiac failure (Fleming, 1989b).

Nearly all studies on anaemia in pregnancy and pregnancy outcome are retrospective in nature. It is possible therefore that some of these reported unfavourable outcomes were due to confounding variables such as infections, micronutrient deficiency and

late arrival at the hospital rather than to anaemia *per se* (Allen, 2000; Brabin *et al.*, 2001; van den Broek, 2003).

Furthermore, there is no internationally accepted haemoglobin threshold below which maternal or foetal mortality or morbidity will almost definitely occur. The absence of internationally accepted 'at risk' haemoglobin threshold has led to arbitrary assignment of haemoglobin values below which an unfavourable event will occur. This has led to difficulty in comparison of data from different researchers (Munasinghe & van den Broek, 2007).

Prospective randomised control double blind studies using maternal morbidity and mortality as outcomes are required but these are not being carried out, since it is considered unethical to deny a severely anaemic woman of the interventions that could potentially save her life.

2.7.2 Effect of malaria in pregnancy

Over 3.2 billion people are at risk of being infected by malaria parasites (Olumese, 2005; Guinovart *et al.*, 2006) and pregnant women as well as children under 5 years of age suffer highest morbidity and mortality. Older children and adults rarely suffer from severe complications of infection. Pregnancy renders previously immune women susceptible to maternal anaemia and low birthweight in response to malaria infection (Brabin, 1991). In regions of low malaria transmission, *Plasmodium falciparum* causes considerable burden on maternal and foetal health and contributes to foetal rejection and severe maternal malaria attacks and death, whereas in high transmission settings, intrauterine growth retardation, pre-term delivery, low birth weight, and maternal anaemia are of particular concern (McCormick, 1985; Menendez, 1995;

Steketee *et al.*, 2001). Women in their first and second pregnancies are particularly vulnerable to malaria (Bouyou-Akotet *et al.,* 2003; Tchinda *et al.,* 2007).

Low birthweight is a result of a combination of intrauterine growth restriction and prematurity (Menendez *et al.,* 2000). Infection is frequently asymptomatic (Steketee *et al.*, 1996c). Consequently, treatment of only symptomatic episodes will fail to treat most infections. Peripheral blood slides are often negative, despite parasites being sequestered in the placenta (Shulman *et al.,* 1999; Ismail *et al.,* 2000).

Epidemiological data have shown that the risk of malaria infection falls with increasing gravidity (McGregor, 1984), with primigravidae (PG) being at particularly high risk of infection. Possible reasons for this may include the development in multigravidae (MG), of strain-specific immunity to parasites which bind to chondroitin sulphate-A (the ligand thought to be the site of parasite binding leading to placental sequestration) (Fried *et al*., 1998; Ricke *et al*., 2000). Primiparity (having only one child) is an independent risk factor for *P. falciparum* infection during pregnancy (Bouyou- Akotet *et al*., 2003; Adegnika *et al*., 2006; Yatich *et al.*, 2009).

It is also known, through randomized controlled trials, that antimalarial interventions in PG can significantly reduce severe anaemia (Shulman *et al.,* 1999) and improve birthweight (Parise *et al.,* 1998). The evidence for benefit in multigravidae (MG), however, remains controversial, as a number of trials have limited recruitment to PG and secundigravidae (Gulmezoglu & Garner, 1998). It is, therefore, not clear whether interventions that are effective in PG should also be given to MG. Although the prevalence of malaria infection is highest in PG, infection in MG is not infrequent, but it is not clear whether the pathological effects of malaria in infected women are also less severe in MG (Parise *et al*., 1998).

Current WHO policy for endemic areas is: 'Intermittent treatment with an effective, preferably one-dose, antimalarial drug provided as part of antenatal care should be made available in highly endemic areas to women in their first and second pregnancies. Such intermittent treatment should be started from the second trimester onwards and not be given at intervals of less than one month apart' (WHO, 2000). These recommendations derive from trials of intermittent treatment with sulphadoxine-pyrimethamine (whereby women receive 2 or 3 doses of sulphadoxine-pyrimethamine in the second and third trimesters of pregnancy) (Schultz *et al*., 1994). This regime has been shown to be operationally feasible, safe and acceptable, reducing severe maternal anaemia in PG by 39% (Shulman *et al*., 1999) and significantly improving birthweight in PG and secundigravidae (Parise *et al*., 1998).

The picture is further complicated by HIV infection, HIV-positive women having a higher prevalence of placental malaria than HIV-negative women (Steketee *et al*., 1996c; Verhoeff *et al*., 1999b). HIV-positive MG often have a similar prevalence of infection to HIV-negative PG. This raises the question as to whether policies need to be adapted according to HIV prevalence rates.

2.7.3 Effects of helminths on pregnancy

More than two billion people are infected with helminth parasites worldwide (Dickson *et al*., 2000). Children (Harhay *et al*., 2010) and pregnant women (Adegnika *et al*., 2007) frequently suffer from such infections, and soil-transmitted helminths (*Ascaris lumbricoides, Necator americanus, and Trichirus trichiura*) cause high morbidity by leading to malnutrition, iron-deficiency anaemia, malabsorption, intestinal obstruction, and mental and physical growth retardation in childhood (Allen & Maizels, 1996; Bethony *et al*., 2006). *Schistosoma haem*a*tobium* causes substantial

morbidity in affected populations, and the considerable burden of genito-urinary pathology in women has recently been acknowledged (Kjetland *et al*., 2008; Ramarakoto *et al*., 2008). Intestinal helminth infections and schistosomiasis are highly prevalent in malaria endemic regions (sub-Saharan Africa, South-East Asia, and South America) but despite their potential clinical interaction, the burden of such co-infections has not yet been evaluated in pregnant women.

Besides their clinical importance, these parasitic co-infections may also be important for immunological studies of malaria during pregnancy because of the possibility that immune responses are modulated by helminths. Indeed, a number of studies have highlighted the immunological interactions upon helminth and malaria co-infections (Druilhe *et al*., 2005; Hartgers & Yazdanbakhsh 2006; Supali *et al*., 2010).

Whereas the association of helminth and malaria parasites has been reported by several authors (Druilhe *et al*., 2005; Hartgers & Yazdanbakhsh, 2006; Supali *et al*., 2010), only few data report such an association in pregnant women (Hillier *et al*., 2008, Yatich *et al*., 2009).While in Uganda, hookworm and *Mansonella perstans* were found to be independently associated with *P. falciparum* (Hillier *et al*., 2008), a Ghanaian study showed that women infected with hookworm and *A. lumbricoides* had a 4.8 times higher risk of having a malaria infection (Yatich *et al*., 2009). The actual association may vary by geographical region, depending on the prevalent species and the common risk factors for the parasites being studied. These risk factors could be immunological but are also very likely related to environmental or socioeconomic variables.

Current public health programmes focus on the preventive treatment of malaria in pregnancy and convincing data support the use of these programmes. A similar approach may be conceivable for helminth infections and schistosomiasis, but large

clinical trials are still lacking. An alternative approach would be to look for second-generation antimalarial drugs with activity against other parasitic diseases. Artesunate is known to act on *Schistosoma spp*. (Borrmann *et al.*, 2001) and recent data showed promising activity of mefloquine – alone or in combination with artesunate – against Schistosoma infection (Keiser *et al*., 2010). Similarly, artemether–lumefantrine was shown to have anthelminthic properties in a prospective study in Nigeria (Adedeji *et al*., 2008). Preventive treatment of multiple infections with a single regimen seems particularly promising and should attract further consideration.

2.7.4 Prevention and control of anaemia in pregnancy

To prevent the development of the adverse effects of maternal anaemia, the diet of all pregnant women in Ghana has for some years now been supplemented with iron and folic acid during antenatal care visits as per the National Anaemia Control Programme's Policy. Anaemia in pregnancy could be prevented by supplementing the diets of pregnant women with iron, folic acid, vitamins A and B12. Lack of compliance by pregnant women has been alleged to impede the success of this programme. The Ghana Demographic and Health Survey 2003, observed that four out of five pregnant women in Ghana were receiving iron and folic acid supplements, yet less than 40% of them took these supplements for up to the recommended 90 days prior to delivery. An alternative is to fortify widely consumed foods such as salt, wheat and bread flour with iron. Pregnant women are also encouraged to consume locally available haem-rich foods such as meat and fish.

Malaria prophylaxis, to prevent *Plasmodium falciparum*-induced anaemia in pregnancy has also been recommended by Munasinghe & van den Broek (2007) and

Fleming (1989a). Screening for and treating parasitic diseases such as schistosomiasis and hookworm infestation also prevents anaemia due to blood loss caused by these pathogens. In cases where the anaemia is severe, blood transfusion is often indicated. This should however, be avoided as much as possible to avoid exposing the mother to HIV and hepatitis B viruses, as well as prevent possible allo-immunisation which might aggravate anaemia by causing haemolysis (Fleming, 1989b).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area.

