THE CAUSES OF ANAEMIA IN AGOGO, ASHANTI REGION, GHANA

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By
AMOS KOTEY

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST and the Agogo Presbyterian Hospital. This work has not been submitted for any other degree.

…… Date: __ / ___ / __
Amos Kotey
(Student)

…… Date: __ / ___ / __
Dr. W.K.B.A. Owiredu
(Supervisor)

…… Date: __ / ___ / __
Prof. (Mrs.) M.T. Frempong
(Co-supervisor)

…… Date: __ / ___ / __
Dr. E.F. Laing
(Co-supervisor)

…… Date: __ / ___ / __
Dr. R.A.Ngala
(Head of Department)

ABSTRACT

Anaemia, an intractable nutritional problem, remains an important public health problem in developing countries including Ghana. This homeostatic imbalance whereby the production of erythrocytes is outpaced by its destruction is an indicator of poor health and nutrition. Despite the social and economic cost of anaemia in Ghana, there is paucity of data on its aetiology, raising questions about the appropriateness of the current strategies being employed to achieve progress in the fight against this public health problem. This study aimed at establishing the aetiology of anaemia in Agogo by identifying the most significant contributors to the burden of the disease. A cross sectional study was conducted among 200 adult patients (18-60years) without any established chronic disease from September 2011 to February 2012 at the Out-patients Department of the Agogo Presbyterian Hospital. The participant selection was based on the WHO definition of anaemia in adults as Hb<12.0g/dl (female) and Hb<13.0g/dl (male). Venous blood samples were drawn for FBC, total iron, ferritin, folate, vitamin B_{12} and malaria parasite tests. Total iron was estimated with the colorimetric method and the Enzyme Immunoassay technique was used for measuring ferritin, folate, vitamin B_{12}. Stool samples were also collected for intestinal helminth screening. The mean age of the study participants was 36.9±13.7 years with the female group outnumbering the male group by a 4:1 ratio. One hundred and forty-eight (74%) participants presented with mild anaemia, 40(20%) had moderate anaemia and 12(6%) had severe anaemia. Study participants with mild anaemia had significantly higher red blood cell count (4.14±0.51 M/µL) when compared to those with moderate (3.71±0.67 M/µL) and severe (2.30±0.73 M/µL) anaemia. The mean MCH and MCHC for participants in the moderate (24.79± 4.39 pg; 31.38±2.12 g/dl) anaemia category were significantly lower than those in the mild (27.62±2.92 pg; 33.01±1.29 g/dl) anaemia category (p<0.001). Of the 200 study participants, 75(37.5%) had microcytosis, 10(5%) had macrocytosis and 115(57.5%) had normocytosis. Hypochromasia was seen in 45.5% of the study participants. Hypochromasia and microcytosis had a significant association with the severity of the anaemia (p<0.05). The odds of hypochromasia (OR=2.9; 95%CI=1.4-6.0) and microcytosis (OR=3.1; 95%CI=1.5-6.4) were three times more in the moderate anaemia group compared to the mild anaemia group. Twenty-five (12.5%) of the study participants had P. falciparum malaria infestation and malaria impacted significant risk (p<0.001) on the severity of the anaemia. Two different intestinal parasites were present in the stool samples of 17 (8.5%) study participants. In all, 51 (25.5%) study participants had folate deficiency (serum folate <5.0ng/ml) and 60 had vitamin B_{12} deficiency (serum vitamin B_{12}<200ng/L). Iron deficiency (serum iron <8.9µmol/L), the most prevalent cause of anaemia, occurred in 69(34.5%) of the study participants and had a statistically significant association with the severity of the anaemia (p=0.0028). Twenty-four (12%) participants had depleted iron stores showing significant association with the severity of the anaemia (p<0.0001). Vitamin B_{12} deficiency, folate deficiency and intestinal parasite infection though may result in anaemia, did not have any significant association with the severity of the anaemia (p>0.05) in the study. This study has succeeded in the advocacy for investigating the cause of anaemia before blindly treating patients with haematinics.

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ABBREVIATIONS

95%CI	-	95% Confidence Interval
AIDS	-	Acquired Immune Deficiency Syndrome
ALA	-	Amino Laevulinic Acid
CD163	-	Cluster of Differentiation 163
CDC	-	Centre for Disease Control
CHRPE	-	Committee on Human Research Publication and Ethics
CO_2	-	Carbon Dioxide
CoA	-	Coenzyme A
DNA	-	Deoxyribonucleic Acid
EDTA	-	Ethylenediaminetetraacetic Acid
FBC	-	Full Blood Count
GDP	-	Gross Domestic Product
GSB	-	Ghana Standards Board
Hb	-	Haemoglobin
HCT	-	Haematocrit
HIV	-	Human Immunodeficiency Virus
HRP	-	Horseradish Peroxidase
IDA	-	Iron Deficiency Anaemia
IF	-	Intrinsic Factor
LCD	-	Liquid Crystal Display
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Cell Haemoglobin Concentration
MCV	-	Mean Cell Volume
MEIA	-	Microparticle Enzyme Immunoassay

MMA	-	Methylmalonic Acid
N	-	North
OPD	-	Out Patient Department
OR	-	Odds Ratio
PBG	-	Porphobilinogen
PHC	-	Primary Health Care
RBC	-	Red Blood Cell
RCPA	-	Royal College of Pathologist of Australasia
RV	-	Reaction Vessel
SD	-	Standard Deviation
SWG	-	Standard Wire Gauge
TMB	-	Tetramethylbenzidine
TRIS	-	Tris(hydroxymethyl)aminomethane
UAE	-	United Arab Emirates
UNICEF	-	United Nations Children's Fund
URO	-	Uroporphyrinogen
W	-	West
WHO	-	World Health Organization
ZnPP	-	Zinc Protoporphyrin

Chapter 1

INTRODUCTION

1.1 BACKGROUND

According to the World Health Organization's (WHO) criteria for screening, a condition may be worthwhile to screen for if it is an important health problem and if there are tests available to detect the condition at an early, treatable stage (Wilson and Jungner, 1968). Anaemia which is defined as haemoglobin concentration below established levels is a significant public health problem with major consequences for human health and socio-economic development (WHO/UNICEF/UNU, 2001). Homeostatic imbalance in the blood haemoglobin concentration arising from the rate of production of red blood cells being outpaced by the destruction of red blood cells results in the phenomenon of anaemia (Patel, 2008). The WHO estimates that about two billion people in the world suffer from anaemia and approximately 50% of all anaemia cases are attributable to iron deficiency. There are more individuals with iron deficiency than any other medical condition at any given moment worldwide (World Health Organization, 2008). In resource limited settings, significant proportions of young children and women of child bearing age suffer from anaemia, although prevalence vary widely and accurate data are often lacking (WHO/UNICEF/UNU, 2001). For many years, anaemia has been recognized as a public health problem but little as been reported by way of progress as the global prevalence rate remains unacceptably high (WHO/UNICEF, 2004).

Anaemia is an indicator of both poor nutrition and poor health. Iron Deficiency Anaemia (IDA) remains the commonest form of anaemia (WHO/UNICEF, 2004). Developing countries carry the most significant proportion of the reported cases of anaemia whose aetiology is often multifactorial. The most important factors that

contribute to anaemia include parasitic infections, HIV infections, chronic inflammatory disorders, micronutrient deficiencies and genetic disorders (Calis *et al.*, 2008; Hinderaker *et al.*, 2002; Koukounari *et al.*, 2006; Mugisha *et al.*, 2008; Muhangi *et al.*, 2007; van Eijk *et al.*, 2002).

Infectious diseases like malaria, helminth infection, tuberculosis and HIV/AIDS are important factors contributing to the high prevalence of anaemia in many populations (Asobayire *et al.*, 2001; van den Broek and Letsky, 2000). Plasmodium *falciparum* infection- related anaemia contributes significantly to maternal and child mortality, hence prevention and treatment of anaemia in at- risk pregnant women and young children is of major importance. Hookworm infections and schistosomiasis cause blood loss and contribute to the burden of anaemia. HIV/AIDS is an increasing cause of anaemia which is recognized as an independent risk factor for early death among HIV/AIDS- infected people.

The contribution of vitamin B_{12}, folate and vitamin A to the burden of anaemia is unclear though they have been documented as causes of anaemia (International Nutritional Anemia Consultative Group (INACG), 2003). It remains therefore important to establish in various populations the role various micronutrient deficiencies play in the overall alarming prevalence of anaemia. The impact of haemoglobinopathies on anaemia prevalence needs equally to be examined (WHO/UNICEF, 2004).

In Ghana, anaemia was ranked as the fourth leading reason for hospital admissions and the second factor contributing to death after a review of the disease profile and pathology reports of selected hospitals. The data showed 59% of pregnant and lactating mothers, 83.5% of pre-school children and 71.3% of school-age children

were anaemic. The Ghanaian female workforce lost over 14.2 million Ghana Cedis in economic activity due to anaemia after economic impact analysis (Agble, 2004). Anaemia has been associated with depressed mental, decreased physical activity and negative consequence on scholastic performance (Grantham-McGregor and Ani, 2001; Schauer and Zlotkin, 2003). The loss of work productivity due to childhood anaemia has been estimated in economic terms by Horton and Ross to be 4.5% of the country's Gross Domestic Product (GDP) (Horton and Ross, 2003).

It is important to recognize the complexity of anaemia so that effective strategies can be mapped out to achieve progress. Approaches designed to combat anaemia as a public health problem must be multifactorial and multisectorial (WHO/UNICEF, 2004)

1.2 PROBLEM STATEMENT

Generally, numerous countries lack national prevalence data on anaemia inspite of the public health importance of anaemia. For countries where some survey data are available, the data are related to three population groups namely preschool-age children, pregnant women and non-pregnant women in their reproductive years (De Benoist *et al.*, 2008). Significant population groups like adult males and elderly are not captured in most of the national prevalence data available.

Iron deficiency has been noted as the most significant contributor to the burden of anaemia (De Benoist *et al.*, 2008). Despite this fact, the prevalence of this deficiency is often lacking in the national prevalence data on anaemia bringing into question the strategies and programmes designed to combat the public health problem of anaemia by health care regulators.

Public health officials have grossly underrated the contribution of factors other than iron deficiency to the burden of anaemia which has influenced their design of strategies directed mainly if not only at IDA. This stems from the confusion of equating iron deficiency anaemia to anaemia (De Benoist *et al.*, 2008)

In Ghana, there is little empirical data clearly outlining the contributing factors to the burden of anaemia at the district, regional and national levels. Hence current strategies being implemented by the Ghana Health Service to control anaemia could be potentially unsuccessful and misdirected.

1.3 JUSTIFICATION

The increased risk of maternal and child mortality due to anaemia has been reported in various studies conducted to assess the impact of anaemia (Bothwell and Charlton, 1981; Macgregor, 1963; Scholl and Hediger, 1994). The negative effects of IDA on cognitive and physical development of children, and work productivity of adults raise serious concern (Stolzfus, 2001).

Vitamin B_{12} deficient patients were often treated with folic acid before the role of vitamin B_{12} was appreciated in causing megaloblastic anaemia. In many of such patients, the anaemia improved initially, but the associated neurological damage progressed steadily. The phenomenon known as masking of vitamin B_{12} deficiency has never been studied systematically for the obvious reason that patients would never knowingly be given the wrong treatment, particularly when the neurological damage associated with vitamin B_{12} deficiency is often irreversible (Mills, 2000).

Folate and Vitamin B_{12} deficiency with the compensatory increase in homocysteine are a significant risk factor for cardiovascular disease (Klee, 2000).

Since limited information is available on the pathogenesis of anaemia in Ghana a study such as this will provide useful data for healthcare authorities to incorporate useful diagnostic techniques in assessing and managing anaemia. This will also make it easier to design new interventions that are more effective and integrative in addressing the multiple contributing factors to anaemia.

The results of this study will help draw the attention of public health authorities on the need to re-evaluate current strategies to control anaemia by making sure that the various factors contributing to anaemia have been identified and addressed properly through an integrated approach.

A drive spearheaded by health authorities to encourage fortified food intake and dietary diversification which are important, sustainable strategies in preventing anaemia due to micronutrient deficiencies could be greatly accelerated by the empirical evidence this study will provide.

1.4 AIM OF STUDY

To determine the causes of anaemia in adult patients presenting at the Agogo Presbyterian Hospital in the Asante Akim North Municipality of the Ashanti region.

1.5 SPECIFIC OBJECTIVES

- To establish the most significant contributor to the burden of anaemia in the Asante Akim North Municipality.
- To assess the contribution of iron, folate, vitamin B_{12}, helminth infection and malaria to the public health importance of anaemia.
- To classify the severity of anaemia at the Out-patient clinic by the WHO guideline.
- To assess the type of anaemia based on the red cell indices.

Chapter 2

LITERATURE REVIEW

2.1 ANAEMIA-PUBLIC HEALTH CONCERN

The world is plagued by anaemia, a common and intractable nutritional health problem affecting billions of people. According to the World Health Organization (WHO) estimates, some two billion people of the world's population are affected by anaemia, which is defined as haemoglobin concentrations below recommended thresholds (World Health Organization and Centre for Disease Control and Prevention, 2007).

Globally, the public health problem of anaemia affects both developing and developed countries with major consequences for human health as well as social and economic development. Anaemia occurs in all age groups, but is most prevalent in pregnant women and in young children. More frequently, it coexists with other causes, such as malaria, parasitic infection, micronutrient deficiencies, and haemoglobinopathies (De Benoist *et al.*, 2008). Iron deficiency anaemia in 2002 was considered to be the most significant contributor to the global burden of the disease (World Health Organization, 2002).

