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NUTRITIONAL ANAEMIA IN PREGNANT ADOLESCENT GIRLS IN SELECTED COMMUNITIES IN THE GREATER ACCRA REGION

BY

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DECLARATION

I hereby declare that this thesis is the result of research undertaken by Freda Dzifa Hogrey towards the award of the Doctor of Philosophy in Dietetics in the Department of Nutrition and Dietetics, University of Ghana. This was done under the supervision of Prof. Edwin K. Wiredu, Prof. George Asare and Dr. Matilda Asante.

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ABSTRACT

Background

Nutritional anaemia in pregnancy still remains a problem of public health concern particularly in developing countries such as Ghana. The period of adolescence and pregnancy are both critical periods that place the individual at risk of developing nutrient deficiencies because of the rapid and increased demands in nutrients. Failure to meet the nutritional demands can affect the growth of the adolescent mother and that of the developing baby. Literature gaps exist in the micronutrient (iron, folate and vitamin B_{12}) status, quality of diets, as well as dietary practices, myths and misconceptions of nutritional anaemia among pregnant adolescent girls in Ghana.

Aim

To determine the prevalence of nutritional anaemia, quality of the diet and to assess the myths and misconceptions concerning nutrition of pregnant adolescent girls in three communities in the Greater Accra Region.

Methods

A mixed method design (cross sectional and qualitative focus group discussions) was employed. The cross sectional component recruited pregnant adolescent girls (N=265) between the ages of 15-19 years from 3 hospitals in the Greater Accra Region. Dietary intake data were obtained based on a 3-day 24 hour dietary recall and a food frequency questionnaire. Minimum Dietary Diversity for women of reproductive ages (MDD-W) was determined based on FAO guidelines (FAO & FHI360, 2016). . Serum concentrations of iron, folate and vitamin B_{12} were determined using VITROS Chemistry Fe Slides method, ID-Vit® ELISA Folate and ID-Vit® ELISA Vitamin B_{12} test kits respectively. Serum TIBC and ferritin were also determined with VITROS Chemistry products Total Iron Binding Capacity (TIBC) kit and AccuBind

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ELISA microwells respectively. Four (4) focus group discussions were held to obtain information on nutrition knowledge, myths and misconceptions and sources of information on nutritional anaemia. Differences between means were tested using independent t-tests and ANOVA. Z-tests were used to test for differences between proportions. Logistic regressions were employed in determining the odds of being anaemic and the odds of obtaining good dietary diversity. Thematic approach of analysis was employed in analysing the qualitative data.

Results

Two hundred and six five (265) and twenty four (24) pregnant adolescents were involved in the cross sectional and qualitative studies respectively. Their mean age was 17.88 (SD = 1.08) years for the cross sectional study and 17.44 (SD = 1.29) years for the qualitative component. The prevalence of anaemia was 71.6%. Deficiencies in iron, folate and vitamin B_{12} were 61.4%, 50.4% and 11.7% respectively. Mean nutrient intakes of iron, folate and vitamin B_{12} were 9.10 (SD = 4.26; CI 8.54 -9.66) mg, 110.90 (SD = 56.36; CI 103.46-118.34) µg and 1.61 (SD = 1.26; CI 1.44-1.78) µg respectively. Proportion of girls with adequate intakes of iron (1.3%); and vitamin B_{12} (22.5%)) were significantly low ($p < 0.001$). None of the pregnant girls had adequate intake of folate. Prevalence of good MDD-W was 24.6% and average MDD-W score was 3.85 (SD = 0.95). The significant predictor of MDD-W was dietary folate ($p = 0.010$). The significant predictors of anaemia were dietary vitamin B_{12} ($p = 0.022$) and dietary iron ($p = 0.039$). Turkey berries (*Solanum torvum*), milk and malt drinks were regarded as foods that promote blood formation. The pregnant girls mentioned inadequate food intakes, drinking a lot of water, paradoxically not drinking a lot of water and exposure to sunlight as the main causes of anaemia.

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Conclusion

Deficiencies of iron, folate and vitamin B_{12} are very prevalent among the pregnant adolescent girls. Dietary diversity was generally poor and therefore much education is needed to encourage diversity in dietary intake. Knowledge on nutritional anaemia is sub-optimal and therefore necessitates targeted nutrition education to prevent pregnancy-related anaemia in the adolescent girl.

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DEDICATION

I dedicate this work to my Father in Heaven for taking me through this long journey. His grace has been my source of strength. To my wonderful husband, Rev. Francis Intiful, my lovely daughters Francyne and Freda and my son Francis Jnr. for enduring the many times I had to neglect them because of this work.

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LIST OF ABBREVIATIONS

ATP- Adenosine Trisphophate

CoA- Coenzyme- A

DD- Dietary Diversity

DDS-Dietary Diversity Score

DNA- Deoxyribonucleic Acid

FAO- Food and Agricultural Organization

GSS- Ghana Statistical Service

H_4F- Tetrahydrofolate

MDD-W- Minimum Dietary Diversity for women of reproductive age

MDG-Millennium Development Goals

MeH_4F-5-Methyl tetrahydrofolate

MOH- Ministry of Health

RDA- Recommended Daily Allowance

SD- Standard Deviation

SDG- Sustainable Development Goals

THF- N-methyly tetrahydrofolate

UN- United Nations

UNICEF- United Nations Children's Fund

WDD- Women's Dietary Diversity

WHO- World Health Organization

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CHAPTER 1

1.0 INTRODUCTION

1.1 Introduction

Anaemia is a global health problem affecting about one fourth of the world's population, mostly children and women of reproductive ages (Balarajan, Ramakrishnan, Özaltin, Shankar, & Subramanian, 2011; Osungbade & Oladunjoye, 2012). The World Health Organization (WHO) estimates that about 38% of all pregnant women are anaemic (WHO, 2015). Anaemia in pregnancy has serious consequences that ultimately increase the risk of both maternal and neonatal mortality (Balarajan et al., 2011). Every year, more than 115,000 maternal deaths and 591,000 perinatal deaths are attributed to anaemia globally, with Africa and Asia being the most affected continents (Ezzati, Lopez, Rodgers, & Murray, 2004).

There are several causes of anaemia. The commonest causes in developing countries, like Ghana, originate from nutritional deficiencies and infections (Osungbade & Oladunjoye, 2012). Nutritional anaemias in pregnancy are associated with deficiencies in iron, folate and/or vitamin B_{12}. This condition arises when the amount of these nutrients absorbed by the gastrointestinal tract or are taken in the diet are insufficient to meet normal physiological requirements (Stoltzfuz, 2001). The physiologic demands in pregnancy and adolescence increase the risk of` developing these deficiencies when dietary intake is unable to meet the additional demands placed on the body by the developing foetus and the adolescent growth spurt respectively. Deficiencies may also arise when there is increased loss of blood by the body resulting in nutrient loss. Iron, folate, and vitamin B_{12} are micronutrients that are very crucial in the development of the foetus in pregnancy (Black, 2008). Iron deficiency can result in anaemia in which the red blood cells become microcytic and hypochromic (DeLoughery, 2014;

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Urrechaga, Hoffmann, Izquierdo, & Escanero, 2015). Folate and vitamin B_{12} deficiencies usually present with megaloblastic anaemia (Aslinia, Mazza, & Yale, 2006).

One of the earliest adaptations of pregnancy is the expansion of blood plasma volume (Weissgerber & Wolfe, 2006). Nutritional anaemia in pregnancy is largely attributed to this physiological expansion of plasma volume which becomes disproportionate to red blood cell mass (Thomas & Thomas, 2002). Alongside this plasma volume expansion, short term inter-pregnancy interval can also compound the problem by depleting these micronutrients (Van Eijsden, Smits, Van der Wal, & Bonsel, 2008).

The deficiency of these nutrients cannot be ignored because of their involvement in major physiologic processes in pregnancy. Previous studies have linked low plasma vitamin B_{12} in pregnancy to recurrent abortions, neural tube defects especially spina bifida, anorexia, irritability, failure to thrive and apathy (Dror & Allen, 2008; Groenen et al., 2004; Molloy, Kirke, Brody, Scott, & Mills, 2008). In pregnancy, between 0.1 - 0.2 ug/day of vitamin B_{12} is transferred to the foetus during the second and third trimesters (Davis, Hanson, Isaacs, & Wright, 2012). The basic biochemical role of the vitamin is in one carbon unit metabolism. It serves as a cofactor for the enzymes, methyl malonyl CoA mutase and methionine synthase (Wu, Imhoff-Kunsch, & Girard, 2012). Malonyl CoA mutase is needed for the pathways involving propionyl-CoA, example being the metabolism of odd-chain fatty acids and branched-chain amino acids. Methionine synthase, on the other hand is responsible for catalysing the methyl transfer from 5-methyltetrahydrofolate (MeH_4F) to homocysteine, resulting in the formation of tetrahydrofolate (H_4F) and methionine (Banerjee & Matthews, 1990). The role of Methionine synthase in both folate and methionine metabolism is indispensable. Reduced levels of this enzyme results in megaloblastic or macrocytic anaemia (anaemia with bigger than normal red blood cells). Furthermore, an anomaly in the synthesis of

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methionine synthase can result in high levels of homocysteine in the blood, which is believed to be a risk factor for developing cardiovascular diseases (Ganguly & Alam, 2015).

Deficiency in folate has also been associated with preterm delivery, low birth weight and foetal growth retardation (Fekete et al., 2010; Fekete et al., 2012). Folate is basically responsible for biosynthesis of DNA and also involved in methylation cycles. When there are reduced levels of folate, there is reduction in DNA biosynthesis (FAO/WHO, 2005). This results in reduction in cell division due to arrest of the cell cycle at the G2 stage leading to continued cell growth without division and macrocytosis. The most affected cells are those that are rapidly dividing such as red blood cell precursors in the bone marrow. Failure for the cells to divide rapidly, results in reduced number of red blood cells and then anaemia. General reduction in DNA synthesis results in increased vulnerability to infections, decreased coagulation of blood and secondary malabsorption.

The primary role of iron in the body involves acting as oxygen carrier to tissues from the lungs via red blood cell haemoglobin, acting as a medium of transport of electrons within cells, as part of iron containing enzymes (cytochromes) with the basic function of transfer of energy within cells, specifically the mitochondria. The cytochrome (P450)'s main function includes the synthesis of hormones, bile acids and detoxification of foreign substances in the liver. It also signals control in some neurotransmitters such as dopamine and serotonin system in the brain (FAO/WHO, 2005).

The body requires iron for the synthesis of haemoglobin (protein responsible for carrying oxygen in the blood). Consequently, in situations where the body is deficient in iron, synthesis of haemoglobin is impaired and this leads to iron deficiency anaemia (Wong, 2017).

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There is no doubt that pregnancy poses an enormous physiological burden on the woman. There is a complex relationship between maternal nutrient intake and foetal development (Allen, 2005; Fall et al., 2003). The interrelationship is such that when nutrients are limited, the placenta and foetus receive priority over other maternal tissue. Thus, for many women, especially in the developing world, when proper dietary measures are not taken, pregnancy can even result in high mortality rates. During pregnancy, a total amount of 1,040 mg of iron is required. This is needed to cater for maternal requirements, foetal/placental requirements; expansion of maternal erythrocyte mass and basal iron loses (FAO/WHO, 2005). The nutritional burden pregnancy places on the adolescent girl are even greater. This is in view of the fact that, the girl who is still growing and in need of additional iron for her own growth, has to share her scarce nutrient resources with that of the developing foetus inside of her.

Growth cessation has been identified as one of the nutritional challenges an adolescent girl may face during pregnancy. In Mexico and rural Bangladesh, Casanueva, Roselló-Soberón, De-Regil, del Carmen Argüelles, and Céspedes (2006) and Rah et al., (2008) respectively showed cessation in linear growth among pregnant adolescent girls studied. Among the Bangladeshi girls, there was an observed loss of about 0.6 cm - 2.7 cm in linear growth. When pregnant adolescent girls were compared with never pregnant girls, the pregnant girls did not gain in stature, irrespective of the birth outcome, however, girls who had never been pregnant increased in height by about 0.36 (SD = 0.04) cm. Katz, Khatry, LeClerq, West, and Christian (2010) studying Nepalese women, saw that girls less than 16 years had significantly higher prevalence of post-partum mid upper arm circumference reduced compared to 20-25 year olds. These anthropometric indices underscore the overall impact of good nutrition on the health of the adolescent girl.

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The effect of adolescent pregnancy on growth can also trickle down to the developing baby. A pregnant woman with adequate dietary intakes is on the path to achieving a good pregnancy outcome. There is evidence to suggest that maternal factors are positively associated with infant anthropometric indices (Ferland & O'Brien, 2003; Okubo et al., 2012). Stewart et al., (2007) made this observation in studying the relationship between maternal age and birth outcome. He observed that for each year of increasing maternal age among primiparous women, there was an association with increases in birth length (0.07 cm; 95% CI = −0.01 to 0.16), head (0.05 cm; 95% CI = 0.01 – 0.09) and chest circumferences (0.07 cm; 95% CI = 0.01– 0.12), but not weight (9.0 g; 95% CI = −2.1 to 21.8) of their offspring. Intrauterine growth retardation is another effect that a developing foetus may face when nutrient stores in the mother are inadequate. The risk of having intrauterine growth retardation has been related to nutritional status of the adolescent mother (Bolzan, Guimarey, & Norry, 1998). In the long term, intrauterine growth retardation coupled with low birth weight can influence physical and mental development (Arcos et al., 1995). The intrauterine environment has been described as a major contributor to intrauterine growth retardation in the developing foetus (Wu, Bazer, Cudd, Meininger, & Spencer, 2004). The factors that affect the intrauterine environment include nutrition. Nutritional factors have been seen to control most of the critical roles influencing placental and foetal growth (Barker & Clark, 1997).

Results in a study conducted in Ghana suggests that, micronutrients that are particularly involved in blood formation are inadequate (Koryo-Dabrah, Nti, & Adanu, 2012). In Finland, 44% of pregnant women studied reported low intakes of folic acid compared with recommendations given for that physiological group (Arkkola et al., 2006). Alwan et al., (2011) reported a very high percentage (80%) of British pregnant women having dietary iron intakes below the 14.8 mg/day recommendation of the reference nutrient intakes. In India 75-100% of

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pregnant women were consuming less than the Recommended Daily Allowance (RDA) in folic acid and iron (Pathak et al., 2004a). Similarly in a study assessing nutrient intakes using a food frequency questionnaire among Western rural Chinese women, inadequate folate and iron intakes were observed to be 97% and 64% respectively among the pregnant women interviewed (Cheng, Dibley, Zhang, Zeng, & Yan, 2009). According to Lee, Talegawkar, Merialdi, and Caulfield (2013) in a systematic review of dietary intakes of pregnant women in low and middle income countries, folate and iron intakes were more likely to be low among the selected micronutrients analysed. Inadequate intakes subsequently results in low serum concentration which can affect the outcome of the pregnancy.

According to the United Nations Population Fund, 95% of all adolescent pregnancies can be found in sub-Saharan Africa with a rate of 143 pregnancies in 1000 pregnancies (Treffers, 2002; United Nations Population Fund, 2013). Many adolescent pregnancies are concentrated among low socioeconomic communities therefore these pregnant teenagers tend to enter pregnancy with low nutrient stores and high risk of nutritional inadequacy (Story & Alton, 1995). It is therefore crucial that in developing countries, special attention is given to adolescent nutrition, and especially during pregnancies to ensure that optimal nutrition for both mother and baby is achieved. This is of utmost interest for the adolescent mother, since the long-term effects of poor nutritional status cannot be overlooked given that adolescence is a critical period during which lifetime habits are established (Hall, 2007). The dietary habits acquired during the period of adolescence have the potential to enhance or undermine health throughout life (Hall, 2007).

1.2 Problem Statement

Anaemia is a condition that can pose serious complications in pregnancy. Several studies have reported low birth weight and preterm delivery among anaemic pregnant women (Balarajan et al., 2011; Bánhidy, Ács, Puhó, & Czeizel, 2011; Ren et al., 2007; Tzur, Weintraub, Sergienko,

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& Sheiner, 2012). In addition to these, there is also reported long term effect of maternal anaemia on child mental development (Chang, Zeng, Brouwer, Kok, & Yan, 2013).

About 38% of pregnant women globally suffer from anaemia (WHO, 2015). Current data available suggests that 44.6% of Ghanaian pregnant women are anaemic (GSS, 2015). Pregnancy in adolescence poses a bigger nutritional challenge because of the dramatic increase in the physical growth and development of the adolescent girl which tends to put pressure on the girl's nutritional needs (Lenders, McElrath, & Scholl, 2000). In fact, the remarkable growth during the adolescent period is regarded as second to the growth that occurs in the first year of life (Story & Stang, 2005). It is also a period when there is a second opportunity for catch up growth if environmental conditions in terms of nutrient intakes are favourable. There are also social and cultural factors that affect the adolescent girl's eating habits (Rasmussen et al., 2006; Story, Neumark-Sztainer, & French, 2002). In the light of these reasons, pregnancy tends to put the adolescent girl's nutrient and energy needs in direct competition with those of her developing baby and hence conditions like severe anaemia can be a major cause of mortality both in the adolescent girl and the baby (Brabin, Hakimi, & Pelletier, 2001; Lenders et al., 2000).

In Ghana, adolescent pregnancies account for as many as 14.2% of all pregnancies (GSS, 2015). Anaemia in pregnancy also contributes to at least 12% of all maternal deaths (MOH, 2008). It is therefore of necessity to pay attention to adolescent pregnant girls. Since the states of adolescence and pregnancy are both considered vulnerable, it is imperative that critical data be obtained to ascertain the enormity of the situation so that appropriate interventional measures can be used to remedy nutrient deficiencies that arise before or during pregnancy in adolescent girls in Ghana.

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Previous studies have established the usefulness of dietary diversity in assessing the micronutrient quality of the diet (Arimond et al., 2010). This has led to the recent development of the new indicator, Minimum Dietary Diversity for Women (MDD-W) of reproductive age (FAO & FHI360, 2016). The MDD-W is a proxy measure that can be used to determine the micronutrient quality of the diet of populations. The use of this new indicator in determining the quality of the diet is lacking in the Ghanaian context. Its successful application in Ghana can affirm the use of a low-cost proxy indicator for assessing micronutrient quality of the diet of women of reproductive age in the country.

The need to develop public health approaches and monitoring programmes are necessary in achieving micronutrient adequacy in women of reproductive ages (Harika et al., 2017). These public health approaches and monitoring programmes can be made based on available data. Data on the micronutrient status in women of reproductive ages particularly in the African region is lacking. In Ghana, there is paucity of current information on the magnitude of the problem and the characteristics of nutritional anaemia in adolescent girls particularly among the pregnant ones. The UN Sustainable development goals (SDGs) recognizes the enormous burden of nutrition in women of reproductive ages and therefore has targeted the need to address the nutritional burdens of women of reproductive ages including adolescent girls (Haddad et al., 2016). In addition, the use of MDD-W to predict micronutrient quality of the diet and the dietary habits of reproductive age women is also understudied in Ghana. This study therefore sought to determine the extent to which pregnant adolescent girls in Ghana have nutritional anaemia and to provide information to researchers, health workers and policy makers on the use of MDD-W as a proxy measure for determining the micronutrient quality of the diet of pregnant girls.

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1.3 Conceptual Framework of Study

The conceptual framework of this study is illustrated on figure 1.1. The causes of anaemia are interrelated and multifactorial. An individual's cultural background may influence the thinking pattern. This frames the individual's myths and misconceptions about what to eat and what not to eat. The individual's socioeconomic status also influences her food intake. The inadequate food intake consequently leads to deficiencies in iron, folate and vitamin B_{12}. In addition, diseases and infections as well as blood loss can lead to nutritional deficiencies. These nutritional deficiencies ultimately end in insufficient haeme production with the resultant effect as nutritional anaemia. The consequences of nutritional anaemia are varied including recurrent abortions, recurrent foetal loss, congenital abnormalities and neonatal and maternal mortalities.

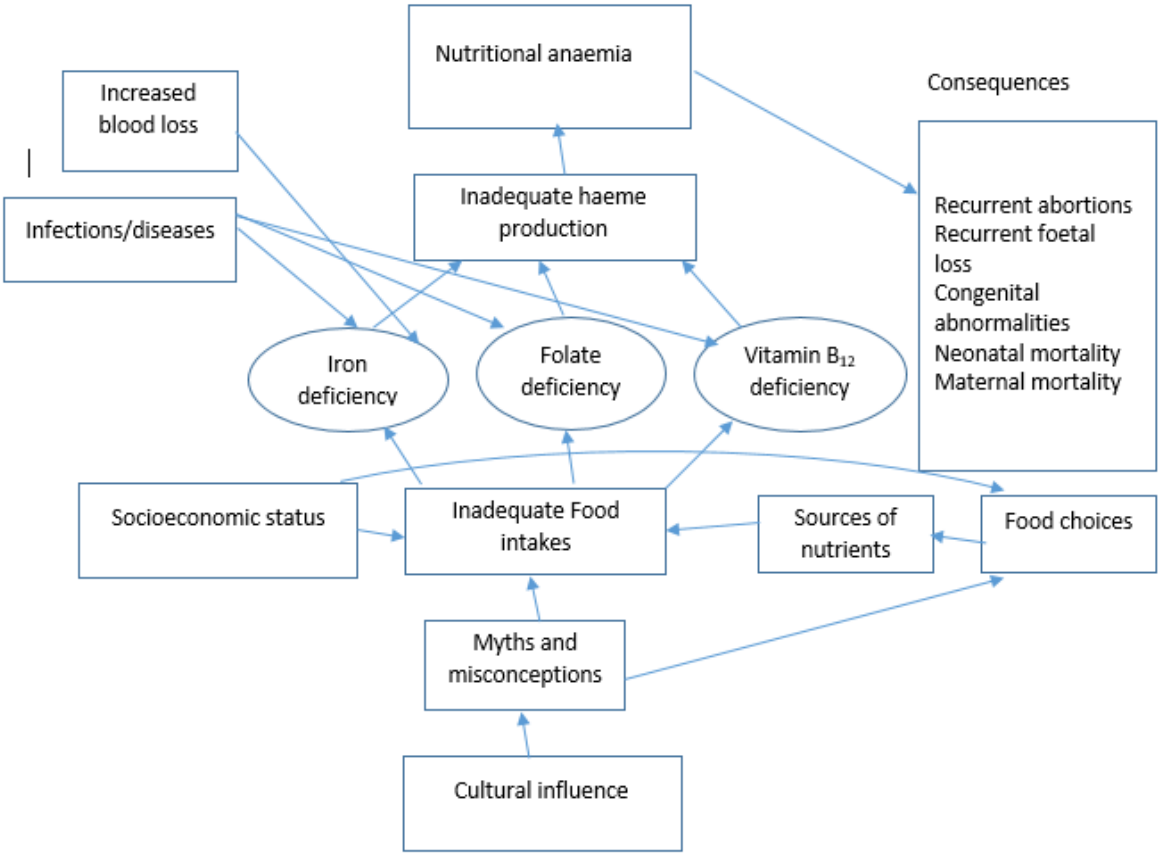

Figure 1.1 Conceptual framework of nutritional anaemia for study

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1.4 Research Questions

The study was guided by the following research questions;

1. What is the prevalence of anaemia among the pregnant adolescent girls?
2. What is the relationship between nutritional anaemia and sociodemographic characteristics of pregnant adolescent girls?
3. What are the mean serum concentrations of iron, folate and vitamin B_{12} among the adolescent girls?
4. What is the nutrient quality of the diet of the pregnant girls?
5. What is the contribution of poor dietary intakes to deficiencies of iron, vitamin B_{12} and folate in pregnant adolescent girls?
6. What is the diversity of their diet based on the newly developed Minimum Dietary Diversity for women of reproductive age indicator (MDD-W)?
7. What is their knowledge and sources of information on nutritional anaemia?
8. What cultural factors (myths and misconceptions about food) can affect nutritional anaemia in pregnant adolescent girls?

1.5 Aims of study

1. To determine the prevalence of nutritional anaemia and the relative contributions of iron, folate and vitamin B_{12} among pregnant adolescent girls.

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2. To assess nutrient intakes (Iron, folate and vitamin B_{12}) and Minimum Dietary Diversity (MDD-W) of the pregnant adolescent girls.

3. To assess nutrition knowledge, myths and misconceptions and sources of information regarding nutritional anaemia in pregnant adolescent girls.

1.5.1 Specific objectives

The specific objectives were to;

1. estimate energy and nutrient intake with a focus on iron, Vitamin B_{12} and folate in adolescent pregnant girls to ascertain their adequacy.
2. determine the minimum dietary diversity score for women of reproductive ages (MDD-W).
3. determine the relationship between dietary intakes (iron, folate, vitamin B_{12}) and serum concentrations (iron, folate, vitamin B_{12}).
4. examine the socio-demographic data for factors that would promote pregnancy-related anaemia in the adolescent pregnant girl.
5. assess their knowledge on nutritional anaemia.
6. assess whether they have any myths or misconceptions that may affect nutrient intake and the reasoning behind their dietary practices.
7. assess their sources of information regarding nutrition and dietary practices during the period of pregnancy.

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1.6 Hypotheses

The study was based on the following hypotheses;

1. Null hypothesis (H_0): Pregnant adolescent girls have no nutritional anaemia.
2. Null hypothesis (H_0): Dietary diversity does not predict nutritional anaemia.
3. Null hypothesis (H_0): Pregnant adolescent girls do not have any myths or misconceptions that will affect nutrient intakes.