Bolgatanga Municipality is located in the centre of the Upper East Region, and is also the regional capital. It has a total land area of 729 sq. km and is bordered to the north by the Bongo District, south and east by Talensi and Nabdam Districts, respectively, and Kassena-Nankana District to the west. The municipality was established by LI 1797 (2004). For the purpose of health delivery, the Municipality has been divided into nine (9) sub-municipalities namely: Bolga Central, Bolga North, Bolga South, Gambibgo, Sherigu, Sumbrungu, Zuarungu, Zuarungu-Moshie and Plaza. The population of the municipality is 136,343, with a growth rate of 1.2% (www.bolga.ghanadistricts.gov.gh). This is lower than the national rate of 2.7%. The population density is 259 persons per sq. km. This is far greater than the national density of 79.3 persons per sq. Km (www.bolga.ghanadistricts.gov.gh).

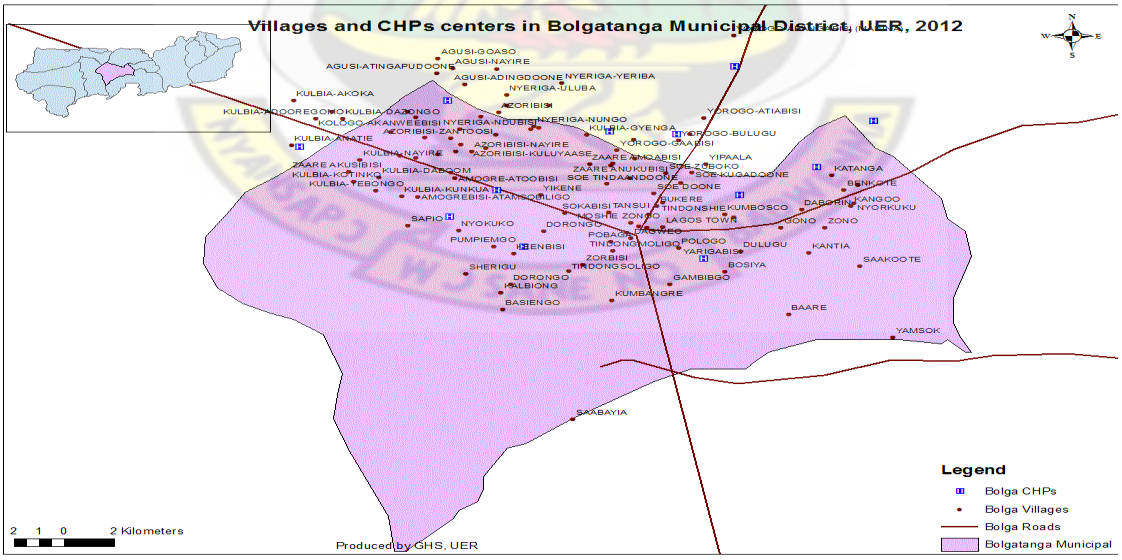

Figure3.1 Map of Bolgatanga Municipality

(Courtesy:www.bolga.ghanadistricts.gov.gh)

The municipality has a regional hospital, 3 health centres, 6 functional community health integrated centres (CHIPs) and 10 clinics (two are privately owned). The Regional Hospital, Bolgatanga (RHB) was established in 1946 to serve the minority white population of the then Gold Coast. It is a regional referral hospital and training centre for housemen and junior doctors, nurses and midwives. It provides both primary level health care and some specialist referral services and it has a catchment area population of about 1,004,243 (according to 2011 annual projections). The antenatal coverage for the Upper East Region for the year 2011 was 84.9% (5,301), and that for the Bolgatanga regional hospital was 19% (1233) of the 6,347 expected pregnancies in the municipality, thus hospital-based samples give a fair representation of the total antenatal population in the region.

3.1.1 Study Period`

The study was undertaken between May, 2013 and May, 2014.

3.1.2 Study Type and Study Population

The study was cross-sectional, and the study population was 200 anaemic pregnant women of haemoglobin concentration <10g/dl as test and 200 non-anaemic pregnant women of haemoglobin concentration >10g/dl as control. The test subjects were attending their first antenatal care at the Bolgatanga Regional Hospital.

3.1.3 Inclusion Criteria.

Pregnant women attending their first antenatal care of ages ranging from 15-48 years.

3.1.4 Exclusion Criteria.

1. Pregnant women in need of emergency care or having an at-risk pregnancy such as gestational diabetes, pre-eclampsia and eclampsia.

2. Antenatal pregnant women reporting for repeat visits during the study period.

3. Subjects who have been confirmed to be HIV positive.

3.1.5 Sampling Strategy

Subjects for the study were sampled consecutively. Every pregnant woman attending her first antenatal care who met the eligibility criteria was selected.

3.1.6 Sample Size

The expected sample size was based on the estimated anaemia prevalence in the Upper East Region, thus 51% (GSS, 2004). Using a CI of 95%, a 5% acceptable margin of error and the need for a sample size large enough to be statistically representative, a total of 400 anaemic pregnant women were to be enrolled, however, due to high cost of reagents and consumables, and the fact that the entire study was self-funded, the sample size was reduced to 200 anaemic pregnant women and 200 non-anaemic pregnant women.

3.1.7 Informed Consent and Subject Recruitment

Pregnant women attending their first antenatal visit at the RHB-ANC were approached and the rationale of the study was explained to them. Informed consent was then sought from subjects. Using a structured questionnaire, their socio-demographic data were obtained. In the process of seeking informed consent, the aims and objectives of the study, as well as the benefits of the proposed study, were explained to the participants.

3.1.8 Specimen Collection and Transfer

About 5mls of participants' venous blood were drawn from the median cubital vein or basilic vein or the cephalic veins of the lower arm for haematological and biochemical analysis. About 2mls were collected into a BD vacutainer, containing EDTA for determination of haematological parameters and 3mls into a BD Vacutainer with SST II Advance Semi-separator gel (BD, Belliver Indusrial Estate, Plymouth, PL6 7BP, United Kingdom) for determination of biochemical parameters. About 2 drops of blood were collected on a slide for the preparation of thick blood film for malaria parasite identification.

About 2g of early morning stool and 10mls midstream urine samples were also collected into sterile containers. The urine was used for the determination of *Schistosoma haematobium* and the stool for the determination of enteric parasites. The collected samples were transferred in a cold box to the Haematology and Biochemistry laboratories of RHB for haematological and biochemical tests respectively.

3.1.9. Ethical Issues

The participants'consent was sought and those who agreed to be part of the study were recruited. The participants from whom information and samples were collected, were given code numbers. No name was recorded on the samples but the code numbers were used. The ethical approval of the research protocol was granted by two review boards. Firstly, the Committee on Human Research, Publication and Ethics of Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital (CHRPE-KNUST/KATH) reviewed and approved it and secondly, it was reviewed and approved by the Institutional Review Board of the Navrongo Health Research Centre (IRB-NHRC).

3.2 Methods of Analysis-Haematological Tests

3.2.1 Full Blood Count Estimation with Sysmex® KX-21N

Figure 3.2 Sysmex® KX-21N Haematology analyser (Courtesy Sysmex KX-21N Operator's manual, 2006).

The Sysmex® KX-21N (Sysmex Corporation, Kobe, Japan) is an automatic multi-parameter blood cell counter for *in vitro* diagnostic use in clinical laboratories. The KX-21N processes approximately 60 samples per hour and displays on the LCD screen the distribution curves of WBC, RBC, and platelets, alongside values of 19 parameters, as the analysis results (Sysmex Corporation, 2006).

3.2.1.1 Principle of Sysmex® KX-21N

The Kx-21N employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC 38 detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the haemoglobin concentration, using the non-cyanide haemoglobin method. Blood is aspirated from the sample probe into the sample rotor valve. Six microlitres (6µl) of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 ml of diluents. At the same time, 1.0 ml of WBC/HGB lyse fluid is added to prepared 1: 500 dilution sample. When the solution is made to react for approximately 10 seconds, the RBC is haemolyzed and platelets shrink, with WBC membrane maintained as they are. At the same time, haemoglobin is oxidized to methaemoglobin (Sysmex Corporation, 2006). Of the diluted/haemolyzed sample in the WBC transducer chamber, approximately 1.0 ml is transferred to the HGB flow cell. Then, 500µl of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection system. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is directed to the sample in the HGB flow cell. Concentration of the sample is measured as absorbance. The absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby quantifying HGB (haemoglobin value) (Sysmex Corporation, 2006)

3.2.1.2 Methodology

The blood sample collected in the Vacuette® K3EDTA tube was mixed thoroughly on a roller and arranged serially according to the pathology numbers. The KX-21N (Sysmex Corporation, Kobe, Japan) was then put in the ready status. The pathology number for each sample was entered on the LCD screen for storage. The Vacuette® K3EDTA tube with the blood sample was mixed and the plug covering the tube was removed gently. The tube was then set to the sample probe for aspiration and the start switch pressed. After analysis, the LCD displayed the results and the print-out was made on a thermal paper.