Numerous countries across the globe implement interventions aimed at reducing the impact of anaemia in groups which are most susceptible to its devastating effects: pregnant women and young children. The periodic collection of data on anaemia in various countries, clearly identifying the aetiology of the disease will help assess the impact of the interventions being implemented, the adequacy of the strategies and the progress made in the fight against anaemia (De Benoist *et al.*, 2008).

The fundamental health issue of anaemia has not been solved and continues to affect the health, quality of life, and working capacity of billions of people all over the world. Most cases of anaemia are due to iron deficiency, which is often associated with folate deficiency and/ or vitamin B_{12} deficiency as well as with infections (Milman, 2011).

2.1.1 Definition

Anaemia is defined as a reduction in the red blood cell count below the lower level of the normal range (Greer *et al.*, 2008). In clinical practice however, anaemia is defined by a haemoglobin concentration which is below the recommended lower thresholds established by epidemiological population surveys or by the local laboratory. Using the WHO recommended haemoglobin thresholds make it convenient to perform useful comparison between different populations and countries on the burden of the disease (De Benoist *et al.*, 2008; World Health Organization and Centre for Disease Control and Prevention, 2007). The thresholds are set at the fifth percentile of the haemoglobin concentration of a normal population of the same sex and age group (World Health Organization and Centre for Disease Control and Prevention, 2007). The definition of anaemia in the context of the WHO criteria is haemoglobin concentration less than 13.0 g/dl in men and less than 12.0 g/dl in women (Wilson and Jungner, 1968).

2.1.2 Aetiology

The different causes of anaemia may work in concert, so in a single individual, various nutrient deficiencies and different infestations may all play a role. These different causes of anaemia influence the choice of treatment and planning of future prophylaxis in the general population (Milman, 2011). The main causes of anaemia

include: dietary iron deficiency; infectious diseases such as malaria, hookworm infections and schistosomiasis; micronutrient deficiencies including folate, vitamin B_{12} and vitamin A; or inherited conditions that affect red blood cells such as thalassaemia and sickle cell disease (World Health Organization and Centre for Disease Control and Prevention, 2007). The most significant contributor to the onset of anaemia is iron deficiency. IDA has often been used as synonymous to anaemia, and the prevalence of anaemia used as a proxy for IDA (De Benoist *et al.*, 2008).

Fifty percent of the cases of anaemia are attributable to iron deficiency (WHO, 2001) but this proportion could vary among population groups in different areas according to local conditions. Low dietary intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high (i.e. growth and pregnancy) are the main risk factors IDA (De Benoist *et al.*, 2008).

Blood loss resulting from menstruation, parasitic infestation such as hookworm, ascaris and schistomiasis contribute to the lowering of haemoglobin concentration resulting in anaemia. Malaria, cancer, tuberculosis and HIV can also contribute to the burden of anaemia. An increase to the risk of anaemia could result from deficiencies of copper and riboflavin. The impact of haemoglobinopathies on anaemia prevalence needs to be considered within some populations (De Benoist *et al.*, 2008).

2.1.3 Signs and Symptoms

A patient with anaemia may present with fatigue and dizziness. However, mild anaemia could produce little by way of clinical signs and symptoms (Karnath, 2004). In severe cases of anaemia with haemoglobin concentration less than 5.0 g/dl, high-output heart failure may develop (Metivier *et al.*, 2000). Anaemia results in increased

cardiac output in order to compensate for tissue hypoxia, and an associated systolic ejection murmur may occur as a result of increased aortic flow (Karnath, 2004). Pica, defined as the compulsive eating of non-food substances is an unusual symptom of iron deficiency anaemia (Rose *et al.*, 2000). Patients with iron deficiency anaemia may develop Plummer- Vision syndrome, characterized by dysphagia and oesophageal webs formed of thin mucosal membranes (Hoffman and Jaffe, 1995).

Signs of anaemia include pallor of the conjunctivae, face, nail beds and palmer creases, although the absence of pallor does not rule out anaemia (Nardone *et al.*, 1990). Physical examination for the presence of anaemia focuses on areas where capillaries are close to the surface (i.e. conjunctivae, nail beds). One study reported a sensitivity of 95% and a specificity of 68% for the detection of pallor in the palms, nail beds or conjunctivae for patients with moderate anaemia defined as haemoglobin concentration less than 8.0 g/dl (Muhe *et al.*, 2000). Another study found a statistically significant correlation between low haemoglobin concentration and pallor of the conjunctivae, nail beds and palmar creases. The presence of the pallor was quite specific and never present in the absence of anaemia (Strobach *et al.*, 1988).

2.1.4 Health Consequence

Anaemia indicates poor nutrition and poor health (De Benoist *et al.*, 2008). Several studies have documented the most dramatic health effects of anaemia, i.e. increased risk of maternal and child mortality due to severe anaemia (Bothwell *et al.*, 1981; Macgregor, 1963; Scholl and Hediger, 1994). Iron deficiency with or without anaemia has important consequences for human health and child development: anaemic women and their infants are at greater risk of dying during the prenatal period (World Health Organization and Centre for Disease Control and Prevention,

2007). The WHO has documented the negative consequence of IDA on cognitive and physical development of children and the physical performance of adults, particularly their work productivity (WHO, 2001).

Before the mechanism by which vitamin B_{12} caused megaloblastic anaemia was understood, patients who presented with vitamin B_{12} deficiency were often treated with folic acid. The anaemia in such patients resolved initially, however, the associated neurological damage progressed steadily. This phenomenon known as masking of vitamin B_{12} deficiency has never been studied systematically for the obvious reason that patients would never knowingly be given the wrong treatment, particularly when the neurological damage associated with vitamin B_{12} deficiency is often irreversible (Mills, 2000). Deficiencies of folate and vitamin B_{12} lead to a compensatory increase in homocysteine , a significant risk factor for cardiovascular disease (Klee, 2000).

Economic impact analysis of anaemia in Ghana indicated that the female work force between 2001 and 2005 lost over 14.2 million Ghana cedis in economic productivity due to the debilitating effects of anaemia (Agble, 2004).

2.2 MECHANISM OF ANAEMIA

Anaemia occurs by three mechanisms: blood loss, increased red blood cell destruction and decreased blood production. One of these mechanisms may be dominant in the anaemia presented, however more than a single cause may occur (Conrad, 1990). The aetiology of anaemia in many patients is obvious and would not require an organized systematic investigation. In some cases however, an intelligent search needs to be undertaken to identify the aetiology of the disease. This can be accomplished by

determining which of the three mechanisms cited may be operative in producing the anaemia (Conrad, 1990).

Increased blood loss will produce anaemia and this may be due to an acute or chronic condition. Trauma and gastrointestinal bleeding leads to this blood loss (Rudolph *et al.*, 2002).

The other mechanism which involves increased destruction of red blood cells occurs in haemolytic anaemia. Extrinsic and intrinsic factors may be responsible for the increased cell destruction (Coyer and Lash, 2008). Hereditary or acquired diseases make up the intrinsic factors. Spherocytosis and elliptocytosis are conditions that cause anaemia because of a disorder in the red cell membrane. Enzyme disorders in the red cell such as glucose-6-phosphate dehydrogenase and pyruvate synthesis diseases contribute to the cause of anaemia. Sickle cell disease and thalassaemia also cause anaemia because of structural abnormality in the red blood cells (Kumar *et al.*, 2003). The extrinsic factors include blood transfusion reactions, haemolytic anaemia, thrombocytopaenia purpura and disseminating intravascular coagulation (Coyer and Lash, 2008).

Impaired erythropoiesis occurs when there is disturbance of proliferation and distribution of stem cells. This is one of the mechanisms by which anaemia occurs. Conditions which cause impaired cell production include reduced erythropoietin, aplastic anaemia, bone marrow dysfunction, renal cell aplasia, renal failure and endocrine disorders. Impaired cell production is also the outcome of defective DNA synthesis, such as in vitamin B_{12} and folate deficiency anaemia. Defective haemoglobin synthesis is the pathological process responsible for iron deficiency

anaemia, thalassaemia and the anaemia of chronic infections (Brill and Baumgardner, 2000; Kumar *et al.*, 2003).

2.3 HAEMOGLOBIN

The formation of normal red blood cells involves a series of diversified processes including the biosynthesis of nucleic acid, porphyrin, haem and proteins. Limitations placed on any of these processes could lead to anaemia (Richert and Schulman, 1959).

2.3.1 Structure and Function of Haemoglobin

The normal adult haemoglobin has a molecular weight of 64,500 Daltons and is capable of reversibly binding to one oxygen molecule (Lehninger *et al.*, 1993). Haemoglobin is made up of four subunits, with each having one polypeptide chain and one haem group. Haemoglobin molecule carry the same prosthetic haem group iron protoporphyrin IX associated with a polypeptide chain of 141 (α) and 146 (β) amino acid residues. The ferrous ion in the haem is linked to the N of a histidine and the prophyrin ring is wedged into its polypeptide chain. The polypeptide chain is made up of two kinds, alpha and beta chains which are similar in length but vary in amino acid sequence. The alpha chains of embryonic and adult haemoglobins are the same. The non-alpha chains include the beta chain of normal adult haemoglobin ($\alpha_2\beta_2$), the gamma chain of fetal haemoglobin ($\alpha_2\gamma_2$) and the delta chain of HbA2 (Marengo-Rowe, 2006).

The oxygen binding property of haemoglobin is determined by the precise sequence of amino acid of the alpha and beta chains. Oxygen binds reversibly to the ferrous iron atom in each haem group. The haem group that is oxygen bound varies with the partial pressure of oxygen (Perutz, 1983). The sigmoid shape of the oxygen equilibrium curve shows a cooperative interaction of oxygen binding sites. Falling

pH, increase in erythrocyte organic phosphate concentration, or a rise in temperature all result in rightward displacement of the oxygen equilibrium and hence affinity is lowered with a resultant release of oxygen (Marengo-Rowe, 2006; Wells, 1999). Compounds such as nitric acid and carbon monoxide are capable of binding to ferrous atom of haemoglobin. Carbon monoxide has a greater affinity for the ferrous atom of haemoglobin than oxygen and once carboxyhaemoglobin is formed, oxygen cannot displace carbon monoxide. The oxygen transport mechanism in the body is dependent on the adequacy of oxygenation of blood in the lungs, blood flow rate and distribution, haemoglobin concentration, and the affinity of haemoglobin for oxygen. Availability of oxygen to the body could be hampered by abnormalities in the physiological pathway (Marengo-Rowe, 2006).

2.3.2 Haem Synthesis

Haem is synthesized by both nucleated red blood cells and reticulocytes. Matured cells are however not capable of synthesizing haem. Glycine and succinyl-coenzyme-A condense to form aminolaevulinic acid with the participation of pyridoxal-5-phosphate, pantothenic acid and ferrous iron in a complex reaction catalysed by the enzyme aminolaevulinic acid synthetase. Two molecules of aminolaevulinic acid combine to form porphobilinogen which is subsequently converted enzymatically to uroporphyrinogen. Successive decarboxylations of propionic and acetic side chains to vinyl and methyl groups on the tetrapyrrole lead to coproporphyrinogen then protoporphyrin-IX. Insertion of iron into protoporphyrin completes the haem portion of haemoglobin (Brown, 1963).

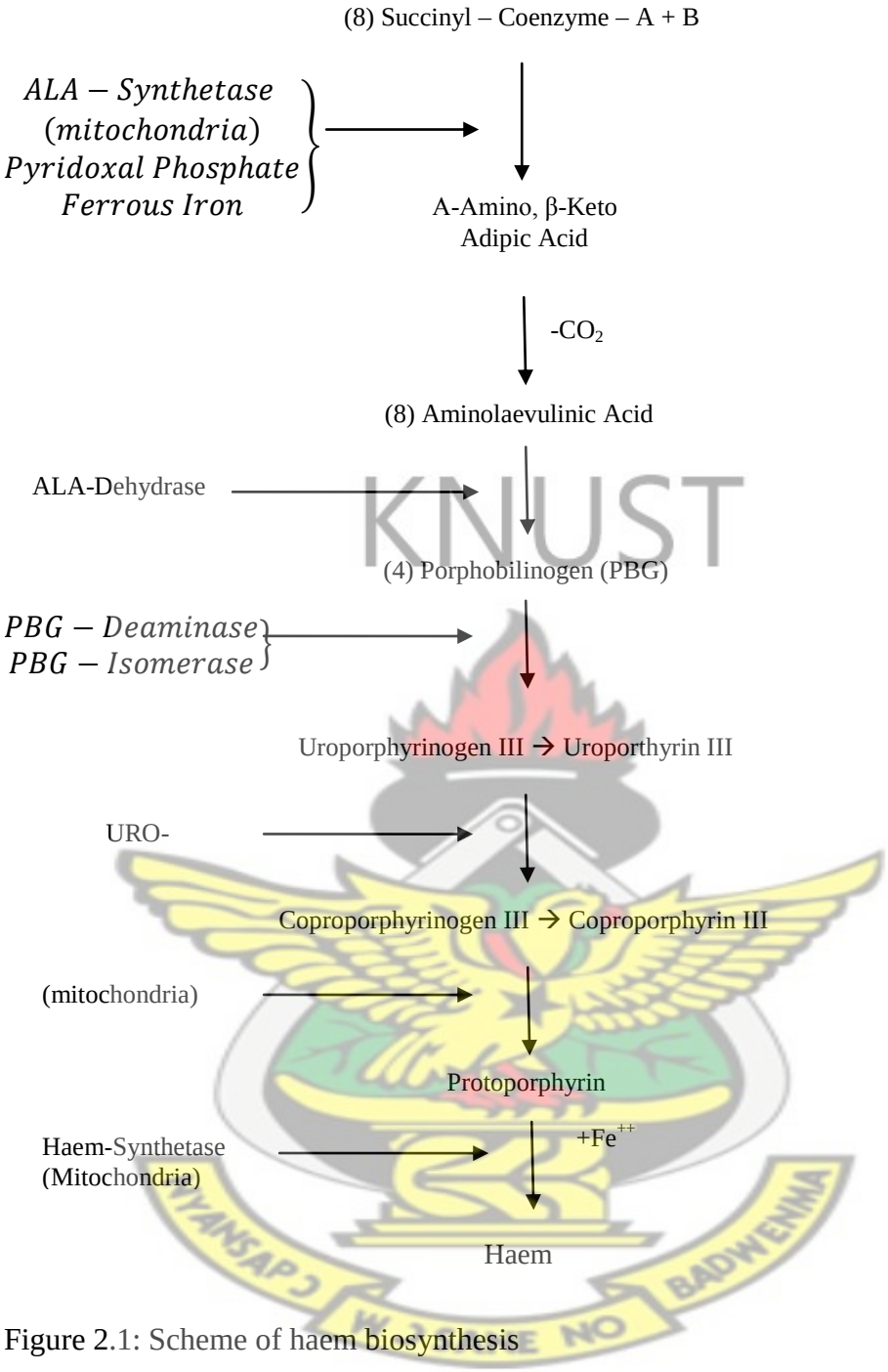

Figure 2.1: Scheme of haem biosynthesis

2.3.3 Globin Synthesis

Globin synthesis occurs at a similar rate as haem synthesis (Srinoun *et al.*, 2009) although under some conditions, such as radiation, prolonged incubation, elevated temperatures, addition of various metals or nucleosides, the ratio of haem to globin synthesis within cells or haemolysates of immature cells is altered from the usual 1:1