1.7 Justification of study

The United Nations (UN) estimated that by 2015, all countries would have successfully achieved the Millennium Development Goals (MDG). Ghana, as a member of the UN, was faced with challenges in meeting these goals, particularly with goals 4 (to reduce child mortality) and 5 (to improve maternal health) which affect maternal and child health. Currently the UN has rolled out a new developmental agenda (Sustainable Development Goals, SDGs) set out to propel the global developmental agenda. The target of the SDG number 3 is to reduce the maternal mortality ratio to less than 70 per 100,000 live births by 2030. Ghana currently has a maternal mortality rate of 340 deaths to 100, 000 live births (UN, 2015). To achieve this daunting task of reducing maternal mortality, it is very necessary to focus on adolescent pregnant girls to ensure that they are provided with optimal attention in terms of health care. This study is therefore of great importance in contributing to the attainment of the SDG 3. It will also bring to fore the usefulness or otherwise of the MDD-W as an indicator to assess diet quality in the Ghanaian adolescent. The study would be expected to provide reliable and current quantitative and qualitative data that will inform health professionals, policy makers and other

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stakeholders on the burden of nutritional anaemia and the micronutrient quality of the diet of adolescent pregnant girls. This will enable appropriate interventions to be instituted to prevent and manage nutritional anaemias in pregnant adolescent girls and improve the outcome of their pregnancies.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Overview of anaemia

Anaemia is generally defined as a lower than normal concentration of haemoglobin (Koury & Ponka, 2004). It is diagnosed based on reference ranges established by the WHO, taking into consideration the age, sex and physiological condition of the individual (WHO, 2011a). Anaemia is defined by the WHO as haemoglobin level less than 13.0 g/dL in men, less than 12.0 g/dL in women and less than 11.0 g/dL in pregnant women and preschool children (WHO, 2011b). The term nutritional anaemia was coined to describe anaemia that results due to deficiency in one or more essential nutrients particularly iron, folate and/or vitamin B_{12} (Stoltzfus, 2001). Production of red blood cells requires iron, folate and vitamin B_{12}. Erythroblasts (immature erythrocyte with nucleus) require vitamin B_{12} and folate for proliferation during the period of differentiation. Therefore, the deficiencies of vitamin B_{12} and folate lead to impairment in DNA synthesis which results in megaloblastic anaemia (Koury & Ponka, 2004).

Iron is essential in the synthesis of haemoglobin which takes place in the mitochondria and ribosomes through series of biochemical reactions. The initial stage in the synthesis of haemoglobin is the formation of the pyrrole molecules formed from two molecules of succinyl CoA and two molecules of glycine. The next stage is the coming together of four molecules of pyrrole to form protoporphyrin IX which further combines with Fe^{2+} to form a haeme molecule. The haeme molecule reacts with a polypeptide to create haemoglobin chain of either alpha or beta. Two chains of alpha and two chains of beta combine to form haemoglobin (Guyton &

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Hall, 2017). The concentration of haemoglobin in the blood can be used to define the level of anaemia or otherwise in an individual.

According to Chulilla, Colás, and Martín (2009), anaemia can be classified based on the following criteria;

1. Aetiology (This can be as a result of increased destruction of red blood cells, increased loss of blood or defects in red blood cell formation).

2. Red blood cell morphology (The red blood cells could be described as normochromic, hypochromic, normocytic, microcytic or macrocytic).

3. Physiological abnormality (This results from defects in maturation of red blood cells or increase in the breakdown of red blood cells).

2.2 Conceptual framework of anaemia in reproductive women

The aetiology of anaemia is multifactorial. Based on the UNICEF conceptual framework on malnutrition, Nguyen et al., (2015) outlined the conceptual framework (figure 2.1) for anaemia in reproductive women as arising from three distinct levels; underlying causes, biological causes and immediate causes. The characterization of the framework includes the micronutrients iron, folate and vitamin B_{12} as part of the significant biological causes of anaemia. Inadequate dietary intakes were included as part of the immediate causes. Poor practice of hygiene, food insecurity, poor healthcare which are largely influenced by socioeconomic status, education, occupation and ethnicity are the underlying causes.

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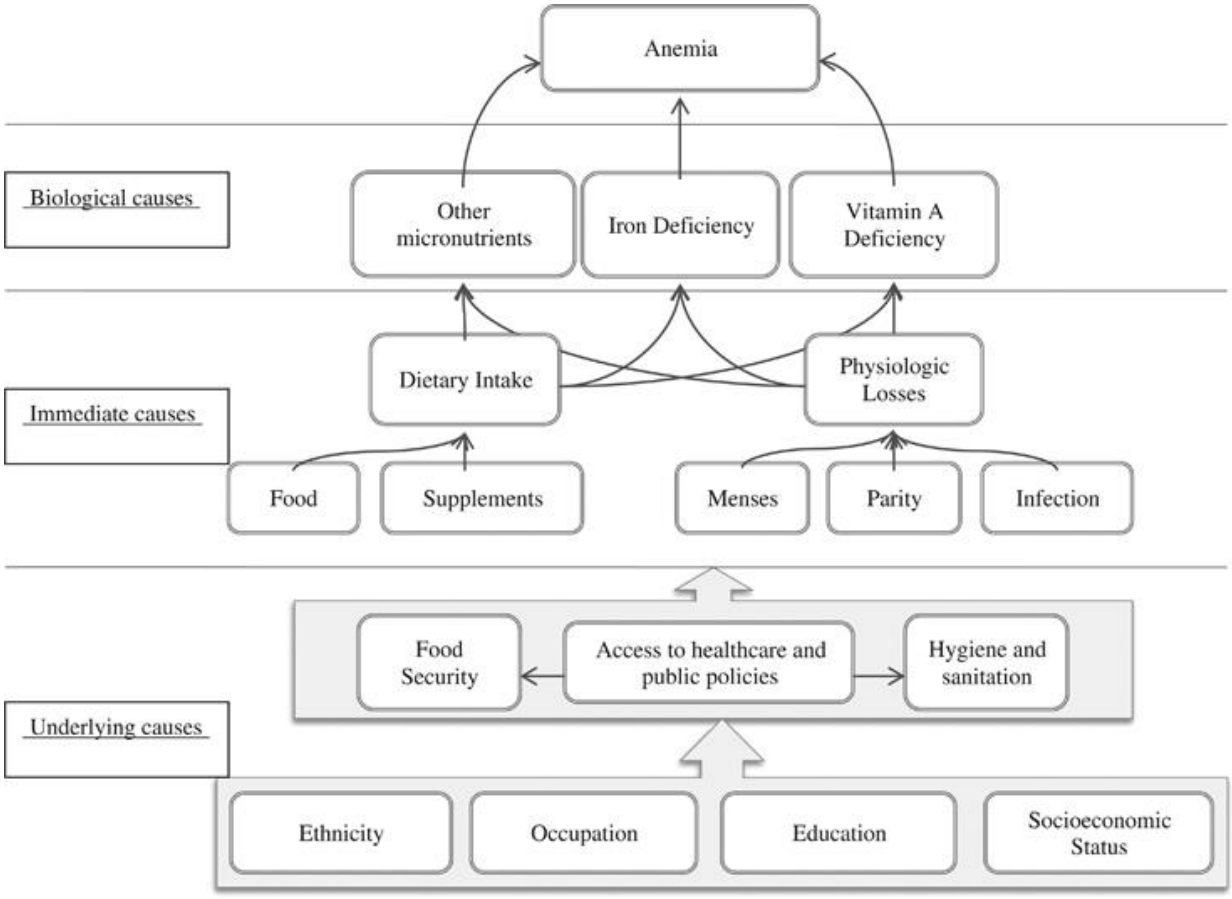

Figure 2.1: Conceptual framework of factors associated with anaemia in female of reproductive age
Source: Nguyen et al., 2015

2.3 Iron

Iron is found in every living cell and involved in various metabolic activities including oxygen transport and storage, adenosine triphosphate production (ATP), deoxyribonucleic acid (DNA) synthesis and the electron transport chain system (Clark, 2008; Schümann, Ettle, Szegner, Elsenhans, & Solomons, 2007; Theil, 2004). An average of 3.8 g and 2.3 g of iron is found in men and women respectively. This is equivalent to 50 mg/kg body weight for a man of about 75 kg and 42 mg/kg body weight for a woman of 55 kg (Theil, 2004). More than 70% of iron in the body is classified as functional iron with the rest stored as ferritin or haemosiderin. About

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80% of functional iron in the body can be seen in red blood cells as haemoglobin. The 20% left is either found in myoglobin or intracellular respiratory enzymes (e.g., cytochromes).

2.3.1 Iron deficiency anaemia

Iron deficiency anaemia is a condition that is globally considered to be of public health significance and regarded as a major public health problem in many parts of the world (Milman, 2011). It can be seen at any stage of the lifecycle but more prevalent during pregnancy and childhood (Al-Sayes, Gari, Qusti, Bagatian, & Abuzenadah, 2011; Stevens et al., 2013). The WHO estimates that about half of anaemia globally is due to iron deficiency (WHO/UNICEF/UNU, 2001). Iron deficiency is seen as one of the most common form of malnutrition affecting about 1.62 billion people representing 28.4% of the world's population (Stoltzfus, 2003; WHO, 2008). About a quarter of the world's women and children are affected by iron deficiency anaemia (Stoltzfus, 2003). From the period 1990-2010, iron deficiency anaemia was ranked among the top 7 causes of anaemia in the world and accounted for 8.8% of the global total of the years lived with disability (Kassebaum et al., 2014). It was also noted to account for 841,000 deaths and 35,057,000 disability-adjusted life years lost (Stoltzfus, 2003).

In Africa, anaemia is prevalent in about 40.7% of the total population with approximately 55.8% of pregnant women being affected (WHO, 2008). Anaemia is of serious public health significance in Ghana because of reported high prevalence. According to the current demographic health survey, 42.4% of Ghanaian women aged 15-49 are anaemic, with teenage girls (15-19 years) recording the highest prevalence of 47.7% (GSS, 2015). Anaemia in pregnancy was reported to be 56% during the same period (GSS, 2015).

The impact of iron deficiency anaemia in pregnancy is of global concern because the most common cause of anaemia in pregnancy has been attributed to iron deficiency (Goonewardene,

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Shehata, & Hamad, 2012; WHO, 2011b). It is estimated that 50% of anaemia in pregnant women can be attributed to iron deficiency anaemia (De Benoist, McLean, Egli, & Cogswell, 2008). Africa is the worst affected continent in terms of severity with between 52.8% and 61.3% of pregnant women suffering from the condition. In Europe, between 18-31% of pregnant women are affected (De Benoist et al., 2008).

The deficiency of iron in the body involves a three-stage route. The first stage occurs when the body records a negative iron balance, followed by depletion of iron which subsequently results in iron deficient erythropoiesis and then finally the occurrence of iron deficiency anaemia (Clark, 2008).

Several parameters are used in diagnosing iron deficiency anaemia in an individual. For many years serum iron concentrations have been used to determine the body stores of iron (Beutler & Waalen, 2006). In situations of iron deficiency, serum iron is usually decreased. Haematological tests which are based on characteristics of red blood cells (i.e haemoglobin concentration, haematocrit, mean cell volume and red blood cell distribution width) as well as biochemical tests (i.e erythrocyte protoporphyrin concentration, serum ferritin concentration, transferrin saturation and transferrin concentration) are used (WHO, 2011b). Biochemical tests are used to detect early changes in iron status. Haemoglobin and haematocrit are indices that reflect the amount of functional iron in the body. Haemoglobin is an indicator of the concentration of iron containing protein in circulating red blood cell. Haematocrit (also called packed cell volume, PCV) is a more direct and sensitive measure of the proportion of whole blood that is occupied by the red blood cells. Both haemoglobin and haematocrit detect iron deficiencies in the later stages (WHO, 2011b).

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In pregnancy, haemoglobin concentration and haematocrit decrease during the first and second trimesters due to the expansion of blood volume (Chandra, Tripathi, Mishra, Amzarul, & Vaish, 2012). Those who take iron supplements however tend to have a gradual rise in haemoglobin concentration and haematocrit in the third trimester (Chandra et al., 2012).

2.3.2 Dietary Iron intakes

Some researchers have recommended oral supplementation of iron in pregnant women because of reported low dietary intakes (ACOG, 2008). Dietary iron is basically found in two forms: haem and non-haem (Singh et al., 2006). Haem iron is mainly from animal source and found in smaller amounts compared to the non-haem. It is more bioavailable to the body and proven to have less effect on the gastrointestinal tract compared to the non-haem (Frykman, Bystrom, Jansson, Edberg, & Hansen, 1994; Hallberg, Björn-Rasmussen, Howard, & Rossander, 1979). The non-haem component forms the larger percentage of dietary iron. In Britain for instance 95% of dietary iron has been found to be non-haem (Singh et al., 2006).

An optimal dietary intake of iron is crucial to ensure protection from iron deficiency anaemia. Low dietary intakes during pregnancy has been reported to generally affect the overall iron status in the body (Alwan et al., 2011). Available data suggest inadequate dietary iron to be common among pregnant women and adolescent girls in low and middle income countries as well as in developed countries (Alwan et al., 2011; Blumfield, Hure, Macdonald-Wicks, Smith, & Collins, 2013; Lee et al., 2013; Vandevijvere et al., 2013). In two separate dietary assessment studies conducted in the Southern part of Thailand, one hospital based and the other community based, the percentage of inadequate dietary iron intake was 45.2% and 98.8% respectively. The wide disparity in the percentages could be attributed to the differences in the cut-off points used. Sukchan et al., (2010) used a cut-off point of 66.7% of the RDA whiles Piammongkol, Marks, Williams, and Chongsuvivatwong (2004) used a cut-off of 80% of the RDA. In some parts of

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China, a low intake of iron was observed in the third trimester of pregnancy with some other women being at risk of excessive iron intakes (Liu et al., 2015).

In a more developed area such as Sydney, about 99.5% of pregnant women assessed did not meet the daily requirement for iron intake (Goletzke, Buyken, Louie, Moses, & Brand-Miller, 2015). Similarly, Rodríguez-Bernal et al., (2013) established inadequate dietary iron intakes among Spanish pregnant women located around the Mediterranean area. Studies in developing areas such as Alexandria, Egypt, reported that pregnant women attending ante-natal care had low mean intakes of dietary iron below 50% of the RDA (Naem, El-Sayed, Nossier, & Abu Zeid, 2014). In Ghana scarcity of published data exist on dietary iron intakes of pregnant women. A literature search found two studies which reported inadequate intakes (Koryo-Dabrah, Nti, & Adanu, 2012; Nti, Larweh, & Gyemfua-Yeboah, 2002). According to Annor, Debrah, and Essen (2016), the iron content of commonly consumed Ghanaian foods ranged between 3.6 mg to 15.5 mg/100 g. Commonly consumed green leafy vegetables such as "kontomire" (*Xanthosoma sagittifolia),* "Aleefu" *(Amaranth cruentus)* and "Bokoboko" *(Talinum triangulare)* also contain 14.64 mg, 40.50 mg and 28.21 mg of per 100g iron respectively (Kwenin, Wolli, & Dzomeku, 2011).

2.3.2.1 Enhancers and inhibitors of iron absorption

Efficient absorption of iron is important for its utilization by the body. Non-haem iron basically occurs in the diet in the ferrous or the oxidized ferric state (Miret, Simpson, & McKie, 2003). It is absorbed very early in the duodenum where the pH is low and therefore favours the solubility of iron. Nevertheless, certain factors enhance or inhibit iron absorption by the body. Absorption is well enhanced by ascorbic acid, haem source of iron and the protein meat factor (Miret et al., 2003). When food is consumed together with phytates, phenols and calcium, absorption of iron can be inhibited.

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Phytate is considered as one of the most important inhibitor of iron in most populations (Hurrell & Egli, 2010). It can hinder non-haem iron absorption by as much as 90% (Mangels, Messina, & Messina, 2011). Several kinds of grains, nuts, vegetables, seeds, legumes, roots, tubers and fruits contain phytates (Weaver & Kannan, 2002). They influence absorption in a dose-dependent manner and therefore small amounts can have clear effects on absorption by the individual (Gillooly et al., 1983; Hallberg, Mats Brune, & Rossander, 1989). Fermentation can however degrade the phytates present in these food components making iron more bioavailable (Brune, Rossander-Hultén, Hallberg, Gleerup, & Sandberg, 1992). Other food processing methods such as soaking, heating, milling and germination have also been useful in reducing the phytate content in foods hence improving the bioavailability of iron (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002).

Polyphenols are also inhibitors of iron. They are generally present in the diet as components of fruits, vegetables, spices, pulses and cereals. They are also high in tea, coffee, red wine and cocoa. Phenolic compounds form complexes with dietary iron in the intestinal lumen thereby making it unavailable for absorption (Hurrell, Reddy, & Cook, 1999).

Calcium present in dairy products and that consumed as a salt has been seen to interfere greatly with absorption of both haem and non-haem iron (Hallberg, Brune, Erlandsson, Sandberg, & Rossander-Hulten, 1991). The mechanism of action for inhibition has not been clearly ascertained. However, it is strongly suggested that the process of inhibition occurs in the mucosal cell, the final transfer step for haem and non-haem iron (Gleerup, Rossander-Hulthen, Gramatkovski, & Hallberg, 1995; Hallberg, Rossander-Hulten, Brune, & Gleerup, 1992). Both calcium and iron are essentially required by the body for optimal wellbeing. This in effect necessitates pragmatic steps to ensure that one nutrient does not compromise the presence of

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the other. To reduce this antagonist action between the two nutrients, it is recommended that foods rich in both minerals are not consumed from the same meal (Hallberg et al., 1992).

Ascorbic acid (vitamin C) and muscle tissue has been named to be the main enhancer of non-haem iron absorption among humans (Hurrell & Egli, 2010). Ascorbic acid whether synthetic or natural remains the most effective enhancer of iron absorption (Zijp, Korver, & Tijburg, 2000). Radioisotope studies conducted among human participant have shown that an addition of ascorbic acid to the meal increases iron absorption (Lynch & Cook, 1980). Conrad and Schade (1968) explained the enhancing effect of ascorbic acid to be mainly due to its ability to chelate iron and therefore reduce ferric iron to a ferrous state. Ascorbic acid has the potential to override the inhibitory effects of inhibitors such as polyphenols, phytates and calcium (Hallberg, Brune, & Rossander, 1989; Siegenberg et al., 1991; Stekel et al., 1986).

The enhancing effect of muscle tissue obtained from meat, fish or poultry has been shown in radioisotope studies. In a study by Bæch et al. (2003), they demonstrated an increase in non-haem iron absorption when pork was included in a high phytate and low ascorbic acid meal. Similarly a 60 g pork meat when added to a vegetarian diet in a 5-day fully controlled diet increased non-haem iron absorption by 50% (Kristensen et al., 2005).

2.3.3 Mechanism of iron absorption in the body

Absorption of both haem and non-haem iron takes place in the upper part of the gastrointestinal tract, specifically the duodenum. Its bioavailability is however influenced by factors such as the type of iron present in the food (i.e. whether it is haem or non-haem) and presence of other dietary components (Sharp & Srai, 2007). The absorption of non-haem is largely affected by the foods consumed at the same time (Semba & Ramakrishnan, 2008). Haem iron is less consumed contributing to about 10-15% of total intake of iron in meat eating populations.

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Notwithstanding, it is able to contribute to greater than 40% of the total iron absorbed by the body (Hurrell & Egli, 2010).

Haem absorption in the initial uptake stages differs from that of non-haem. Haem goes through the mucosal cells unchanged via the brush border membrane possibly by endocytosis (Parmley, Barton, Conrad, Austin, & Holland, 1981). From this stage, haem iron goes through the same intracellular pool as fresh non-haem iron that has been absorbed and hence affected by the same factors (Morgan & Oates, 2002). Non-haem iron on the other hand goes through the cell in the ferrous state via the carrier divalent metal transporter I. The first step is termed the uptake step which involves the transport of iron into the enterocytes across the brush border membrane. The second step is the movement of iron through the cell and then the transfer step, which is the transportation across the basolateral membrane. The fourth step involves the passage of the non-haem iron through the intestinal space and capillary wall (Currie et al., 2004).

The absorption of haem iron ranges from about 40% in periods of deficiency to about 10% when the body is replete of iron stores (Hallberg, Hultén, & Gramatkovski, 1997). Its absorption can also be negatively affected by the presence of calcium in the diet. In situations where haem iron is cooked at very high temperatures for too long, it can be converted to non-haem iron (Lombardi-Boccia, Martinez-Dominguez, & Aguzzi, 2002). In the case of non-haem iron, ascorbic acid, fermentation and the presence of meat or chicken in the diet all enhance its absorption.

2.3.4 Regulation of iron absorption

In spite of the essentiality of iron to the human body cells, it can be highly toxic to the cells when present in high levels (Cassat & Skaar, 2013). The human body does not have the ability to dispose of excess iron. This therefore makes it mandatory for the absorptive process to be

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tightly regulated within the confines of physiological limits to avoid conditions of iron overload and deficiency (Cassat & Skaar, 2013). The body is able to maintain iron balance via three distinctive and important mechanisms (FAO/WHO, 2005). The initial stage is the continual re-use of iron from that which had been catabolised from red blood cells in the body. The natural lifespan of red blood cells is 120 days after which the iron present in them is released to transferrin in the plasma. This iron then gets back to the red blood cell precursors in the bone marrow or other cells in different tissues. This procedure controls both transport of iron to tissues in the body and also the formation of free radicals (FAO/WHO, 2005). The second mechanism has to do with the storage of the iron. The protein ferritin stores and releases the iron when the body requires it for metabolic activities. The third mechanism is the regulation of absorption at the intestinal level (FAO/WHO, 2005). Iron absorption increases with decreased body stores and decreases when body stores are adequate.

2.3.5 Role of iron in erythropoiesis

Erythropoiesis is the production of mature red blood cells from the bone marrow. It requires a continuous supply of iron and singly consumes most of the iron that enters the body (Arvedson & Sasu, 2009; Beckman, Silberstein, & Aldoss, 2010). Iron is needed in the formation of the nucleus of the iron-porphyrin haem ring. This, together with globin chains forms haemoglobin. The critical stage for oxygen delivery from the lungs to the tissues is the binding of haemoglobin to oxygen. When sufficient iron is absent, small erythrocytes (red blood cells) and insufficient haemoglobin are formed. This in effect results in microcytic (small) hypochromic (pale) anaemia (Beckman et al., 2010).

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2.3.6 Causes of iron deficiency

The most common cause of iron deficiency is attributable to inadequate intakes which are unable to meet physiological requirements (FAO/WHO, 2005). For instance, in pregnancy and adolescence, nutrient requirements increase dramatically to meet the physiological needs of the body. Several studies have established the link between low dietary intakes of iron and anaemia. Ma et al. (2002) and Seck & Jackson, (2010) reported low dietary iron intake among Chinese and Senegalese pregnant women who were anaemic in comparison to those who were not anaemic respectively. In Tanzania, although other factors such as parasitic infections and malaria were seen to be associated with anaemia, low dietary iron intake was reported to be the major cause of anaemia among the pregnant women investigated (Tatala, Svanberg, & Mduma, 1998). A study conducted in two communities in Ghana further demonstrated low dietary iron intakes compared to RDA. The researchers further attributed the high prevalence of anaemia to the low dietary intakes of iron (Nti et al., 2002).

In addition, excessive blood loss and poor body utilization have also been established to result in iron deficiency. The aetiology of blood losses has been attributed to certain disease conditions characterized by internal bleeding that could be related to peptic ulcers and GIT cancers. In addition, excessive menstrual loss and fibroids can contribute to iron loses. Iron loses are also reported in diseases such as coeliac and Crohn's which are characterized by malabsorption. Conditions like congenital atransferrinaemia, which is as a result of a polymorphism that affect normal transferrin function, are also causes (Shamsian et al., 2009).

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2.3.7 Role of iron deficiency in pathogenesis of anaemia

As earlier on alluded to, iron deficiency anaemia can be classified into 3 stages involving deficiency of storage iron, iron deficient erythropoiesis and iron deficient anaemia (Clark, 2008). The absence of iron promotes anaemia because red blood cells or erythrocytes cannot be formed. About 200 billion erythrocytes are produced daily from the bone marrow and this requires nearly 25 mg of iron (Camaschella, Pagani, Nai, & Silvestri, 2016). In situations of blood loss, there is a preferential use of body iron stores to speed up erythropoiesis (Naigamwalla, Webb, & Giger, 2012). When body stores are effectively depleted, erythropoiesis and production of myoglobin (an iron containing protein) become limited (Anderson, Aronson, & Jacobs, 2000). This subsequently leads to iron deficiency anaemia. Anaemia is worsened because erythrocytes that are iron deficient are fragile and have a shortened lifespan. Iron deficient erythrocytes leads to defective haemoglobin synthesis resulting in hypochromic and microcytic cells (Naigamwalla et al., 2012).

2.3.8 Indices for determining iron deficiency anaemia

In the diagnosis of iron deficiency anaemia, standard traditional markers, serum and plasma iron, transferrin saturation and ferritin, are used. Another marker that has been used is the serum soluble transferrin receptor (Thomas & Thomas, 2002). Iron deficiency anaemia is diagnosed based on the presence of anaemia and red blood cell morphology (i.e hypochromia and microcytosis) in addition to low serum ferritin, decreased transferrin saturation or increased serum soluble transferrin receptor (Thomas & Thomas, 2002). In cases where red blood cell morphology is used as an index in determining anaemia, it is usually useful to provide information on the iron status of the individual over a long period. It is therefore a late indicator of iron restricted erythropoiesis (Macdougall, 1998). Hypochromic red cells that are more than

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10%, together with a low serum ferritin is assumed to be indicative of little supply for erythropoiesis to the extent that it is not enough to maintain normal red cell haemoglobinization. In this case, the body's iron stores may have been considerably depleted (Schaefer & Schaefer, 1998).