3.2.2 Haemoglobin Electrophoresis on Cellulose Acetate Using Whole Blood Sample

Whole blood sample was centrifuged and plasma removed. Lysate of each sample was made by washing the sample four times in saline, and haemolysed in carbon tetrachloride. About 100ml of tris buffer pH 8.5 was poured into each of the outer sections of the electrophoresis chamber. Two wicks were wetted in the buffer and each draped over each support bridge, ensuring each made contact with the buffer and that there were no air bubbles under the wicks. A strip of cellulose acetate paper was wetted in the buffer and afterwards placed on a horizontal support and 5µl each of haemolysate of each sample was loaded on the cellulose acetate paper along with control samples of haemoglobin SS, AS and AC. The cellulose acetate membrane was immediately placed over the two wicks in the electrophoresis chamber with the sample end nearest to the cathode (negative pole). The lid of the electrophoresis tank was replaced and the terminals secured. The power source was switched on, for the

electrophoresis to be run for 20 minutes at 250-350 Volts. The relative mobilities of haemoglobins A, S, and C were determined.

3.2.3 Parasitological Tests

3.2.3.1 Thick Blood Film Preparation for Malaria Parasite Examination

Only thick blood film was used for malaria parasite examination since it is more sensitive. It allows the detection of very mild infection with scanty number of parasites.

- The blood sample in the EDTA tube was mixed gently and the slide for each study participant was labeled with their unique identity number.
- Two drops (6µl) of whole blood were spotted in the middle of a microscope slide using a WHO standardized template. Using the corner of the glass spreader, the blood was spread clockwise within the diameter of the template.
- The film was air-dried.

3.2.3.2 Giemsa Staining Technique

The Gurr® Giemsa working solution was filtered before use. The Giemsa stain was checked with positive quality control slides to assess its staining characteristics, prior to it being used. The stain was diluted 1:10 with a buffer pH of 7.2.

- The slides with the dried films were placed on a rack with space between the slides to avoid cross-contamination.
- The slides were flooded with the Giemsa working solution and stained for 10 minutes.

- The stain was rinsed gently off the slide with water from the tap. This was done carefully in order not to wash the thick film preparation off the slide.
- The smears were air-dried before microscopic examination.

3.2.3.3 Microscopic Examination of Blood Film

The examination of the blood film for malaria parasites was done by two certified microscopists independently. Non-concurrence in presence of parasite and level of parasite density between the primary readers was referred to a third expert microscopist whose determination of parasitaemia was considered final. The thick smear was used to examine each slide so as to detect very mild infection with scanty number of parasites. The slides were examined using the Primo Star (Carl Zeiss MicroImaging GmbH, Germany) microscope with the ×100 objective lens. The parasitaemia for positive slides was determined using the plus (+) system of quantification. The results were categorised as follows:

1-9 parasites per 100 microscopic fields (+); 10-99 parasites per 100 microscopic fields (++); 1-9 parasites per microscopic field (+++); more than 10 parasites per microscopic field (++++). Slides were declared negative when 100 high power fields were scanned without any parasite being seen.

3.2.4 Stool Analysis for parasitic infections (Cheesbrough, 2004)

Microscopic identification of ova in the stool is the most common method for diagnosing enteric parasitic infections. About 2g early morning stool specimen was collected from each participant. About 1g specimen was fixed in 6ml of 10% formalin and the resulting mixture was filtered. Afterwards, diethylether was added to the 10ml

mark of the mixture. The resulting suspension was mixed and centrifuged at 3000 r.p.m. for 10 minutes. Afterwards, the diethyl ether layer (supernatant) was decanted and a wet mount preparation of the sediment was made on a microscopic slide. The wet mount preparation of the sediment was examined microscopically using the ×10 objective with the condenser iris closed sufficiently to give good contrast.

A wet mount preparation of specimen was done to identify intestinal flagellates by emulsifying about 1g stool specimen with a reasonable amount of normal saline. A wet mount preparation of the specimen was made on a microscopic slide. The specimen was examined microscopically for identification of enteric parasites at low power objective.

3.2.5. Urine analysis for *Schistosoma haematobium* (Cheesbrough, 2004)

The urine sedimentation technique was used to detect the presence of *S. haematobium* ova in the urine samples. An aliquot of the collected urine sample was dispensed into a centrifuge tube for centrifugation at 3000 r.p.m. for 10 minutes and a wet mount preparation of the sediment was made on a microscopic slide. The specimen was examined microscopically for identification of *S. haematobium* ova. The observation was done at low power magnification (× 40 objective).

3.2.6 Biochemical Tests

3.2.7 Ferritin

Estimation of serum ferritin was done using the Abbot AxSYM® System (Abbot Laboratories, Lisnamuck, Longford, Ireland). The AxSYM® Ferritin assay provides a quantitative, automated methodology for ferritin determination as a useful indicator of body iron stores. The biological principle behind the AxSYM® system of determining ferritin is based on microparticle enzyme immunoassay (MEIA) technology.

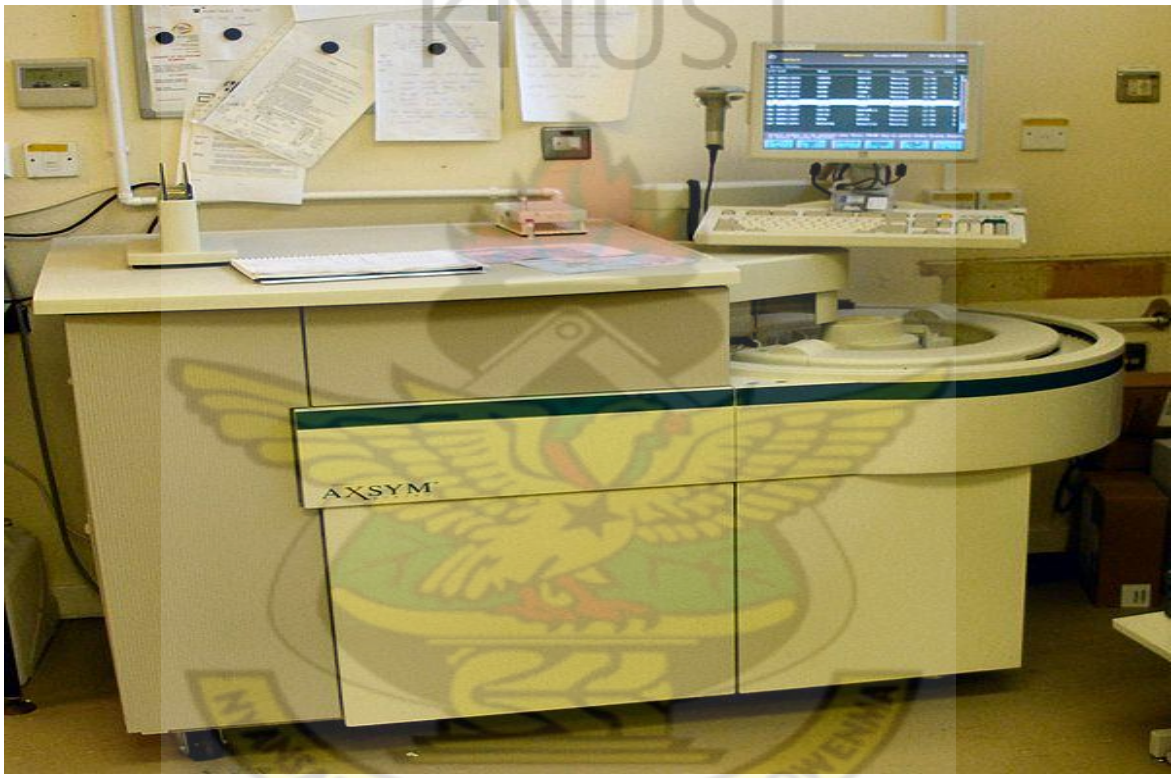

Figure 3.3 Abbot AxSYM® System (Abbot Laboratories, Lisnamuck, Longford, Ireland)

3.2.7.1 Principle of MEIA

The microparticle enzyme immunoassay (MEIA) is a technique in which the solid-phase support consists of very small microparticles in liquid suspension. Specific reagent antibodies (anti-ferritin) are covalently bound to the microparticles. The

antigen of interest (ferritin) is sandwiched between the bound antibodies and antigen-specific, enzyme-labelled antibodies. Antigen-antibody complexes generate a signal which is detected and quantified by analysis of fluorescence from the enzyme-substrate interaction.

3.2.7.2 Assay Procedure

The AxSYM® Ferritin file was installed on the AxSYM® system from a software disk prior to performing the ferritin assay. The system inventory of matrix cells, bulk solutions and waste levels were confirmed to be acceptable before the analysis was initiated. AxSYM® Ferritin standard calibrators and controls were requested on the system and confirmation was made that assay control values were within concentration ranges specified in the package insert.