ratio (Kassenaar *et al.*, 1957; Richmond *et al.*, 1951). The incorporation of amino acids from the plasma occurs at a concentration gradient (Christensen *et al.*, 1952; Riggs *et al.*, 1952) and their synthesis into the protein globin occurs primarily in the microsomal portion of cells. The specificity of the globin synthesized is dependent on the microsomal DNA (Lamfrom, 1961).

The exact mechanism by which haem and globin are joined require further investigation as there is evidence that these two can be separated and recombined nonenzymatically (Ponka, 1997). In the presence of globin however, there seems to be enhanced haem synthesis from protoporphyrin (Schwartz *et al.*, 1961) and the suggestion has been made that protoporphyrin and globin combine before the insertion of iron (Ericksen, 1957).

2.4 IRON

Iron remains an essential mineral for man and an important component of metalloproteins involved in oxygen transport and metabolism. It is approximated that in a well nourished individual, the body contains 3-4 g of iron. Nearly two thirds of all the iron present in the body is contained in the haemoglobin, the protein in the red blood cells that carries oxygen to the tissues (Milman, 2011). The contribution of iron deficiency to the overall burden of anaemia is approximately 50%. This proportion may however vary according to different population groups influenced by the unique demographic variables (WHO, 2001).

Iron is required for the production of haemoglobin in progenitor red blood cells. Deficiency of iron supply to the bone marrow leads to impairment in haemoglobin synthesis and a decline in the circulating red blood cells. This subsequently leads to IDA with a low haemoglobin concentration. IDA characteristically can be corrected

or cured by treatment with iron, either by the oral route or intravenously (Milman, 2011). Anaemia is typically microcytic with a low mean red blood cell volume and hypochromic with low haemoglobin concentration in the red blood cells, referred to as low mean corpuscular haemoglobin and low mean corpuscular haemoglobin concentration.

The four major factors which control iron absorption in the healthy individual are physiological need of iron, dietary iron intake, iron bioavailability in dietary intake and adaptation (Cook, 1990). Iron deficiency is mainly as a result of insufficient dietary iron intake. Pedersen *et al* pointed out that even in developed countries; dietary iron intake in some population groups was extremely low. Danish women of reproductive age for example had a mean iron dietary intake of 9 mg/ day (Pedersen *et al.*, 2010). This dietary survey meant that more than 90% of the women had iron dietary intake below the recommended daily allowance of approximately 18 mg/ day (Nordic Council of Ministers, 2004).

Dietary iron is made of the haem and non-haem iron. Haem iron has a good bioavailability with a favourable gastrointestinal absorption, and is present in food products of animal origin like meat, poultry and fish. Non-haem iron which has its origin in plant food products however has a poor bioavailability with decreased absorption. Non haem iron is present in vegetables, cereals, grains and legumes (Milman, 2011).

Continuing blood loss from the gastrointestinal tract due to infections, intestinal parasites and inflammatory bowel contribute to IDA in many parts of the world. Women in their reproductive years who experience heavy blood losses during their menstrual periods are at risk of developing IDA. Recurrent uterine bleeding

associated with some gynaecological diseases increase the risk of IDA (Milman, 2011).

2.5 FOLATE

Folate deficiency remains the common vitamin deficiency producing anaemia (Al Khatib *et al.*, 2006; De Benoist *et al.*, 2008). The extent to which folate deficiency contributes to the overall burden of anaemia in various populations has not been intensively investigated as iron deficiency. Further studies are therefore required to provide a clearer picture of folate deficiency in many populations. Folate deficiency causes megaloblastic anaemia with high MCV and is associated with a raised homocysteine level which is a significant factor for the development of cardiovascular disease (Milman, 2011).

In a few studies, folate nutritional status has a direct correlation with risk of cardiovascular disease. In the Nutrition Canada Survey which involved over 5000 men and women, there was a significant association between serum folate levels and the risk of fatal coronary heart disease. In the same study, there was a 69% increase in the relative risk of fatal coronary heart disease among a third of the population with the lowest serum folate compared with the third that had the highest serum folate (Morrison *et al.*, 1996). It has been estimated that, in developing countries, folate deficiency occurs in as many as 25% to 72% of women of reproductive age (Al Khatib *et al.*, 2006; De Benoist *et al.*, 2008). In the fetus and new baby, folate deficiency is associated with a high risk or neural tube defects as well as other organ defects (De Benoist *et al.*, 2008).

Folate is present in food of plant like green leafy vegetables and grains. However, food processing procedure (cooking, milling, frying, and baking) destroys a high percentage of the folate content in the food (Milman, 2011).

2.5.1 Biochemical Roles of Folate

Folate functions as cofactors and cosubstrates for biological methylation and nucleic acid synthesis. Folate also functions as regulatory molecules. They act as mobile cofactors in several key enzymatic reactions, not being tightly bound to the apoenzyme, and carry one-carbon residues. In effect, folates are cofactors in the reactions they are involved in. The intracellular concentrations of the different folates are in general much lower than their Michaelis constant values for the enzymes, and so the rate or steady state of the reaction can change over quite a large range of cellular folate concentrations (Nijhout *et al.*, 2004).

Measurement of the plasma total homocysteine concentration, which reflects the intracellular homocysteine concentration, can be used as a surrogate marker for the possible range of folate. Homocysteine is converted to methionine by methionine synthase, and 5-methyltetrahydrofolate is the cosubstrate that donates the methyl group. The concentration of plasma total homocysteine falls as plasma folate concentrations increase from <2 nmol/L to >15 nmol/L (Refsum *et al.*, 2006; Selhub *et al.*, 1993). Over this range, changes in the blood folate concentration could influence the methylation of tissues of the body.

There is no convenient marker to evaluate the status of nucleic acid synthesis, but it is likely this can change over similar range of blood folate concentrations (Nijhout *et al.*, 2004). The misincorporation of uracil into DNA, because of the inadequate synthesis of thymidine, a folate-requiring step, is inversely related to the blood concentration of

folate (Blount *et al.*, 1997). Cellular folate acting as regulatory molecules exert allosteric effects on several enzymes in the folate and methionine cycles, such as methylenetetrahydrofolate reductase, glycine-*N*-methyltranferase and serine hydroxymethyltransferase (Matthews and Daubner, 1982; Shane, 1995; Wagner, 1995). Plasma concentration of folate influence the cellular concentrations of folates although the concentration of folates within cells is subject to many regulatory processes (Shane, 1995; Wagner, 1995). Folates enter mammalian cells as monoglutamates, but are rapidly modified by the addition of 4-8 glutamate residues to form long side chains. Polyglutamation greatly increases the affinity of folates as both substrates of their own enzyme and inhibitors of other enzymes in the folate pathway (Matthews and Daubner, 1982; McGuire and Bertino, 1981). Polyglutamation also constitutes a mechanism to trap folates within cells because the long-chain folylpolyglutamates are poorly accepted by the membrane carriers responsible for efflux across the cell membrane (Shane, 1995).

2.6 VITAMIN B_{12}

Vitamin B_{12} deficiency is the second most common vitamin deficiency causing anaemia. It is characterized by a megaloblastic anaemia with high MCV and morphological features such as hyperlobulation of the nuclei of the granulocytes (Greer *et al.*, 2008). Vitamin B_{12} deficiency is a significant public health problem, particularly among the elderly. In the United States, conservative estimates indicate that 2-3% of the elderly population above the age of sixty five have or will develop pernicious anaemia caused by failure of gastric intrinsic factor production and consequent vitamin B_{12} malabsorption (Carmel, 1996; Chanarin, 1979).

In developing countries, vitamin B_{12} is a significant problem (De Benoist *et al.*, 2008), and studies done in Lebanon and Turkey revealed that approximately 40% of women of reproductive age had vitamin B_{12} deficiency (Al Khatib *et al.*, 2006; Karaoglu *et al.*, 2010). This could be attributable to insufficient dietary vitamin B_{12} intake and food cobalamin malabsorption syndrome or pernicious anaemia (Al Khatib *et al.*, 2006). In the last decade, a high prevalence of vitamin B_{12} deficiency has been observed in diverse locations, such as Guatemala, India and Israel (Gielchinsky *et al.*, 2001; Refsum *et al.*, 2001b; Rogers *et al.*, 2003). The causes of deficiency in these population groups may be related to low intake and unrecognized malabsorption (Carmel *et al.*, 2003).

Vitamin B_{12} is a coenzyme in a methyl transfer reaction that converts homocysteine to methionine and in a separate reaction converts L-methylmalonyl-CoA to succinyl-CoA. This explains why increased homocysteine and/or methylmalonic acid in the blood are measures of impaired cobalamin status, which may occur in the presence of normal serum cobalamin concentrations and the absence of the classic signs myelopathy and megaloblastosis (Allen *et al.*, 1993).

Although low serum vitamin B_{12} concentrations are a sensitive indicator of vitamin B_{12} status, the risk of vitamin B_{12} deficiency associated with as intermediate range of vitamin B_{12} concentrations is unclear. Plasma methylmalonic acid (MMA) is a useful diagnostic test in persons with a low or low normal serum vitamin B_{12} concentration (Allen and Casterline, 1994; Baik and Russell, 1999). Plasma total homocysteine concentrations could be elevated as a result of low folate, B_{12} or B_6 intakes, or because of renal insufficiency, methylene tetrahydrofolate reductase polymorphism, or the use of certain medications. Elevation of plasma MMA concentration could be

as a result of renal insufficiency. Severe vitamin B_{12} deficiency causes anaemia, although haematologic signs are not always present. Haematologic and neurologic abnormalities are inversely correlated in vitamin B_{12} deficiency (Baik and Russell, 1999).

Vitamin B_{12} deficiency causes neuropathy with pronounced neurological symptoms and in the newborn baby has been associated with an increased risk of neural tube defects (Groenen *et al.*, 2004). Masking of vitamin B_{12} has been shown to occur in clinical practice. However, knowledge of how frequent masking occurs and of the lowest dose of folic acid that produces masking is limited. Little knowledge is also known about how often patients with vitamin B_{12} deficiency have symptoms of neurological damage without anaemia in the absence of folic acid therapy. Anaemia is an important clue to the diagnosis of vitamin B_{12} deficiency, particularly in the elderly, who show neurologic signs such as confusion, parenthesias, and dementia. Data on the rate of low vitamin B_{12} concentrations occurring without anaemia are urgently needed given the uncertain folic acid exposure and the lack of good data on maximal safe exposures to folic acid (Mills *et al.*, 2003).

Vitamin B_{12} is a vitamin that is synthesized exclusively by microorganisms. The dietary sources of vitamin B_{12} are primarily from animal origin, including meat, dairy products and eggs (Position of the American Dietetic Association and Dieticians of Canada., 2003). Individuals consuming food of predominantly plant origin, such as vegetarians may not be getting enough vitamin B_{12} and are therefore at a high risk of deficiency (Milman, 2011). Vitamin B_{12} is not present in plant foods unless they have been exposed to specific bacterial action (Elmadfa and Leitzmann, 2004b; Green and Miller, 2007). Vegans may be able to obtain small amounts of vitamin B_{12} from the

ingestion of special microbially fermented products, although the bioavailability of vitamin B_{12} in these products is yet to be clarified. In rural areas, individuals may obtain some vitamin B_{12} through the ingestion of cobalamin contained in bacteria-contaminated plant foods (Elmadfa and Fritzsche, 2004a; Elmadfa and Leitzmann, 2004b).