Serum iron tests are usually ordered in addition to other iron tests to evaluate iron deficiency and also when there is suspicion of iron overload. Tests that show low levels of serum iron, high transferrin or total iron binding capacity, low percent transferrin saturation and a low ferritin level are indicative of iron deficiency. Essentially the transferrin test, transferrin saturation and total iron binding capacity (TIBC) all measure the same things. They reflect the body's iron stores and its ability to bind and transport iron.

2.3.9 Managing iron deficiency anaemia

The diagnostic criteria for managing iron deficiency anaemia vary between studies and dependent on various parameters including the cost, the sensitivity and specificity of the test, the invasiveness of the test and whether it is a late or early indicator of the deficiency (Cook, 2005). According to Cook, (2005), haemoglobin concentration, transferrin saturation, mean corpuscular haemoglobin, zinc protoporphyrin and reticulocyte haemoglobin could all be used as screening tools to screen for iron deficiency. However, these tests may be characterised by low sensitivity and specificity. On the other hand, they could be inexpensive. Serum ferritin, serum transferrin receptor and bone marrow iron are used as definitive tools (Bashiri, Burstein, Sheiner, & Mazor, 2003; Cook, 2005). Serum ferritin concentration is regarded as the most accurate and recommended test for defining iron deficiency by most studies even though it has been established to be affected by inflammation, malignancy or hepatic disease (Cook, 2005; Peyrin-Biroulet, Williet, & Cacoub, 2015; Puolakka, Jänne, Pakarinen, & Vihko, 1980). Iron

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is a crucial mineral needed in pregnancy and therefore its deficiency should be prevented if possible and treated in the case of deficiency as early as possible. In a review of several guidelines for the diagnosis and management of anaemia, more than half of the guidelines recommended oral administration of iron as the first line treatment for iron deficiency (Peyrin-Biroulet et al., 2015). Administering oral iron in pregnancy is particularly favourable in treating iron deficiency anaemia. Some have also complained of adverse reactions attributed to the dosage (Bashiri et al., 2003). Slight to moderate anaemia could be treated with ferrous iron of not more than 100 mg/day (Zhou, Gibson, Crowther, & Makrides, 2009). Zhou et al., 2009, showed that ferrous iron was effective in treating iron deficiency anaemia when pregnant anaemic women were given doses of ferrous iron between 20-80 mg/day between meals. The effect of the ferrous iron on the iron status of the women increased with increase in dosage with the highest being 80 mg/day.

A second option to the treatment of iron deficiency anaemia is the use of intravenous iron. This option is faster in increasing haemoglobin and replenishing body stores of iron in contrast to oral iron (Bashiri et al., 2003). It should be considered in situations where oral iron administration fails to increase haemoglobin within two weeks.

The WHO recommends iron supplementation in pregnancy in order to prevent anaemia. The recommended dosage stands at 60 mg/day during the course of pregnancy. However a higher dosage is recommended if the period of supplementation is short (Stoltzfus, Dreyfuss, & WHO, 1998). Some data have provided beneficial effects of prophylactic supplementation in pregnancy. The benefits brought reduction in anaemia, improved haemoglobin and produced high Apgar scores in babies of supplemented mothers (Preziosi et al., 1997; Singh, Fong, & Arulkumaran, 1998; Zhao et al., 2015). On the contrary, in non-anaemic women, routine iron

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supplementation proved not to be beneficial (Ziaei, Norrozi, Faghihzadeh, & Jafarbegloo, 2007).

Dietary approaches have also been employed in managing iron deficiency. Dietary modification and diversification where people are encouraged to consume foods from good and different sources of iron have been beneficial and most preferable in preventing iron deficiency anaemia (Lynch, 2005; Stoltzfus, 2003). Fortification of frequently consumed foods and condiments are also encouraged. Cereal based foods, curry powder, milk and wheat flour are some of the vehicles that have been used for the fortification of foods with iron (Ballot, MacPhail, Bothwell, Gillooly, & Mayet, 1989; Gaucheron, 2000; Hurrell et al., 2010; Hurrell, Reddy, Burri, & Cook, 2000). Even though diversification and modification are more preferred, getting people to have dietary changes often proves to be difficult. Also, food sources that are of high iron content tend to be expensive and therefore not consumed by most people in the lower socioeconomic bracket. Fortification therefore becomes a more or less expensive method with remarkable results (Olsson, Väisänen, Konar, & Bruce, 1997).

2.4 Vitamin B_{12} and Folic Acid

The metabolic roles of vitamin B_{12} and folic acid are intertwined and therefore discussed under the same section. Folic acid is needed mainly for synthesis, repair and methylation of DNA, whiles vitamin B_{12} is essential in supplying methyl groups for protein and DNA synthesis (Mahmood, 2014).

2.4.1 Sources and intakes of vitamin B_{12}

Vitamin B_{12} or cobalamin is an important component of red blood cells. The vitamin is basically obtained from the diet that is of animal origin (Allen, 2012). In spite of the copious amount of vitamin B_{12} producing bacteria colonizing the colon, its location in the body is too distal to

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allow normal absorption (Antony, 2003). Plants generally do not synthesize vitamin B_{12} however, certain blue-green algae called cyanobacteria are able to produce inactive cobalamin which is biologically unavailable to the body (Antony, 2003). This leaves the main food sources of vitamin B_{12} to animal source foods such as meat, eggs, fish, milk and liver, with the latter being one of the richest sources. For groups, such as vegetarians, who do not take animal source foods, their best sources of vitamin B_{12} could be obtained from fortified products such as cereals and soy products. The content of vitamin B_{12} in foods has been estimated by microbiologic and competitive binding assays. These are good for the estimation of vitamin B_{12} in muscles, meat, eggs and dairy products (Stabler & Allen, 2004).

The average dietary intake of vitamin B_{12} in developed countries has been reported to be between 5-30 µg/day (Moll & Davis, 2017). In the UK, average dietary intakes of vitamin B_{12} among women of childbearing age was reported to be 3.82 µg/day. This was seen to positively correlate with levels in the blood (Sukumar et al., 2016). In developing countries, more than 50% of reproductive age women do not meet the dietary intake recommendations for vitamin B_{12} (Arsenault et al., 2013; Nguyen et al., 2014). In Ghana, vitamin B_{12} dietary intakes of 2.22 µg/day were reported among reproductive age women in a rural community (Akwetea, 2015).

2.4.2 Vitamin B_{12} deficiency and prevalence

The deficiency of the vitamin has become a very common micronutrient deficiency problem especially among vegetarians and some individuals in the developing world. In most developing countries, because animal products are expensive, people in the middle and lower income groups cannot afford it regularly (Antony, 2003). In effect, even individuals who classify themselves as non-vegetarian tend to have low vitamin B_{12} status due to low purchasing power. Their vitamin B_{12} status therefore tends to be only marginally better than that of lacto-ovo-vegetarians who only consume dairy products and eggs (Antony, 2003).

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Deficiency of vitamin $B_{12,}$ particularly in pregnancy, is still a major public health problem across the globe (McLean, de Benoist, & Allen, 2008). In a recent meta-analysis of 57 studies, Sukumar et al., (2016) showed that the overall worldwide prevalence of vitamin B_{12} insufficiency was about 25%. However, when the results were analysed according to trimester, the prevalence was presented as 21%, 19% and 29% for the first, second and third trimesters respectively. Very high prevalence of insufficiency was observed from the Eastern Mediterranean and Indian sub-continents. In an analysis of a nationwide survey among Colombian pregnant women, about 18.6% were reported to have serum vitamin B_{12} below 200 pg/ml (Ramírez-Vélez, Correa-Bautista, Martínez-Torres, Meneses-Echávez, & Lobelo, 2016). Baron, Solano, Pena, and Moron (2003) reported a lower prevalence (8.3%) of vitamin B_{12} deficiency among adolescent pregnant girls in Valencia, Venezuela. A similar observation was made in a study conducted in the Limpopo province in South Africa among pregnant teenage girls. This study reported 7% prevalence of vitamin B_{12} deficiency (Bopape, Mbhenyane, & Alberts, 2008). Published baseline studies reporting on the prevalence of vitamin B_{12} deficiency among pregnant adolescent girls is scarce in Ghana. Nonetheless in a recent study, Dei-Adomakoh, Acquaye, Ekem, and Segbefia (2014) reported on anaemia in pregnant Ghanaian women in the second trimester. In the study, 36 pregnant women representing 100% of participants who were tested for serum vitamin B_{12} levels because they exhibited MCV greater than 90 fl had normal serum levels of vitamin B_{12}.

2.4.3 Causes and consequences of vitamin B_{12} deficiency

Globally the most common cause of vitamin B_{12} deficiency is attributable to inadequate dietary intake (Stabler & Allen, 2004). Intake is solely dependent on intake of animal source foods since that is where it can be found. On the other hand, other foods can be fortified with vitamin B_{12} to provide for the dietary needs of the body. Individuals who constantly have no or low

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intakes of animal source foods such as vegetarians and lacto-ovo vegetarians, are at constant risk of developing deficiency symptoms of the vitamin. It is well documented that strict vegetarians and lacto-ovo vegetarians are at high risk of vitamin B_{12} (Zeuschner et al., 2012. Allen, 2008). This especially involves babies who are breastfed by vegan mothers (Allen, 2012). For instance in Netherlands, when some pregnant women were studied, 40% of those who were lacto-ovo vegetarians were not able to meet their estimated average requirements of vitamin B_{12} compared to 6% of those who consumed small amounts of meat (Koebnick et al., 2004).

Malabsorption causes depletion of vitamin B_{12} stores in the body faster than low dietary intakes would do. Depletion is even more rapid in situations of both low dietary intakes and malabsorption (Allen, 2008). Malabsorption may occur due to the lack of intrinsic factor or other intestinal abnormalities. Disorders occurring in the ileum resulting in loss of receptors for vitamin B_{12} intrinsic factor causes deficiency of the vitamin. Hence, loss of the ileum through surgical means and diseases such as ulcerative colitis, tropical sprue, crohn's disease, regional enteritis and ileal lymphoma may hamper vitamin B_{12} absorption (Nagalla & Schick, 2017). Gastric atrophy is usually common in the elderly. Atrophic gastritis is usually accompanied by low or no secretion of gastric acid and hence overgrowth of bacteria in the upper small intestine (Allen, 2008).

In developing countries, the high cost of animal source foods usually affects the daily consumption of vitamin B_{12}. In addition cultural and religious beliefs may affect consumption of animal source foods negatively (Gittelsohn & Vastine, 2003). Further evidence suggest inadequate intake of vitamin B_{12} rather than malabsorption to be the main cause of the deficiency, although the malabsorption cannot be totally ruled out (Allen, 2008). Infants who

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are born to mothers deficient in vitamin B_{12} stand a high risk of developing a deficiency because of low stores at birth (Allen, 2005).

The lack of intrinsic factor, which is needed in vitamin B_{12} absorption, could also result in deficiency. *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium that is implicated in the development of gastric ulcers and gastric atrophy in the elderly. Infection of *H. Pylori* causes a series of changes to the gastric mucosa and gastric function (Jones, Day, Jennings, & Sherman, 1999). Infection of *H. Pyroli* may lead to inflammation of the gastric mucosa hence elevation in serum gastrin and pepsinogen with reduced somatostatin concentration (Oliveira, Queiroz, Rocha, & Mendes, 1994). In autoimmune atrophic gastritis, antibodies produced by the immune system destroy the parietal cells resulting in decreased acid production and intrinsic factor. This effectively causes malabsorption of vitamin $B_{12.}$ The reduction in acid production leads to an increase in production of gastrin by the G cells in an attempt to get the parietal cells to secrete more acid (Shane, 2008).

The development of the brain begins from the prenatal period and progresses through the school age period (Black, 2008). Deficiency of vitamin B_{12} can have serious consequences on a mother and her developing child. The deficiency is usually associated with peripheral neuropathy and myelopathy which can progress to severe motor problems if untreated (Black, 2008). In infants, deficiency of vitamin B_{12} has been associated with brain atrophy and demyelination (Lövblad et al., 1997). Several reports have been presented on the neurological effect of vitamin B_{12} deficiency during the developmental stages of life. The developing brain is highly sensitive to vitamin B_{12} deficiency than the matured brain (Stabler & Allen, 2004). This explains the reason for complications like anencephaly in off-springs of pregnant women with low vitamin B_{12} status (Molloy et al., 2008). Stollhoff and Schulte (1987) presented a case report of the neurological effect of vitamin B_{12} on a one and half year old boy. The deficiency was seen to

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be characterized by megaloblastic anaemia with a progressive neurological disorder which appeared similar to leukodystrophy. In two case reports of the neurological effect of vitamin B_{12} deficiency in 9 months and 6 months old infants, the researchers showed that babies who were exclusively breastfed by mothers who were strict vegetarians exhibited symptoms including serious psychomotor retardation (Casella, Valente, de Navarro, & Kok, 2005). However, the situation was reversed after administration of vitamin B_{12} supplements. Other researches made similar observations (Mariani et al., 2009; Roed, Skovby, & Lund, 2009). However contrary to the belief that low levels of vitamin B_{12} is associated with poor cognitive development, in a cohort of Indian children, higher maternal folate, but not vitamin B_{12}, concentrations during pregnancy was observed to be a better predictor of childhood cognitive ability (Veena et al., 2010). In older populations, vitamin B_{12} has been associated with cognitive performance. In a prospective study involving the aged between 61-87 year olds, decrease in brain volume was greater among those with low vitamin B_{12} status (Vogiatzoglou et al., 2008).

A link between vitamin B_{12} deficiency and early loss of pregnancy and infertility has been proposed (Molloy et al., 2008). The authors cited several anecdotal studies which over the years had reported a link between low vitamin B_{12} levels in pregnancy to early loss of pregnancy and infertility. These studies adopted two main approaches to arrive at these conclusions. The first involved following women with recurrent and severe vitamin B_{12} deficiency and observing them after sometime. The outcome of this observation was a high recurrent foetal loss and periods of infertility. The second approach looked at vitamin B_{12} deficiency prevalence in pregnant women who had had recurrent foetal loss and concluded that early vitamin B_{12} deficiency in pregnancy may be involved in their foetal losses.

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Studies in animal models have also shown severe deficiency of vitamin B_{12} to be linked to increased body fat, induced adiposity and altered lipid profile and subsequent reductions in frequency of conception and litter size of the animals (Ghosh, Sinha, Putcha, & Raghunath, 2016; Kumar et al., 2014). Additionally, there are confirmed reports of increased adiposity, insulin resistance and gestational diabetes in human studies being associated with low vitamin B_{12} levels (Krishnaveni et al., 2009). The study hypothesized that low plasma vitamin B_{12} and increased folate concentration during pregnancy will be associated with high incidence of gestational diabetes. The pregnant women were followed up during pregnancy and up to 5 years after delivery. The results of the study indicated positive association between low vitamin B_{12} during pregnancy with skinfold thickness, insulin resistance and prevalence of diabetes after the 5 years.

Other studies have reported of suboptimal levels of vitamin B_{12} concentration in pregnancy being independently associated with low birthweight and lower cord blood in India and UK respectively (Adaikalakoteswari et al., 2015; Muthayya et al., 2006).

2.4.4 Absorption and function of vitamin B_{12}

Vitamin B_{12} found in food is generally bound to some proteins. However, cooking may free some of the vitamin B_{12}. Pepsin which is present in gastric juice also frees the remaining protein bound to vitamin B_{12}. The freed vitamin B_{12} attaches itself to a mixture of glycoproteins that are secreted by the stomach and salivary glands (WHO/FAO, 2005). The glycoproteins, which are also known as R-binders, protect vitamin B_{12} from chemical denaturation in the stomach. The free vitamin B_{12} gets attached to intrinsic factor. The complex (vitamin B_{12} and intrinsic factor) then binds to cubilin receptors on the brush border of the terminal ileal mucosa resulting in the crossing of the complex into the intestinal mucosa. Vitamin B_{12} is then separated from intrinsic factor and transferred into transcobalamin II. Here a pH greater than six is needed to

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transport vitamin B_{12} into the mucosal enterocytes by way of active transport (WHO/FAO, 2005). The cobalamin-TCII complex binds to cell surfaces followed by endocytosis into the cytoplasm. The TCII is degraded and the cobalamin released into the cytoplasm, with the cobalt undergoing enzymatic reduction followed by methylation to form methylcobalamin or mitochondrial adenosylation to form adenosylcobalamin (WHO/FAO, 2005).

The bulk of vitamin B_{12} is stored in the liver (Shipton & Thachil, 2015). Other organs of the body such as the kidney, bone, brain, spleen and muscle also contain significant amounts of vitamin B_{12}. The first chemical reaction vitamin B_{12} is involved in is the conversion of homocysteine to methionine. In this reaction, homocysteine is bound to vitamin B_{12} and then N methyl Tetrahydrofolate (THF) transfers the methyl moiety to the cobalt part of the vitamin. When this happens the methyl group that was attached to N^I- methyl THF is transferred to homocysteine to produce methionine. In circumstances where there is deficiency of vitamin B_{12}, accumulation of N^I- methyl THF occurs, a situation termed as the methyl folate trap. This in effect causes the lack of folate and THF that is needed for nucleic acid metabolism. The lack of folate and THF results in megaloblastic anaemia which is very characteristic of vitamin B_{12} and folate deficiency (Shipton & Thachil, 2015).

The second major role of vitamin B_{12} is the conversion of L-methymalonic to succinyl CoA. It is necessary in the form of 5-deoxyadenosyl cobalamin to convert L-methymalonic to succinyl CoA. This biochemical step is necessary in obtaining energy from fats and proteins. Succinyl CoA is also important in the production of haemoglobin (Mahmood, 2014).

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2.4.5 Stages of vitamin B_{12} deficiency

Herbert (1994)) identified four stages of vitamin B_{12} depletion. The first stage is characterised by reduction in serum concentrations of holo-transcobalamin II, the protein transporter of vitamin B_{12}. When the deficiency has progressed to stage II, there is the reduction in the glycoprotein holo-haptocorrin. In both stages I and stage II there is remarkable depletion in plasma and cell stores of vitamin B_{12}. Stage III shows elevated plasma levels in homocysteine or methyl malonic acid. The exhibition of clinical signs such as numbness, reduced cognitive function, loss of position and difficulty in walking occurs in stage IV (Reenan, 2006). Anaemia becomes evident by stage IV where erythrocytes become macrocytic and there is a corresponding rise in mean corpuscular volume, serum homocyteine and methylmalonic acid levels. At this stage, physical evidence of vitamin B_{12} deficiency is exhibited by the affected individual.

2.4.6 Folate/Folic acid

Folic acid and folate are synonymous for the water-soluble vitamin once known as vitamin B9. Folate is basically made up of a group of compounds known as pteroylglutamates (Kurosawa et al., 2017). These compounds occur naturally in foods. The coenzymes of folate perform very important functions in metabolic pathways. These pathways usually involve one-carbon unit transfer in methylation reactions that take place during the synthesis of protein, neurotransmitters, phospholipids and nucleotides (Ebara, 2017). This gives folate a very important role in DNA synthesis and repair. Another essential role of folate is to act in conjunction with vitamin B_{12} as an essential co-factor in the re-methylation cycle that converts homocysteine to methionine. This role is very much related to that of vitamin B_{12}. In effect deficiency of vitamin B_{12} can be mistaken for folate deficiency (Selhub, Morris, & Jacques, 2007).

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2.4.7 Folate metabolism, absorption and control

Folate is an essential nutrient to the body with regards to cell division and homeostasis. For normal metabolism to take place, folate coenzymes are usually involved in nucleic acid synthesis regeneration of methionine and the reduction and oxidation of one carbon units (Wagner, 1995).

The particular role of folate in metabolic reaction is to accept or donate one carbon units in very key metabolic pathway reactions hence earning folate-requiring reactions the name one carbon metabolic reactions. In spite of the very important role of one carbon units in metabolic reactions, its synthesis together with synthesis of methyl groups are highly controlled in the body (Wagner, 1995). Coenzymes of folate and other pertinent enzymes are divided between the mitochondria and the cytosol. Products of metabolism easily cross between the cytosol and the mitochondria but the enzymes of folate do not cross. The knowledge in the area of nutrition in the regulation of one carbon metabolism is scanty. Studies however indicate that diet influences the intracellular concentration of folate (Dietrich, Brown, & Block, 2005). Hence poor folate status would impair the metabolism of one carbon units especially when there is severe deficiency.

The intestines receive folate from two main sources; the first is the dietary source where absorption occurs in the small intestines (Said et al., 2000). The absorption of folate occurs when the polyglutamates of dietary folate are hydrolysed in the brush border by conjugates (Allen, 2008). The second is via a bacterial source from the large intestines. Here folate is synthesized by the usual microflora in the large intestine. Absorption takes place afterwards (Said et al., 2000). The absorption of folate is reduced when intake is high. This is as a result of saturation that occurs in the folate transport system in the intestines (Allen, 2008).

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2.4.8 Causes, prevalence of folate deficiency and food sources

Low dietary intake is ranked the leading cause of folate deficiency (Allen, 2008). Folate deficiency tends to be high in populations where there is high consumption of refined cereals and low intakes of leafy vegetables and fruits which are high in folate (Shils & Shike, 2006). It is therefore likely that populations in developing countries may consume more folate than those in industrialized countries because of the high folate content of certain legumes, fruits and vegetables relative to refined cereals. The ratio of body stores to the daily requirements is given as 100:1. Hence just a few weeks of inadequate intake can give rise to folate deficiency (Shils & Shike, 2006). Establishing nutrient intake values for folate varies across different countries. This is based on the bioavailability of folate, the model indicator for folate adequacy, variability in requirements across different physiological groups and genetic variations in requirements (Stamm & Houghton, 2013). Generally, estimating folate intake in the population tends to be quite problematic due to the unreliability of folate content in food composition tables (Black, Paul, & Hall, 1985). Data available suggest that many women in their reproductive ages are not meeting the desired folate requirement (Gernand, Schulze, Stewart, West Jr, & Christian, 2016). According to Sherwood, Houghton, Tarasuk, and O'Connor (2006) one third of pregnant and lactating women may not be meeting their folate requirements based on dietary intakes alone. In a review of dietary intakes of pregnant women in high income countries, intakes of folate were found to be below recommendations (Blumfield et al., 2013). Lee et al., (2013), in a systematic review among pregnant women in low and middle income countries, reported that folate intakes among the women were below the estimated average requirements. Recommendations for folate requirements have been set to ensure that there is enough to maintain red blood cell levels and to meet physiological demands in pregnancy. The required amount needed for the one carbon metabolism to support maternal and placental tissue

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expansion and for foetal growth. The WHO recommends that 400 mg supplementation of folate in pregnancy is adequate to meet the physiologic demands (Gernand et al., 2016).

Conditions that will hinder or impair absorption and utilization of folic acid have also been implicated in folate deficiency. Chronic alcoholism for example can impair the absorption of folate (Wani et al., 2013). Absorption of folate in chronic alcoholism is associated with abnormalities in its metabolism, which may include decreased hepatic storage and inhibition of bone marrow response to folic acid. In effect, alcohol tends to decrease the intestinal absorption of both dietary polyglutated folate and its monoglutamyl folic acid (Halsted, Villanueva, Devlin, & Chandler, 2002). The effect is more intense in alcoholics who are malnourished.

Certain drugs are known to interfere with folate absorption. These medications include anti-epileptic drugs. Drugs such as phenytoin and carbamazepine can reduce the absorption of folate by up to 90% in patients receiving such medications (Morrell, 2002). The mechanisms by which these drugs reduce folate absorption is not fully understood. But it is believed that these drugs have anti-folate properties that tend to increase the homocysteine levels in the blood by decreasing folate absorption (Paknahad, Chitsaz, Zadeh, & Sheklabadi, 2012). In conditions such as coeliac disease where the disease is characterized by malabsorption, folate deficiency is also likely to be present (Halfdanarson, Litzow, & Murray, 2007). Polymorphisms in genes resulting in methylenetetrahydrofolate reductase deficiency and glutamate forminotransferase deficiency would also result in folate deficiency. Methylenetetrahydrofolate reductase is an enzyme that converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the active form of folate, and regulates intracellular folate levels (Leclerc, Sibani, & Rozen, 2013). This reaction is essential for the process that converts the amino acid homocysteine to methionine. Methionine is utilized by the body to produce proteins and other vital compounds.

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During physiologic periods of accelerated growth such as pregnancy, infancy and adolescence, there is a rise in the body demands of folic acid. During the period of pregnancy for instance, there is an increased production of the red blood cells and then growth of foetal tissues, resulting in increased demands for folic acid. The need for folate is most essential in the first month of pregnancy when the development of the neural tube takes place (Talaulikar & Arulkumaran, 2011)). In adolescence, the age of onset, velocity of change and the time of completion of the adolescent growth spurt affect the need and also the utilization of the nutrient. Thus, in the absence of methylenetetrahydrofolate reductase deficiency, 5-methyltetrahydrofolate cannot be synthesised especially during periods of physiologic accelerated growth, resulting in active folate deficiency (Daniel & Bennett, 1975).