The AxSYM® Ferritin reagents and samples were pipetted in the following sequence:

- Sample and all AxSYM® ferritin reagents required for one test were pipetted by the sampling probe into various wells of a Reaction Vessel (RV).
- Sample was pipetted into one well of the RV.
- The Anti-ferritin coated microparticles, Anti-ferritin Alkaline Phosphatase Conjugate, Specimen Diluent and Tris(hydroxymethyl)aminomethane (TRIS) buffer were pipetted into another well of the RV.
- The RV was immediately transferred into the processing centre by the processing probe.
- An aliquot of the specimen diluent, conjugate, microparticles and TRIS buffer mixture were then pipetted and mixed with the sample.

- The ferritin, enzyme-labeled antibody and microparticles were bound forming an antibody-antigen-antibody complex.
- An aliquot of the reaction mixture containing the antibody-antigen-antibody complex bound to the microparticles was transferred to the matrix cell. The microparticles were irreversibly bound to the glass fiber matrix.
- The matrix cell was washed to remove the unbound materials.
- The substrate, 4-methylumbelliferyl phosphate, was finally added to the matrix cell and the fluorescent product was measured by the MEIA optical assembly.

AxSYM® ferritin utilized a point-to-point reduction to generate a standard calibration curve. The concentration of ferritin in the serum was obtained from the standard curve.

3.2.8 Colorimetric methods of iron indices determination (Henry, 1984)

3.2.8.1 Principle:

The iron in serum is dissociated from its Fe^{3+}– transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the $Fe^{3+}$to Fe^{2+}. The chromogenic agent, Ferrene, forms a highly coloured Fe^{2+}– complex that is measured photometrically at 560 nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe^{2+}ions to serum to facilitate their binding to the unsaturated iron binding sites on transferrin. The excess Fe^{2+}ions are reacted with Ferrozine to form the colour complex, which is measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding. The total iron

binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value. The Transferrin saturation (TfS) is calculated as a percentage of serum iron and total iron binding capacity as follows:

$$TfS = \frac{Serum\ iron \times 100}{TIBC}$$

3.2.8.2 Manual Procedure:

Serum Iron:

- Test tubes/cuvettes were labeled: "Blank", "Standard", "Control", "Sample", etc.
- 2.5 millilitres of iron buffer reagent was dispensed into all tubes.
- 0.5 millilitres (500µl) of sample was added to respective tubes and mixed. Blanking was done by adding 500µl of iron-free water.
- Spectrophotometer was zeroed at 560nm with the reagent blank.
- Absorbances of all tubes were read and recorded as A_1 reading.
- Iron colour reagent was added at a volume of 0.05ml (50µl) to all the tubes and mixed.
- Tubes were placed in the water bath at 37^{O}C for 10 minutes.
- Instrument was zeroed at 560nm with the reagent blank.
- Absorbances of all the tubes were read and recorded as (A_2 reading).

Calculations:

A=Absorbance

Std= Standard

Conc. of Std = Total Iron (µg/dl)

$\frac{A2Test-A1Test}{A2Test-A1Std}$× Conc. of Std = Total Iron (µg/dl)

UIBC (Unsaturated Iron Binding Capacity):

- Test tubes/cuvettes were labeled: “Blank”, “Standard”, “Control”, “Test”, etc.
- Two millilitres of UIBC buffer reagent was added to all tubes.
- One milliliter iron-free water was added to “Blank”.
- 0.5milliliters (500µl) iron- free water was added to “standard” plus 0.5 ml (500µl) standard and mixed.
- Respective sample of volume 0.5ml (500µl) was added to "test” plus 0.5 ml (500µl) IronStandard and mixed.
- Spectrophotometer was zeroed at 560nm with the reagent blank.
- Absorbances of all tubes were read and recorded as A_1 reading.
- Iron colour reagent was added at a volume of 0.05 (50µ) to all the tubes and mixed.
- Tubes were placed in the water bath at 37^{O}C for 10 minutes.
- Instrument was zeroed at 560nm with the reagent blank.
- Absorbances of all the tubes were read and recorded as (A_2 reading).

Calculations:

A=Absorbance

Std= Standard

$\frac{Conc\ of\ Std-(A2Test-A1TEST)}{(A2Test-A1Std)}$× Conc. of Std = UIBC (µg/dl)

3.2.9 Total and direct bilirubin (Pearlman & Lee, 1974)

The most common method for the laboratory determination of bilirubin is the coupling of serum bilirubin with diazotized sulfanilic acid (p-diazobenzenesulfonic acid) to produce an azobilirubin dye.

3.2.9.1 Total bilirubin assay procedure

The JAS total bilirubin method is based on a modification of the Pearlman and Lee method in which a surfactant is used as a solubiliser. Sodium nitrite was added to sulfanilic acid to form diazotized sulfanilic acid. Bilirubin in the sample reacted with the diazotized sulfanilic acid to produce azobilirubin which absorbs strongly at 550 nm. The absorbance measured at 550nm was directly proportional to the total bilirubin concentration in the sample.

3.2.9.2. Direct bilirubin assay procedure

Most methods currently used for assaying bilirubin are based on the reaction between bilirubin and diazotized sulphanilic acid solutions. In aqueous solution, without the solubiliser, only the direct (conjugated) bilirubin would react in this manner. The JAS direct bilirubin reagent uses an acid diazo method. Conjugated bilirubin reacts with diazotized sulphanilic acid to produce an acid azobilirubin, the absorbance of which is proportional to the concentration of direct bilirubin in the sample and is measured at 550 nm.

3.3 C-Reactive protein assay procedure (CRP)(Tillet and Francis, 1930):

The CRP Latex test is a rapid slide agglutination test for the quantitative and semi-quantitative detection of C-reactive protein in serum. The reagent containing particles coated with specific anti-human C-reactive protein antibodies agglutinate in the presence of CRP in the patient's serum.

3.3.1 Test Procedure:

- Reagents and samples were brought to room temperature
- Fifty microlitres of the sample and 1 drop of the control were placed into separate circles on the card.
- The latex was resuspended gently.
- One drop of the latex reagent was added to each circle next to the sample to be tested.
- Disposable pipette/stirrer was used to mix and spread over the entire area enclosed by the ring. A new stirrer was used for each sample.
- Cards were rotated at 300 r.p.m. for 2 minutes.

3.3.2 Reading and Interpretation:

- Card was examined visually for the presence or absence of agglutination or clumps within 1minute of removing it from the rotator.
- Positive Results - the presence of agglutination which indicates a level of CRP≥6mg/L.
- Negative Results – no agglutination would indicate a level of CRP <6mg/L.

Dilution	CRP mg/L
1:1	6
1:2	12
1:4	24
1:8	48
1:16	96

3.3.3 Limitations of the Procedure:

- CRP levels in the range of 15mg/L or above may cause false negative results due to prozone effects.
- A final diagnosis of inflammation should not be made on the result of this test alone, but should be based on a correlation of this test results with other clinical findings.

3.4 Statistical Analysis.

Data were entered into Microsoft Excel worksheet. Results were presented as mean ± standard deviation (SD) and frequency (percentage) where necessary. The Fischer's exact test or Chi-square (X^2) was used to assess the statistical significance of categorical variables. Unpaired sample t-test was used to compare between two means of continuous variables. P-value less than 0.05 was considered statistically significant. Logistic regression was used to predict associated risk factors. Serum iron, TIBC and %TfS were $\log_{10}$-transformed because their data were not normally distributed. Analysis was performed using GraphPad Prism 5 Project software (GraphPad software, San Diego California USA, www.graphpad.com)

CHAPTER FOUR

RESULTS

4.1 Socio-Demographic and Obstetric Characteristics of Study Population

According to table 4.1, the mean ages of the anaemic and non-anaemic pregnant women were similar, thus 27.43±6.47 and 27.52±6.48 years respectively. There was no statistically significant difference with regards to age, $p>0.05$. Regarding the anaemic pregnant women recruited for the study, the proportion of younger women dominated (76.5%), compared to only 23.5% older women who were anaemic. While a higher number of the non-anaemic subjects were from the urban setting, in the case of the anaemics, those from rural settlement (60.0%) predominated.

For both the anaemic and non-anaemic, the majority had basic education, followed by those who were illiterate. Participants with higher education were significantly non-anaemic, $p<0.05$. The test population and control were both predominantly self-employed. Whereas there was a significantly higher proportion of unemployed subjects in the anaemic pregnant women (27.0%), there was a lower proportion (5.5%) of civil servants who had anaemia.

While a majority of the non-anaemic pregnant women were multigravida, a significantly higher percentage of the anaemic pregnant women were primigravida (39.5%). Even though significantly higher percentage of both test and control were multiparous, the non anaemic had the higher multiparity. On the other hand, the anaemic pregnant women were significantly primiparous. In the case of those who were non-anaemic, the highest percentage reported to the ANC in the first trimester of their pregnancy, but for those who were anaemic, the majority reported in the second trimester. Between the test and control populations, there was no difference in percentages recruited in the third trimester of pregnancy.