2.6.1 Biochemical Role of Vitamin B_{12}

Vitamin B_{12} is a relative large and complex micronutrient that plays a fundamental role in cell division and in one-carbon metabolism (Elmadfa and Leitzmann, 2004b; Green and Miller, 2007). Its absorption occurs by both an active and passive mechanism. In food, vitamin B_{12} is protein bound and liberated from the protein by an active mechanism in the stomach where it binds to a salivary R-binder (family of haptocorrins). It is released again in the upper small intestine and attaches to the intrinsic factor (IF). The vitamin B_{12}-IF complex move to the lower end of small intestines, where it is absorbed by ileal receptors. The two main transport proteins of vitamin B_{12} in human plasma are haptocorrin and transcobalamin. Vitamin B_{12} absorption by the passive mechanism occurs equally across the absorptive surface of the gastrointestinal tract. This is an ineffective absorption mechanism as only 1-2% of an oral dose can be utilized. An important component of cobalamin absorption is the partial reabsorption and conservation of biliary vitamin B_{12} by the enterohepatic circulation (Green and Miller, 2007). Vitamin B_{12} acts as cofactors in two important intracellular metabolic reactions. It is involved in the mitochondrial reaction in which the enzyme methylmalonyl-CoA mutase requires cobalamin in the form of 5'-deoxyadenosylcobalamin (converting methylmalonyl-CoA to succinyl-CoA). The second is a cystolic reaction that requires methylcobalamin for the folate-dependent methylation of the sulfur containing amino acid homocysteine to form methionine,

catalyzed by methionine synthase. Methionine metabolism is regulated by vitamin B_{12}, folate and vitamin B_6. The methionine synthase reaction is necessary for normal DNA synthesis (Elmadfa and Leitzmann, 2004b; Green and Miller, 2007).

Vitamin B_{12} deficiency may result in defective DNA synthesis, homocysteine accumulation and impaired regeneration of methionine (Friso and Choi, 2002; Geisel *et al.*, 2005; Green and Miller, 2007; Stabler, 2001). The assimilation, transport and metabolism of vitamin B_{12} can be affected by genetic factors. These genetic factors include; congenital IF deficiency or functional abnormality, congenital transcobalamin deficiency, congenital haptocorrin deficiency, cobalamin mutations resulting in hyperhomocysteinemia and methylmalonic academia. Severe disorders involving gene deletion or mutation generally result in serious complication during childhood, whereas milder but more prevalent conditions can arise at any age as a result of polymorphisms of genes involved in the vitamin B_{12} pathway (Aléssio *et al.*, 2007; von Castel-Dunwoody *et al.*, 2005).

2.7 HELMINTH INFECTIONS/ INFESTATIONS

Helminth infections considered chronic or acute are associated with anaemia (Greer *et al.*, 2008). Intestinal helminthes are among the most common and widespread of human infections contributing to poor nutritional status, anaemia and impaired growth in children of school going age (Dickson *et al.*, 2000) Intestinal parasites like hookworms, which are prevalent in tropical and subtropical regions are a significant cause of the high prevalence of anaemia in many countries (Smith and Brooker, 2010). Hookworm and other soil-transmitted helminthes contribute significantly to the chronic loss of blood and iron in the tropics (Brooker *et al.*, 2008). Hookworm infection has been singularly named as a strong predictor of iron deficiency and

anaemia in many populations (Ayoya *et al.*, 2006; Stoltzfus *et al.*, 1997). In a Kathmandu hospital, Nepal, hookworm infection was associated with anaemia among women receiving antenatal care (Bondevik *et al.*, 2000).

The anaemia which results from helminth infections is primarily due to iron deficiency anaemia but is often associated with vitamin B_{12} and folate deficiencies. Epidemiological surveys have revealed the predisposing factors to intestinal helminth infestations as poor sanitation, inappropriate environmental conditions coupled with indiscriminate defaecation, geography and contamination of water bodies (Brooker *et al.*, 2008). Developing countries report a high prevalence and intensity of the infection, particularly among population groups with poor environmental sanitation (Van Eijk *et al.*, 2009). Important practices like hand washing, disposal of refuse and personal hygiene when not done properly may contribute to individuals contracting the infection from the environment (Stoltzfus *et al.*, 1997).

Some studies have indicated that there is a worm threshold above which clinically significant anaemia is likely to occur, with the precise threshold dependent on the iron status of the host (Lwambo *et al.*, 1992).

Worm burden in influencing morbidity, is also a key determinant of transmission dynamics and hence the rate of reinfection following anti-helminthic treatment (Anderson and May, 1985). The intensity of the infection may also influence the efficacy of the treatment (Bennett and Guyatt, 2000). As the intensity of hookworm infection varies considerably between populations, the risk of anaemia attributable to hookworm and the impact of treatment will differ among populations (Smith and Brooker, 2010).

2.8 MALARIA

Malaria infection places a huge economic burden on developing countries. There are over 500 million episodes of malaria annually and is responsible for about 18% of childhood deaths in sub-Saharan Africa (Snow *et al.*, 2005).

Malaria, considered as a rural disease in Africa, contributes significantly to morbidity and mortality in many urban African populations (Donnelly *et al.*, 2005). The United Nations has projected a doubling of the African urban population by 2030 (United Nations Population Division, 2004), with an estimated 200 million urban residents at risk of malaria (Keiser *et al.*, 2004). There is therefore an urgent need to identify the risk factors in such urban populations (Donnelly *et al.*, 2005; Klinkenberg *et al.*, 2005).

Anaemia has been shown to be prevalent in areas where malaria is endemic and the introduction of antimalaria programmes has proven to be effective in reducing the burden of anaemia (Le Hung *et al.*, 2005). Anaemia resulting from malaria is associated with factors which involve increased destruction and reduced production of red blood cells (Menendez *et al.*, 2000). The direct mechanism involves increased spleenic clearance of infected and uninfected red blood cells and induced dyserythropoiesis (Crawley, 2004; McDevitt *et al.*, 2004). Depending on the degree immunity of the host, the infected red blood cells are destroyed before the schizonts mature and release merozoites. There has been suggested evidence that as many as ten uninfected red blood cells are removed from circulation for every one parasitized red blood cell (Jakeman *et al.*, 1999). In the acute and convalescent phases of malaria infection, the life span of both the infected and uninfected red blood cells is shortened (Looareesuwan *et al.*, 1987; Rosenberg *et al.*, 1973).

An acute episode of malaria usually induces anaemia of varying severity, which in extreme cases could be fatal. The post malarial anaemia has characteristics similar to IDA. The IDA arises from the redistribution of iron because there is minimal iron excretion after the lysis of infected red blood cells caused by malaria. The potentially toxic haemoglobin released from the ruptured red blood cells is complexed to haptoglobin and hemopexin. Specific receptors on circulating macrophages (CD 163) recognize the haptoglobin-haemoglobin complex and internalize them. The iron-loaded macrophages migrate to the reticulo-endothelial system and the hemopexin-haemoglobin complex undergoes receptor-mediated uptake by hepatocytes where the iron can persist for a long time (Prentice *et al.*, 2010).

Malaria being an inflammatory disease, blocks the recycling of sequestered iron from the liver and macrophages (Nweneka *et al.*, 2009). The redistribution of the body iron is central to the iron-limited suppression of erythropoiesis, because under normal conditions, 95% of the iron supply to the erythron comes from the recycled iron and only 5% from recent absorption from diet (Andrews and Schmidt, 2007). An increase in erythropoietic activity signaled by a raised erythropoietin level is seen during the acute and convalescent phase of malaria. In the absence of sufficient supply of iron, there is microcytosis and an increase in the proportion of prophyrin moieties in which zinc is substituted for iron, thus creating elevated concentrations of zinc protoporphyrin (ZnPP). A raised ZnPP may indicate iron deficiency. It may be considered to be independent of the confounding effects of malaria inflammation, but in actuality reflects functional iron supply to the erythron. ZnPP has been shown to have anti-malarial effects (Iyer *et al.*, 2003).

2.8.1 Mechanism of Malaria Infection

Malaria infection in the human host is initiated by a bite from the female anopheles mosquito during which the sporozoites which enter the body migrate to the liver initiating the hepatic phase of the disease. The erythrocytic phase of the parasite life cycle is initiated when the merozoites emerge from the ruptured liver schizonts. The parasites in the infected red blood cells follow one of two developmental pathways, the asexual phase characterized by the classical fever and the sexual phase allowing transmission of the parasite in future mosquito bites. In the asexual phase, the merozoites develop into a ring stage parasite that matures to a trophozoite and then into a schizont. The schizont on rupturing releases numerous merozoites, thus initiating the cycle of infection in red cells. The cycle takes 48hrs in P. *falciparum* infection and is a synchronous process. As the parasites continue to infect other red blood cells, the parasitaemia increases unrestricted unless the individual is able to mount an immune response. The rupture of schizonts produces cytokine-inducing toxins which result in the fever associated with the malaria (Robson and Weatherall, 2009).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study on the Causes of Anaemia in Agogo, Ashanti Region, Ghana, was a cross-sectional hospital based study conducted at the Agogo Presbyterian Hospital in the Asante Akim North Municipality between the months of September 2011 and February 2012. The study took place at the Out- patients Department of the hospital among adult (18 to 60 years) patients presenting clinically with anaemia without any established chronic condition. Pregnant women were excluded from the study. Adult patients who presented clinically with anaemia were referred by their clinicians to the laboratory for the estimation of their haemoglobin level. Patients who met the pre-inclusion criteria (Hb concentration <13.0 g/dl in men, and <12.0 g/dl in women) were guided through an informed consent process. The protocol of the study was thoroughly explained to the prospective participants and they were made to voluntarily sign the study consent form. 5 mls of blood was drawn from each participant for Full Blood Count (FBC), malaria parasite, total iron, ferritin, vitamin B_{12} and folate assays. Stool samples were also collected from each participant for screening of intestinal parasites.

3.2 STUDY AREA

The Asante Akim North Municipality is one of the 27 Districts in the Ashanti Region. The Ashanti Region is the third largest of 10 administrative regions in Ghana, occupying a total land surface of 24,389 square kilometers or 10.2% of the total land area of Ghana. In terms of population, however, it is the most populated region with a population of 3,612,950 in 2000, accounting for 19.1% of Ghana's total population. The Ashanti region also harbors the capital city of Kumasi. The Ashanti region is

centrally located in the middle belt of Ghana. It lies between longitudes 0.15W and 2.25W, and latitudes 5.50N and 7.46N. The region shares boundaries with four of the ten political regions, Brong-Ahafo Region in the north, Eastern region in the east, Central region in the south and Western region in the South west.

The Asante Akim North Municipality was carved out of the erstwhile Asante Akim District Council in 1988 as part of Ghana's Decentralization Process. It has Konongo-Odumase as its twin Capital Town. It is located in the eastern part of Ashanti Region and covers a land area of 1,160 sq. km with an estimated population of 142,434 for 2006 (projection from 2000 Population Census). The Municipality shares boundaries with Sekyere East on the north, Kwahu South on the east, Asante Akim South on the south and Ejisu-Juaben Municipal on the west. The sub-districts are Konongo-Odumasi, Agogo, Juansa, Dwease-Praaso and Amanteman.

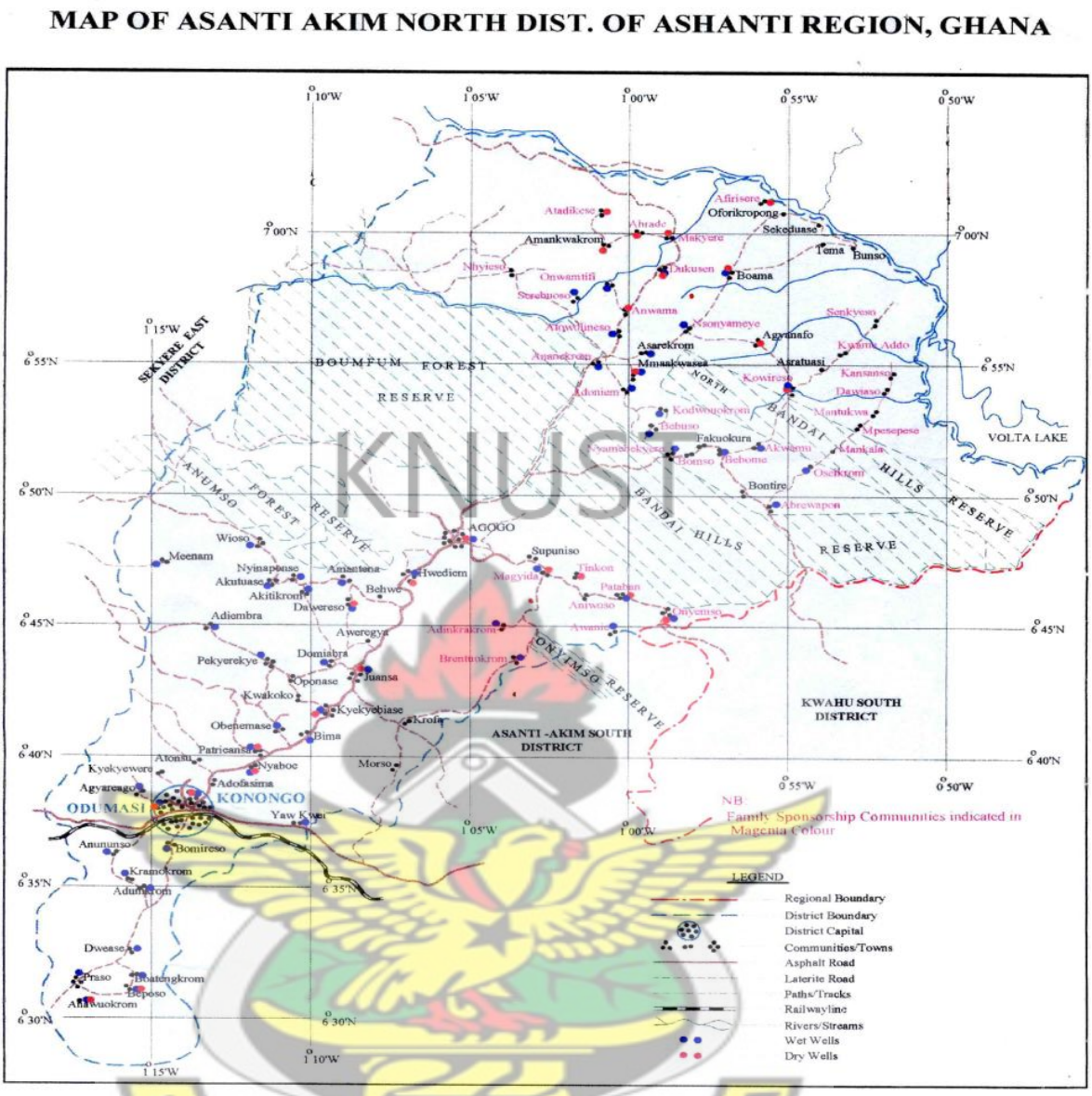

Figure 3.1 Map of Asante Akim North Municipality
(Courtesy:www.Ashanti_districts.png)

3.3 STUDY SITE

Agogo Presbyterian Hospital, the oldest mission hospital in Ghana was established by the Basel Mission on 21st March 1931. On December 1st, 1961, it was handed over to the Presbyterian Church of Ghana. It is a municipal hospital that serves the Asante Akim North catchment area and has a bed complement of 250. The hospital has been accredited for Housemanship Training in Surgery, Paediatrics and Obstetrics/ Gynaecology; Diploma in Ophthalmology programme for Doctors and Ophthalmic

Nursing Training. The hospital has been designated as a collaborative centre for the University of Ghana School of Public Health and is one of two sites in Ghana where a Phase III, double blind (observer-blind), randomized, controlled multi-center study to evaluate, in infants and children, the efficacy of the RTS,S/ASO1E malaria candidate vaccine trial is taking place.