Globally, only a small number of countries have national data covering folate status (de Benoist, 2008). The situation is even worse in developing countries. In Ghana, only a small number of studies have been conducted on the folate status of some sections of the population (Akwetea, 2015; Dei-Adomakoh et al., 2014; Owusu, Thomas, Wiredu, & Pufulete, 2010). Akwetea, 2015 reported folate deficiencies of 71% among respondents in a rural community. Females formed 30% of this percentage. Dei-Adomakoh et al., 2014 reported 16.7% prevalence of folate deficiency among pregnant women in Accra. In a study that investigated the folate status of Ghanaians living in London and those living in Accra, it was revealed that those living in London had higher serum levels of folate compared to those living in Accra. The authors explained that the differences could be due to the fact that the population in London consumed more food products fortified with folic acid as compared to those living in Accra (Owusu et al., 2010). Assessment of folate status of pregnant adolescent girls and adult women is lacking globally. However, few studies have been reported. Haidar (2010) reported 31.3% prevalence of folate deficiency in Ethiopian women. In Nepal, a study assessed the folate status of 1165

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pregnant women in their first trimester of pregnancy. Twelve percent of the women were found to be deficient in folate as compared to riboflavin (33%), vitamin B_6 (40%) and vitamin B_{12} (28%) (Jiang, Christian, Khatry, Wu, & West, 2005). In another study where the pregnancy was 28 weeks, about 26% had low deficiency status (Pathak et al., 2004b).

Dietary sources of folate are mainly green vegetables, legumes and some fruits such as orange and papaya (Allen, 2008). Other sources of folate that have been identified in Ghana include traditional soups and stews such as peanut (groundnut) soup, okro stews/soup, beans stew, kontomire (*Colocasia esculenta*) and fermented maize dishes (banku, kenkey, porridges) (Owusu et al., 2010).

2.4.9 Consequences of folate deficiency in pregnancy

As earlier stated, folate plays an essential role in DNA synthesis. The disturbance in DNA synthesis could result in abnormality in the division of cells (Scholl & Johnson, 2000). Pregnancy is one of the periods characterized by rapid and widespread cell division due to synthesis of red blood cell, enlargement of the uterus and the growth of both the foetus and the placenta. These are all heavily dependent on folate (Scholl & Johnson, 2000) and so folate requirements tend to increase during this period because of its peculiar role in synthesis of nucleic acids. Studies have reported associations between low serum concentrations of folate and low birth weight, preterm delivery, foetal growth retardation and spontaneous abortion. In a study to investigate the impact of folate from dietary sources and supplements on pregnancy outcome of 832 women, Scholl, Hediger, Schall, Khoo, & Fischer (1996) found that the women who had low folate intakes (less than 240 mg) and those with low serum folate had increased risk of delivering preterm and low birthweight babies. In another study, the researchers sought to examine the link between maternal B-vitamins concentrations in pregnancy and outcomes such as gestational age, birthweight and length among Singaporean women. The results of the

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study indicated that higher plasma concentrations of folate were associated with longer gestational age and reduced risk of preterm delivery but this was not the case for vitamin B_{12} and vitamin B_6.

There is an established link between folate and the development of neural tube defects (Pitkin, 2007). The mechanism by which the neural tube defect occurs and how folate tends to prevent this is still not well explained (Pitkin, 2007). In a review of several studies, an association between folate status of pregnant women and neural tube defects of their offspring was seen (Pitkin (2007). These studies led to the recommendations to supplement pregnant women with folic acid. In a meta-analysis Blom (2009) showed how the risks of occurrence of neural tube defect were reduced with peri-conceptional folic acid supplementation.

2.4.10 Roles of vitamin B_{12} and Folate in erythropoiesis and development of anaemia

The absence of folate and vitamin B_{12} in erythropoiesis results in megaloblastic anaemia. Megaloblastic anaemia is mainly characterized by pancytopenia (i.e reduced red blood cell, white blood cells and platelets) and reticulocytopenia (reduced reticulocytes) (Koury & Ponka, 2004). In situations of vitamin B_{12} and folate deficiency, there is a decreased de novo synthesis of deoxy nucleotides. This consequently leads to impairment in the synthesis and repair of DNA eventually leading to cell death. Under deficiency conditions of vitamin B_{12} and folate, erythropoiesis becomes ineffective due to the inability of the erythroid cells to become mature (Koury & Ponka, 2004). Vitamin B_{12} is required for the transfer of methyl group from the compound 5-methyl tetrahydrofolate in the conversion of homocysteine to methionine. In situations of vitamin B_{12} deficiency, folate remains bound to the methyl group creating a pseudo folate deficiency in a situation termed the 'methyl folate trap' preventing folate from being released (Scott, 1999). This interferes with methionine synthesis and synthesis of erythrocytes. Folate, also required for erythrocyte maturation and DNA synthesis when trapped, cannot be

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used for this function resulting in decreased and immature production of erythroid cells. The decrease in the numbers of erythroid cells survival in the terminal stages of erythropoiesis results in anaemia (Koury & Ponka, 2004).

2.4.11 Indicators of folate and vitamin B_{12} deficiency

Detecting the presence of folate or B_{12} deficiency in an individual without a proper indicative measure can lead to wrong diagnosis because, folate and vitamin B_{12} deficiencies manifest with the same symptoms, making it very difficult to distinguish between them clinically. Detecting the presence of folate or vitamin B_{12} and distinguishing one from the other depends largely on laboratory testing (Green, 2011).

Methods for assessing folate and vitamin B_{12} status are categorized into two. The first is measuring the concentrations of the vitamins directly in the blood and the second involves measuring the metabolites that accumulate in the blood as a result of deficiencies in the vitamin (Green, 2011). In the case of measuring the concentration of vitamins, blood cells, plasma or serum concentrations are used. Microbiological or radio-dilution methods in measuring the concentration can be applied (Mangels et al., 2011). The vitamin B_{12} can be carried by two different binding proteins. The first is transcobalamin which binds just a small portion of the total plasma vitamin B_{12} (20-30%) and is generally responsible for delivering vitamin B_{12} to cells in the body and therefore considered to be functionally important (Green, 2011). Haptocorrin is the second glycoprotein vitamin B_{12} gets attached to. Generally, using serum or plasma vitamin B_{12} gives a result of good sensitivity however poor in specificity. They are also good indicators for assessing long term and short term status of the nutrient. An individual with serum plasma concentration less than 200 pg/ml is said to be deficient.

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Plasma and serum folate on the other hand undergo daily changes which relate to frequent dietary intakes. The most reliable measurement that may be used in determining deficiency status is red cell folate. The second approach of assessing deficiency of vitamin B_{12} and folate as mentioned earlier on is the measuring of the metabolites homocysteine and methyl malonate (Allen, Stabler, Savage, & Lindenbaum, 1993; Carmel, Green, Rosenblatt, & Watkins, 2003; Green, 1995). The body requires the presence of both vitamin B_{12} and folate to utilize homocysteine. In their absence there is accumulation of homocysteine in the blood. On the contrary, methyl malonate needs only vitamin B_{12} for its conversion to succinate. This implies that vitamin B_{12} deficiency alone causes the rise in plasma methylmalonate (Carmel et al., 2003; Green, 2008).

2.5 Other nutrients related to nutritional anaemia

2.5.1 Copper

Copper is essential for electron transfer in oxidative-reductase activities. An adult of 70 kg has about 110 mg of copper in the body (Uauy, Olivares, & Gonzalez, 1998). The brain and liver contains about 25% of the amount of copper in the body (Uauy et al., 1998). A large proportion of copper is absorbed in the duodenum, the rest being absorbed in the stomach and jejunum (Linder & Hazegh-Azam, 1996). The mechanism by which copper is absorbed is not well understood. It is transported from the intestinal mucosa to the blood in the form of copper albumin complex. Approximately 15-80% of copper is absorbed by the body from dietary sources.

The absorption of copper is dependent upon dietary intakes and the individual's nutritional status (Turnlund, Keyes, Anderson, & Acord, 1989). Human milk, proteins from animal sources and histidine enhance copper absorption considerably. On the other hand, phytates, zinc, ascorbic acid and cow's milk negatively affect copper absorption (Mills, 1985).

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Copper deficiency arises mainly as a consequence of low stores at birth, poor absorption, inadequate dietary intake and increased requirement as a result of the physiological state and malabsorption conditions like celiac disease, short bowel syndrome and cystic fibrosis (Olivares, Hertrampf, & Uauy, 2007). One of the common clinical manifestations of copper deficiency is anaemia. Copper is a component of ceruloplasmin which is needed in the conversion of Fe^{2+} to Fe^{3+}, which is essential in converting Fe^{2+} to Fe^{3+}, a vital step needed for the incorporation of iron into transferrin (Olivares et al., 2007). It is hypothesised that anaemia occurs as a result of defective mobilization of iron due to the reduction in the activity of ceruloplasmin.

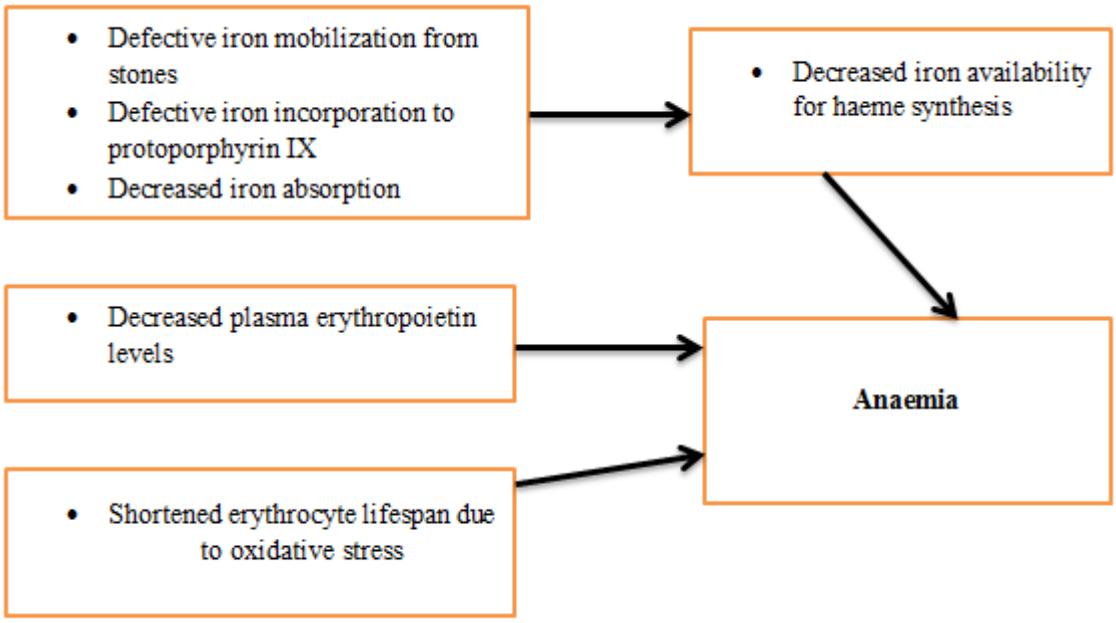

Figure 2.2: Possible mechanism by which copper deficiency can cause anaemia

Source: Olivares et al, 2007

2.5.2 Zinc

Zinc is one of the common minerals in the body. The body contains about 1.5 to 2.5g of zinc. The element has three distinct functional roles classified as catalytic, structural and regulatory (Olivares et al., 2007). These specific roles make zinc essential in metabolism, differentiation

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and cellular growth. The concentration of zinc in the body affects the outcome of pregnancy, growth and development of the foetus, linear growth and exposure to infections and neuro-behavioural development (Salgueiro et al., 2002).

The main absorption site of zinc is in the small intestine (Cousins, 1985). Absorption of zinc is dependent on the physiological state, condition of stress such as disease infection, dietary content of zinc, zinc status of the individual as well as its bioavailability (Olivares et al., 2007). Absorption is generally enhanced by animal proteins such as eggs, beef and cheese. The main inhibitory factors are phytates. However, processes such as soaking, germination and fermentation improves the absorption (Roohani, Hurrell, Kelishadi, & Schulin, 2013).

Zinc deficiency is mainly as a result of increased requirements that may arise due to physiologic states, low dietary intakes, malabsorption and impaired utilization. Zinc deficiency is suspected to result in anaemia by altering erythropoiesis. However the mechanism by which zinc contributes to erythropoiesis thereby contributing to anaemia is not fully explained (Olivares et al., 2007). However, three potential mechanisms have been suggested. These could be as a result of decreased erythroid proliferation, decreased plasma levels of erythropoietin or due to a shortened life span of erythrocytes as a result of oxidative stress because zinc is a cofactor for red blood cell superoxide dismutase (Olivares et al., 2007).

2.5.3 Vitamin A

Previous studies have drawn a link between vitamin A deficiency and anaemia. Studies involving supplementation of pregnant women with either vitamin A alone or in combination with some other nutrients reported increases in haemoglobin concentrations (Panth, Shatrugna, Yasodhara, & Sivakumar, 1990; Suharno, Karyadi, West, & Hautvast, 1993). Three main mechanisms have been proposed as the possible means by which vitamin A could cause

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anaemia. These are through modulation of erythropoiesis, modulation of metabolism of iron and modulation of immunity to infectious diseases and the anaemia of infection (Semba & Bloem, 2002).

2.5.4 Selenium

Selenium is an essential trace mineral found in the body in the form of selenomethionine and selenocysteine. The human body is unable to synthesize selenium. Selenium is one of the components of several of the antioxidant enzymes, 25 of which had been identified as of 2006 (Moghadaszadeh & Beggs, 2006). Sea foods and organ meats such as liver, kidney and the heart are some of the richest sources of selenium (Semba, 2007). There is also considerable amount of selenium in cereals and grains depending on the soil content of selenium from where the plant was grown. The body absorbs about 50-100% of the selenium consumed (Semba, 2007).

The possible mechanism by which selenium could contribute to the pathogenesis of anaemia is through the function of the enzyme glutathione peroxidase. Selenium is a key component of this enzyme which is known to protect haemoglobin from oxidation in the red blood cells (Semba, 2007). Low selenium content is also reported to be associated with decreased life span of the red blood cells (Fontaine, Valli, & Young, 1977).

2.6 Nutrient intakes and dietary diversity in achieving micronutrient intakes

Poor dietary intakes of pregnant adolescent girls have adverse consequences for both the mother and the foetus. Dietary intakes of both macro and micronutrients should be adequately met by the pregnant adolescent to ensure a safe pregnancy outcome.

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2.6.1 Energy and protein intakes in pregnancy

Associations have been reported between dietary energy intake and pregnancy weight gain. A systematic review that explained whether energy and other macronutrient intakes were associated with pregnancy weight gain, found a positive association. However, the contribution of macronutrients could not be established (Tielemans et al., 2015). In fact, pregnancy weight gain has been associated with pregnancy outcomes. The risk of low birth weight and preterm delivery increases with too little pregnancy weight gain whiles too much weight gain has also being linked to caesarean delivery, gestational diabetes, large for gestational age and increased retention of weight gained after delivery (Tielemans et al., 2015). There is also a further link of excessive weight gain in pregnancy to childhood obesity which has a further established association with chronic diseases later in life (Herouvi, Karanasios, Karayianni, & Karavanaki, 2013; Nehring, Lehmann, & von Kries, 2013).

Energy intake of varying degrees have been reported among adolescent pregnant girls. The estimated energy requirement for adolescent girls is about 2,368 kcal. In pregnancy, an additional 340 kcal and 452 kcal are required in the second and third trimesters of pregnancy respectively (DRI, 2004). Generally, dietary intakes in pregnant girls have been reported to be inadequate in energy both in developed and developing countries (Derbyshire, 2009; Oguntona & Akinyele, 2002; Pena, Sanchez, Portillo, & Solano, 2003; Shipala, Wafula, Ettyang, & Were, 2012). The main reasons ascribed to these inadequate intakes bordered around perceived food shortage, cultural restrictions, poor nutrition knowledge and low educational levels.

Several studies have reported on balanced protein and energy supplementation in pregnancy. Balanced protein supplementation is defined as supplementation in which the protein portion of the diet provides less than 25% of overall energy content (Kramer & Kakuma, 2003). Providing balanced protein energy supplementation to both nourished and undernourished

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women has proven to be beneficial. It provides significant improvement in foetal growth and development in multiple ways including reduction in low birth weight, small for gestational age and still births (Imdad & Bhutta, 2012). In a systematic review by Liberato, Singh, and Mulholland (2013), balanced protein energy supplementation with proteins providing at least 20% of energy improved birthweight by 95-324 g and height by 4.6-6.1mm. However, the benefits were seen to be more evident in undernourished women (Imdad & Bhutta, 2012). Contrary to earlier beneficial reports, protein energy supplementation did not improve pregnancy outcome among Indian women who were underweight in a recent publication. The authors explained that the effect could not be seen probably due to the small sample size used in their study compared to other reports (Dwarkanath et al., 2016).

2.6.2 Dietary diversity and nutritional status of reproductive age women

Dietary diversity (DD) is a qualitative low cost tool that gives a reflection of access to variety of foods in the household (Household Dietary Diversity, HDD) and also serves as an alternative tool that can be used to assess the nutrient adequacy of diets of individuals (Individual Dietary Diversity, IDD) (FAO, 2007). It evaluates two main components of diet quality; the first is the nutrient adequacy of the diet and the second the balance or variety of the diet (FAO & FHI360, 2016). It has been useful in accessing the macro and micronutrient adequacy of the diet in population groups such as infant and young children and women of reproductive ages (FAO, 2007). This has led to the recent development of the Minimum Dietary Diversity for Women of reproductive age (MDD-W).

The MDD-W is an indicator which describes whether or not women of reproductive ages (15-49 years) have been able to consume a minimum of five out of ten defined food groups from the previous day. This indicator can be used as an alternative to describe the minimum proportion of women in a population who achieve micronutrient adequacy (FAO & FHI360,

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2016). It is a newly developed tool that has been designed to reflect the micronutrient adequacy component of the determination of diet quality (FAO & FHI360, 2016). The Minimum Dietary Diversity for Women of reproductive age (MDD-W) indicator covers 11 micronutrients (iron, folate, vitamin B_{12}, vitamin C, vitamin B_6, niacin, riboflavin, vitamin A, thiamine, calcium and zinc) in its assessment. The MDD-W assessment is obtained based on 10 main food groups whiles the preliminary developed Women's Dietary Diversity (WDD) covered 9 food groups. Women's Dietary Diversity (WDD) was developed as an initial step towards the development of a more dichotomous tool (FAO & FHI360, 2016). MDD-W became necessary with the continuous call for a more dichotomous tool. Reproductive women who consume foods in five or more food groups out of the 10 are more likely to have a better micronutrient adequacy when compared to those who consumed less (Martin-Prével et al., 2015). The food groups as described by the FAO (FAO & FHI360, 2016) are fully expanded in the methodology section. They are the grains, tubers and white roots and plantains group, pulses (lentils, beans and peas) group, nuts and seeds group, dairy group, poultry, fish and meat group, eggs, dark green leafy vegetable group, other vitamin A rich fruits and vegetable group, other vegetables group and other fruits group.

Reproductive age women living in poor countries are at a greater risk of deficiency in micronutrients mainly as a result of low intakes arising from the lack of dietary diversity in their diet (Arimond et al., 2010). Dietary diversity has been used as an indicator to assess the micronutrient quality of females in their reproductive years. Zerfu, Umeta, and Baye (2016) in a longitudinal study carried out among pregnant women, reported low dietary diversity to be associated with low birth weight, preterm delivery, still birth and maternal anaemia. Among Mozambican adolescent girls, dietary diversity was found to be positively associated with serum zinc. However, this was only observed during the season of hunger. In a similar study

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conducted in Kenya, dietary diversity correlated positively with serum retinol (Fujita, Lo, & Baranski, 2012). On the contrary, Ali, Thaver, and Khan (2014) reported no association between dietary diversity and haemoglobin status although there was a positive association with weight gain during the pregnancy period. In Ghana, published studies reporting on dietary diversity in reproductive age women is lacking. However in a very recent publication, Saaka, Oladele, Larbi, and Hoeschle-Zeledon (2017) observed among rural Ghanaian women no association between dietary diversity and anaemia regardless of the socioeconomic status of the women even though in an earlier study an association was observed among urban women (Saaka & Rauf, 2015). The authors explained that the disparities might have occurred due to the fact that analysis were only carried out in the third trimester for the urban study while the rural study analysed all participants irrespective of their trimester. In a related study, also among reproductive Ghanaian females (15-49 years), no significant association was observed between Dietary Diversity Score (DDS) and prevalence of iron deficiency anaemia. However, DDS was significantly associated with haemoglobin levels but not with anaemia (Kubuga, Lee, Song, & Song, 2016).

2.7 Nutritional knowledge, myths and misconceptions of nutritional anaemia

Nutrition knowledge is imperative especially during the period of pregnancy. According to Blondin & LoGiudice (2018), the level of nutrition knowledge in women increases when they get pregnant. Also, Szwajcer, Hiddink, Maas & Koelen, et al., (2008), found that among Dutch women, interest in nutrition related information heightened when they were pregnant compared to the pre-pregnancy period. Those in their first trimester were found to be particularly more interested in obtaining nutrition related information. This may be mainly because they recognised how critical the period of pregnancy is and the impact of nutrition on their health

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and that of their developing baby. It is therefore important for pregnant women to be provided with the necessary counselling or education that can improve their knowledge in nutrition.

Nutrition education and counselling is crucial as it has been found to be a common strategy that can be used to improve the nutritional knowledge of women during the course of pregnancy (Girard & Olude, 2012). The need for pregnant women and the adolescent girl to be knowledgeable in nutrition related issues cannot be overemphasised because of the strong evidence linking nutrition knowledge during pregnancy to the outcome of pregnancy. Nutrition knowledge decreases the risk of preterm delivery as well as some foetal complications (Girard & Olude, 2012). Also, counselling on nutritional issues in the course of pregnancy have been found to lower the risk of maternal anaemia by 30% (Darnton-Hill, 2013). Blondin & LoGiudice (2018), in studying pregnant women's knowledge and their awareness of nutrition, found that, in their search for nutrition related information, their sources of information may be unreliable. Additionally, even though some pregnant women may be eager to acquire nutrition related information, they may not be adequately fed with the required information. Similarly, in a suburban rural region of central Pennsylvania, pregnant women thought they were not given enough nutrition education from their healthcare professionals especially when they have had a previous pregnancy. This was on the assumption that if they have had a previous pregnancy then they were more likely to know what should be important to eat in terms of their nutrition with the current pregnancy (Downs, Savage & Rauff, 2014).

In their eagerness to obtain nutrition information, many pregnant women resort to various channels to obtain the information. In a qualitative study, Blondin & LoGiudice (2018), reported that majority of the pregnant women revealed that their primary source of information was the use of the internet search engine GOOGLE. They maintained that with just a click of the bottom they were able to retrieve the kind of information they needed. Among some low

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income pregnant women in the South Eastern part of the United States, pregnant women's main sources of information were from health professionals, family members and through reading (Lewallen, 2004). In Ethiopia, Daba, Beyene, Fekadu, & Garoma (2013), found that the pregnant woman's ability to obtain nutrition related information was significantly associated with educational level, occupational status and nutritional attitude. Those with higher educational status were more likely to have higher levels of nutrition knowledge compared to those with lower levels of the education. This they explained could be attributed to their access to the internet and other educative materials.

In some jurisdictions there are taboos or myths and misconceptions associated with certain foods that have an impact on the anaemia situation of the pregnant woman. Different types and forms of misconceptions and cultural beliefs about certain kinds of foods exist in several countries (Quiroz & van Andel, 2015). A number of these myths and misconceptions have been identified to be practiced especially in pregnancy.

In some parts of Nigeria, pregnant women are forbidden to consume snails because they believe if they do, their babies will become sluggish (Ekwochi et al., 2016). Also consumption of grasscutter is forbidden because it is believed to prolong and make labour difficult (Ekwochi et al., 2016; Maduforo, 2010). Leafy vegetables which may contribute to good sources of iron are also considered as harmful because they are believed to form particles which when attached to the baby's head could be harmful (Zerfu et al., 2016b). In another study that investigated the barriers to the prevention of anaemia among Indonesian nurse-midwives, cultural beliefs concerning the consumption of fish, meat and eggs were forbidden in pregnancy. This is because they were believed to produce bad odour in the blood. This was stated by the nurse-midwives as one of the three main reasons that made it difficult to prevent anaemia during pregnancy (Widyawati et al., 2015). In the Kalenjin community in Kenya, animal organs, meat

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and eggs were seen to be restricted by 96%, 87% and 68% of pregnant women respectively. The reasons given for the restrictions included baby talking too much (if the organ meat consumed is the tongue of the animal), baby becoming too big thereby bringing misfortune to the baby or mother (when meat is consumed) and high blood pressure or colic pain in babies when eggs are consumed (Riang'a, Broerse, & Nangulu, 2017).

In Ghana, there are some documentation of myths and misconceptions about food during pregnancy that can impact on anaemia development in the pregnant woman. In a study conducted in the Western Region of Ghana, mainly among rural folks, meat, beans, 'kontomire' (taro leaves) and turkey berries (*Solanum Torvum*) were strongly recommended for pregnant women to consume as it was believed they promote blood formation and also makes the woman deliver strong babies (Otoo, Habib & Ankomah, 2015). On the contrary, in the Upper East Region of Ghana among the Kassena and the Nankana people, the diet of the pregnant woman was restricted to the consumption of vegetables. Meat and groundnuts are prohibited as it is believed to lead to the birth of "spirit children" (Nti, Laweh, & Gyemfua-Yeboah, 2002; Arzoaquoi, Essuman, Gbagbo, Tenkorang, et al., 2015). Consumption of snails is also widely prohibited in pregnancy across many of the tribes in Ghana. The major reason given for the prohibition is the belief that children born to pregnant women who ate snails will become sluggish or tend to drool (de-Graft Aikins, 2014; Otoo et al., 2015; Gadegbeku , Wayo, Ackah-Badu, & Nukpe et al., 2013).