Table 4.1 - Socio-Demographic and Obstetric Characteristics

Variables	Anaemic (n=200) n (%)	Non-anaemic (n=200) n (%)	p- value
Maternal age	27.43±6.47	27.52±6.48	0.883
<30 years	153 (76.5%)	132 (66.0%)	0.0269
≥30 years	47 (23.5%)	68 (34.0%)	0.0203
Residency			
Urban	80 (40.0%)	137 (68.5%)	<0.0001
Rural	120 (60.0%)	63 (31.5%)	<0.0001
Level of education			
No formal education	67 (33.5%)	58 (29.0%)	0.3882
Basic	100 (50.0%)	90 (45.0%)	0.3675
Higher	33 (16.5%)	52 (26.0%)	0.0274
Occupation type			
Unemployment	54 (27.0%)	33 (16.5%)	0.0151
Self-employment	135 (67.5%)	133 (66.5%)	0.9153
Civil servant	11 (5.5%)	34 (17.0%)	0.0004
Gravidity			
Primigravidity	79 (39.5%)	48 (24.0%)	0.0012
Multigravidity	121 (60.5%)	152 (76.0%)	0.0012
Parity			
Primiparity	82 (41.0%)	54 (27.0%)	0.0043
Multiparity	118 (59.0%)	146 (73.0%)	0.0043
Trimester of pregnancy			
1st	69 (34.5%)	105 (52.5%)	0.0004
2nd	99 (49.5%)	74 (37.0%)	0.0153
3rd	32 (16.0%)	21 (10.5%)	0.1397

Values are presented as n (%). Comparisons between proportions in anaemic and non-anaemic groups were performed using Fischer's exact test. p<0.05 was considered statistically significant.

4.2 Risk factors associated with anaemia

Table 4.2 shows logistic regression of factors associated with anaemia in pregnancy. Logistic regression indicated that maternal age <30years {(OR=1.677, 95% CI (1.081 to 2.601); p=0.0269}, unemployment {(OR=5.058, 95% CI (2.258 to 11.33); p< 0.0001}, Primigravidity {(OR=2.067, 95% CI (1.344 to 3.181); p= 0.0020}, Nulliparity {(OR=1.879, 95% CI (1.234 to 2.861); p= 0.0043}, first time visit to ante-natal clinic in the second trimester {(OR=2.036, 95% CI (1.327 to 3.123); p= 0.0013} and visit in third trimester of pregnancy {(OR=2.319, 95% CI (1.236 to 4.349); p= 0.0012} were significantly independent risks factors of anaemia.

Table 4.2-Logistic regression of factors associated with anaemia in pregnancy

Variable	Odds ratio (OR)	95% CI	p-value
Maternal age			
<30 years	1.677	(1.081 to 2.601)	0.0269
≥30 years	Reference		
Residency			
Urban	0.3066	(0.2032 to 0.4625)	< 0.0001
Rural	Reference		
Level of education			
None	1.820	(1.039 to 3.188)	0.4850
Basic	1.751	(1.040 to 2.948)	0.3740
Higher	Reference		
Occupation status			
Unemployment	5.058	(2.258 to 11.33)	< 0.0001
Self-employment	0.137	(0.0526 to 1.452)	0.0012
Civil servant	Reference		
Gravidity			
Primigravidity	2.067	(1.344 to 3.181)	0.0020
Multigravidity	Reference		
Parity			
Nulliparity	1.879	(1.234 to 2.861)	0.0043
Multiparity	Reference		
Trimester of pregnancy			
1st	Reference		
2nd	2.036	(1.327 to 3.123)	0.0013
3rd	2.319	(1.236 to 4.349)	0.0012
Source of water			
Pipe borne water	0.2908	(0.1921 to 0.4401)	< 0.0001
others (well, bore hole)	Reference		
PHA			
Yes	1.567	(0.9315 to 2.638)	0.1159
No	Reference		

PHA: Previous history of anaemia; CI: Confidence interval

4.3 Nutrition, Source of drinking water and Past Anaemic Condition of Study Participants

From Table 4.3, whether in the anaemic pregnant women or non-anaemic pregnant women, most of them took meals three times daily. However, a higher proportion of anaemic pregnant women ate from both home and street, compared to non-anaemic controls (59.5% vs 41.5%; $p<0.0001$). Those who ate meals from their homes predominated in the non-anaemic control.

A higher proportion of the anaemic pregnant women (70.5%) drank from well and borehole and other sources, compared to 41.0% non-anaemic control ($p<0.0001$), but the main source of water supply for the non-anaemic control was pipe-borne. The presence of domestic livestock was reported in the test and control subjects, but it was more common in the anaemic pregnant women. Body mass index and previous history of anaemia were similar in the two groups of pregnant women ($p>0.05$) **(Table 4.3).**

Table 4.3-Nutrition, BMI, Source of Water and Past Anaemic Condition of Study Participants.

Variables	Anaemic (n=200) n (%)	Non anaemic (n=200) n (%)	p- value
Number of meals			
Three times	183 (91.5%)	172 (86%)	0.1128
Others (breakfast, etc)	17 (8.5%)	28 (14%)	0.1128
Source of food			
Home only	76 (38.0%)	105 (52.5%)	0.0048
Street	5 (2.5%)	12 (6.0%)	0.1349
Both home and street	119 (59.5%)	83 (41.5%)	< 0.0001
Source of water			
Pipe-borne water	59 (29.5%)	118 (59.0%)	< 0.0001
others (well, bore hole)	141 (70.5%)	82 (41.0%)	< 0.0001
Presence of domestic livestock			
Yes	172 (86.0%)	147 (73.5%)	0.0027
No	28 (14.0%)	53 (26.5%)	0.0027
BMI categorisation			
< 22.0 kg/m^2	99 (49.5%)	81 (40.5%)	0.0874
≥ 22.0 kg/m^2	101 (50.5%)	119 (59.5%)	0.0874
Previous history of anaemia			
Yes	42 (21.0%)	29 (14.5%)	0.1159
No	158 (79.0%)	171 (85.5%)	0.1159

Values are presented as n (%). Comparisons between proportions of anaemic and non-anaemic groups were performed using Fischer's exact test. p<0.05 was considered statistically significant.

4.4 Haematological and biochemical profiles

Table 4.4 shows the haematological and biochemical profiles of study participants. Anaemic pregnant women had a significantly lower mean HGB, HCT, MCHC and serum iron levels, compared to their non-anaemic counterparts ($p<0.0001$). Conversely, there was a significantly higher mean WBC, MCV, RDW and Log_{10} %TfR saturation amongst anaemic pregnant women, compared to controls ($p<0.05$).

Table 4.4-Haematological and biochemical profile of study subjects

Parameters	Anaemic (n=200)	Non-anaemic (n=200)	p-value
Haematological profile			
HGB (g/dl)	8.93 ± 0.91	11.28 ± 1.01	<0.0001
WBC (/µL)	6.41 ± 1.88	6.05 ± 1.32	0.0290
HCT (%)	28.29 ± 2.90	33.79 ± 4.71	<0.0001
MCHC (g/dl)	31.58 ± 2.02	33.14 ± 1.58	<0.0001
MCV (fl)	85.85 ± 11.29	80.60 ± 12.19	<0.0001
RDW (%)	16.81 ± 5.95	14.83 ± 3.55	<0.0001
Biochemical profile			
Log_{10} Serum Ferritin (ng/ml)	1.88 ± 0.09	1.84 ± 0.01	0.6520
Serum iron (µg/dl)	135.6 ± 88.4	179.5 ± 78.9	0.0120
Log_{10}TIBC (µg/dl)	2.05 ± 0.08	1.93 ± 0.01	0.0581
Log_{10} %TfS (%)	1.64 ± 0.03	0.53 ± 0.001	<0.0001
Total Bilirubin (µmol/l)	18.09 ± 6.63	18.64 ± 6.33	0.5640
Direct Bilirubin (µmol/l)	5.45 ± 2.13	5.82 ± 2.37	0.2490
Indirect Bilirubin (µmol/l)	12.79 ± 6.08	13.26 ± 6.06	0.5930

Values are presented as mean ± standard deviation. HGB: haemoglobin; WBC: White blood cells; HCT: Haematocrit; MCHC: Mean corpuscular haemoglobin concentration; MCV: Mean corpuscular volume; RDW: Red cell distribution width; TIBC: Total iron binding capacity; TfS: Transferrin saturation. The comparisons were between anaemic and non-anaemic groups, using unpaired t-test (independent sample t-test). p <0.05 was considered statistically significant.