The Agogo Presbyterian Hospital recorded a total of 167,739 outpatient attendance and 11,449 admissions in 2010. The top ten causes of outpatient attendance at the Agogo Presbyterian Hospital for the 2010 fiscal year were malaria (13.53%), hypertension (11.31%), acute eye infection (11.23%), Cough & Cold (6.78%), Diabetes Mellitus (6.45%), Pregnancy & associated conditions (3.39%), Skin diseases & Ulcer (3.21%), P.U.D. (2.81%), Cataract (2.20%) and Gynaecological conditions (1.81%) (Agogo Presbyterian Hospital, 2010).

The hospital has both a diagnostic and research Laboratory which undertakes Haematology, Biochemistry and Microbiology assays. The laboratory is equipped with a Sysmex® Automated Haematology Analyzer KX-21N and XS-1000i (Sysmex Corporation, Kobe, Japan). There are also two Vital Scientific® Flexor E chemistry analyzers, scientific refrigerators for storing samples up to -80^{0}C, safety cabinets and microscopes. The laboratory has enrolled in External Proficiency Schemes with the the United Kingdom National External Quality Assessment Scheme for General Haematology and the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Programs for General Chemistry.

3.4 STUDY POPULATION

Adult patients between the ages of 18 and 60 years presenting with anaemia, defined by the WHO criteria, as haemoglobin (Hb) concentration <13.0 g/dl in men, and

<12.0 g/dl in women (Wilson and Jungner, 1968) without any established chronic condition were approached to participate in the study. The participants were made to understand that it was voluntary to partake in the study and that all information obtained will be treated confidentially. Participants who met the predetermined inclusion criteria were made to endorse an informed consent form before any study procedure was performed.

3.5 SAMPL SIZE

The prevalence rate of anaemia in adults in a study conducted in Buea, Cameroun with climatic conditions similar to that in Agogo was 14.8% (Takem *et al.*, 2010). Adjusting for a non-response rate of 2%, with a confidence interval of 95%, the estimated sample size for the study using the formula $N=z^2pq/d^2$ was 200 study participants. Demographic data variables were collected through interview of study participants using a well structured questionnaire.

3.6 ETHICAL APPROVAL

An application for ethical approval of the study was submitted to the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST/Komfo Anokye Teaching Hospital, Kumasi. Approval to commence the study was given by the CHRPE (CHRPE/184/10) in February 2011. A written approval was obtained from the Agogo Presbyterian Hospital management to use their facility as a study site.

3.7 INCLUSION CRITERIA

- Adult patients, between 18 and 60 years , presenting with anaemia (Hb concentration <13.0 g/dl in men, and <12.0 g/dl in women) at the Out-patient

department of the Agogo Presbyterian Hospital were eligible to participate in the study

- An informed consent form was administered to prospective participants, explaining in detail the purpose of the research, potential risk or discomfort to the participants and potential benefits to the participants. Participants on agreeing to take part were made to sign or thumbprint the consent form to provide proof of their willingness to be involved in the study without undue influence. The consent process was a prerequisite before any study procedure was commenced.

3. 8 EXCLUSION CRITERIA

- All patients below 18 years and above 60 years were excluded from the study
- All pregnant women were non-eligible to participate in the study
- All adult patients who were shown to have chronic conditions or infections from their medical records like Sickle Cell, thalassaemia, leukaemia, HIV, Tuberculosis, chronic renal and liver diseases were excluded from the study
- All emergency cases requiring patient admission were excluded from the study
- Patients who failed to give consent to the study were excluded

3.9 SAMPLE COLLECTION

5 mls of blood was drawn from each study participant by qualified laboratory personnel into Vacuette® serum clot activator and K3EDTA tubes for the laboratory assays to be done. The tubes were appropriately labeled with unique pathology numbers assigned each participant on the study. 1 to 3 g of stool sample was requested from each study participant to examine for intestinal parasites.

3.9.1 Blood Collection Technique

- A sterile, dry, plastic syringe with 5 ml capacity was selected and attached to a disposable 20 SWG needle.
- A soft tubing tourniquet was applied to the upper arm of the participant to enable the veins to be seen and felt. The participant was asked to make a tight fist to make the veins more prominent.
- The index finger was used to feel a suitable vein, one which was sufficiently large, did not roll and with a direction that could be felt.
- The puncture site was cleansed with 70% ethanol and allowed to dry.
- With the thumb of the left hand holding down the skin below the puncture site, the venepuncture was made with the bevel of the needle directed upwards in the line of the vein. The plumber of the syringe was steadily withdrawn at the speed it was taking the vein to fill.
- When sufficient blood had been collected, the tourniquet was released and the participant instructed to open his or her fist.
- The needle was removed and the puncture site immediately pressed with a piece of dry cotton wool.
- The needle was removed from the syringe and the clean dry plain tubes were carefully filled with the required volume of blood. The blood in the EDTA tube was thoroughly mixed.

3.10 LABORATORY PROCEDURE

The blood sample in the Vacuette® serum clot activator was spun in a Thermo Scientific® centrifuge (Thermo Electron Industries SAS, Chateau-Gontier, France) at 3000 rpm for 10 minutes at room temperature after allowing sufficient time for the sample to clot. The serum samples were aliquoted into Eppendorf® tubes and stored

in a Thermo Scientific® refrigerator (Thermo Fisher Scientific Inc., Asheville, United States), calibrated by the Ghana Standards Board (GSB), at -80^{0}C prior to performing the biochemical assay. The Vacuette® K3EDTA sample was used in preparing blood film for malaria parasite examination, performing a complete blood count on the Sysmex® Automated Haematology Analyzer KX-21N (Sysmex Corporation, Kobe, Japan) and stored in a thermometer monitored 2-8^{0}C scientific refrigerator (Thermo Fisher Scientific Inc., Asheville, United States).

3.10.1 Malaria Parasite Examination

3.10.1.1 Blood Film Preparation

- The blood sample in the EDTA tube was mixed gently and the slide for each study participant was labeled with their unique identity number.
- 2 µl of blood was placed on the spot indicated for the thin film and 6 µl in the middle of the larger circle for thick film using a WHO standardized template.
- Thin film: the spreader was placed at 30^0 angle at the small drop blood, allowing the blood to run the width of the spreader and gently spread to a feather finish away from the larger drop.
- Thick film: using the corner of the glass spreader, the blood for the thick smear was spread clockwise into a homogenous diameter circle using the diameter of the template.
- The slides were air-dried and the thin film was fixed for 15 seconds with absolute methanol. This was done carefully to avoid fixing the thick smear in the methanol.

3.10.1.2 Giemsa Staining Technique

The Gurr® Giemsa stain was controlled 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 which was established by the internal quality control in the laboratory.

- A filter paper was fluted and the Giemsa working solution was filtered before use.
- The slides 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. This was done carefully in order not to wash the thick preparation off the slide
- The smears were air-dried before microscopic examination

3.10.1.3 Microscopic Examination of Blood Film

The examination of the blood film for malaria parasites was done by two certified microscopist independently. The thick smear was used to examine each slide so as to detect very mild infection with scanty number of parasites. The thin smear which consisted of a blood film spread in a layer with the thickness progressively decreasing toward a feathery end was used for the malaria parasite species identification by their characteristic features. The slides were examined using the Primo Star (Carl Zeiss MicroImaging GmbH, Germany) microscope with the X 100 objective lens. The parasite density was estimated by counting the number of parasites against a minimum of 200 white blood cells. The results were calculated as follows:

$$\frac{Number\ \ of\ parasites\ \ counted}{Number\ \ of\ WBC\ counted} \times Total\ WBC\ count = Parasites/\mu l\ blood$$

Slides were declared negative when 50 high power fields were scanned without any parasite being seen.

3.10.2 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 an hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and platelets along with data of 19 parameters, as the analysis results (Sysmex Corporation, 2006)

3.10.2.1 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

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. 6 µl 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 is added to prepare 1: 500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, the RBC is haemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, haemoglobin is converted into red coloured 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. 500 µl 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 method.

In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. The absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby calculating HGB (haemoglobin value) (Sysmex Corporation, 2006)

3.10.2.2 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 inputted on the LCD screen and the [enter] button was

pressed to store the pathology number. 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 issued on a thermal paper.

3.10.3 Iron Colorimetric Method

3.10.3.1 Principle

Iron is dissociated from the transferrin-iron in a weak acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions with Nitro-PAPS (2-(5-nitro-2-pyridyl-azo) - 5- (n-propyl-n- (3-sulfopropyl-amino-phenol) give a coloured complex:

$$\text{Tranferrin } (Fe^{3+})_2 \xrightarrow{Ascorbic\ Acid} 2Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} \xrightarrow{Nitro-PAPS} \text{Coloured complex}$$

The intensity of the coloured complex formed is proportional to the iron concentration in the sample.

3.10.3.2 Manual Procedure

Wavelength	**Temperature**	**Cuvette**	**Measurement**
595 nm (590-610 nm)	20-25^{0}C	1 cm light path	Against Reagent Blank

Pipetting into cuvettes was done as follows:			
	Sample	**Standard**	**Reagent Blank**
Iron free water	-	-	0.5 ml
Sample	0.5 ml	-	-
Buffer	2.0 ml	2.0 ml	2.0 ml
Reductant	0.1 ml	0.1 ml	0.1 ml
Standard	-	0.5 ml	-
Mix and read the initial absorbance of the sample and the standard against the reagent blank. Then add:			
Chromogen	0.1 ml	0.1 ml	0.1 ml
Mix and incubate for 5 min at 20-25^{0}C. Read final absorbance against reagent blank. Subtract initial absorbance from final absorbance to give ΔA for sample and standard.			

Calculation:

Concentration = $\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times$ conc. of standard

3.10.4 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 iron body stores.

The biological principle of the AxSYM® Ferritin is based on Microparticle Enzyme Immunoassay (MEIA) technology.

3.10.4.1 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 are covalently bound to the microparticles. The antigen of interest is sandwiched between the bound antibodies and antigen-specific, enzyme-labeled antibodies. Antigen-antibody complexes generate a signal which is detected and quantitated by analysis of fluorescence from the enzyme-substrate interaction.

3.10.4.2 Assay Procedure

The AxSYM® Ferritin file is 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 are confirmed to be acceptable before the analysis is initiated. AxSYM® Ferritin standard calibrators and controls are requested on the system and confirmation is made that assay control values are within concentration ranges specified in the package insert.

The AxSYM® Ferritin reagents and samples are 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 Center 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 generated from the standard curve.

3.10.5 Folate and Vitamin B_{12}

3.10.5.1 Principle

The assay employs a solid-phase enzyme immunoassay to detect folic acid and vitamin B_{12} level in serum using purified human folic acid and vitamin B_{12} antibodies (Human Folic Acid ELISA® kit, Human Vitamin B12 ELISA® kit, Xiamen, China) respectively coated to microtiter plate wells to make a solid phase antibody. The analyte present in the serum combines with the horseradish peroxidase (HRP) labeled antibody in the plate wells to form an antibody-antigen-enzyme-antibody complex. After a complete wash, a colour developing reagent, Tetramethylbenzidine (TMB) substrate solution is added to produce a visible blue colour. This step is catalysed by

HRP enzyme. The reaction is terminated by the addition of sulphuric acid and the intensity of the colour change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of folic acid and vitamin B_{12} in the serum are calculated by comparing their optical density to a standard curve.