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CHAPTER 3

3.0 METHODOLOGY

3.1 Study area

The study was carried out in three health facilities in the Accra Metropolis of the Greater Accra Region of Ghana. According to the Ghana Statistical Service (GSS, 2015), the population of the Metropolis is 1, 665,086. Females formed 51.9% of the total population. The population of adolescent girls between 15-19 years is 88,278 forming about 10.2% of the total population. About 5% of the adolescents are married with another 1.5% cohabiting. Some (20.7%) of the adolescent girls engage in various economic activities. The informal private sector employs the largest number of the total populace (74%). The private formal sector forms the second largest employer (16.9%) with the government employing 7.8%. Females are largely employed in the private formal sector (82.9%). In terms of religious affiliations, Christians are predominant (78.7%), followed by Islam (17.0%) (GSS, 2014). The three health facilities where the study was carried out are located in three different municipalities in the Accra Metropolis (Figure 3.1), namely Ablekuma South (South-West), Ga South (South-Central) and La Dade-Kotopon (South-East).

The Ussher Polyclinic is located in James Town, one of the oldest towns in the Ga South Municipal Assembly. The polyclinic serves several communities in the catchment area such as James Town, Accra Central, and Abossey Okai. It has a special clinic for ante natal services where pregnant women are required to regularly attend ante natal care and education. Pregnant women are usually provided with nutritional education and education on other pregnancy related issues. The La General Hospital is located in the La Dade-Kotopon Municipal Assembly with various departments serving the populace in the area. The hospital has a bed capacity of

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about 166 and serves various communities including La, Osu, Labone and Teshie. It also has a special clinic for ante natal services. The Mamprobi Polyclinic is located at the Ablekuma South metro of Accra. It also serves a wide community of people including people in areas such as Dansoman, Laterbiokorshie, Korle-Bu and Mateheko.

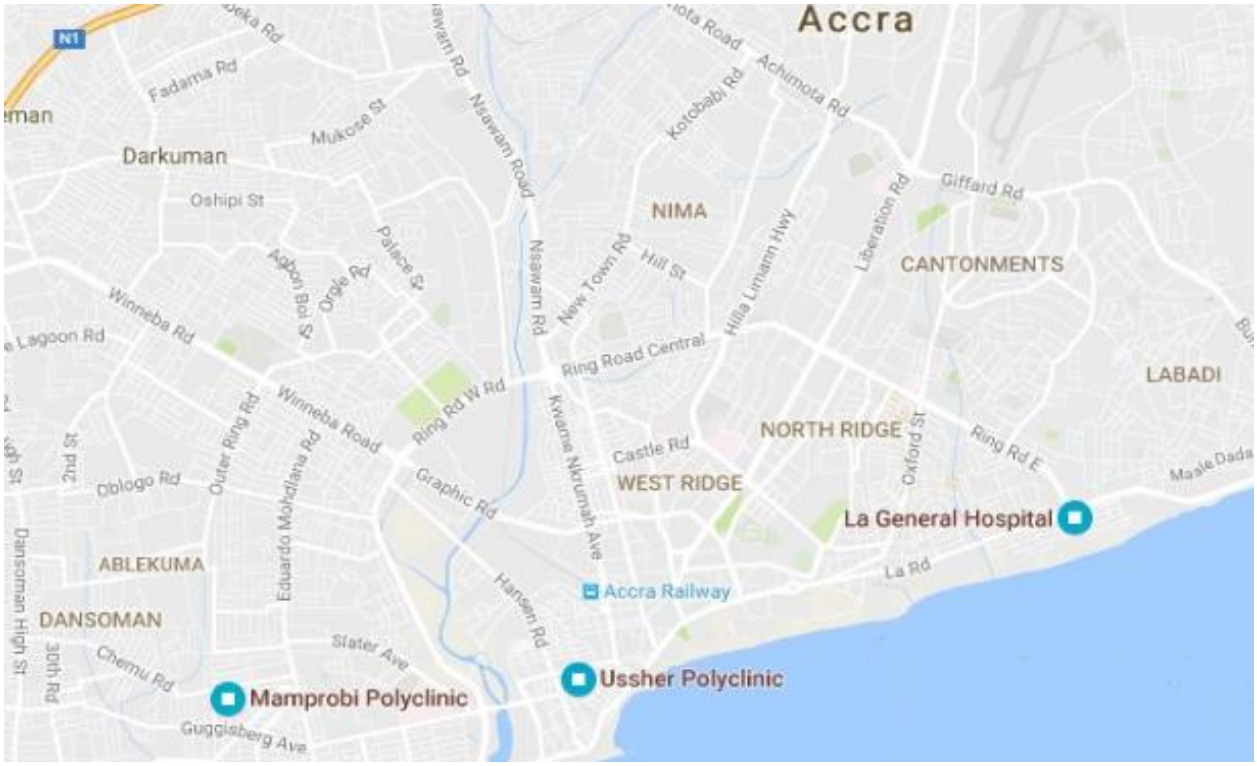

Figure 3.1: Location of the three health facilities on google map

3.2 Study design

The design for this study was cross-sectional. Background information, blood samples and dietary information were collected from participants on the same day they were recruited. Follow-up calls were made to collect remaining information on dietary intakes where necessary.

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3.3 Study population

The study population was made up of pregnant adolescent girls between the ages of 15-19 years. These girls should have been confirmed to be pregnant and scheduled to start ante-natal care. The pregnant girls should also be attending anyone of the three health facilities for ante-natal care.

3.4 Sample size determination

The sample size to ascertain the number of pregnant adolescent girls recruited into the study was determined using Epi-Info version 7.2 StatCalc calculator. The following parameters were imputed to compute the sample size;

Population size- 88,278 adolescent girls (GSS, 2015)

Expected Frequency- 50% based on deficiency prevalence in adolescent girls (Kapil & Bhadoria, 2014).

Margin of error- 5%

Confidence interval- 95%

The calculated minimum sample size was 245. However a total of 265 pregnant girls were recruited.

3.5 Inclusion criteria

Apparently healthy pregnant adolescent girls between the ages of 15-19 years who had reported to the ante-natal clinic for ante-natal services for the first time during the pregnancy. They should not have been on any form of haematinics at the time of recruitment.

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3.6 Exclusion criteria

The exclusion criteria were pregnant adolescent girls who tested positive for any kind of haemoglobinopathy and already on any haematinics. Those who had worm infestations or occult stool blood were also excluded after screening.

3.7 Participant Recruitment

A period of about 4-5 months was used at each hospital to recruit participants for the study. A two-stage sampling procedure was used. The first involved the selection of health facility for the study and the second the selection of the pregnant girls. Health facilities for the study were selected based on the location (West, Central and East) and the frequency of cases of adolescent pregnancies reporting for antenatal care in the facility. The three health facilities that tended to have the highest prevalence of adolescent pregnancies in the area were selected. The second stage was the selection of participants from the selected health facilities. All adolescent girls who came for ante-natal care for the first time were approached and asked of their willingness to participate in the study after they had been given information on what the study entailed. Those who agreed and met the selection criteria were recruited after filling and signing the informed consent form.

3.8 Data Collection

Data were obtained through interviewer administered questionnaires and results of laboratory investigations.

3.8.1 Background Information

A semi- structured questionnaire was used to collect socio- demographic information from the participants (Appendix A). Data collected included participant age, educational level, marital

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status and occupation if they engaged in any. Other relevant information such as use of food supplements and pica practice were also obtained.

3.8.2 Dietary intakes and nutrient intake assessment

Two retrospective methods for dietary assessment were used to determine nutrient intakes and dietary patterns among the girls. These were the 24-hour dietary recall and the food frequency questionnaire. The questionnaires were administered by a trained research assistant and the researcher.

3.8.2.1 24-hour dietary recall

The 24-hour dietary recall method is a retrospective way of quantitatively obtaining dietary intake information from individuals (Johnson, 2002). It can be used to assess the total dietary intake or some aspects of the diet. It can also be used to evaluate the total nutrient intake if dietary supplement information is obtained during the interview. The dietary information gathered can also be used to assess the diversity of the diet consumed. Unlike other methods for obtaining dietary information, this method is economical, easy to use and puts little burden on the respondent. It can be used in situations where respondents cannot read nor write because the trained interviewer does the recording (Johnson, Soultanakis, & Matthews, 1998).

The major limitation of this method is its dependence on the memory of the respondent. Nonetheless, a good interviewer could adopt memory joggling techniques that can help improve the recall to obtain very reliable information. There have also been concerns over the number of recalls that could provide more accurate estimate of dietary intakes. Some researchers assert that between 2-7 times of recalls are acceptable (Bernard, 2006; Posner, Martin-Munley, Smigelski, & Cupples, 1992). Another school of thought also suggest another recall method

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such as the food frequency to be added to the 24-hour recall to compliment the information obtained (Posner et al., 1992).

A three-day (2 week days and a weekend) 24-hour dietary recall method was used to quantitatively determine the nutrient intakes of the girls. The first recall was taken on the day of the interview. Subsequent recalls were made through telephone interviews. The girls were made to recall all foods and drinks they had taken in the past 24-hours. They were provided with familiar household measures such as spoons, ladles, sardine tins etc. to aid with a more accurate estimation. The estimated foods were further converted to grams. These were then inputted into the Microdiet dietary analysis software (Downlee Systems, UK) to generate data on the nutrient intakes. The mean intake of three (3) completed days was computed for each participant. Dietary data analysis were carried on individuals with three (3) full days 24-hour dietary recall.

3.8.2.2 Food frequency questionnaire

The food frequency questionnaire (FFQ) is also a retrospective method used to obtain dietary information on an individual's usual intake or pattern. It consists of a list of food items and beverages with categorized responses for the respondent to indicate their frequency of consumption. The FFQ is also economical and easy to use. It can also be self-administered. The advantages of this method include little training required to administer, information can be obtained in one time visit and it can be used to obtain information on inadequate intakes in any of the food groups and so doing identify possible nutrient deficiencies. The FFQ is also disadvantaged in its major reliance on memory. The administration of the questionnaire can also be boring since it involves a long list of foods and repetitive responses (Adamson et al., 2009).

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The FFQ used for this study was adopted from a validated checklist developed for a Ghanaian population to determine dietary patterns and intakes of micronutrients (Owusu, 2008). The checklist was developed from the Ghanaian food composition tables (Eyeson, 1975) and also incorporated a validated folate food frequency questionnaire (Pufulete, Emery, Nelson, & Sanders, 2002). The girls were asked to indicate the number of times they had consumed particular foods over specified periods. The details of the checklist is provided in Appendix B.

3.9.0 Determination of dietary diversity

The Food and Agricultural Organization (FAO) defines dietary diversity as a qualitative tool used to measure food consumption that reflects household access to a variety of foods. It also serves as a proxy measure for nutrient adequacy of the diet of the individual (FAO & FHI360, 2016). It is an easily administered low cost tool but effective in determining diet quality. It is also useful in determining micronutrient quality of the diet.

The dietary diversity is determined based on the data obtained from the 24-hour recall. Foods consumed were grouped into 10 food groups based on the Minimum Dietary Diversity Score indicator for women of reproductive age (MDD-W) groupings (FAO & FHI360, 2016). The minimum 15 g rule is used as indicative of food consumption from a group. This implies that food is deemed to have been consumed if an individual consumed at least 15 g. A score of one is given if the food is consumed and zero when not consumed. The MDD-W covered the following food groups;

1. Grains, tubers and white roots and plantains
2. Pulses (lentils, peas and beans)
3. Nuts and seeds
4. Dairy
5. Poultry, fish and meat

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6. Eggs
7. Dark green leafy vegetables
8. Other Vitamin A-rich fruits and vegetables
9. Other vegetables
10. Other fruits

Detailed description of the food groups is given in Appendix C.

3.10.0 Laboratory procedures

The serum concentrations of folate, vitamin B_{12}, iron, total iron binding capacity (TIBC) and ferritin in the blood of the pregnant adolescent girls were determined. Serum folate and vitamin B_{12} were determined using the ELISA test kits; ID-Vit folic acid and ID-Vit B_{12} kits (Immundiagnostic AG, Germany) respectively. Serum iron and TIBC were determined using the VITROS Fe Slide and VITROS Chemistry Calibrator Kit 4 on VITROS Chemistry systems (Ortho-Clinical Diagnostics, UK). Serum ferritin was determined with the AccuBind ELISA microwells test kit (Monobind Inc. USA). Analysis were carried out at the Virology laboratory of the School of Biomedical and Allied Health Sciences, University of Ghana and the Biochemistry Department of the Korle-Bu Teaching Hospital. A detailed description of procedures are outlined in appendix D.

3.11.0 Determination of dietary practices, knowledge on nutritional anaemia and myths/misconceptions of dietary intakes in pregnancy

A total of four (4) focus group discussions were conducted to assess in-depth views of the pregnant adolescent girls on dietary practices, knowledge on nutritional anaemia and myths/misconceptions of dietary intakes in pregnancy. Their sources of information relating to nutritional anaemia (iron, folate and vitamin B_{12} deficiencies) were also obtained. To ensure uniformity of questions asked at every meeting, a discussion guide was employed (Appendix

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E). An average of 5-7 adolescent girls were purposively organized for each focus group (Figure 3.3). Those who satisfied the inclusion criteria were invited to partake in the focus group discussions.

At least one focus group discussion was conducted at each of the study sites. For each discussion, adolescent girls were assigned codes to ensure confidentiality. Discussions were audio taped after obtaining consent from participants.

Figure 3.2: Sections of the pregnant adolescent girls being prepared for the focus group discussions

3.12 Ethical considerations

3.12.1 Ethical clearance

The proposal for this study was reviewed and granted ethical clearance by the Ethics and Protocol Review Committee of School of Biomedical and Allied Health Sciences, University of Ghana (Appendix F). The study adhered to the required ethical protocol for studies involving human subjects.

3.12.2 Permission and Informed consent

Permission to carry out the study in the various health facilities was obtained first from the Municipal health directorate and then the various administrations of the health facilities. The girls were provided information about the study (Appendix G). The information provided

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included the objectives of the study, procedures involved, the benefits, confidentiality and voluntariness of the study participants. Those who provided consent were included in the study.

3.13 Data Management/ confidentiality

During the individual interviews, privacy was ensured and questionnaires kept away from unauthorized persons. Data collected were kept confidential and only accessible to investigator. Participants were assigned codes to hide their identity from unauthorized individuals. The data on questionnaires and laboratory analysis were transferred into a statistical software and password protected. Hard copies of the questionnaires and laboratory reports were kept under lock and key to protect them from unauthorized individuals.

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3.14 Data capture and analysis

The IBM SPSS version 20.0 software was used for data entry, cleaning and analysis. Preliminary analysis was done to check for the normality of data. In addition, categorizations of data according to standardized reference values were done as follows;

3.14.1 Nutrient intakes and MDD-W determination

Nutrient intakes were reported with means, standard deviations and confidence intervals set at 95%. To determine the adequacy of nutrient intakes, the means were compared with the Estimated Average Requirements (EAR) reference values from the Institute of Medicine (Food and Nutrition Board, 2005). The comparison was done taking into consideration the ages of the girls and the trimester of pregnancy. The cut-off point for the estimation of energy intakes was based on the Estimated Energy Requirements (EER).

Frequencies of consumption of foods were defined as follows;

More frequently: greater or equal to five times a week

Frequently: 1-4 times in a week

Sometimes: 1-2 times in a month

Rarely/never: less than 1 in a month

With the determination of MDD-W, a score of one was awarded when a food is consumed from a food group and zero awarded when food was not consumed from a food group. The averages of the scores were computed. The highest possible total score of MDD-W to achieve was ten and the lowest score was zero. A score greater or equal to five was considered good and a score less than five was considered poor.

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3.14.2 Biochemical analysis

Categorization of anaemia was based on WHO reference classification of anaemia as follows;

Determination of girls who were below recommendations for serum folate and serum vitamin B_{12} were classified as shown below;

Classification	**[a]Folate**	**[b]Vitamin B_{12}**
Normal	≥ 4µg/L	≥ 250 ng/L
Low/deficient	<4 µg/L	<250 ng/L

[a]deBenoist (2008)
[b]Berg & Shaw (2013)

Serum ferritin reference values were compared to Monobind-Inc. Laboratory USA, values for females of reproductive ages. Those with serum ferritin concentrations between 10-124 ng/ml were considered to have normal serum concentrations. Serum iron and TIBC concentrations were compared with reagent manufacturers reference values as follows;

Classification	**Serum Iron**	**Serum TIBC**
Low	<37µg/L	<47.4 µmol/L
Normal	≥37 µg/L	47.4-89.0 µmol/L
High	-	>89.0 µmol/L

(Ortho-Clinical Diagnostics, UK**)**

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3.15 Descriptive statistical analysis

Descriptive variables were used to summarize data either on tables or bar charts. Prevalence of anaemia and nutrient deficiencies of iron, folate and vitamin B_{12} were determined using frequencies (percentages). Means and medians were used to estimate central tendencies. Standard deviations and confidence intervals were used to measure the spread of data.

3.16 Inferential statistics

Data that were not normally distributed were transformed in SPSS using the logarithmic transformation method. Statistical significance was set at $p < 0.05$. Pearson's correlations were used to determine relationships between various variables (e.g haemoglobin, dietary iron, serum iron etc).

Z-test for proportions were used to test for statistical differences between proportions: Differences between proportions of the background characteristic variables of the girls, the differences between the proportion of girls who had adequate dietary intakes and those who did not and differences between the proportion of girls with good MDD-W and those with poor MDD-W were all determined using Z-tests.

Differences between continuous variables were determined using ANOVA (In cases where post hoc analysis were done, the Bonferroni method was used) and independent t-tests.

Chi-square test was used to test for association between proportion of girls with good and poor MDD-W and trimester of pregnancy.

ANOVA and independent t-tests were used to determine differences between means of dietary intakes in comparison with various socio-economic variables and the trimester of pregnancy. Differences between the means of the various biochemical indicators of nutritional anaemia

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(iron, vitamin $B_{12,}$ folate, ferritin and TIBC) were determined by T-tests/ANOVA. ANOVA was used in the case of comparisons of more than two means and independent t-tests in the case of comparison between two means. One sample t-tests were used to compare differences between mean nutrient intakes and EARs or RDAs.

Pearson's correlations were used to determine possible relationship between variables. Logistic regression modelling was used to predict MDD-W and girls who were more likely to be anaemic. The Hosmer-Lemeshow goodness of fit test was used to assess how good the model was fitted to the data in all of the logistic regression analysis. Confidence intervals were set at 95% and odds ratios were used in describing the strength of association observed.

3.17 Qualitative analysis

The audio taped data were transcribed and typed into Microsoft word, coded and entered into NVIVO version 11 (QSR International, Australia). The thematic approach was employed in the analysis of data obtained from the focus group discussions. This followed the six (6) main steps described by Braun and Clarke (2006) in conducting thematic analysis namely familiarisation of oneself with data, generation of codes, searching for themes, reviewing themes, defining and naming themes and the production of the report. In the production of report, relevant and enthusiastic responses were underscored.

3.18 Quality assurance

A pilot study was initially carried out at the Madina Polyclinic (Kekele) to assess the research instrument to be used. Questionnaires were pre-tested to ensure that participants understood the questions and provided appropriate responses. Three (3) data collection personnel were taken through a day of training to ensure effective administration of questionnaires. Their educational backgrounds were senior high school and first degrees. Females were used to administer

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questionnaires to adolescent girls to ensure participants felt at ease in their responses. The training involved how to administer the questionnaires and obtaining dietary information using the 24-hour dietary recall method. Focus group discussions were held in enclosed rooms to ensure the privacy of the girls and to encourage openness in the discussions. All laboratory analyses were performed by qualified laboratory trained personnel from the University of Ghana and the Korle-Bu Teaching Hospital using both internal and external quality control measures.

Data entry were carried out by two research assistants. Data were cleaned and cross checked by principal investigator to minimize errors with data entry.

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CHAPTER 4

4.0 RESULTS

4.1 Background characteristics of pregnant adolescent girls

The background characteristics of the adolescent girls are presented in tables 4.1a and 4b. Their ages ranged between 15 -19 years. The mean age was 17.88 (SD = 1.08) years. For the focus group participants the mean age was 17.44 (SD = 1.29) years. The age distribution of the girls is presented in figure 4.1.

Table 4.1 describes the sociodemographic characteristics of the pregnant girls. A lower proportion (2.6%) of the girls had Senior High School as their highest level of formal education. About 27.5% had no formal education. The vast majority (96%) were single. Christians were 75.9%. The proportion that were unemployed was 47.5%. The highest proportion (41.5%) lived with their parents compared to living with husband/partner, alone or with other relative/friend. The use of supplements before pregnancy was very low (0.4%). A few of the girls (9%) practised pica.

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Table 4.1: Background characteristics of pregnant adolescent girls (N=265)

Variable	Total
Age (years)	
Mean (SD)	17.88 (1.08)
Range	15- 19
Educational level	**N (%)**
No education	73 (27.5)
Primary	89 (33.6)
Junior High School	96 (36.2)
Senior High School	7 (2.6)
Marital status	
Married	10 (3.8)
Single	255 (96.2)
Religion	
Christian	201 (75.9)
Islam	64 (24.1)
Occupation	
Unemployed	126 (47.5)
Artisan	50 (18.9)
Trader	89 (33.6)
Lives with	
Parents	110 (41.5)
Husband/partner	90 (34.0)
Alone	13 (4.9)
Other relative/friend	52 (19.6)
Previous pregnancy	
Yes	32 (12.1)
No	233 (87.9)
Supplement use before pregnancy	
Yes	1 (0.4)
No	264 (99.6)
Pica Practice	
Yes	24 (9.1)
No	241 (90.9)

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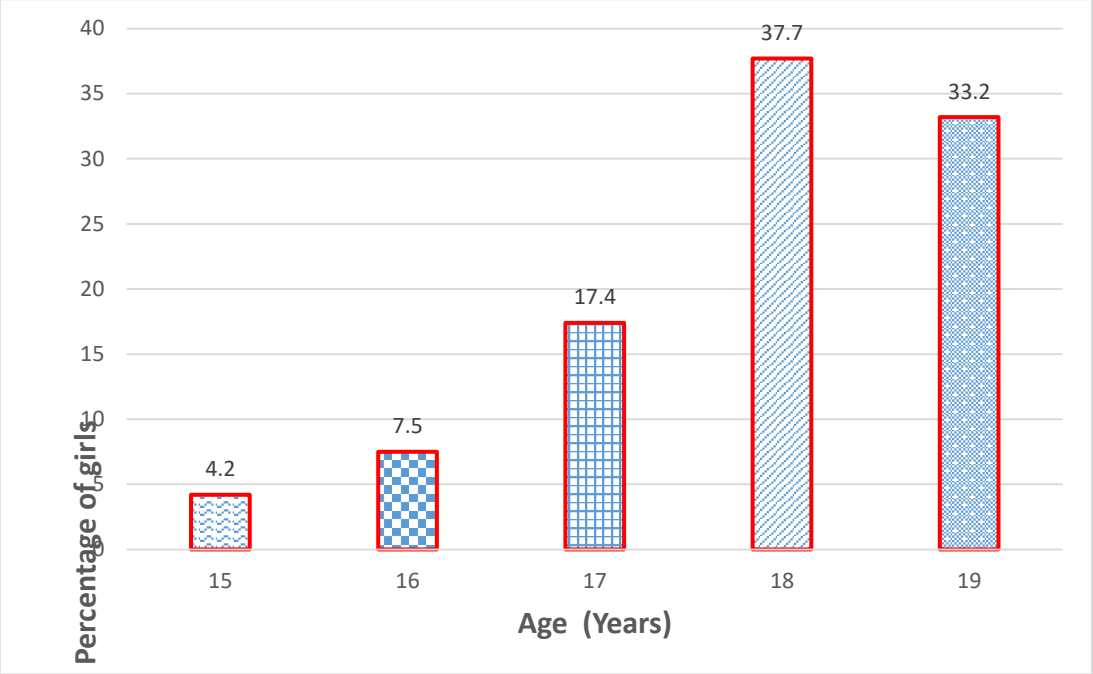

Figure 4.1: Age distribution of pregnant adolescent girls

4.2 Nutrient intakes of the pregnant adolescent girls

Tables 4.2a and 4.2b describe the energy, macronutrients and selected micronutrients of the pregnant adolescent girls. Complete three days dietary recall data could be obtained from 223 pregnant adolescent girls. Mean intakes for energy, protein, carbohydrate and fat were 1622 (SD=551 kcal), 40.25 (SD=13.65 g), 276.39 (SD=111.07 g) and 46.37 (SD=23.11 g) respectively. The energy contributed by protein (9.9%) was less than the recommended amount (10-35%) (DRI, 2004). With the exception of iodine (p = 0.043) and vitamin B_{12} (p = 0.008), there were no significant differences in the mean intakes between girls in the various trimesters. There was a general trend of reduced mean intakes of energy and nutrients with the trimesters of the pregnancies although the differences did not reach statistical significance.

Table 4.2c shows the comparison of the mean intake of the selected nutrients to the EAR or RDA using a one sample t-test. The mean intakes of all the nutrients were significantly lower

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than the EAR/RDA. For the nutrients under consideration, only vitamin B_{12} (73.2 %) was above 50% of the EAR. The girls were only able to meet 39.1 % and 21.3 % of the EAR of iron and folate respectively.