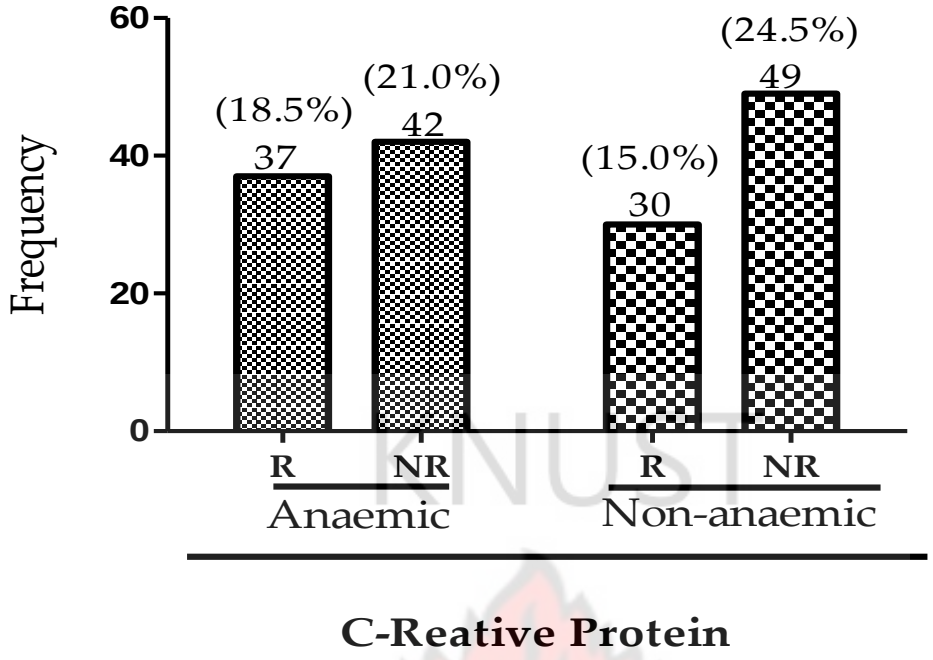

Figure 4.1 C-Reactive Protein of study participants.

R: Reactive; NR: Non-reactive. Values in parentheses represent proportion of various frequencies

Subjects who had anaemia were more likely to report with a positive response to C-reactive protein (18.5%) than their non-anaemic counterparts (15.0%) **(Figure 4.1).**

Table 4.5-Haemoglobin genotypes of the subjects

Hb Genotype	Anaemic n (%)	Non-Anaemic n (%)	p-value
AA	129(62.6%)	142 (71.0%)	0.1992
AC	17 (8.5%)	39 (19.5%)	0.0023
AS	32(16.0%)	15 (7.5%)	0.0180
CC	9(4.5%)	4 (2.0%)	0.2589
SC	12(6.0%)	-	-
SS	1(0.5%)	-	-

Values are presented as n (%). Comparisons between proportions of anaemic and non-anaemic groups were performed using Fischer's exact test. p<0.05 was considered statistically significant

From Table 4.5, the AA and CC genotypes were of similar proportions between the anaemic and non-anaemic subjects ($p \geq 0.05$). A significantly greater percentage (19.5) of the non-anaemic had AC genotype than the anaemic, who had only 8.5% of this genotype. Conversely, a significantly greater percentage of AS genotype was found among those who were anaemic. Additionally, SC and SS genotypes were found in only women who were anaemic.

4.5 Association between anaemia and parasitaemia

The overall occurrence of malaria parasites, and levels of the parasite densities in the test and control subjects were not significantly different, as shown in Table 4.6 (Chi-square = 0.4611, p=0.7941)

Similarly, urine and stool parasites prevalence were not significantly different, although 0.5% of 200 anaemic pregnant women sampled had *S. mansoni* and *S. haematobium* (Chi-square = 3.977, p=0.1369) **(Table 4.7).**

Table 4.6 Malaria parasitaemia in anaemic and non-anaemic pregnant women

Parasitaemia	**Anaemic (n=200) n (%)**	**Non-anaemic (n=200) n (%)**	**X^2, df (p- value)**
No Mps seen	161 (80.5%)	166 (83.0%)	0.4611, 2 (0.7941)
+1	35 (17.5%)	30 (15.0%)	
+2	4 (2.0%)	4 (2.0%)	
+3	-	-	

Values are presented as n (%). Mps: malaria parasite. Comparisons between proportions in anaemic and non-anaemic groups were performed using Chi-square test. p<0.05 was considered statistically significant.

Table 4.7 Stool and urine-related parasites in the pregnant women

Parasites	Anaemic (n=200) n (%)	Non-anaemic (n=200) n (%)	X^2, df (p- value)
Stool			
Intestinal flagellates			
NAD	145 (72.5%)	158 (79%)	3.977, 2 (0.1369)
+1	54 (27%)	39 (19.5%)	
+2	1 (0.5%)	3 (1.5%)	
+3	-	-	
S. mansoni (++)	1 (0.5%)	-	-
Urine			
S. haematobium	1 (0.5%)	-	-

Values are presented as n (%): NAD: No Abnormality Detected. S: *Schistosoma.* Comparisons between proportions in anaemic and non-anaemic groups were performed using Chi-square test (X^2). df: degree of freedom.

CHAPTER FIVE

DISCUSSION

5.1 Demographic, Obstetric and Socio-economic Background of Study Participants in Relation to Anaemia.

Anaemia in pregnancy is the major cause of maternal morbidity and mortality in developing countries. The use of haemoglobin level alone is insufficient in determining the status of anaemia in pregnancy. This study therefore aimed to evaluate the biochemical and haematological profiles of anaemic pregnant women attending Antenatal Clinic at the Bolgatanga Regional Hospital. The study was cross-sectional, and involved two hundred anaemic pregnant women as test population and two hundred non-anaemic pregnant women as control population.

According to Table 4.1, there is a significant association between maternal age and anaemia, as a higher proportion of younger pregnant women (76.5%) were anaemic than older pregnant women. The greater susceptibility of younger women to anaemia can be attributed to the fact that younger pregnant women belong to the physically active group, undergoing rapid growth and having increased nutritional requirements (Centres for Disease Control and Prevention, 1998; Wharton, 1999). Due to the increased iron requirements of pregnancy and growth, pregnant women, especially the younger ones and infants are recognized as the groups most vulnerable to iron deficiency anaemia.

It was also observed that 33.5% of the anaemic women were illiterates having no formal education and 50.0% had only basic education, hence did not have adequate knowledge of proper nutrition and balanced diet. Subjects from the rural settlements

were more anaemic (60.0%), compared to those from urban settlements (40.0%). Living in the rural areas had a significant association with anaemia (Table 4.1). This might be probably due to, although not significant, frequent exposure to malaria infections (Table 4.6). The smaller sample size (200 anaemic population) recruited for the study greatly influenced the findings on malaria infection. Similar research conducted elsewhere had significant association between malaria infection and anaemia partly due to larger sample size used (Ricardo 1999). Malaria infections were predominant in the anaemic pregnant women because most rural settings in the Upper East Region have houses made of mud walls and thatch roof (Data on housing not shown). The association between housing and malaria has been described previously in Africa and elsewhere (Banguero, 1984; Koram *et al.,* 1995; Ghebreyesus *et al.*, 2000). In Matola, a peri-urban area of Maputo, Mozambique, houses with reed roofs and clay walls were associated with significantly higher risks of clinical episodes of malaria (Ricardo 1999). In Eritrea, walls made from mud increased an individual's risk for malaria parasitaemia, compared to individuals living in houses with walls made of other construction materials (Sintasath *et al.,* 2005). An earlier study in Eritrea also revealed an association between mud walls and malaria infection (Ong'echa *et al.,* 2006). These types of housing construction provide microenvironments for mosquitoes and may ensure their chance of survival and feeding opportunities (Schofield & White, 1984).

Thus, living in urban areas significantly decreases the risk of anaemia in pregnancy. Most of the inhabitants of urban areas, approximately 68.5% were non-anaemic pregnant women having higher educational qualification and well-paid jobs.

A significant association was also found between the prevalence of anaemia and gravidity (Table 4.1). According to Desalegn (1993), there is a two-fold decrease in

the risk of anaemia as the number of pregnancies increase. From Table 4.1 there was a significant relationship between the prevalence of anaemia and parity, as the number of children increases, there was a decrease in the risk of anaemia. This suggests that the behaviours and attitudes of primiparous and primigravid women may differ significantly from women with children or with previous pregnancies. This finding is contrary to the study by Desalegn (1993) in Southwestern Ethiopia which showed that the prevalence of anaemia increased with parity (nulliparity = 28%, parity 1-4 = 43.6%, and parity ≥ 5 = 53.5%; $p < 0.01$). He related his findings to depleted iron stores and other nutrients during increased and repeated pregnancies and also the possibility of sharing of resources with the foetus.

Compared to the non-anaemic pregnant women, a relatively high proportion of anaemic pregnant women (49.5%) made their first antenatal visit in the second trimester (Table 4.1) whereas only 34.5% anaemic pregnant women enrolled in their first trimester of pregnancy. The development of anaemia is gradual and progressive, if the causative factors are not identified and treated. Therefore, pregnant women who report to the ante-natal clinic in the first trimester are expected to have any factor that predisposes them to anaemia to be timely managed, hence the less tendency for them to be anaemic, compared to their counterparts who report beyond the first trimester.

According to Desalegn (1993), anaemia increases with gestational age, indicating that women who wait until the third trimester to seek prenatal care are more likely to develop anaemia during pregnancy than those who seek it at an earlier gestational age. It has also been reported that women who had their first antenatal visit within the first trimester demonstrated higher compliance with recommended antenatal care (Majoko *et al*., 2004).