3.10.5.2 Method

1. Standard was diluted as follows:

Folic Acid	**Vitamin B_{12}**		
40 µg/l	600 ng/l	Standard 5	150 µl standard+ 150 µl standard diluent
20 µg/l	300 ng/l	Standard 4	150 µl standard 5+ 150 µl standard diluent
10 µg/l	150 ng/l	Standard 3	150 µl standard 4+ 150 µl standard diluent
5 µg/l	75 ng/l	Standard 2	150 µl standard 3+ 150 µl standard diluent
2.5 µg/l	37.5 ng/l	Standard 1	150 µl standard 2+ 150 µl standard diluent

2. Blank wells were set separately and 40 µl of sample diluent was added to testing wells. 10 µl of serum was to wells and mixed gently.
3. The wells were covered with closure plate membrane and incubated for 30 minutes.
4. Reconstitution of wash solution was done with distilled water 20 folds.
5. Plates were uncovered and washed 5 times. Wells were pat to dry.
6. 50 µl of HRP-conjugate reagent was added to each well except the blank well.
7. Incubation of plate was done for 30 minutes and washed 5 times. Wells were pat to dry.
8. 50 µl of chromogen solution A and solution B were added to each well. Wells were evaded from light and incubated for 15 minutes at 37^0C.
9. 50 µl of stop solution was added to each well. The blue colour changed to yellow colour.
10. Absorbance was read spectrophotometrically at 450 nm within 15 minutes after adding stop solution.

CALCULATION: The standard density was taken as the horizontal and the optical density for the vertical. A standard curve was drawn on a graph paper and the optical density of each sample was traced to obtain the corresponding density which was then multiplied by the dilution factor.

3.11 DATA ENTRY AND ANALYSIS

Analysis of the encoded data was done using GraphPad Prism version 5 (San Diego California, USA, www.graphpad.com). Data was expressed as mean ± standard deviation (SD). Categorical variables were displayed as frequencies and percentages obtained by univariate analysis. Groups of anaemia were compared using chi-square and Fisher exact tests of significance at 95% confidence interval. Micronutrient parameters were expressed as geometric mean (95% CI) and groups compared using one-way ANOVA and Bonferroni pairwise analysis. Odds ratio and 95% CI were calculated for the aetiology of anaemia. Relationship between variables was established using Spearman rank correlation coefficient.

Chapter 4

RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Two hundred adult participants whose haemoglobin levels were less than 12.0 g/dl and 13.0 g/dl for females and males respectively seeking treatment at the Agogo Presbyterian Hospital, Agogo, were enrolled into the study. Of the total number of participants in the study, 40 (20%) were males and 160 (80%) were females. The ages of the study participants ranged from 18 to 60 years and the mean age was 36.9 ± 13.7 years. The 21-30 age group had the highest number of study participants, 50, representing 25% of the study population.

Table 4.1 Personal and demographic characteristics of study participants

Personal Characteristic	Total (n=200)	Anaemia			p value
		Mild (n=148)	Moderate (n=40)	Severe (n=12)	
Age	**n(%)**	**n(%)**	**n(%)**	**n(%)**	
≤20	30(15.0)	24(16.2)	4(10.0)	2(16.7)	0.7050
21-30	50(25.0)	36(24.3)	12(30.0)	2(16.7)	
31-40	39(19.5)	27(18.2)	10(25.0)	2(16.7)	
41-50	34(17.0)	25(16.9)	6(15.0)	3(25.0)	
51-60	47(23.5)	36(24.3)	8(20.0)	3(25.0)	
Sex					
Male	40(20)	27(18.2)	9(22.5)	4(33.3)	0.4117
Female	160(80)	121(81.8)	31(77.5)	8(66.7)	
Marital status					
Married	96(48.0)	70(47.3)	22(55)	4(33.3)	0.8189#
Single	74(37.0)	55(37.2)	13(32.5)	6(50.0)	
Divorced	17(8.5)	12(8.1)	4(10.0)	1(8.3)	
Widowed	13(6.5)	11(7.4)	1(2.5)	1(6.3)	
Education					
None	40(20.0)	27(18.2)	9(22.5)	4(33.3)	0.1623#
Primary	78(39.0)	56(37.8)	11(27.5)	6(50.0)	
Secondary	66(33.0)	55(37.2)	9(22.5)	2(16.7)	
Tertiary	16(8.0)	10(6.8)	6(15)	0(0.0)	

Data are given as number (percentage) of persons. Percentages are based on totals within each category and may not total 100 because of rounding. Groups are compared using chi-square tests. # indicates p-value when 'moderate' and 'severe' are combined into one group.

Table 4.1 shows the marital status, education level and the occupation of the study participants. Ninety six (48%) were married and 78(39%) had education to the primary school level.

4.2 LABORATORY RESULTS

4.2.1 Anaemia Categorization of Study Participants

According to the classification developed by the World Health Organization (1968), anaemia was categorized as mild, moderate or severe based on the haemoglobin concentration in the blood. Mild anaemia was defined as haemoglobin concentration

of 10.0-12.9 g/dl (males) and 10.0-11.9 g/dl (female). Moderate and severe anaemia corresponded to haemoglobin concentration of 7.0-9.9 g/dl and less than 7.0 g/dl respectively.

One hundred and forty-eight (74%) individuals had mild anaemia, 40(20%) had moderate anaemia and 12(6%) had severe anaemia. The 21-30 and 51-60 age groups each had 36 study participants with mild anaemia. Twelve (30%) in the 21-30 age group had the highest number of study participants with moderate anaemia. Severe anaemia was more prevalent in the 41-50 and 51-60 age groups, constituting 25% of each age group from Table 4.1. The mean ages of the participants in the mild, moderate and severe anaemia groups were 38.83 ± 4.06 years, 36.05 ± 2.03 years and 36.92 ± 1.15 years respectively.

4.2.2 Red Cell Morphology in Mild, Moderate and Severe Anaemia

Figure 4.1 shows the absolute red blood cell count (A), mean cell volume (B), haematocrit (C), mean corpuscular haemoglobin (D) and mean corpuscular haemoglobin concentration (E) expressed as mean ± SD.

There were significant differences in the red blood cell count of study participants presenting with moderate and severe anaemia compared to mild anaemia ($p<0.001$). Study participants with mild anaemia had significantly higher red blood cell count (4.14 ± 0.51 M/μL) when compared to those with moderate (3.71 ± 0.67 M/μL) and severe (2.30 ± 0.73 M/μL) anaemia.

From figure 4.1, the mean MCV of participants within the mild (82.92 ± 9.34 fL) anaemia category was significantly higher when compared to those in the moderate (78.45 ± 9.56 fL) and severe (76.43 ± 11.01 fL) category ($p<0.001$). The mean haematocrit of moderate (28.42 ± 2.94 %) anaemia patients was significantly lower

than those with mild (34.37 ± 1.97 %) anaemia patients ($p<0.05$). The mean haematocrit in the severe (17.8 ± 4.25 %) anaemia category though lower than those in the mild category was not significant ($p>0.05$).

The mean MCH and MCHC for participants in the moderate (24.79 ± 4.39 pg; 31.38 ± 2.12 g/dl) anaemia category were significantly lower than those in the mild (27.62 ± 2.92 pg; 33.01 ± 1.29 g/dl) anaemia category. There was however no significant difference between the mean MCH and MCHC of participants in the severe (25.75 ± 5.69 pg; 32.07 ± 1.56 g/dl) anaemia category compared to those in the mild anaemia category ($p>0.05$).

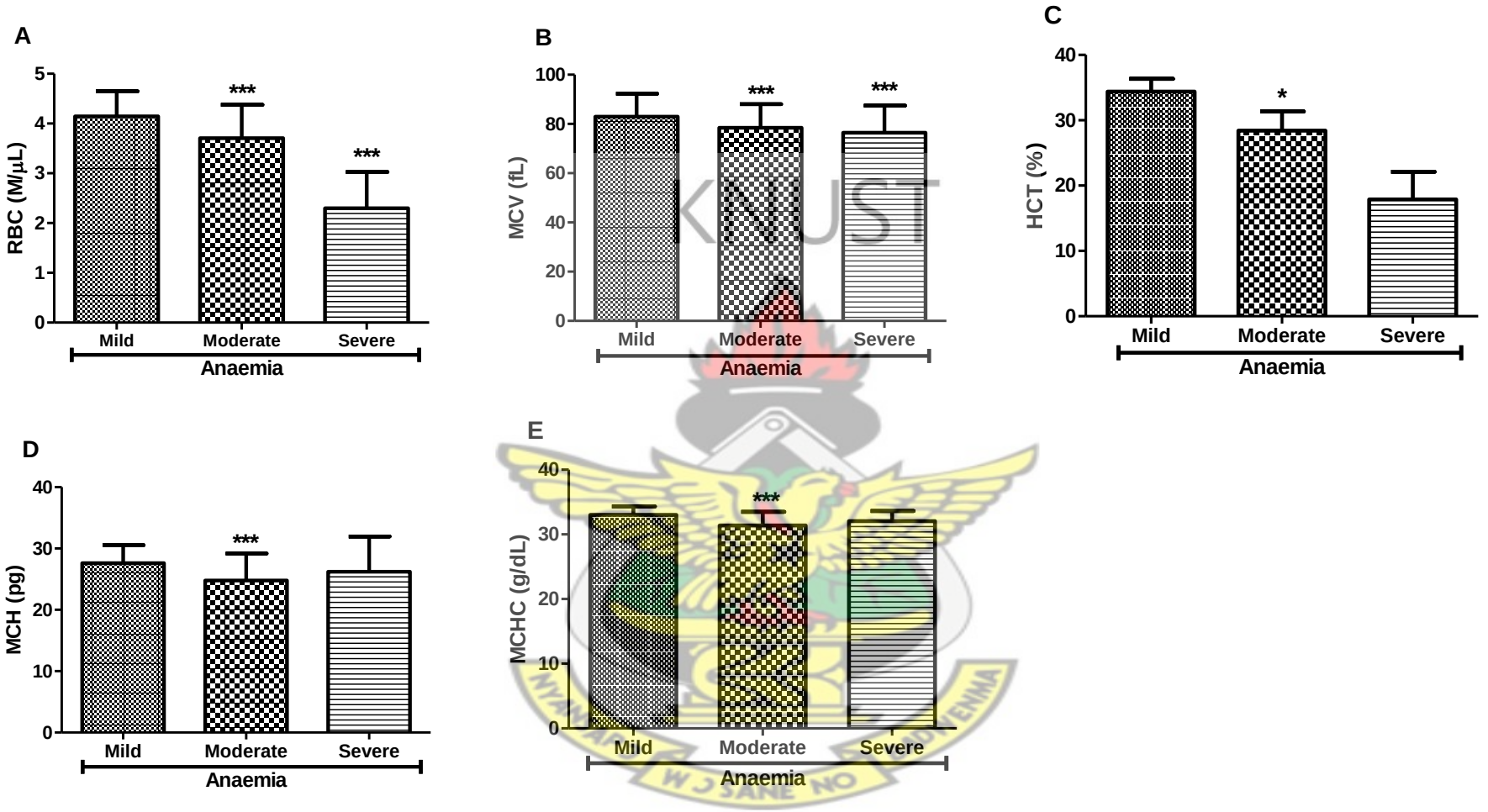

Figure 4.1 RBC(A), MCV(B), HCT(C), MCH(D) and MCHC(E)) in mild, moderate and severe anaemia patients. Results are are expressed as means±SD. Values significantly different from mild anaemia. *=p <0.05, *** =p<0.001.

Table 4.2 Clinical characteristics associated with severity of anaemia.

		Anaemia						
	Total	Mild	Moderate	Severe		OR (95% CI)		
	(N=200)	(N=148)	(N=40)	(N=12)	P Value	Mild vs Moderate	Mild vs Severe	Moderate vs Severe
Clinical characteristics								
Malaria	25(12.5)	10(6.8)	11(27.5)	4(33.3)	**0.0002**	**5.2(2.0-13.5)*** **	**6.9(1.8-26.9)***	1.3(0.3-5.3)
Intestinal Parasite	17(8.5)	13(8.8)	4(10.0)	0(0.0)	0.5364			
Blood In Stool	9(4.5)	5(4.1)	2(5.0)	1(11.1)	0.4308#			
Folate deficiency	51(25.5)	37(25.0)	13(32.5)	1(8.3)	0.2331			
Vitamin B_{12} deficiency	60(30.0)	47(31.7)	8(20.0)	5(41.7)	0.2346	1.9(0.8-4.3)	0.7(0.2-2.2)	0.4(0.1-1.4)
Iron deficiency	69(34.5)	41(27.7)	22(55.0)	6(50.0)	**0.0028**	**3.2(1.6-6.6)****	2.6(0.8-8.6)	0.8(0.2-3.0)
Low ferritin	24(12.0)	8(5.4)	14(35.0)	2(16.7)	**<0.0001**	**15(5.6-42.1)*****	3.5(0.7-18.7)	0.2(0.0-1.2)
Microcytosis	75(37.5)	45(30.4)	23(57.5)	7(58.3)	**0.0022**	**3.1(1.5-6.4)****	3.2(1.0-10.6)	1.0(0.3-3.8)
Macrocytosis	10(5.0)	8(5.4)	2(5.0)	0(0.0)	1.0000#	1.1(0.2-5.3)	1.5(0.1-27.8)	1.6(0.1-36.2)
Hypochromasia	91(45.5)	58(39.2)	26(65.0)	7(58.3)	**0.0095**	**2.9(1.4-6.0)****	2.2(0.7-7.2)	0.8(0.2-2.8)
Normocytic hypochromasia	20(10.0)	16(10.8)	3(7.5)	1(8.3)	0.8094	1.4(0.4-5.4)	1.3(0.2-11.0)	0.9(0.1-9.5)
Microcytic hypochromasia	70(35.0)	41(27.7)	23(57.5)	6(50.0)	**0.0011**	**3.5(1.7-7.3)*****	2.6(0.8-8.6)	0.7(0.2-2.7)

Data are given as number (percentage) of persons. Percentages are based on totals within each category and may not total 100 because of rounding. Anaemia groups are compared using chi-square tests and Fisher exact tests. # indicates p-value when 'moderate' and 'severe' are combined into one group. *p < 0.05,*p < 0.01,**p < 0.001,p < 0.0001. OR-odds ratio.***

4.3 CLINICAL CHARACTERISTICS OF STUDY PARTICIPANTS STRATIFIED BY SEVERITY OF ANAEMIA

4.3.1 Parasitic Infection

From Table 4.2, 25 (12.5%) of the study participants had P. *falciparum* malaria infestation. Ten of these malaria cases were associated with mild anaemia, 11 with moderate anaemia and four with severe anaemia. Malaria infection impacted significant risk ($p<0.001$) on the severity of the anaemia. The odds of malaria infection were five times more in moderate anaemia (OR=5.2; 95%CI=2.0-13.5) compared to mild anaemia and seven times more in severe anaemia (OR=6.9; 95%CI=1.8-26.9) compared to mild anaemia.