4.2.1 Nutrient intakes according to socioeconomic variables – Between subgroup comparisons

The mean nutrient intakes against socioeconomic variables are reported in table 4.3. In this table, comparisons were made between subgroups in each demographic characteristic. Educational status of the girls did not influence intakes of all the nutrients. Girls who were married had low intakes for all the nutrients analysed but these were not statistically significant except for iodine (51 µg versus 56 µg; $p = 0.048$). Muslim girls had significantly higher intakes of selenium compared to the Christians (32 µg versus 34 µg; $p = 0.008$). Girls who had had previous pregnancies had significantly higher intakes of iron (11µg verses 8.9µg; $p = 0.041$) and iodine (72 µg versus 54 µg; $p = 0.042$) compared to those who had not. Vitamin B_{12} (2.11 µg versus 1.56 µg; $p = 0.003$) and vitamin C (46 mg versus 29 mg; $p = 0.027$) intakes were significantly higher in girls who practiced pica compared to those who did not.

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Table 4.2a: Macronutrient intake of pregnant adolescent girls (N=223)

Nutrient	1ST Trimester (n=65)	2ND Trimester (n=142)	3RD Trimester (n=16)	TOTAL	Percentage contribution to energy (%)	*p*-Value
Energy (Kcal)						
Mean ± SD	1630 ± 517	1627 ± 573	1540 ± 504	1622 ± 551	-	0.828
C.I	1502 - 1759	1532 – 1722	1271 – 1809	1549 - 1694		
Protein (g)						
Mean ± SD	41.38 ± 11.39	40.00 ± 15.00	37.90 ± 9.44	40.25 ± 13.68	9.92	0.620
C.I	38.55 – 44.20	37.51 – 42.49	32.87 - 42.93	38.44 – 42.06		
Carbohydrates(g)						
Mean ± SD	273.65 ± 105.76	279.54 ± 114. 64	259.58 ± 104.23	276.39 ± 111.07	68.16	0.772
C.I	247.45 – 299.86	260.52 – 298. 56	204.04 – 315.13	261.74 – 291.05		
Fats (g)						
Mean ± SD	47.86 ± 22.98	45.76 ± 23.23	45.70 ±22.90	46.37 ± 23.11	25.73	0.828
C.I	42.17 -53.56	41.89 – 49. 63	33.50 - 57.90	43.32 – 49.42		

ANOVA test for significant differences in means set at $p < 0.05$.

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Table 4.2b: Micronutrient intake of pregnant adolescent girls (N=223)

Nutrient	1ST Trimester (n=65)	2ND Trimester (n=142)	3RD Trimester (n=16)	Total	P-Value
Iron (mg)					
Mean ± SD	9.09 ± 4.32	9.17 ± 4.36	8.53 ± 2.95	9.10 ± 4.26	0.849
C.I	8.02 – 10.17	8.44 – 9.89	6.95 – 10.10	8.54 – 9.66	
Folate (µg)					
Mean ± SD	115.87 ± 52.63	110.61 ± 59.49	93.33 ± 38.71	110.90 ± 56.36	0.358
C.I	102.84 – 128.91	100.74 – 120.48	72.70 – 113.96	103.46 – 118.34	
Vitamin B_{12} (µg)					
Mean ± SD	2.02 ± 1.83[a]	1.44 ± 0.92[b]	1.48 ± 0.87[b]	1.61 ± 1.28	0.008*
C.I	1.57 – 2.47	1.28 – 1.59	1.02 – 1.95	1.44 – 1.78	
Selenium (µg)					
Mean ± SD	31.72 ± 16.61	34.46 ± 19.39	26.44 ± 14.64	33.08 ± 18.34	0.199
C.I	27.60 – 35.84	31.24 – 37.67	18.64 – 34.24	30.66 – 35.51	
Iodine (µg)					
Mean ± SD	58.01 ± 42.08[a]	57.89 ± 38.57[a]	31.95 ± 37.40[b]	56.06 ± 39.94	0.043*
C.I	47.58 – 68.44	51.49 – 64.29	12.03 – 51.89	50.79 – 61.33	
Vitamin C (mg)					
Mean ± SD	28.35 ± 17.62	30.58 ± 29.44	37.79 ± 27.89	30.45 ± 26.41	0.441
C.I	23.99 – 32.72	25.69 – 35.46	22.93 – 52.65	26.96 – 33.93	
Calcium (mg)					
Mean ± SD	434 ± 313	426 ± 313	403 ± 359	427 ± 315.10	0.935
C.I	354 – 512	374 – 477	211 – 594	385– 468s	

*ANOVA (Post hoc-Bonferroni) significant difference set at $p < 0.05$. [a] and [b] Significantly different from each other

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Table 4.2c: Comparison of means to EAR/RDA and percentage of nutrient intakes

Nutrient	EAR	Mean intakes	[a]Percentage of intakes (%)	*p*-Value
Iron (mg)	23	9.10	39.1	<0.001
Folate (µg)	520	110.90	21.3	<0.001
Vitamin B_{12} (µg)	2.2	1.61	73.2	<0.001
Selenium (µg)	60	33.08	55.1	<0.001
Iodine (µg)	220	56.06	25.5	<0.001
Vitamin C (mg)	80	30.45	38.1	<0.001
Calcium (mg)	1300	426.84	32.8	<0.001

One-sample t-test significant at $p < 0.05$. [a]Mean intakes/EAR or RDA x 100

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Table 4.3: Mean nutrient intakes according to socioeconomic variables (N=223) – Between subgroup comparisons

Characteristic	Energy (kcal)	Protein (g)	Carbohydrate (g)	Fat (g)	Calcium (mg)	Iron (mg)	Zinc (mg)
Educational level							
No education (n=63)	1478 (507)	39.6 (14.5)	247.2 (96.2)	42.9 (19.2)	416 (313)	8.4 (4.7)	4.8 (2.0)
Some Education (n=160)	1678 (559)	40.5 (13.4)	287.9 (114.7)	47.7 (24.4)	431 (317)	9.4 (4.1)	4.8 (2.0)
p-value	*0.848*	*0.918*	*0.393*	*0.131*	*0.999*	*0.566*	*0.899*
Marital status							
Married (n=9)	1369 (584)	36.1(12.9)	234.3 (100.3)	37.7 (23.9)	260 (216)	8.1 (6.2)	4.5 (2.1)
Single (n=214)	1632 (549)	40.4 (13.7)	278.2 (111.4)	46.7 (23.1)	434 (317)	9.1 (4.1)	5.0 (1.9)
p-value	*0.547*	*0.716*	*0.735*	*0.564*	*0.229*	*0.237*	*0.793*
Religion							
Christian (n=165)	1610 (529)	36.8 (12.7)	276.1 (111.6)	45.4 (21.7)	418 (303)	9.0 (4.2)	4.9 (1.8)
Islam (n=56)	1642 (594)	41.4 (16.5)	274.1 (104.8)	48.9 (26.9)	452 (354)	9.2 (4.4)	4.9 (2.4)
p -value	*0.357*	*0.064*	*0.871*	*0.478*	*0.210*	*0.754*	*0.127*
Previous pregnancy							
Yes (n=23)	1770 (581)	41.3 (13.2)	296.2 (116.1)	54.2 (28.7)	411 (296)	11.0 (5.6)	5.2 (1.7)
No (n=200)	1605 (546)	40.1 (13.8)	274.1 (110.6)	45.5 (22.3)	429 (319)	8.9 (4.0)	4.9 (2.0)
p -value	*0.475*	*0.947*	*0.521*	*0.504*	*0.482*	*0.041**	*0.630*
Living with							
Parents (n=85)	1591 (412)	39.7 (10.5)	268.0 (80.9)[a]	46.7 (22.4)	466 (330)	8.7 (3.5)	4.9 (1.6)
Husband/partner (n=77)	1637 (574)	41.1 (15.1)	281.2 (116.6)[a]	45.5 (21.1)	423 (328)	9.1 (3.4)	5.0 (2.1)
Alone (n=13)	1347 (459)	36.2 (15.4)	202.4 (71.1)[a]	48.7 (20.7)	308 (187)	8.6 (5.6)	4.5 (2.2)
Other relative/friend (n=48)	1725 (714)	41.0 (15.0)	303.6 (144.4)[b]	46.5 (28.2)	393 (289)	9.9 (5.6)	5.1 (2.1)
p -value	*0.155*	*0.647*	*0.025**	*0.969*	*0.286*	*0.428*	*0.849*
Pica practice							
Yes (n=24)	1765 (463)	40.5 (9.9)	305.0 (102.2)	50.3 (20.8)	409 (258)	10.4 (4.0)	4.9 (1.2)
No (n=241)	1609 (557)	40.2 (14.0)	273.9 (111.7)	46.0 (23.3)	428 (320)	9.0 (4.3)	5.0 (2.0)
p -value	*0.898*	*0.128*	*0.777*	*0.745*	*0.149*	*0.987*	*0.132*

*ANOVA and T-test. Significant differences set at $p < 0.05$. Data is presented as means (SD)
Values with superscripts a and b significantly different from each other

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Table 4.3: continued. Mean nutrient intakes according to socioeconomic variables – Between subgroup comparisons

Characteristic	Copper (µg)	Selenium (µg)	Iodine (µg)	Vitamin B_{12} (µg)	Vitamin C (mg)	Folate (µg)
Educational level						
No education (n=63)	1.02 (0.44)	32 (20)	55 (36)	1.56 (0.89)	28 (21)	108.15 (45.37)
Some education (n=160)	1.13 (0.56)	33 (18)	56(41)	1.63 (1.40)	31 (28)	111.99 (60.24)
p-value	*0.105*	*0.905*	*0.264*	*0.293*	*0.233*	*0.158*
Marital status						
Married (n=9)	0.97 (0.37)	26 (9)	51 (55)	1.54 (0.97)	26 (17)	93.71 (41.47)
Single (n=214)	1.11 (0.53)	33 (18)	56 (39)	1.61 (1.29)	31 (27)	111.62 (56.86)
p -value	*0.185*	*0.172*	*0.048**	*0.921*	*0.739*	*0.286*
Religion						
Christian (n=165)	1.10 (0.50)	32 (16)	57 (40)	1.65 (1.34)	31 (27)	108.22 (52.83)
Islam (n=56)	1.07 (0.56)	34 (23)	54 (40)	1.49 (1.06)	30 (24)	116.37 (64.45)
p -value	*0.997*	*0.008**	*0.713*	*0.866*	*0.845*	*0.319*
Previous pregnancy						
Yes (n=23)	1.29 (0.53)	30 (20)	72 (50)	1.61 (0.94)	38 (35)	111.36 (52.50)
No (n=200)	1.07 (0.52)	33 (18)	54 (38)	1.60 (1.31)	30 (25)	110.85 (56.91)
p -value	*0.962*	*0.864*	*0.042**	*0.805*	*0.129*	*0.938*
Living with						
Parents (n=85)	1.10 (0.52)	31(15)	60 (40)	1.66 (1.57)	29 (23)	102.49 (46.01)
Husband/partner (n=77)	1.90(0.50)	34 (21)	58 (39)	1.56 (1.06)	28 (22)	113.77 (62.38)
Alone (n=13)	0.80 (0.34)	27 (16)	36 (44)	1.32 (1.00)	26 (16)	96.91 (41.68)
Other relative/friend (n=48)	1.20 (0.60)	37 (20)	51 (39)	1.68 (1.08)	39 (37)	124.90 (63.83)
p -value	*0.145*	*0.237*	*0.184*	*0.779*	*0.078*	*0.117*
Pica Practice						
Yes (n=24)	1.05 (0.34)	29 (15)	34 (27)	2.11 (2.93)	46 (38)	90.85 (41.95)
No (n=241)	1.10 (0.54)	33 (18)	58 (40)	1.56 (1.01)	29 (25)	112.66 (57.00)
p -value	*0.083*	*0.424*	*0.085*	*0.003**	*0.027**	*0.123*

*ANOVA and T-test. Significant differences set at $p < 0.05$. Data is presented as means (SD)
Values with superscripts a and b significantly different from each other

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4.2.2 Adequacy of nutrient intakes in pregnant adolescent girls

Individual mean intakes were compared to the Estimated Average Requirements (EAR) reference values of the Institute of Medicine, Food and Nutrition Board 2005. The EAR is the estimated average daily nutrient intake necessary to meet the nutritional requirements of healthy individuals for nutrients with established Recommended Dietary Allowance (RDA) (Food and Nutrition Board, 2005). The percentages of pregnant adolescent girls with adequate intakes were determined using the cut-off point method. Thus, those with intakes above the EAR were categorized as having adequate intakes. The percentage of girls who met the requirements is shown in table 4.4. With the exception of carbohydrates (mean of 94.2%), less than a third of the girls met their dietary intakes for each of the selected nutrients. Iron and vitamin B_{12} intakes were met by 1.3% and 22.5% of the girls respectively. A Z-test to compare the percentage of girls who met nutrient requirements and those who did not, showed significant difference for all the selected nutrients ($p < 0.001$). Z-test for proportions was not performed for folate because all the girls did not meet the daily requirement.

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Table 4.4: Percentage of pregnant adolescent girls with adequate intakes (N=223)

Nutrient	1st Trimester (n=65) n (%)	2nd Trimester (n=142) n (%)	3rd Trimester (n=16) n (%)	Total (N=223) n (%)	*p*-value
[1]Energy	7 (10.7)	7 (4.9)	0 (0.0)	14 (6.3)	<0.001
Protein	20 (30.8)	41 (28.9)	2 (12.5)	63 (28.3)	<0.001
Carbohydrates	64 (98.5)	131 (92.3)	15 (93.8)	210 (94.2)	<0.001
Calcium	6 (9.2)	13 (9.2)	2 (12.5)	21(9.4)	<0.001
Iron	**1 (1.5)**	**2 (1.4)**	**0 (0.0)**	**3 (1.3)**	**<0.001**
Zinc	1 (1.5)	3 (2.1)	0 (0.0)	4 (1.8)	<0.001
Selenium	10 (15.4)	26 (18.3)	1 (6.3)	37 (16.6)	<0.001
Iodine	3 (4.6)	3 (2.1)	0 (0.0)	6 (2.7)	<0.001
Vitamin B_{12}	**20 (31.3)**	**27 (19.0)**	**3 (18.8)**	**50 (22.5)**	**<0.001**
Vitamin C	2 (3.1)	9 (6.3)	1 (6.3)	12 (5.4)	<0.001
Folate	**Not determined**	**Not determined**	**Not determined**	**Not determined**	**-**

[1] Cut-off based on Estimated Energy Requirement. Z-test for proportions significant at $p < 0.05$. P-value is the difference between percentage with adequate intake and those with inadequate intake.

Within demographic subgroup comparisons of girls who had adequate and inadequate intakes of iron, folate and vitamin B_{12} nutrients are presented in tables 4.5a-c. The rest of the other nutrients are presented in Appendix G. The socio-demographic status of the girls did not have any impact on the adequacy of their intakes of these nutrients. Among the married girls, no significant difference was observed in vitamin B_{12} intakes between the proportion

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that had adequate intakes and those who had inadequate intakes (44.4% versus 55.6%; p = 0.739). However, a significantly higher proportion of single girls had inadequate intakes compared to those who had adequate intakes (78.4% versus 21.6%; $p < 0.001$).

The proportions of both Christian and Muslim girls who had inadequate intakes of iron (98.8% and 98.2%) and vitamin B_{12} (76.8% and 78.6%) were significantly higher than those who had adequate intakes ($p < 0.001$). Both groups of girls also had significantly inadequate intakes of folate.

More girls who had had previous pregnancies had significantly inadequate intakes of iron (95.7%, $p < 0.001$) and vitamin B_{12} (73.9%, $p < 0.001$) than those who had adequate intakes. A similar observation was also made of those who were pregnant for the first time.

There was no significant difference in intakes of vitamin B_{12} among girls who lived alone (69.2% versus 30.8%; p = 0.166). Significantly higher proportion had inadequate intakes of iron and vitamin B_{12} for both those who responded YES and NO for pica practice. None of the girls had adequate intakes for folate.

Within each employment status group there was significantly higher proportion of those with inadequate intakes of iron, vitamin B_{12} and folate.

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Table 4.5a: Percentage of girls with adequate and inadequate intakes of iron according to socioeconomic status (N=223) – Between subgroup comparisons

Socioeconomic characteristics	Adequate intake n (%)	Inadequate n (%)	P-value
Educational level			
No Formal Education	2 (3.2)	61 (96.8)	<0.001
Formal Education	1 (0.6)	159 (99.4)	<0.001
Marital status			
Married	0 (0.0)	9 (100)	-
Single	3 (1.4)	211 (98.6)	<0.001
Religion			
Christian	2 (1.2)	163 (98.8)	<0.001
Islam	1 (1.8)	55 (98.2)	<0.001
Previous Pregnancy			
Yes	1 (4.3)	22 (95.7)	<0.001
No	2 (1.0)	198 (99.0)	<0.001
Lives with			
Parents	0 (0.0)	85 (100)	-
Husband/Partner	0 (0.0)	77 (100)	-
Alone	0 (0.0)	13 (100)	-
Friend/relative	3 (6.3)	45 (93.8)	<0.001
Pica Practice			
Yes	0 (0.0)	18 (100)	-
No	3 (1.5)	202 (98.5)	<0.001
Occupation			
Unemployed	1 (1.0)	102 (99.0)	<0.001
Artisan	0 (0.0)	40 (100)	-
Trader	2 (2.5)	78 (97.5)	<0.001

Z-test for proportions significant at $p < 0.05$

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Table 4.5b: Percentage of girls with adequate and inadequate intakes of vitamin B_{12} according to socioeconomic status (N=223)- Between subgroup comparisons

Socioeconomic characteristics	Adequate intake n (%)	Inadequate n (%)	*p*-value
Educational level			
No Formal Education	15 (23.8)	48 (76.2)	<0.001
Formal Education	35 (22.0)	124 (78.0)	<0.001
Marital status			
Married	4 (44.4)	5 (55.6)	0.739
Single	46 (21.6)	167 (78.4)	<0.001
Religion			
Christian	38 (23.2)	126 (76.8)	<0.001
Islam	12 (21.4)	44 (78.6)	<0.001
Previous Pregnancy			
Yes	6 (26.1)	17 (73.9)	0.022
No	44 (22.1)	155 (77.9)	<0.001
Lives with			
Parents	14 (16.7)	70 (83.3)	<0.001
Husband/Partner	17 (22.1)	60 (77.9)	<0.001
Alone	4 (30.8)	9 (69.2)	0.166
Friend/relative	15 (31.3)	33 (68.8)	0.009
Pica Practice			
Yes	3 (17.6)	14 (82.4)	0.008
No	47 (22.9)	158 (77.1)	<0.001
Occupation			
Unemployed	20 (19.6)	82 (80.4)	<0.001
Artisan	9 (22.5)	31 (77.5)	<0.001
Trader	21 (26.3)	59 (73.8)	<0.001

Z-test for proportions significant at $p < 0.05$

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Table 4.5c: Percentage of girls with adequate and inadequate intakes of Folate according to socioeconomic status (N=223)-Between subgroup comparison

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No Formal Education	0 (0.0)	63 (100)	-
Formal Education	0 (0.0)	160 (100)	-
Marital status			
Married	0 (0.0)	9 (100)	-
Single	0 (0.0)	214 (100)	-
Religion			
Christian	0 (0.0)	165 (100)	-
Islam	0 (0.0)	56 (100)	-
Previous Pregnancy			
Yes	0 (0.0)	23 (100)	-
No	0 (0.0)	200 (100)	-
Lives with			
Parents	0 (0.0)	85 (100)	-
Husband/Partner	0 (0.0)	77 (100)	-
Alone	0 (0.0)	13 (100)	-
Friend/relative	0 (0.0)	48 (100)	-
Pica Practice			
Yes	0 (0.0)	18 (100)	-
No	0 (0.0)	205 (100)	-
Occupation			
Unemployed	0 (0.0)	103 (100)	-
Artisan	0 (0.0)	40 (100)	-
Trader	0 (0.0)	80 (100)	-

Z-test for proportions significant set at $p < 0.05$

P-values could not be computed because none of the participants had adequate intakes of folate.

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4.2.3 Multiple inadequate nutrient intakes

Figure 4.2 shows the percentage of girls with multiple inadequate intakes of folate, iron and vitamin B_{12}. Combined iron and folate had the highest level of inadequate intakes as about 98% of the girls had failed to have adequate intakes of these two nutrients together. Those with inadequate intakes for all three nutrients combined (folate, iron and vitamin B_{12}) were 75.8%. Inadequate intakes of combined folate and vitamin B_{12} compared with iron and vitamin B_{12} were 76.7% and 75.8% respectively.

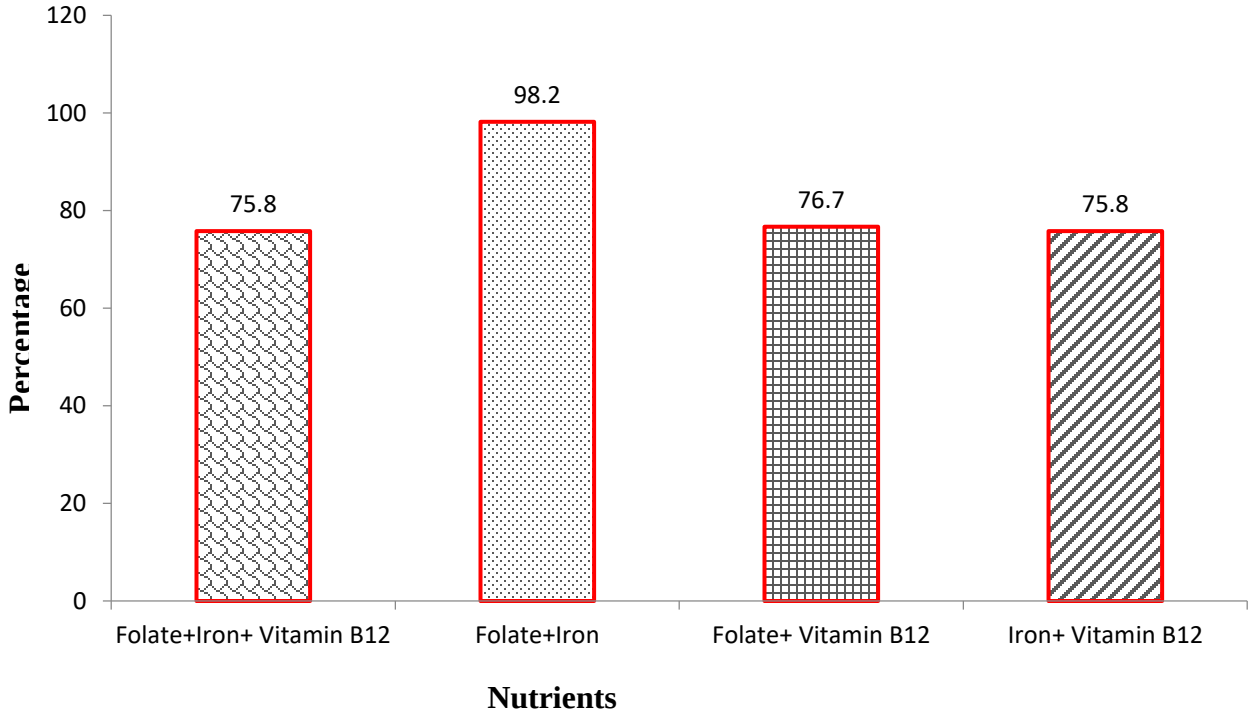

Figure 4.2: Percentage of girls with multiple nutrient inadequate intakes

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4.2.4 Frequency of consumption of foods

A detailed table reporting on the frequency of consumption of foods is presented in (Appendix I). The summary of the findings is reported below. Foods listed on the food frequency checklist were classified and discussed under thirteen main food groups. Frequencies of consumption were grouped as very frequent (≥ 5 times a week) frequent (1 - 4 times a week), sometimes (1-2 times a month), rarely or never.

4.2.4.1 Frequency of consumption of hot and cold beverages

Beverages prepared from cocoa (chocolate) and tea bags were consumed at least 1-4 times in a week by 69.5% and 64.9% of the girls respectively. Horlicks was never or rarely consumed by 94.0% of the girls. About 49.4% never consumed coffee and 27.2% rarely consumed it. Fruit juices and soft drinks (coke, fanta, sprite etc) were consumed by 75% and 23.2% respectively for 1-4 times a week among the girls. Diet drinks, beer, wine and hard liquor were rarely consumed by 75.4%, 91.7%, 93.5% and 87.9% of the girls respectively.

4.2.4.2 Frequency of consumption of bread, cereals and staples

A range of 33.2% - 36.2% of the girls consumed at least one type of the listed breads (sugar, tea, butter) for about 1-2 times in a month. “Sugar” bread was the most frequently consumed (48.7%) for about 1- 4 times per week.

About 11% and 68.3% of the girls consumed rice more than 5 times per week and between 1- 4 times per week respectively. *Banku* and *kenkey* both prepared from fermented maize dough were consumed frequently (1-4 times/week) by 82.6% and 69.8% of girls respectively.

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Fufu flour (prepared from cassava, cocoyam or plantain) was never consumed by 64.4% of the girls as opposed to pounded *fufu* which was frequently (1-4 times/week) consumed by 61.1% of the girls. About 56% and 47.9% frequently (1-4 times/ week) consumed plantains and yams respectively.

4.2.4.3 Frequency of consumption of porridges

Corn porridge locally referred to as "*koko*" was consumed by 2-6% of the girls more than 5 times in a week. Sixteen percent consumed it 1- 4 times in a week. "*Hausa koko"* which is prepared from millet was frequently consumed (1-4 times/week) by 65.7% of the girls. About 32% consumed oats 1-2 times in a month. However, 12.8% consumed it frequently (1 – 4 times/week). Fortified breakfast cereals were never or rarely consumed by 81.9% and 13.6% of the girls respectively. A few (2.3%) of the girls consumed wheat porridge more than 5 times a week.