From Table 4.3, there was no significant difference in BMI between anaemic and non-anaemic pregnancy, although most of the anaemic pregnant women were underweight (BMI<22.0 Kg/m^2). This predisposed them to anaemia due to the fact that they were malnourished (Kramer *et al*., 2000). Reports of past history of anaemia were similar in the test and control populations. The test population, though not significant, had a higher proportion of anaemic pregnant women with past history of anaemia (Table 4.3). The study found that a major risk factor for the development of subsequent anaemia in the current pregnancy was a history of anaemia in the previous pregnancy (Table 4.3). Women who had anaemia in previous pregnancies tend to have persistent anaemia in the next pregnancy (Fleming *et al*., 1974). Most women have inadequate iron stores to start with when they embark upon a new pregnancy (Puolakka *et al.*, 1980; Romslo *et al.*, 1983) and when their iron stores become depleted at the end of their pregnancy, they often do not have time to replenish their stores, which may take more than a year to return to pre-pregnancy levels (Letsky, 1995). The situation is made even worse with multiparity, and when the interval between pregnancies is too close for the women to recover their iron stores.

Compared to the non-anaemic pregnant women, a higher percentage of anaemic pregnant women (70.5) used sources of water, other than pipe-borne water. There was also a higher percentage (86) of the anaemic pregnant women reporting of the presence of domestic animals in their homes (Table 4.3).

Intestinal worm infections are common worldwide, but thrive in poor communities in the tropics, where poor water supply and poor sanitation are common (Crompton, 2000). Presence of domestic animals can result in zoonotic transfer of microscopic

parasites from intermediate host (eg. ticks) to humans (Centres for Disease Control and Prevention, 2014).

5.2 Haematological parameters and their relationship with anaemia in pregnancy.

From Table 4.4, the mean haemoglobin concentration of the anaemic pregnant women was 8.93±0.91g/dl which fell within the moderate anaemia category, as defined by the World Health Organisation (WHO, 2008) as (7.0<HGB<9.9)g/dL. The mean haemoglobin concentration of the non-anaemic pregnant women was 11.28±1.01g/dl. There was a statistically significant difference in mean haemoglobin concentration between the anaemic and the non-anaemic pregnant women recruited in this study.

According to Table 4.4 the mean WBC level of the anaemic pregnant women was significantly higher than that of the non-anaemic pregnant women. This may be due to the fact that white blood cells are responsible for body defence during pregnancy. This agrees with previous work by (Luppi 2003), who asserted that a total lymphocyte count rises in early pregnancy and will remain elevated throughout pregnancy. This is attributed to the body building the immunity of the foetus and it is achieved by a state of selective immune tolerance, immunosuppression, and immunomodulation in the presence of a strong antimicrobial immunity (Luppi, 2003).

The MCV of the anaemic pregnant women was higher than that of the non-anaemic pregnant women (Table 4.4). This finding is contrary to a similar study conducted in Sokoto, Nigeria, by Erhabor *et al*. (2013), who found a significantly lower MCV in the anaemic pregnant women recruited. He related his finding to microcytosis and

hypochromia, suggestive of iron deficiency anaemia. There was a significantly lower mean MCHC of the anaemic pregnant women than the non-anaemic pregnant women. Low MCHC values are found in iron deficiency anaemia and other conditions in which the red cells are microcytic and hypochromic (Cheesbrough, 2004).

Table 4.4 also shows that the mean HCT of the anaemic pregnant women was significantly lower (28.29±2.90) % than that of the non-anaemic pregnant women (33.80±4.71) %. This finding is similar to that of James *et al.* (2008). The decrease in HCT may be due to increase in plasma volume during pregnancy, which causes haemodilution and hormonal changes, and conditions that promote fluid retention and iron deficiency. During pregnancy, an increased plasma volume with the lack of an adequate increase in erythrocyte mass results in a decrease in haemoglobin level and the development of anaemia, which is defined as dilution anaemia (www.pregnancy.com.au › Pregnancy › Pregnancfy Problems). In addition, the general decrease in different blood indices is more likely explained by increased needs during pregnancy. Therefore, the increase in these blood indices in the non-anaemic pregnant women is a reflection of adequate iron supply resulting in increased haemoglobin production.

The observation that the mean RDW of the control was significantly lower than the test ($p<0.05$; Table 4.3), agrees with the findings of Andrews (2009), that in latent iron deficiency, RDW would be expected to increase because a microcytic population of cells appear in the blood. RDW is a better diagnostic haematological test of early iron deficiency than the conventional iron status markers such as ferritin, TIBC, serum iron, transferrin saturation. Red cell indices (MCV, MCH and MCHC) are mean values, which cannot express the small variation of red cells size, which accompanies early iron deficiency (Viswanath *et al.*, 2001) whereas RDW can

express the small variation of different population of red cell size (Bain & Bates, 2001).

5.3 Biochemical profiles of anaemic and non-anaemic pregnant women.

With exception of serum iron and transferrin saturation, there were no statistically significant changes between the anaemic and non-anaemic groups with regard to the biochemical parameters (ferritin, TIBC, total bilirubin, direct bilirubin and indirect bilirubin). Serum iron was low in the anaemic group (Table 4.4). A low serum iron is an indicator of iron deficiency anaemia (Brittenham, 2005). Transferrin saturation was elevated in the anaemic group (Table 4.4). This finding is contrary to a similar study (Nuzhat, *et al.,* 2011) conducted in Mansehra, Pakistan. The study found a low transferrin saturation level in the anaemic pregnant women, which is suggestive of iron deficiency anaemia.

Ferritin is the actual gold standard of iron status testing, but in certain conditions such as infections, ferritin is not valuable, since it is falsely elevated as an acute phase reactant (Cunningham *et al*, 2001). During pregnancy, ferritin shows weak correlations with other iron parameters and the severity of anaemia; therefore additional tests are helpful (Cook *et al.*, 1976; van den Broek, 1998). It was realised from this study that the levels of ferritin were not normally distributed, hence the values were $\log_{10}$-transformed, before the t-test. This study showed that the mean ferritin level for the anaemic pregnant women before $\log_{10}$-transformation was 76.30ng/ml and that for the control was 69.98ng/ml. The WHO has recommended a cut-off serum ferritin of <50µg/l for developing countries, with high prevalence of inflammatory conditions (Mburu *et al*., 2008). The elevation of mean ferritin level in

both the anaemic pregnant women and the non-anaemic pregnant women beyond the reference (≤50ng/ml), in cases where C-reactive protein level was >6ng/ml is suggestive of the presence of inflammation, caused by parasitic infections or haemoglobinopathies (Mburu *et al.*, 2008). The reactive response to C-reactive protein level in the anaemic pregnant women was 18.5% which was higher than that for the non-anaemic pregnant women that was 15.0%.

5.4 Haemoglobin genotype and anaemia in pregnancy

From table 4.5, haemoglobin genotypes AA and CC were of similar proportions in both the anaemic and non-anaemic pregnant women. The proportion of AC genotype was higher in the non-anaemic women (19.5%) than in the anaemic women who only had 8.5% of the genotype. The presence of the AC genotype in individuals does not predispose them to haemolytic tendency; this accounts for the higher proportion of this genotype in the non-anaemic.

On the other hand, haemoglobin genotypes AS, SC and SS contributed greatly to anaemia in this study, since a significant proportion of the anaemic pregnant women were having AS, SC and SS genotypes. Anaemia is one of the major complications of sickle cell disease and may be caused by haemolysis or trapping of the red blood cells in the spleen (Yu *et al.*, 2009).

5.5 Effect of parasitic infections on anaemia in pregnancy

According to Table 4.6, although statistically insignificant, the prevalence of malaria, *Plasmodium falciparum,* infection among pregnant women attending antenatal clinic at the Bolgatanga Regional Hospital, Upper East Region was higher (19.5%) in the anaemic women than in the non-anaemic women (17%). Additionally, relatively more anaemic pregnant women were having intestinal flagellates in their stool samples, as compared to the non-anaemic group. Only one stool and one urine sample of the anaemic pregnant women contained *Schistosoma mansoni* (0.5%) and *Schistosoma haematobium* (0.5%) respectively. *Plasmodium falciparum, Schistosoma haematobium, Schistosoma mansoni* and intestinal flagellates, according to Table 4.7 contributed marginally to anaemia. This finding agrees with that of Richard (2003), that parasitic infections (malaria and intestinal helminthes) coexist widely with micronutrient deficiencies and contribute importantly to anaemia and a cycle of retarded growth and development. Parasitic infections are well pronounced in poorer families, adolescent girls and pregnant women (WHO, 2002). According to the findings of Pell *et al*. (2013), most pregnant women could not afford subsidised insecticide-treated bed nets in the Northern part of Ghana. This predisposed them to the *Plasmodium falciparum* infection, leading to anaemia in pregnancy.