Two different intestinal parasites were present in the stool samples of 17 (8.5%) study participants (Table 4.2). Fourteen of these parasites were intestinal flagellates and three were hookworm infestation. Red blood cells were present in 9 of the stool samples presented to the laboratory out of the 200 received. Intestinal parasites did not impact significantly to the severity of the anaemia ($p=0.5364$).

4.3.2 Type of Anaemia

The type of anaemia using red cell morphology was segregated using the MCV (80-95fL) and MCH (27-33pg). Microcytosis, normocytosis and macrocytosis were defined as MCV<80fL, MCV=80-95fL and MCV>95fL respectively. Hypochromasia was also defined as MCH<27pg. Of the 200 study participants, 75(37.5%) had microcytosis, 10(5%) had macrocytosis and 115(57.5%) had normocytosis. Hypochromasia was seen in 45.5% of the study participants. Hypochromasia and microcytosis had a significant association with the severity of the anaemia ($p<0.05$). The odds of hypochromasia (OR=2.9; 95%CI=1.4-6.0) and microcytosis (OR=3.1; 95%CI=1.5-6.4) were three times more in the moderate anaemia group compared to the mild anaemia group. The impact of macrocytosis on the severity of anaemia was not however statistically significant ($p>0.05$).

Normocytic hypochromasia had no significant association with the severity of the anaemia (p=0.8094). Microcytic hypochromasia as expected had a significant association with the severity of the anaemia (p=0.001) and the odds were four times more in the moderate anaemia (OR=3.5; 95%CI=1.7-7.3) compared to mild anaemia.

4.3.3 Micronutrient Parameters Stratified by Severity of Anaemia

In all, 51 (25.5%) study participants had folate deficiency (serum folate <5.0ng/ml). Thirty seven had mild anaemia, 13 had moderate anaemia and only one presented with severe anaemia. Sixty out of the 200 study participants had vitamin B_{12} deficiency with 47, eight and five in the mild, moderate and severe anaemia categories respectively.

Iron deficiency (serum iron <8.9 µmol/L) occurred in 69(34.5%) of the study participants and had a statistically significant association with the severity of the anaemia (p=0.0028). Grouping of these 69 iron deficient participants by the severity of their anaemia showed that 41 had mild anaemia, 22 presented with moderate anaemia and half of the participants who presented with severe anaemia (12) were iron deficient. The odds of iron deficiency in the moderate anaemia group was three times that of the mild anaemia group (OR=3.2; 95%CI=1.6-6.6). Serum ferritin levels less than 12 ng/ml referred to depleted iron stores as defined in the WHO report on serum ferritin concentration for the assessment of iron stores and iron deficiency in populations (WHO, 2011). By the WHO definition, 24(12%) participants had depleted iron stores. Eight of them had mild anaemia, 14 had moderate anaemia and 2 had severe anaemia. The depleted iron stores had a significant association with the severity of the anaemia (p<0.0001). The odds of depleted iron stores were 15 times more in the moderate anaemia group compared to the mild anaemia group (OR=15; 95%CI=5.6-42.1).

Vitamin B_{12} deficiency, folate deficiency and intestinal parasite infection did not have any significant association with the severity of the anaemia ($p>0.05$).

Table 4.3 Geometric means of micronutrient parameters of study participants

		Anaemia					
	Total	Mild (A)	Moderate (B)	Severe (C)	P value		
Parameter	**(n=200)**	**(n=148)**	**(n=40)**	**(n=12)**	**All groups**	**AvsB**	**A vs C**	**B vs C**
Ferritin (ng/ml)	79.9(65.2-98.0)	91.8(74.4-113.2)	43.8(24.5-78.2)	108.0(35.2-331.2)	**<0.0001**	**<0.0001**	0.1344	**<0.0001**
Vitamin B_{12} (ng/L)	236.1(211.8-263.1)	222.0(195.1-252.7)	298.1(233.0-358.8)	256.1(155.6-421.3)	0.1546	0.0582	0.5535	0.6010
Folate (ng/ml)	8.9(7.7-10.2)	9.3(7.9-11.0)	7.2(5.3-9.9)	9.5(5.7-15.8)	0.3588	0.1621	0.3973	0.9667
Iron (µmol/L)	11.8(10.7-13.0)	13.6(12.3-14.9)	7.7(5.8-10.1)	8.7(4.7-16.0)	**<0.0001**	**0.0018**	0.2334	0.5968

Data are presented as geometric mean (95% CI). Groups are compared using one-way ANOVA and Bonferroni pairwise analysis

Table 4.4 Spearman rank correlation coefficients of age and laboratory features of subjects.

Parameter	HB	Age	HCT	MCV	MCH	MCHC	Iron	Ferritin	Vit B12	Folate
RBC	0.1139	-0.1313	**-0.5854*** **	**-0.4928*** **	**-0.5306*** **	-0.2667	0.0062	**-0.3341*** **	0.0113	0.1141
HB		0.1935	0.0054	0.0199	-0.0565	**-0.1428***	0.0067	0.0144	-0.0324	0.0137
Age			0.0096	**0.1775***	0.1261	-0.0034	-0.0351	**0.2917*** **	-0.0194	-0.0224
HCT				**0.1841** **	0.1343	0.0224	0.2428	0.0403	0.0054	0.0752
MCV					**0.8807*** **	**0.3421*** **	**0.1928*** **	**0.3837*** **	0.0199	**-0.1426***
MCH						**0.7120*** **	**0.2141** **	**0.2931*** **	-0.0437	-0.106
MCHC							**0.2747*** **	**0.2768*** **	**-0.1428***	0.103
Iron								0.0533	0.0067	**0.3248*** **
Ferritin									0.0144	-0.0296
Vitamin B_{12}										0.1274

**** Correlation is significant at 0.05 level (2-tailed). ** Correlation is significant at 0.01 level(2-tailed). *** Correlation is significant at 0.001 level (2-tailed).***

4.4 GEOMETRIC MEANS OF MICRONUTRIENT LEVELS STRATIFIED ACCORDING TO SEVERITY OF ANAEMIA

Table 4.3 shows data of the geometric means and 95% confidence interval of iron, vitamin B_{12}, folate and ferritin of all 200 study participants. The table also shows the association between the micronutrient deficiencies and the severity of the anaemia. The data in Table 4.3 were log transformed to normal, and the geometric means and 95% confidence calculated for each group of anaemia.

As expected, the geometric mean of ferritin in the mild anaemia group (43.8 ng/ml) was lower compared to the moderate anaemia group (91.8 ng/ml). In the severe anaemia group however, the geometric mean of ferritin was highest (108.0 ng/ml). The trend analysis showed that ferritin levels were associated with the severity of the anaemia ($p<0.0001$). Similarly, the severity of the anaemia occasioned by the level of serum iron was significantly increased ($p<0.0001$). The geometric means and 95% CI for the mild, moderate and severe anaemia were 13.6 µmol/L (95% CI=12.3-14.9), 7.7 µmol/L (95% CI=5.8-10.1) and 8.7 µmol/L (95% CI=4.7-16.0) respectively. The geometric mean of iron in moderate anaemia category was significantly lower than in the mild anaemia category ($p=0.0018$).

4.5 CORRELATION COEFFICIENT OF CLINICAL VARIABLES

Table 4.4 shows the Spearman correlation coefficients of age and other laboratory results of study participants. RBC was negatively correlated with HCT ($r=-0.5854$; $p<0.001$), MCV ($r=-0.4928$; $p<0.001$), MCH ($r=-0.5306$; $p<0.001$) and ferritin (**(**$r=-0.3341$; $p<0.001$). MCV was however positively correlated with MCH ($r=0.8807$; $p<0.001$), MCHC ($r=0.3421$; $p<0.001$), iron ($r=0.1928$; $p<0.001$) and ferritin ($r=0.3837$; $p<0.001$) but negatively correlated with folate ($r=-0.1426$; $p<0.05$). Iron was positively correlated with folate ($r=0.3248$; $p<0.001$).

Chapter 5

DISCUSSION

5.1 INTRODUCTION

Globally, anaemia persists as a major health problem. The World Health Organization estimates that two billion people suffer from anaemia (Underwood, 1996). According to epidemiological data collected in developing countries by the World Health Organization, about half of women and young children are anaemic (DeMaeyer *et al.*, 1989; Scrimshaw, 1984). In the United States and Europe however, 7-12 % of women and children are anaemic (Dallman *et al.*, 1984; Hallberg, 1981).

Anaemia remains a major concern because of its association with impaired mental and physical development. Morbidity and mortality risk also increase in individuals with anaemia (Sharmanov, 1998). Nutritional deficiency, primarily dietary iron deficiency remains the major cause of anaemia; although other factors including haemorrhage, infection, genetic disorders and chronic diseases contribute to the burden of anaemia (Hercberg and Galan, 1992; International Nutritional Anemia Consultative Group (INACG), 1979; International Nutritional Anemia Consultative Group (INACG), 1989; Yip and Dallman, 1988). Anaemia associated with infectious diseases increase the risk of morbidity because several immune mechanisms are affected (Scrimshaw, 1990).

The causes of anaemia are multifactorial. Micronutrient deficiencies of iron, folate and vitamin B_{12} are important causes of anaemia (Provan and weatherall, 2000). Iron deficiency which is the most important cause of nutritional anaemia accounts for almost one million deaths annually (Cook *et al.*, 1994; Hercberg and Galan, 1992). Parasitic infections such as malaria and hookworm have been shown to be associated with anaemia. The chronic intestinal blood loss associated with hookworm infestation and the

induced iron deficiency causes anaemia. Studies have established the strong relation between chronic anaemia and malaria in malaria endemic areas like Sub-Saharan Africa and Papua (Guyatt, 2000; Hopkins *et al.*, 1997; Premji *et al.*, 1995; Verhoef *et al.*, 2002)

Despite this global view, little has been done in developing countries by way of identifying the aetiology of anaemia and the contribution of each factor to the burden.

5.2 DEMOGRAPHY OF ANAEMIA

Our data showed no association ($p>0.05$) between age, gender, marital status and education level with anaemia. From our study data, 80% of the study population were female and 20% were male confirming earlier reports of women being more susceptible to anaemia than men (Stoltzfus *et al.*, 1998). In a study conducted at the Ayub Teaching Hospital, Abbottabad, Pakistan, 60.29% of the anaemic population were females with 39.71% being males (Idris, 2005). The Abbottabad study differed from this study but confirmed that there were more women with anaemia than men. In another study conducted among University of Peshewar students, women were 2 to 3 times more likely to present with anaemia than men (Khan *et al.*, 2010b). This study results were also consistent with other studies (Hashmi *et al.*, 1973; Karim *et al.*, 1994; Paracha *et al.*, 1997). The greater susceptibility of women to anaemia can be attributed to the increased requirement of iron due to their monthly blood loss from menstruation and a lower iron intake from food (Cheong *et al.*, 1991; Hallberg *et al.*, 1995).

From the data 48.5% of the study population fell between the 21-30 and 51-60 year groups. A similar study in Ayub Teaching Hospital showed that 40.1% of the anaemic patients fell within these two age groups (Idris, 2005). The 21-30 age group has the physically active individuals undergoing rapid growth and have increased nutritional requirement (Centers for Disease Control and Prevention, 1998; Wharton, 1999). The

older adult group (51-60) had an increased incidence of chronic illness and poor nutritional status (Choi *et al.*, 2004; Smith, 2000), and may account for why they have a sizeable percentage. Aging has also been shown to physiologically decrease the haemopoietic activity of the marrow (Lipschitz *et al.*, 1981).

This study revealed that 20% of the study population had no formal education, 39%, 33% and 8% had primary, secondary and tertiary education respectively. There was no statistically significant association between the levels of education and the severity of anaemia. The results of these study are corroborated by a study of socio-demographic factors of anaemia in South-western Nigeria which also found no association between educational level and anaemia (Owolabi *et al.*, 2012). Okwu and Ukoha (2008), and Haniff *et al* (2007) similarly reported results comparable to this study. The findings of this study were however at variance with the results of a study conducted in a primary health centre in Rivers state, Nigeria (Ndukwu and Dienye, 2012), which found a statistically significant association between the severity of anaemia and educational status.