4.2.4.4 Frequency of consumption of milk and milk products

Generally, consumption of milk and milk products were low. The percentage of girls who consumed "carnation" milk, semi-skimmed milk, skimmed milk and condensed milk frequently (1- 4 times/week) ranged from 2.3% to 8.7%. "Ideal" milk (full cream milk) was the most frequently consumed (51.7%) milk. Soymilk and cheese were never consumed by 48.3% and 43.0% of the girls respectively.

4.2.4.5 Frequency of consumption of fats, spreads and oils

Palm oil and white oils (sunflower, frytol etc) were consumed frequently (1-4 times/week) by 73.6% of the girls respectively. Coconut oil was consumed about 1- 4 times /week by

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15.8% of the girls. Consumption of palm kernel and groundnut oil was not common. More than 90% of the girls reported that they never consumed these oils. Similarly, consumption of various spreads (e.g margarine, butter, peanut, marmalade/jam) was not frequent. Only a few of the girls (1.1% to 12.8%) reported that they consumed these about 1-2 times/month).

4.2.4.6 Frequency of consumption of deep fried foods

French fries were never consumed by 83.8% of the girls. About 23% and 21.5% consumed fried yam and fried plantain frequently (1- 4 times/week) respectively.

4.2.4.7 Frequency of consumption of vegetables

Less than 4% of the girls consumed any of the assessed vegetables more frequently (≥ 5 times/week). Frequent consumption (1 - 4x a week) of Turkey berries (*abedru or kantosi*), cabbage, green leafy vegetables, mixed vegetables and okro) was observed in 11.3%, 18.1%, 30.6%, 19.6% and 30.6% of the girls respectively. Carrots were rarely or never consumed by 45.2% of the girls. Cucumber was rarely or never consumed by 25.7% and 31.3% of the girls respectively.

4.2.4.8 Frequency of consumption of fruits

Citrus fruits and banana were the most consumed fruits. About 18% and 23% of the girls reported that they consumed these foods 1- 4 times a week. Other fruits like pawpaw, pineapple and mango, were also consumed frequently by 18.5%, 14.7% and 13.2% of the girls, respectively.

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4.2.4.9 Frequency of consumption of snacks and sweets

Among snacks, plantain chips and biscuits were respectively consumed by 14.0% and 15.1% of the girls frequently (1-4 times/week). Imported crisps and savoury snacks were rarely consumed by 48.6% of the girls.

4.2.4.10 Frequency of consumption of legumes

Baked beans, soy flour/soya beans and bambara beans (*Vigna subterranea*) were never consumed by 63.8%, 81.5% and 44.5% of the girls respectively. Black eye beans was however frequently consumed 1- 4 times in a week by 25.3% of the girls.

4.2.4.11 Frequency of consumption of meat and meat products

Beef, salted beef and chicken were the most frequently consumed meat and meat products for at least 1-4 times in a week by 29.1%, 21.5% and 26.0% of the girls respectively. Eggs were consumed by 6.8% of the girls for at least more than 5 times in a week. Turkey, pork, goat, pig feet, unsalted pig feet and snails were never consumed by 60.8%, 54.7%, 44.6%, 62.3%, 65.3% and 72.1% of the girls respectively.

4.2.4.12 Consumption of fish and sea foods

Fish was consumed more frequently (≥ 5 times/week) consumed by 5.7% of the girls. About 62% consumed fish 1-4 times in a week. More than half of the girls never consumed shell fish such as shrimps and lobsters (61.1% and 76.6% respectively).

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4.2.4.13 Frequency of consumption of soups and stews

Palm soup (prepared from palm nuts and some vegetables), groundnut soup (prepared from groundnut/peanuts and some vegetables) and light soup (prepared from vegetables mainly tomatoes, onions and garden eggs) were consumed 1- 4 times in a week by 51.3%, 63.4% and 58.5% of girls respectively. Girls who consumed palm soup, groundnut soup, light soup and okro soup more than 5 times in a week were 4.5%, 4.9%, 5.7% and 5.3% respectively.

Among the stews, *kontomire* stew (cocoyam leaves) without *agushie* (melon seeds) was consumed 1- 4 times in a week by 41.1% of the girls. Tomato stew was frequently consumed by 75.1% of the girls 1- 4 times per week. Garden egg stew and *agushie* (melon seeds) stew were never consumed by 26.5% and 29.8% of girls respectively.

4.3 Determination of Minimum Dietary Diversity (MDD-W) among pregnant adolescent girls

Minimum Dietary Diversity (MDD-W) was assessed by determining consumption from each of the food groups. The percentage of consumption from each food group is presented in figure 4. 3. The highest consumption was of the grain, white roots, tubers and plantain food group (100%). The second food group with the highest consumption was the 'meat, fish and poultry' group (86.2%). The least consumed food group was the 'other fruits' group (3.1%). Only about 9% of the girls consumed from the dark green vegetable group

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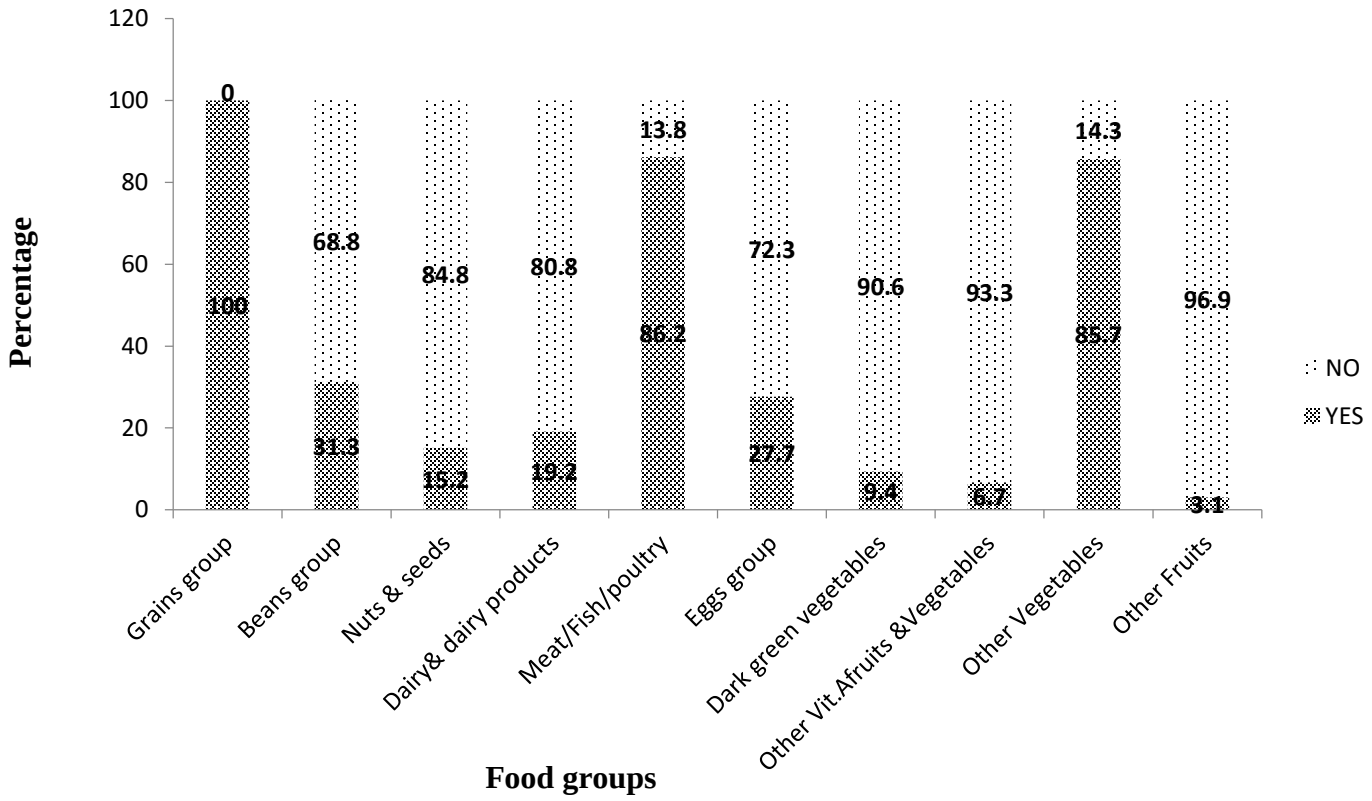

Figure 4.3: Percentage of consumption from each food group.

4.3.1 Percentage of girls with good or poor MDD-W score

Figure 4.4 shows the percentage of pregnant girls who had good MDD-W and those with poor MDD-W. A chi-square test for association was used to test for association between MDD-W and trimester of pregnancy. The test involved a 2x3 design where trimester was measured on 3 levels (i.e 1st, 2nd and 3rd trimesters) and MDD-W measured on two levels (i.e poor dietary diversity and good dietary diversity). The test showed no significant association between trimester and MDD-W ($p = 0.744$). Overall, 75.4% had poor diversity whiles 24.6% had good diversity. When a Z- test was performed to test for the differences in the overall

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percentage of MDD-W for poor diversity and good diversity, a significant difference was observed between the two proportions (p = 0.001).

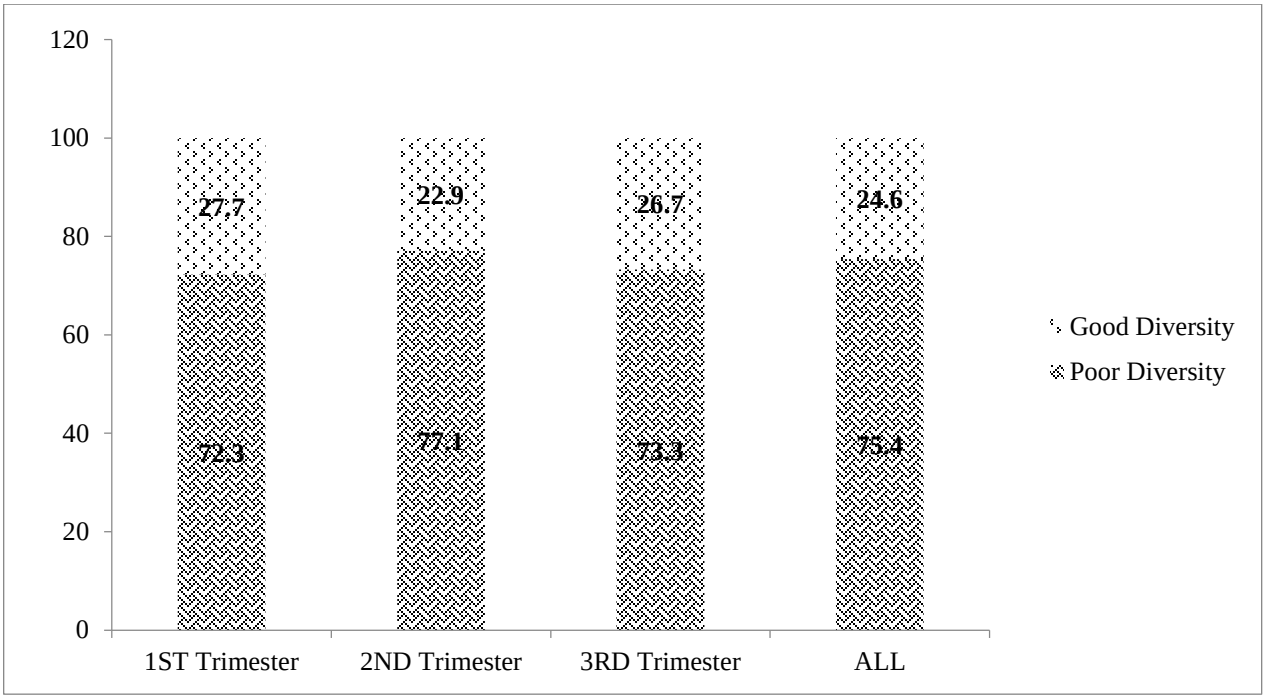

Figure 4.4: Percentage of pregnant girls with poor dietary diversity and good dietary diversity

Z- test showed significant differences between poor diversity and good diversity (p = 0.001)

4.3.2 MDD-W score of pregnant adolescent girls according to socioeconomic variables

The mean score of MDD-W for the girls was 3.85 (SD = 0.95). The minimum score obtained was 1 and the maximum score was 6. Independent sample t-test and ANOVA were used to test for differences in the means of the MDD-W scores according to socio-economic characteristics (table 4.6). No significant differences were observed in the mean scores when the girls were categorized according to educational level, marital status, religious affiliation, previous pregnancy, those they live with, practice of pica and occupational status.

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Table 4.6: Mean Women's Minimum Dietary Diversity Score (MDD-W) according to socio- economic characteristics

Characteristic	Mean (SD)	*p*-value
Total	3.85 (0.95)	-
Educational level		
No education (n=63)	3.76 (0.86)	0.748
Some Education (161)	3.88 (0.98)	
Marital status		
Married (n=8)	3.13 (0.99)	0.728
Single (n=216)	3.88 (0.94)	
Religion		
Christian (n=166)	3.76 (0.97)	0.169
Islam (n=56)	4.09 (0.84)	
Previous pregnancy		
Yes (n=23)	4.13 (0.97)	0.767
No (n=201)	3.82 (0.94)	
Living with		
Parents (n=86)	3.84 (0.94)	0.637
Husband/partner (n=77)	3.89 (0.94)	
Alone (n=13)	3.54 (1.19)	
Other relative/friend (n=48)	3.89 (0.90)	
Pica practice		
Yes (18)	4.00 (0.91)	0.577
No (206)	3.94 (0.95)	
Occupation		
Unemployed (100)	3.84 (0.96)	0.189
Artisan (36)	3.64 (0.93)	
Trader (72)	3.97 (0.94)	

T-test significant at $p < 0.05$
ANOVA significant at $p < 0.05$

The girls were further categorized according to those who had good or poor dietary diversity and analysed based on their socioeconomic variables. Z-test for proportions was used to test

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for significant differences between proportions (Table 4.7). Formal education had no effect on dietary diversity as significantly higher proportions of girls who had some formal education (73.9% versus 26.1%; $p = 0.001$) and those who had no formal education (79.4% versus 20.6%; $p < 0.001$) had poor diversity.

Higher proportion of single girls had poor dietary diversity in comparison to those with good diversity (74.5% versus 25.5%; $p < 0.001$). Poor diversity was also observed among Christians and Muslims.

A significantly greater proportion of girls who had not had other previous pregnancies had poor dietary diversity (75.6% versus 24.4%; $p < 0.001$). Among those who had had previous pregnancies, significant differences were observed in the proportions of girls who had poor and good dietary diversity (73.9% versus 26.1%; $p = 0.022$) respectively. When analysis was done based on who the girls lived with, it was seen that, with the exception of girls who lived alone, there were significant differences in proportions for those who lived with their parents, husband/partners and living with other relatives. Poor dietary diversity was observed among a significantly higher proportion (75.7% versus 24.3%; $p < 0.001$) of girls who did not practice pica. Differences in dietary diversity among girls who practiced pica (72.2% versus 27.8%; $p = 0.058$) was not significant. The proportions with poor diversity were significantly higher across the occupation categories ($p < 0.05$) compared to good diversity.

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Table 4.7: Percentage of girls who had good and poor dietary diversity according to socioeconomic variables (N=224)

Variable	Poor Diversity n (%)	Good Diversity n (%)	*P*-value
Educational level			
No formal education	50 (79.4)	13 (20.6)	<0.001
Formal education	119 (73.9)	42 (26.1)	<0.001
Marital Status			
Married	8 (100)	0 (0.0)	-
Single	161 (74.5)	55 (25.5)	<0.001
Religion			
Christian	130 (78.3)	36 (21.7)	<0.001
Islam	38 (67.9)	18 (32.1)	0.008
Previous pregnancy			
Yes	17 (73.9)	6 (26.1)	0.022
No	152 (75.6)	49 (24.4)	<0.001
Living with			
Parents	67 (77.9)	19 (22.1)	<0.001
Husband/partner	59 (76.6)	18 (23.4)	<0.001
Alone	9 (69.2)	4 (30.8)	0.166
Other relative/friend	34 (70.8)	14 (29.2)	0.004
Pica practice			
Yes	13 (72.2)	5 (27.8)	0.058
No	156 (75.7)	50 (24.3)	0.001
Occupation			
Unemployed	78 (75.0)	26 (25.0)	<0.001
Artisan	33 (84.6)	6 (15.4)	<0.001
Trader	58 (71.6)	23 (28.4)	<0.001

Z-test for proportions significant at $p < 0.05$.

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4.4 Biochemical indices of pregnant adolescent girls

Descriptive statistics of the biochemical indices according to trimester of pregnancy are presented in table 4.8. The mean haemoglobin, iron, TIBC and vitamin B_{12} were 10.32 (SD = 1.45, CI 10.13-10.51) g/dl, 20.62 (SD = 13.09, CI 18.67- 22.57) µg/L, 77.16 (SD = 15.24, CI 74.88 – 79.43) µmol/L and 552.09 (SD = 348.43, CI 501.96 – 602.22) ng/l respectively. No significant differences were seen between the trimesters with the exception of ferritin concentrations. The median and interquartile ranges for ferritin was 14.16 (IQR = 16.76) ng/ml and folate 3.72 (IQR = 9.15) ng/l.

Percentage of pregnant adolescent girls with low, normal and high serum concentrations of biochemical indices are presented in table 4.9. Analysis of biochemical indices were not done on all participants due to insufficient serum sample. On the whole, anaemia defined as haemoglobin (Hb) level less than 11g\dl, was present in 71.6% of the girls while about 89% had low serum iron concentrations. High serum TIBC was seen in 76.6% of the girls and serum ferritin was low in 76.5%. Low serum concentration of vitamin B_{12} was recorded in 21.3% but low serum folate was found in 51.6% and iron in 89.2%.

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Table 4.8: Mean/Median serum concentrations of biochemical indices in pregnant adolescent girls

Variable	1ST Trimester	2nd Trimester	3rd Trimester	Total	CI	P-value
Haemoglobin (g/dl) (n=222)	10.56 ± 1.38	10.18 ± 1.46	10.63 ± 1.51	10.32 ± 1.45	10.13-10.51	0.137
Iron (µg/L) (n=176)	22.66 ± 16.06	19.35 ± 11.21	22.62 ± 13.27	20.62 ± 13.09	18.67-22.57	0.267
TIBC (µmol/L) (n=175)	80.11 ± 12.99	75.02 ± 16.49	81.86 ± 10.36	77.16 ±15.24	74.88 – 79.43	0.066
Ferritin (ng/ml) (n=153) (median ± IQR)	16.13 ± 15.08	13.64 ± 16.72	12.59 ± 304.02	14.16 ± 16.76	25.24 – 50.37	0.001*
Folate (ng/l) (median ± IQR) (n=188)	3.4 ± 7.53	4.12 ± 11.17	2.35 ± 6.22	3.72 ± 9.15	7.19 – 10.10	0.412
Vitamin B_{12} (ng/l) (n =188)	486.28 ± 327.41	586.43 ± 364.60	549.17 ± 251.07	552.09 ± 348.43	501.96 – 602.22	0.196

*ANOVA significant at $p < 0.05$. Data is presented as means ± standard deviation unless otherwise stated.

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Table 4.9: Percentage of pregnant adolescent girls with low, normal and high serum concentrations of biochemical indices

Anaemia Index	1ST Trimester n (%)	2ND Trimester n (%)	3RD Trimester n (%)	Total n (%)
[a]**Haemoglobin (n=222)**				
Normal (≥11.0g/dl)	21 (35.0)	35 (24.6)	7 (35.0)	63 (28.4)
Mild anaemia (10.0-10.9g/dl)	22 (36.7)	55 (38.7)	7 (35.0)	84 (37.8)
Moderate anaemia (7.0-9.9g/dl)	17 (28.3)	50 (35.2)	6 (30.0)	73 (32.9)
Severe anaemia (<7.0g/dl)	0 (0.0)	2 (1.4)	0 (0.0)	2 (0.9)
Total anaemia	39 (65.0)	107 (75.4)	13 (65)	159 (71.6)
[b]**Iron (n=176)**				
Low serum iron (<37 µg/L)	42 (76.4)	103 (95.4)	12 (92.3)	157 (89.2)
Normal serum iron (≥37µg/L)	13 (23.6)	5 (4.6)	1 (7.7)	19 (10.8)
[b]**TIBC (n=175)**				
Low (<47.4 µmol/L)	1 (1.8)	6 (5.7)	0 (0.0)	7 (4.0)
Normal (47.4-89.0 µmol/L)	17 (30.4)	14 (13.2)	3 (23.1)	34 (19.4)
High (>89.0µmol/L)	38 (67.9)	86 (81.1)	10 (76.9)	134 (76.6)
[b]**Ferritin (n=153)**				
Low (<30ng/ml)	34 (22.2)	75 (49.0)	8 (5.2)	117 (76.5)
Normal (≥30ng/ml)	9 (5.9)	23 (15.0)	4 (2.6)	36 (23.5)
[c]**Vitamin B_{12} (n=188)**				
Normal (≥250 ng/L)	42 (28.4)	95 (64.2)	11 (7.4)	148 (78.7)
Low (<250 ng/L)	18 (45.0)	21(52.5)	3 (2.5)	40 (21.3)
[d]**Folate (n=188)**				
Normal (≥ 4 µg/L)	26 (46.4)	61 (51.7)	4 (28.6)	91 (48.4)
Low (< 4 µg/L)	30 (53.6)	57 (48.3)	10 (71.4)	97 (51.6)

[a] based on WHO reference classification (WHO, 2011a)
[b]based on WHO reference classification (WHO, 2011b)
[c]Berg & Shaw (2013)
[d]deBenoist (2008)

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4.4.1 Prevalence of anaemia and serum nutrient deficiency of iron, folate and vitamin B_{12}

Figures 4.5 and 4.6 shows the prevalence of anaemia and the nutrient deficiencies of iron, folate and vitamin B_{12}. Anaemia prevalence based on haemoglobin concentration as has been reported earlier was 71.6%. Girls who had haemoglobin concentrations less than 11 g/dl and were deficient in iron, folate and vitamin B_{12} were 61.4%, 50.4% and 11.7%% respectively. About 28% were found to be deficient in both iron and folate, 11.9% deficient in iron and vitamin B_{12}, 8.5% in folate and vitamin B_{12} and 5.1% in all the three micronutrients (figure 4.6).

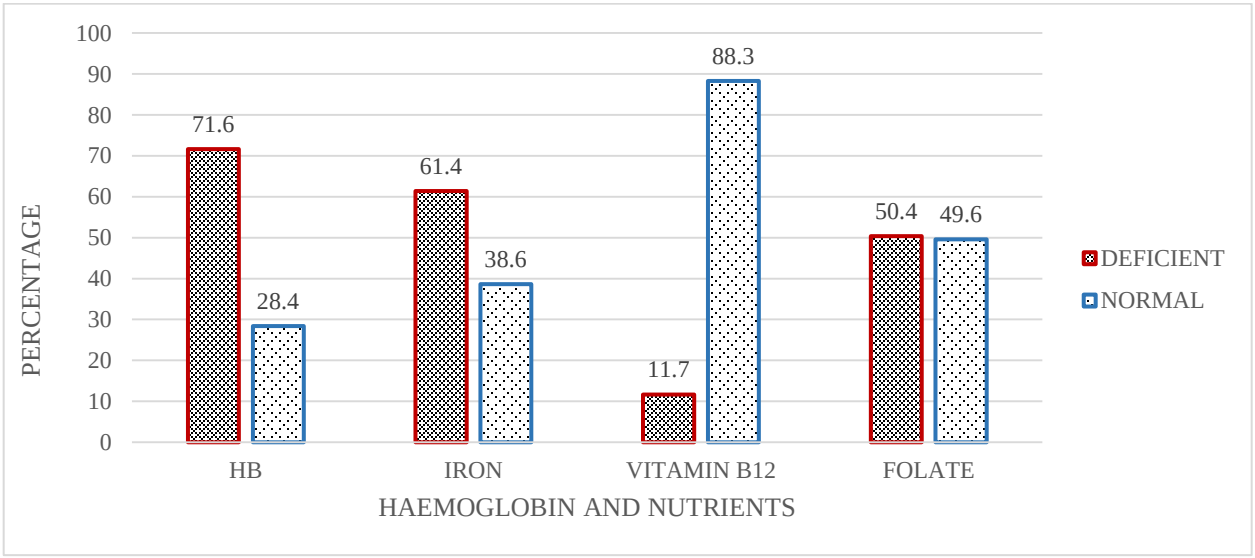

Figure 4.5: Anaemia prevalence and serum nutrient deficiencies of iron, folate and vitamin B12

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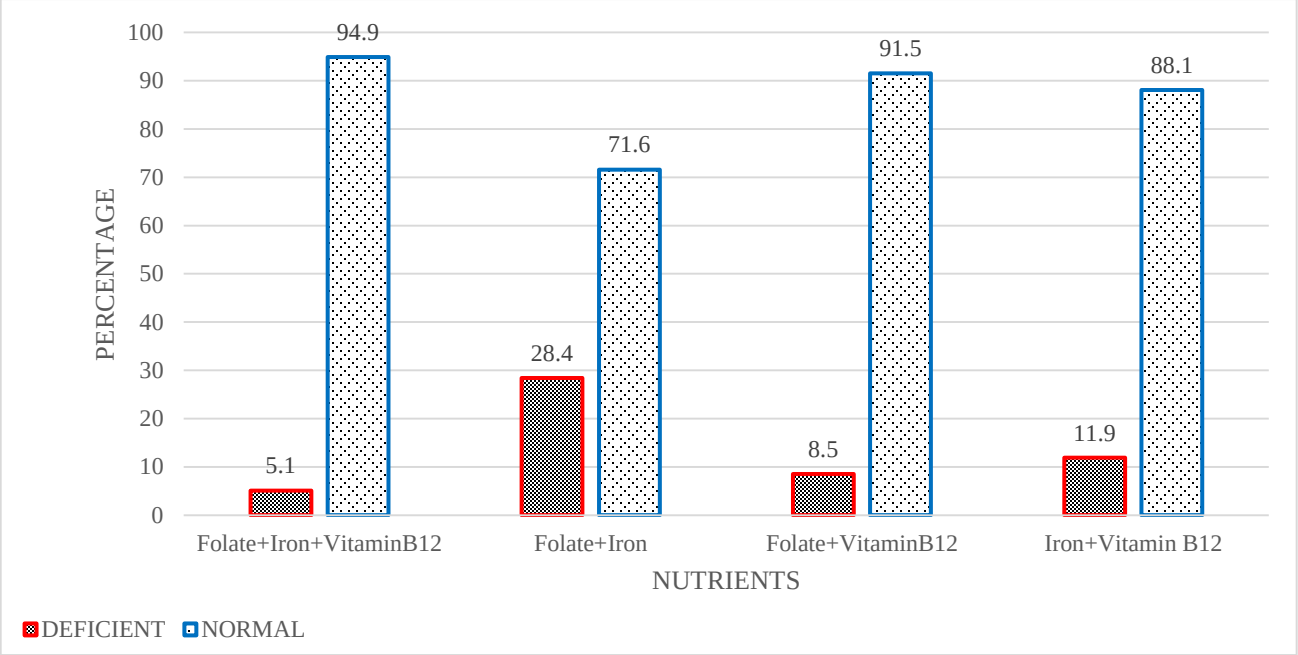

Figure 4.6: Multiple micronutrient deficiencies of iron, folate and vitamin B_{12}

4.4.2 Biochemical indices of girls according to socioeconomic variables

The proportion of girls with normal or high biochemical indices are presented in table 4.10a and 4.10b. The proportion of girls who were anaemic according to haemoglobin classification were greater in both the girls who had no formal education (34.2% versus 65.8%; $p = 0.007$) and those who had formal education (21.9% verses 78.1; $p < 0.001$). Significantly, greater proportion had low serum concentrations of iron both in the no formal education (4.0% versus 96.0%; $p < 0.001$) and the formal education group (13.5% versus 86.5%; $p < 0.001$).