Due to the fact that Bolgatanga Region Hospital (RHB) serves as a referral centre for the various districts including the rural communities, it is not surprising that most of these anaemic pregnant women were from these rural settlements. The various rural settlements drink from irrigation dams and rivers. Some examples of these dams are the Tono dam in Navrongo and the Vea dam in Gowrie. There are uncountable numbers of dug-out dams, in addition to the irrigation facilities that serve as water sources for the people, as well as livestock during the long dry seasons. Incidentally,

the dams have also created a problem by providing ideal conditions for certain types of snails of the genus, Biomphalaria and Bulinus which serve as vectors in the life cycle of the schistosoma species, thus increasing the risk of parasitic infections such as Plasmodium and Schistosoma. (http://www.ghanaexpeditions.com/regions/highlight_detail.asp?id=4&rdid=311).

The occurrence of helminth infection at high rates among pregnant women is mostly indicative of faecal pollution of soil and domestic water supply around homes, due to poor sanitation and improper sewage disposal (Bundy *et al*., 1995; van Eijk *et al*., 2009). Pregnant women are at high risk of infection because of their close relationship with children (Bundy *et al*., 1995; van Eijk *et al*., 2009). Most of these worms are transmitted through the soil, whilst the practice of soil-eating (geophagy) is common amongst pregnant women in many communities in developing countries (Brooker *et al*., 2008).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.0 CONCLUSIONS

Anaemia in pregnancy produces various ill effects, both for mother and foetus, hence for prevention of anaemia, early diagnosis is essential. This study found that younger pregnant women had increased risk of anaemia. It also found that the risk of anaemia decreased as the number of children or pregnancies increased. Rural dwelling increased the risk of anaemia.

The study found a relation between trimester of pregnancy and anaemia. It was found that a higher proportion of the anaemic pregnant women reported to the ante-natal clinic in the second trimester, compared to the higher percentage of non-anaemic women, reporting in the first trimester. Therefore, the risk of anaemia increases with delay in accessing ante-natal care in pregnancy. Hence it is recommended that pregnant women seek early antenatal care since early diagnosis is important in curbing any potential future complications that can affect maternal and foetal health.

It was also found that environmental factors such as source of water (borehole, well), presence of domestic animals increased the risk of anaemia. Hence midwives are to educate pregnant women during antenatal sessions to depend solely on pipe-borne water, and water from wells and boreholes must be treated.

There were significant differences in all the haematological parameters (HGB, WBC, MCV, MCHC, HCT and RDW) between the anaemic and the non-anaemic pregnant women. All the changes were suggestive of anaemia, hence the above parameters can be used to determine the status of anaemia in pregnancy.

Considering the biochemical parameters, it was found that serum iron can be used to determine the status of iron deficiency anaemia in pregnancy since it was low in the anaemic pregnant women compared to the non-anaemic pregnant women. Transferrin saturation was elevated in the anaemic group.

Due to the fact that anaemia in pregnancy has been a major public health concern associated with maternal morbidity and mortality, this study has succeeded in determining the possible causes of anaemia in pregnancy before blindly treating with haematinics.

6.1 LIMITATIONS OF STUDY

The limitations of the study include small sample size, cross-sectional nature of the study and the general lack of reference intervals specific to the local condition in Bolgatanga compelled the comparison of the results of this study with data from countries whose socio-demographic variables vary from this local setting.

6.2 RECOMMENDATIONS

It is recommended that a follow-up study should be done on anaemic pregnant women who have been administered haematinics to determine the impact of the haematinics on anaemia and also the relationship between haematological and biochemical parameters from the first trimester to the time of delivery .

There may be other factors other than what this study found contributing to the burden of anaemia. Other micronutrients such as vitamin A, folate, cyanocobalamin, and zinc were not assessed in this study. Their influence on the burden of anaemia in this setting can therefore become a subject for further scientific investigation.

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APPENDIX

1.0 Questionnaire for the Study :

Biochemical and haematological profiles of anaemic pregnant women attending antenatal clinic at the Bolgatanga Regional Hospital.

Basic Data

1. Participant Study ID-……

Name…….Age……

Weight……..…….Height……

2. Location-

……

……

Socio-economic Factors

4. Educational qualification. A. BECE [ ] B. SSSCE [ ] C. HND [ ] D. Bsc/BA [ ]

E. Msc./PhD [ ] E. others Specify……

5. Occupation? A. Farming [ ] B. Fishing [ ] C. Trading [ ] D. Office work [ ] E.

Others [Specify]……..

6. Source of drinking water. A. Pipe-borne water [ ] B. Borehole [ ] C. Well [ ] D.

Rain water [ ] E. Sachet water [ ].

7. Family size?.....

Nutritional Information

8. Source of food? A. Cooked at home only [ ] B. Bought on Street [ ] C. Both cooked at home and sometimes obtained from street [ ] D. Others [ ]

Specify…….

Preferred meals? A. Breakfast…….. B. Lunch…..…… C. Supper ……...

Presence of Domestic Animals

9. Presence of animals at home? Yes [ ] No [ ]

Specify type of animal A. Dog [ ] B. Cat [ ] C. Goat [ ] D. Sheep [ ] E. Fowl [ ]

Others (specify)…….

Clinical Information/Gastrointestinal Symptoms (To be completed by Nurse/Doctor)

10. Does the patient have any of the following conditions? A. Nausea [ ] B. Abdominal pain [ ] C. General Malaise [ ] D. Fever [ ] E. Others [ ]

(Specify)

……

Specify any treatment given……..…….

11. Has the patient been diagnosed by a doctor to be anaemic before? Yes [ ] No [ ]

If yes, how was it treated?

.....

2.0 PARTICIPANT INFORMATION LEAFLET

Study title: "Evaluation of biochemical and haematological profiles of anaemic pregnant women attending antenatal clinic at the Bolgatanga Regional Hospital.

Researcher: This study is being conducted by Benjamin Ahenkorah, a Biomedical Scientist , Bolgatanga Regional Hospital.

Background: Anaemia remains a major public health problem in Ghana. High prevalence of anaemia in Ghana can be attributed to poor bioavailability of iron in the diet due to low intake of foods that enhance absorption of iron, such as meat and vitamin C-rich foods, as well as the effect of malaria and parasitic infections, such as hookworm and schistosomiasis.

Anaemic pregnant women exhibit low physical activity and increased risk of maternal morbidity and mortality, especially in those with severe anaemia (Scholl & Hediger, 1994; Allen, 2000; de Benoist *et al*., 2008). Anaemia in pregnancy is also associated with negative effects on the neonate.

Hence the study is to determine the possible causes of anaemia in pregnancy, by evaluating the biochemical and haematological profiles of pregnant women attending antenatal clinic at RHB and also provide possible treatment and preventive guidelines.

Purpose: This research is to evaluate the biochemical and haematological profiles of anaemic pregnant women attending antenatal clinic at the Bolgatanga Regional Hospital.

What we require from you: You will be engaged in the study for a period of 10 minutes. We will take 10mls early morning midstream urine, 2g early morning stool and 5mls blood samples from you. A trained person will take about a quarter of a

teaspoon of blood sample using a sterile disposable syringe and needle. Taking of the blood sample may cause a little temporary pain or discomfort. The samples collected will be used to determine your biochemical and haematological profiles.

Risk(s): You may develop a slight skin discomfort during the blood sample taking. You may also have a slight skin swelling which is temporary.

Benefits: You will be screened free of charge and in case found to be anaemic, you will be referred to a doctor for appropriate treatment and counselling. Also you will be educated on the risk factors which contribute to anaemia and how to reduce exposure to these risk factors.

Privacy and confidentiality: We will ask you a few questions. The information you give us will be used only for the study and will not be used in any way that will harm or embarrass you. Your participation and test results will remain confidential. Your samples will be labeled with a code number and not your name. Your name will also not be identified in reporting the study results. You are at liberty to withdraw unconditionally from this research after enrolment and this will not affect you in any way.

Voluntariness: Taking part in this study should be out of your own free will. You are not under obligation to participate in the study. Research is entirely voluntary.

Alternatives to participation:

If you choose not to participate, this will not affect your treatment in this hospital/institution in any way.

Withdrawal from the research:

You may choose to withdraw from the research at any time without having to explain. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers (name, etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.)

Costs/Compensation: For your time, inconvenience and transport to the hospital, you will be compensated with GH₵ 5.00 to show our appreciation for your participation. Also, when you are found to be ill during screening you will be treated free of charge and by participating in this research you will be well informed on the causes of anaemia, and how it can be prevented and treated.

Contacts: (If you have any question concerning this study, please do not hesitate to contact Benjamin Ahenkorah (Principal Investigator) on 0246852572).

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman
Institutional Review Board
Navrongo Health Research Centre
Navrongo.
Tel: 024 4564120 or 020 8161394

3.0 CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____

and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain.I have received a copy of the information leaflet and consent form.

NAME:

_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing the consent-seeking process (in cases involving non-literate participants):

---------- (Name of Witness) certify that information given to

---------- (Name of Participant), in the local language (Frafra), is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (non-literate participant): _____

MOTHER'S SIGNATURE (participant under 18 years): _____

MOTHER'S NAME:

_____

FATHER'S SIGNATURE (participant under 18 years): _____

FATHER'S NAME: _____