5.3 ANAEMIA CLASSIFICATION

In this study, 74% had mild anaemia (female Hb=10.0-11.9g/dl; male Hb=10.0-12.9), 20% had moderate anaemia and 6% had severe. In a study of the socio-demographic factors in anaemia in pregnancy in South-western Nigeria, Owolabi and co-workers reported 80.8%, 16.7% and 2.5% for mild, moderate and severe anaemia respectively (Owolabi *et al.*, 2012) A report of a study among female students attending the University of Sharjay, UAE put mild anaemia at 88.4%, moderate anaemia at 5.8% and severe anaemia at 4.3% (Sultan, 2007). A study conducted by Mishra and co-workers among 598 females in the Barara village of the Ambala district, India, found 75.5% of

the anaemic subjects having mild anaemia, 16.7% having moderate anaemia and 7.8% having severe anaemia (Mishra *et al.*, 2012). These results concur with this present study. Panigrahi and co-workers in their study in Bhubaneswar, Orissa among women in their reproductive age found that of the 146 subjects who were anaemic, 65%, 33% and 2% had mild, moderate and severe anaemia respectively confirming mild anaemia as the most prevalent type, similar to the results of this study (Panigrahi and Sahoo, 2011). Similarly, Biradar *et al* (2012) reported 84%, 15% and 1% for mild, moderate and severe anaemia respectively in a one year cross-sectional study they conducted among adolescent girls in Vantamuri Primary Health Care (PHC).

In identifying the type of anaemia, this study graded the anaemia based on the MCV and MCH values of each study participant. Our results indicated that 37.5% of the anaemia cases were microcytic which is attributable to iron deficiency and malaria infection, 5% macrocytic resulting from vitamin B_{12} or folate deficiency and 45% hypochromasia. Normocytic hypochromasia was 10% of the total anaemia cases and 35% were microcytic hypochromic. There was no study participant who presented with macrocytic hypochromasia. The study in the University of Sharjah among female students reported a higher prevalence rate of microcytosis (59%), hypochromasia (59%) and microcytic hypochromasia (41%) compared to this study except for macrocytosis (4%) among the anaemic population (Sultan, 2007).

The low prevalence of macrocytic anaemia has been reported in other studies (Mohamed and Monir Madkour, 1984; Mohammed and Al-Karawi, 1986). In the north-western Saudi province, 3.4% of the children screened reported with macrocytosis, similar to our current study (El-Hazmi and Warsy, 1999).

5.4 IRON DEFICIENCY AND ANAEMIA

The association between iron deficiency and anaemia, and its indication as the most significant contributor to the public health importance of anaemia has been well documented (Agyei-Frempong *et al.*, 2001; Bagchi, 2004; Cook *et al.*, 1994; Hercberg *et al.*, 1992; WHO, 2001; World Health Organization, 1968; World Health Organization and Centre for Disease Control and Prevention, 2007). There is however paucity of data on the prevalence of iron deficiency among adult anaemic patients in Agogo, Ghana. This study found that of the 200 anaemic patients screened at the Agogo Presbyterian Hospital, 34.5% were iron deficient (serum iron<8.9µmol/L). Measurement of the serum ferritin level in this study showed that 12.0% of the anaemic patients had depleted iron stores. Studies have reported the rise in concentration of ferritin in the first two months of life and its fall in late infancy. It however begins to rise again at about one year of age and continues to adulthood (Domellof *et al.*, 2002; Gibson, 2005). The paucity of data on the aetiology of anaemia in Ghana compelled a comparison of this data with studies done in other parts of the world. A prevalence rate of 85.7%, more than twice of what this study found was reported among anaemic patients in Phan Tien village, southern Vietnam (Le Hung *et al.*, 2005). Idris (2005) also reported 68% as the prevalence rate of iron deficiency. Umeta and co-workers reported a prevalence rate of 17% (Umeta *et al.*, 2008) compared to the 34.5% reported by this study. A prevalence rate of 16.5% was reported among adolescent girls in New Halfa, Eastern Sudan (Abdelrahim *et al.*, 2009). In a study to evaluate the iron status of adults in the capital area of Finland (Lahti-Koski *et al.*, 2003), 16% of the women were observed to have depleted iron stores using their serum ferritin levels as reference, a little higher than the results of this study. The economic cost of iron deficiency is very high especially in developing countries. The impaired work performance and limitation placed on intellect development by iron

deficiency could constrain social and economic development (Edgerton *et al.*, 1979; Pollitt *et al.*, 1986). Iron deficiency has been estimated to decrease work productivity in adults by 5-17% depending on the nature of work (Horton, 1999). Micronutrient deficiency has been suggested by studies to cost India US$2.5 billion annually (Alderman, 2005). Bangladesh also reported an annual per capita productivity loss of 1.9% of their Gross Domestic Product (GDP) to the economic consequence iron deficiency anaemia (Ross and Horton, 1998).

The results of this study which indicated iron deficiency as the most significant contributor to anaemia may possibly be attributed to insufficient dietary intake of iron or low dietary bioavailability of iron, malaria and the loss of blood through menstruation.

5.5 FOLATE, VITAMIN B_{12} AND ANAEMIA

The diet of many in Sub-Saharan Africa is limited to cereals, lacking in green leafy vegetables and animal source food (VanderJagt *et al.*, 2000) which are rich in folate and vitamin B_{12}. Deficiency of any of these vitamins will affect red blood cell maturation and compromise immune function (Gross *et al.*, 1975; Osifo *et al.*, 1984). The elevation of serum homocysteine concentration associated with folate and vitamin B_{12} deficiencies increase the risk of cardiovascular disease and neural tube defect (Kalra, 2004; Mangoni *et al.*, 2002; Wald *et al.*, 2002).

5.5.1 Vitamin B_{12} and Anaemia

Vitamin B_{12} deficiency was the second highest contributor to the aetiology of anaemia in Agogo with 30% of the study participants expressing low serum vitamin B_{12} concentration. The possible reason for this level of deficiency could be assigned to inadequate dietary intake especially as this study was focused on young adults without any established chronic condition. Several Jordanian studies have published prevalence

rates of between 16 to 50% (Abu-Samak *et al.*, 2008; Barghouti *et al.*, 2009). In a study in India where there is a low intake of animal source food, 47% of the adults in the study reported with low serum vitamin B_{12} concentration (Refsum *et al.*, 2001a). In Nigeria, 9% of 162 girls screened had low vitamin B_{12} concentration (VanderJagt *et al.*, 2000) in contrast to a few children who reported with low vitamin B_{12} serum concentration (Abrams *et al.*, 2003) in Botswana. Yajnik and co-workers reported vitamin B_{12} deficiency of 67% among healthy Indian men (Yajnik *et al.*, 2006), a prevalence rate much higher than the results of this study. Reports of surveys done in Latin America, estimates that approximately 40% of children and adults have deficient or marginal vitamin B_{12} status (Allen, 2004). Low serum vitamin B_{12} concentration has been well documented to be associated with anaemia and altered neurological function (Lindenbaum *et al.*, 1988; Rosenthal and Goodwin, 1985). Dietary supplementation with meat has greatly improved cognitive performance in Kenyan school children (Whaley *et al.*, 2003) whereas fortfication of food with vitamin B_{12} in the Netherlands has proved very useful. In an earlier study undertaken in the Netherlands, participants aged 50-65 years were made to consume bread fortified with 9.6 µg of vitamin B_{12} for 12 weeks. The results indicated that the proportion of participants with low serum vitamin B_{12} reduced from 8% to a remarkable 0% (Winkels *et al.*, 2008).

5.5.2 Folate and Anaemia

In this study, low serum folate concentration was observed in 25.5% of the study participants. Several studies have provided scientific evidence of the importance of folic acid supplementation for the prevention of neural tube defects in women of child bearing age (Berry *et al.*, 1999; Vergel *et al.*, 2005; Wilson *et al.*, 2007). VanderJagt and co-workers reported a much lower prevalence rate of 2.4% among female adolescents in Northern Nigeria (VanderJagt *et al.*, 2000). A study of anaemic pregnant females in a

tertiary care centre at Rawalpindi reported a folate deficiency prevalence rate of 20%, a little lower than what this study established (Khan *et al.*, 2010a). Folate deficiency of 11.98% was reported among anaemic pregnant women (Marti-Carvajal *et al.*, 2002) whereas in New Halfa, Eastern Sudan, 69% of adolescent girls reported with folate deficiency (Abdelrahim *et al.*, 2009). An equally high prevalence rate was reported by Thoradeniya *et al* (2006) in Colombo, Sri Lanka. In their study among urban adolescent girls and women of child bearing age, they reported a prevalence rate of 43.9% for folate deficiency. In the United States, there was an introduction of mandatory folic acid fortification of enriched cereal grains which took effect in 1998. Results of the first report indicated a marked increase of folic acid status with a reduction of folic acid deficiency from 22.0% to 1.7% (Choumenkovitch *et al.*, 2001; Jacques *et al.*, 1999).

5.6 PARASITES AND ANAEMIA

In Sub-Saharan Africa, malaria and intestinal parasite infections are endemic in both the rural and urban areas (De Silva *et al.*, 2003; Snow *et al.*, 2005). The association between malaria and anaemia has been well documented (Adam *et al.*, 2005; Huddle *et al.*, 1999; Kagu *et al.*, 2007; Mayor *et al.*, 2007; Muhangi *et al.*, 2007; Ouma *et al.*, 2007; Rogerson *et al.*, 2000; Tarimo, 2007). The anaemia from malaria infection is primarily due to increased red blood destruction. The results of this study showed that 12.5% of the anaemic adult patients attending the OPD clinic had malaria confirmed by a positive Giemsa stained blood slide. In Buea, Cameroon, a malaria prevalence rate of 21.88% was reported among adults enrolled in the study (Takem *et al.*, 2010). Mayor *et al* (2007) reported that 14.4% of their study participants reported with malaria by microscopy. All the parasitaemia reported was due to P. *falciparum* similar to what this study reported.

In Sub-Saharan Africa were malaria is endemic, the association between chronic anaemia and malaria is so strong that anaemia is often taken as a proxy indicator of the malaria control programmmes (Le Hung *et al.*, 2005). Takem and co-workers demonstrated that by clearing parasite of patients, the burden of anaemia could be reduced by more than one third (Takem *et al.*, 2010). Although this study established a significant association between malaria and anaemia, some studies have reported no association (Le Hung *et al.*, 2005; Stoltzfus *et al.*, 2000; Stoltzfus *et al.*, 1997). In contrast to this study, Mato (1998) found no strong association between malaria and anaemia in a study he conducted among Yanomami Amerindian population from the Southern Venezuelan Amazon.

In this study, 8.5% presented with intestinal parasite infection. Only three had hookworm infestation. This study did not detect any association between intestinal parasite infection and anaemia in Agogo similar to what Takem *et al* (2010) reported. There was no hookworm infection reported in a study conducted among adults in Kassala, Eastern Sudan (Abdallah *et al.*, 2012) but this study reported three hookworm infections among the study participants. A study in Ghana conducted among pregnant women by Baidoo *et al* (2010) reported an intestinal helminth infection rate of 17.6%, about twice what our study reported. Husain and his colleagues reported that there was twice an increased risk of developing anaemia with a hookworm infestation and the risk of developing anaemia was also increased with a *Gardia lamblia* infection (Husain and Ali, 1997). The massive campaign in the electronic media on the use of antihelminthics may account for the low incidence of intestinal parasite infection.

Chapter 6

CONCLUSION

The social and economic cost of anaemia remains very high in developing countries with Ghana not being an exception. In developing countries the lack of adequate health facilities and the difficulty in accessing these facilities could further aggravate the severity of anaemia. Every effort at identifying the aetiology of anaemia needs to be vigorously accelerated by governmental agencies to ensure the overall well being of individuals in society is safeguarded. This study has identified that the most significant contributors to the burden of anaemia in Agogo are iron deficiency, vitamin B_{12} deficiency, folate deficiency and malaria. The study identified that gender and level of education did not play any significant role in the development of anaemia although there were more women than men as expected. This study has also succeeded in the advocacy for investigating the cause of anaemia before blindly treating patients with haematinics. This will help in avoiding situations of nutritional overload and deficiencies which could lead to irreversible neurological damage as in the case of the masking of vitamin B_{12} deficiency. In Agogo, a tropical malaria endemic area, there has been a concerted effort in introducing insecticide treated nets over the last one year by the Ghana Health Service supported by the Global Fund. This may have influenced the lower prevalence rate of malaria we recorded in our study compared to other studies in Sub-Saharan Africa. Compliance to the usage of these nets and its effect at ameliorating the burden of anaemia may become an area for scientific evaluation. The period of this study covered the dry climatic season and may also account for the lower prevalence rate recorded in this study.

Fortification of foods with iron, folate and vitamin B_{12} could significantly reduce the disease burden of anaemia in Agogo as was done in the 1990's in the United States.

6.1 LIMITATION

The paucity of published data on the prevalence of anaemia, a cut-off point for determining who is anaemic and the general lack of reference intervals specific to the local condition in Agogo compelled the comparison the results of this study with data from countries whose socio-demographic variables could vary from this local setting.

This study could not assess the dietary intake of the study participants and their possible impact on nutritional status.

The selection of the study participants was purely based on their clinical history to eliminate those with chronic conditions. There may therefore be other minor factors other what this study found contributing to the burden of anaemia.

Other micronutrients such as vitamin A and zinc were not assessed in this study. Their influence on the burden of anaemia in this setting can therefore become subject for further scientific investigation.

6.2 RECOMMENDATION

To allow for the results of this study to influence the policy direction of the Ministry of Health in Ghana, we recommend that a similar project be conducted on a larger scale to give a national picture of the aetiology of anaemia. Such a study will evaluate current strategies in addressing the public health problem of anaemia.

A study that takes in account the socioeconomic background of the study participants could help to explain the trends detected in this study and the type of intervention that will be useful in the treatment and prevention of anaemia.

The current peri-urban status of Agogo (a rural community only two decades ago) and the rapidly increasing sedentary lifestyle of the inhabitants could become a subject of investigation when anaemia is placed in the context of this change.

Given the high prevalence of folate and vitamin B_{12} deficiencies, and the associated compensatory increase in homocysteine level, a subsequent study on anaemia could assess homocysteine levels and the associated cardiovascular risk.

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