When the girls were grouped according to marital status, no statistical difference was observed in the proportion of those with anaemia and those with normal haemoglobin among the married girls (20.0% versus 80.0%; $p = 0.058$). However, significant difference in

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proportion was observed in those who were anaemic and those who were not among the single group (28.6% versus 71.4%; $p < 0.001$). A similar observation was made with the serum iron concentrations. Although there were variations in proportions of girls with normal and low serum folate levels within the socioeconomic groups, there were no statistical differences in serum folate concentrations within the groups.

4.4.3 Relationship between nutrient intakes and serum concentrations

Relationship between dietary intakes (iron, folate, vitamin B_{12}) and serum concentrations (iron, folate, vitamin B_{12}, HB) are shown in table 4.11. Significant positive correlations were seen between dietary folate and dietary iron ($r = 0.509$, $p < 0.001$), dietary vitamin B_{12} and dietary iron ($r = 0.156$, $p = 0.021$), serum vitamin B_{12} and dietary iron ($r = 0.156$, $p = 0.021$) and significant negative correlation for serum TIBC and serum ferritin ($r = -0.243$, $p = 0.004$). Haemoglobin also positively correlated significantly with dietary vitamin B_{12} and serum vitamin B_{12} ($r = 0.161$, $p = 0.027$).

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Table 4.10a: Percentage of girls with low/anaemic, normal and high biochemical indices according to socioeconomic characteristics

	Haemoglobin			Iron			TIBC		
	Normal n (%)	Anaemic n (%)	*p*-Value	Normal n (%)	Low n (%)	*p*-value	High n (%)	Low n (%)	Normal n (%)
Educational level									
No formal education	25 (34.2)	48 (65.8)	0.007	2 (4.0)	48 (96.0)	<0.001	39 (78.0)	1 (2.0)	10 (20.0)
Formal education	42 (21.9)	150 (78.1)	<0.001	17 (13.5)	109 (86.5)	<0.001	95 (76)	6 (4.8)	24 (19.2)
Marital Status									
Married	2 (20.0)	8 (80.0)	**0.058**	1 (20.0)	4 (80.0)	**0.180**	2 (40.0)	0 (0.0)	3 (60.0)
Single	73 (28.6)	182 (71.4)	<0.001	18 (10.5)	153 (89.5)	<0.001	132 (77.6)	7 (4.1)	31 (18.2)
Religion									
Christian	58 (29.1)	141 (70.9)	<0.001	17 (13.5)	109 (86.5)	<0.001	92 (73.6)	7 (5.6)	26 (20.8)
Islam	10 (25.0)	48 (75.0)	<0.001	2 (4.1)	47 (95.9)	<0.001	41 (83.7)	0 (0.0)	8 (16.3)
Previous pregnancy									
Yes	5 (15.6)	27 (84.4)	<0.001	21 (100)	0 (0.0)	-	14 (66.7)	1 (4.8)	6 (28.6)
No	70 (30.0)	163 (70.0)	<0.001	19 (12.3)	136 (87.7)	<0.001	120 (77.9)	6 (3.9)	28 (18.2)
Pica Practice									
Yes	4 (16.7)	20 (83.3)	<0.001	1 (12.5)	7 (87.5)	0.034	5 (62.5)	0 (0.0)	3 (37.5)
No	71 (29.5)	170 (70.5)	<0.001	18 (10.7)	150 (89.3)	<0.001	129 (77.2)	7 (4.2)	31 (18.6)
Occupation									
Unemployed	38 (30.2)	88 (69.8)	<0.001	11 (15.3)	61 (84.7)	<0.001	52 (73.2)	4 (5.6)	15 (21.1)
Artisan	16 (32.0)	34 (68.0)	0.011	1 (2.6)	37 (97.4)	<0.001	31 (79.5)	1 (2.6)	7 (17.9)
Trader	21 (23.6)	68 (76.4)	<0.001	7 (10.6)	59 (89.4)	<0.001	51 (78.5)	2 (3.1)	12 (18.5)
Live with									
Parents	34 (30.9)	76 (69.1)	<0.001	10 (14.9)	57 (85.1)	<0.001	48 (72.7)	4 (6.11)	14 (21.2)
Husband/partner	24 (26.7)	66 (73.3)	<0.001	5 (8.2)	56 (91.8)	<0.001	51 (83.6)	1 (1.60)	9 (14.8)
Alone	3 (23.1)	10 (76.9)	**0.052**	0 (0.0)	10 (100)	-	10 (100)	0 (0.0)	0 (0.0)
Relative/friend	14 (26.9)	38 (73.1)	0.001	4 (10.5)	34 (89.5)	<0.001	25 (65.8)	2 (5.3)	11 (28.9)

Z test for proportions significant at $p < 0.05$

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Table 4.10b: Percentage of girls with low and normal anaemia indices according to socioeconomic characteristics

	Ferritin			Vitamin B_{12}			Folate		
	Normal n (%)	Low levels n (%)	*p*-Value	Normal n (%)	Low n (%)	*p*-value	Normal n (%)	Low n (%)	*p*-value
Educational level									
No formal education	10 (21.3)	26 (78.7)	<0.001	40 (75.5)	13 (24.5)	<0.001	26 (48.1)	28 (51.9)	0.785
Formal education	26 (24.5)	80 (75.5)	<0.001	108 (80.0)	27 (00.0)	<0.001	65 (48.5)	69 (51.5)	0.730
Marital Status									
Married	1 (25.0)	3 (75.0)	-	6 (100)	0 (0.0)	-	3 (60.0)	2 (40.0)	0.655
Single	35 (23.5)	114 (76.5)	<0.001	142 (78.0)	40 (22.0)	<0.001	88 (48.1)	95 (51.9)	0.605
Religion									
Christian	27 (24.3)	84 (75.7)	<0.001	109 (80.1)	27 (19.9)	<0.001	66 (33.2)	69 (51.1)	0.796
Islam	9 (22.5)	31 (77.5)	<0.001	38 (76.0)	12 (24.0)	<0.001	23 (45.1)	28 (54.9)	0.484
Previous pregnancy									
Yes	5 (27.8)	13 (72.2)	<0.001	16 (69.6)	7 (30.4)	0.061	15 (65.2)	8 (34.8)	0.144
No	31 (23.0)	104 (77.0)	<0.001	132 (80.0)	33 (20.0)	<0.001	76 (46.1)	89 (53.9)	0.312
Pica Practice									
Yes	1 (10.0)	9 (90.0)	-	7 (70.0)	3 (30.0)	0.203	5 (55.6)	4 (44.4)	0.739
No	35 (24.5)	108 (75.5)	<0.001	141 (79.2)	37 (20.8)	<0.001	86 (48.0)	93 (52.0)	0.601
Occupation									
Unemployed	15 (24.6)	46 (75.4)	<0.001	63 (81.8)	14 (18.2)	<0.001	42 (52.5)	38 (47.5)	0.655
Artisan	9 (24.3)	28 (75.7)	<0.001	31 (75.6)	10 (24.4)	<0.001	14 (35.0)	26 (65.0)	0.058
Trader	126 (21.8)	43 (78.2)	-	54 (77.1)	16 (22.9)	<0.001	35 (51.5)	3 (48.5)	0.808
Live with									
Parents	17 (26.6)	47 (73.4)	<0.001	51 (73.9)	18 (26.1)	<0.001	36 (49.3)	37 (50.7)	0.907
Husband/partner	9 (18.4)	40 (81.6)	<0.001	56 (83.6)	11 (16.4)	<0.001	31 (48.4)	33 (51.6)	0.803
Alone	2 (22.2)	7 (77.8)	-	7 (70.0)	3 (30.0)	0.206	6 (60.0)	4 (40.0)	0.527
Relative/friend	8 (25.8)	23 (74.2)	<0.001	34 (81.0)	8 (19.6)	<0.001	18 (43.9)	23 (56.1)	0.435

Z test for proportions significant at $p < 0.05$

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Table 4.11: Correlations between dietary intakes of (iron, folate, vitamin B12) and serum concentrations of (iron, folate, vitamin B12, ferritin, TIBC, HB)

Variables	1	2	3	4	5	6	7	8
1. Dietary iron	-							
2. Dietary folate	0.509**	-						
3. Dietary vitamin B_{12}	0.156*	0.093	-					
4. Serum Iron	-0.120	0.065	0.006	-				
5. Serum Folate	0.150	0.004	-0.106	0.59	-			
6. Serum vitamin B_{12}	0.156*	0.093	1.000**	0.066	-0.106	-		
7. Serum ferritin	0.044	0.033	0.088	0.126	0.017	0.088	-	
8. Serum TIBC	-0.017	0.035	-0.045	-0.109	-0.137	-0.045	-0.243**	-
9. HB	0.082	0.074	0.161*	0.009	0.075	0.161*	-0.127	-0.035

**Correlation significant at $p < 0.01$

*Correlation significant at $p < 0.05$

4.4.4 Logistic regression analysis of MDD-W as a function of dietary iron, dietary folate, dietary vitamin B_{12}, serum ferritin, serum iron and serum TIBC

A binary logistic regression analysis was performed to predict girls who had good or poor dietary diversity (MDD-W) on the basis of dietary iron, dietary folate, dietary vitamin B_{12}, serum ferritin, serum iron and serum TIBC. The variance in MDD-W accounted for was 0.135 (Negelkerke R^2), indicating that the predictors explained 13.5% of the variance. Table 4.12, shows that dietary folate was the most significant (p = 0.010) predictor of MDD-W. The odds (42.247) of achieving a good MDD-W was very high for dietary folate. The odds for dietary vitamin B_{12} and serum TIBC were 2.102 and 4.975 respectively.

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Table 4.12: Logistic regression analysis of MDD-W as a function of dietary iron, dietary folate, dietary vitamin B_{12}, serum ferritin, serum iron and serum TIBC

Variables	B	Wald	Sig.	Odds Ratio Exp (B)	95% C.I. for EXP (B) (Odds ratio)	
					Lower	Upper
Dietary Iron	-0.947	0.359	0.549	0.388	0.018	8.592
Dietary folate	3.744	6.556	0.010*	42.247	2.406	741.882
Dietary vitamin B_{12}	0.743	1.055	0.304	2.102	0.509	8.672
Serum ferritin	-0.207	0.098	0.754	0.813	0.222	2.976
Serum TIBC	1.604	0.371	0.542	4.975	0.029	866.315
Serum iron	-1.028	1.372	0.241	0.358	0.064	1.998
Constant	-9.581	2.593	0.107	0.000		

*significant at $p < 0.05$

Another logistic regression analysis to predict adolescent girls who were anaemic or had normal haemoglobin in relation to dietary iron, dietary folate, dietary vitamin B_{12}, serum vitamin B_{12}, serum ferritin, serum iron and serum TIBC was conducted. The Negelkerleke R^2 was equal to 0.154 indicating that the model explained 15.4% of the variance. The results of the regression analysis are shown in table 4.13. Dietary iron (χ^2 = 4.254, p = 0.039) and dietary vitamin B_{12} (χ^2 = 5.266, p = 0.022) were the most statistically significant predictors of anaemia. The odds ratio in favour of the girls not being anaemic increases by a factor of 20.3 for a unit change of the girls' ability to have adequate dietary iron intake. The odds for the other variables were lower as indicated in the table 4.13.

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Table 4.13 Logistic regression analysis of anaemia as a function dietary iron, dietary folate, dietary vitamin B_{12}, serum ferritin, serum iron and serum TIBC

Variables	B	Wald	Sig.	Odds ratio Exp (B)	95% C.I.for EXP (B) (odds ratio)	
					Lower	Upper
Dietary Iron	3.009	4.254	0.039*	20.272	1.161	353.877
Dietary folate	-1.809	2.105	0.147	0.164	0.014	1.887
Dietary vitamin B_{12}	-1.908	5.256	0.022*	0.148	0.029	0.758
Serum ferritin	-0.173	0.106	0.744	0.841	0.297	2.382
Serum TIBC	-2.910	1.342	0.247	0.054	0.000	7.483
Serum iron	-0.987	1.335	0.248	0.373	0.070	1.988
Constant	8.708	5.624	0.122	6052		

4. 5 Dietary practices, myths/misconceptions and nutritional knowledge of anaemia among the pregnant adolescent girls

This section presents the views of the pregnant adolescent girls on matters relating to nutritional anaemia. A total of twenty four (24) girls were engaged in the discussions.

The background characteristics of the girls are described in table 4.14. About 8.3% had no formal education. The rest (91.7%) had attained some level of formal education up to the senior high school level. About (12.5%) were married. Forty two percent (42%) were engaged in trading. Close to half (45.8%) were living with their parents and about 21% had had a previous pregnancy.

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Table 4.14: Background characteristics of pregnant adolescent girls (Focus group) (N=24)

Variable	Total
Age (years)	
Mean (SD)	17.44 (1.29)
Range	15- 19
Educational level	**N (%)**
No education	2 (8.3)
Primary	3 (12.5)
Junior High School	16 (66.7)
Senior High School	3 (12.5)
Marital status	
Married	3 (12.5)
Single	21(87.7)
Religion	
Christian	18 (75.0)
Islam	4 (16.7)
Other	2 (8.3)
Occupation	
Unemployed	8 (33.3)
Artisan	6 (25.0)
Trader	10 (41.7)
Lives with	
Parents	11 (45.8)
Husband/partner	7 (29.2)
Other relative/friend	6 (25.0)
Previous pregnancy	
Yes	5 (20.8)
No	19 (79.2)
Supplement use before pregnancy	
Yes	1 (0.4)
No	24 (99.6)

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The results are provided under three (3) main themes; dietary practices/modifications during pregnancy, myths and misconceptions about food that impact on anaemia and nutritional knowledge on anaemia. Various sub-themes were identified under the three (3) main themes.

4.5.1 Dietary practices/modifications during pregnancy

Many of the participants reported that they had made some changes to the kinds of foods they ate and the amount they consumed when they became pregnant. A few however indicated that they had not made any changes to their dietary intakes. The modifications that were cited to have occurred had to do with consumption of oily foods, quantity of foods consumed, fruit and vegetable intake and the practice of pica or cravings for particular foods.

Consumption of oily foods

For each of the focus group discussions, consumption of oily foods came up as one of the top foods to avoid during the pregnancy period. Two key statements that underscored this assertion are as follows;

"*I was told that oily foods are not good so we must rather eat fruits*" (Pregnant adolescent -01A).

"*We need to stop eating oil because when the oil is too much it can affect you. Sometimes when you eat oil you can regurgitate. Sometimes it makes you feel very nauseous, it means that we need to reduce our oil intake."*(Pregnant adolescent 016C).

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Quantity of foods consumed

A number of the girls indicated that the quantities of the foods they consumed had generally increased since they became pregnant. Some however had not made any changes in terms of the amount of foods consumed. The statements expressed by the girls put their views into perspective;

"*As for me I eat a lot, at first I wasn't eating but now I eat a lot because I started to feel hungry*"

[giggling]*"*(Pregnant adolescent, 01A)

"*Before I got pregnant, I wasn't eating too much but now, I'm eating a lot*" (Pregnant adolescent, 06B)

"*My eating habits are the same. I buy from food joints because by the time I return from town, I'm already tired. I just buy rice or something but vomit it"* (Pregnant adolescent, 05C)

Consumption of fruits and vegetables

Increased fruit and vegetable consumption came out as one of the main new practices they had started including in their diet and expected all pregnant women to be doing the same because they believed it had many health benefits. Some of the typical responses they gave include the following;

"*fruits like mango, tiger nuts, black velvet tamarind (yooyi) and watermelon. I take tiger nuts because I just like it. …… Now that I'm pregnant, I take tiger nuts which I was not taking before*." (Pregnant adolescent 05A).

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" *fruits like mango, watermelon, apple and carrots when eaten will make the foetus healthy. I was told that every morning you have to exercise and add fruits to your diet*." (Pregnant adolescent 07A).

Pica and food cravings

Few of the girls alluded to the practise of pica and some food cravings since they became pregnant. One of the striking comments by a girl was " *ei!, I forgot, one more thing; for 'ayilor'(white clay). When I pass by it and inhale its aroma, I can't leave without eating some. I started eating it when I became pregnant"* (pregnant adolescent 017C).

4.5.2 Myths and misconceptions about foods that impact on anaemia

The myths and misconceptions reported by the girls were grouped under 2 main thematic areas namely foods that promote blood formation and foods recommended to eat or avoid during pregnancy.

Foods that promote blood formation

The girls reported various myths and misconceptions with regards to foods that aid in blood formation. Top among the list of foods that were believed to promote blood formation were a mixture of milk and malt drink and mixture of malt drink, milk and blended turkey berries. However, in one example, a girl reported that the consumption of malt drink can lead to the baby being too big and therefore difficult for the mother to push during delivery. The following statements capture some of the myths and misconceptions expressed by the girls;

"*we were told to be eating fruits. For the 'kantosi' (turkey berries) too it's not just for cooking. Sometimes you can grind, mix it with milk and drink. It helps to increase your blood"* (Pregnant adolescent 016C).

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"*an elderly woman taught me that if you have anaemia, you must grind turkey berries, sieve and drink it. She added that it should be taken in the morning and evening to normalize your blood and make you healthy. She also said it protects you against other diseases such as BP or fever. When I was told at the hospital that I had anaemia, I informed this woman and she told me not to buy any medication and that she will prepare one for me using turkey berries. After taking it I felt okay and my blood level was back to normal"* (Pregnant adolescent 05B)

"a woman told me that if you are pregnant, you can mix malt and milk and add blended turkey berries. She said it is good, it gives you blood." (Pregnant adolescent 03A)

Foods to avoid or take during pregnancy

The girls had varied myths and misconceptions about foods that should be avoided in pregnancy. Many of their myths and misconceptions had been picked from what they had heard mostly from older people. The most mentioned among the foods they think should be avoided were okro, mango, oranges, snails and energy drinks. The main reasons given for the avoidance of okro and snails were because of its slimy nature and the belief that mothers who ate them would either have babies who drool or have babies with slimy bodies. A number of the participants also indicated that palm nut soup was very good and therefore should be eaten frequently in the course of the pregnancy. Some of the statements that better echoed their myths and misconceptions were as follows;

"Yesterday I was at the store and one of my mum's friends came, she was eating mango and asked if I will eat but I said no. She also said that if you are pregnant and you eat mangoes, you can get diarrhoea. This can affect your baby" (Pregnant adolescent 02A)

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*"I heard that if you are pregnant you should not eat mangoes else it can give your child fever. It will give your child a yellow face. I was eating mango and my mum snatched it from my hand and told me not to eat it as well as sweets and sugary foods" (*Pregnant adolescent, 06B*)*

"I heard that a pregnant woman cannot take orange else when she gives birth her baby will get stomach ulcer" (Pregnant adolescent 07B).

"if a pregnant woman eats snails, her baby will drool when she gives birth so they are not good for pregnant women……..eating okro frequently can make your baby's body slimy." (Pregnant adolescent 016C)

4.5.3 Knowledge on nutritional anaemia

Knowledge on nutritional anaemia was widely discussed and grouped under four (4) main sub-themes. These are the causes of nutritional anaemia, symptoms of nutritional anaemia, consequences of nutritional anaemia and remedies for nutritional anaemia.

Causes of nutritional anaemia

Views expressed regarding the causes of anaemia were generally inaccurate and of no scientific merit. The common causes attributed to anaemia were expressed as inadequate food intakes, too much exposure to the sun and drinking of too much water. Another group were of the view that not drinking a lot of water can cause anaemia. These were some of the statements that provide the description of the causes of anaemia as expressed by the pregnant adolescent girls;

"not eating, drinking a lot of water and walking in the sun can cause anaemia. ……. Also, when you wake up at dawn and you don't get enough rest, you can get anaemia." (Pregnant adolescent 05A).

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"……. *If you don't rest or are always working, not drinking water and walking in the sun, you can get anaemia*." (Pregnant adolescent 06B).

Symptoms of Nutritional Anaemia

Many of the adolescent girls gave a fair description of what they thought the symptoms of anaemia were. The views they expressed came out of their own experiences or what they had heard from older people in the community. Some of the symptoms of anaemia mentioned included weight loss, pale eyes, dizziness and numbness. These statements describe some of the views of the girls;

"the person feels dizzy when walking, it's as if he/she is about to fall, so the individual must sit for a while before getting up. Sometimes it happens to me, so I just sit down for a while before getting up but when my mum prepared that medicine for me, it has stopped" (Pregnant girl, 013D).

"one frequently feels dizzy or sometimes you realize that you feel light when walking, as if you are ill" (Pregnant girl, 016C)

"…….one feels dizzy after walking for a short while. You don't really sweat too and also you can collapse when walking. People might think you have epilepsy but it is because of the anaemia" (Pregnant girl, O5A)

"……the individual changes. Upon seeing the person you'll ask whether the person is ill. You will see that the person is ill. I have observed that elderly people check under the eye to see if the person has anaemia" (Pregnant girl, 010C)

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Consequences of nutritional anaemia

Many of the girls outlined two (2) main consequences of anaemia in pregnancy. These were the death of the mother and that of the baby. The following puts their views into perspective;

"When a pregnant woman gets anaemia it can give her problems. For instance, she can lose her child during delivery or she can even lose her life because she does not have enough blood" (Pregnant girl, 016C)

"they said sometimes if you do not have enough blood you can't give birth and even if you give birth sometimes you or the child can die" (Pregnant girl, 018D)

Remedies for anaemia

The girls provided several remedies that they thought could be used to treat anaemia in pregnancy. Among the top mentioned remedies were drinking a lot of water, exercises, herbal medications, turkey berry juice and consumption of fruits. The following statements capture some of their enthusiastic views;

"you should let your diet concern you every day so you can grind and eat only 'kontomire' (cocoyam leaves) or can take only "kantosi" (turkey berry) juice only if you don't want to add it to malt. It can also help you to prevent that problem" (Pregnant girl, 016D).

"…… when you have anaemia, you must exercise, eat plenty of heavy foods like 'banku' (prepared from fermented maize dough) and after that you take fruits" (Pregnant girl, 07B).

"you should take medications given to you by the doctor and eat well but I don't know the name of the medicine". (Pregnant girl, 017D).

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Knowledge and sources of information on the micronutrients (iron, folate and vitamin B_{12})

The knowledge of the adolescent girls on the micronutrients were varied. Some had heard of the names of the nutrients in school while others had no idea what these nutrients were. A few could name some of the food sources for these nutrients but generally many of the girls had no idea what the food sources were. When asked whether they had received any education on the micronutrients (iron, folate, vitamin B_{12}), many of them replied in the negative, reflecting the low level of education on these micronutrients. The few who had obtained some knowledge on these nutrients mentioned school, grandmothers and nurses as their sources of information.

4.6 Summary of findings

The key findings are presented below;

4.6.1 Nutrient intakes of pregnant adolescent girls

- The mean energy intake was 1622 (SD = 551; CI 1549 – 1694) Kcal. Protein, carbohydrate and fat intakes were 40.25 (SD = 13.68; CI 38.44 – 42.06) g, 276.39 (SD = 111.7; CI 261.74 – 291.05)g and 46.37 (SD = 23.11; CI 43.32 – 49.42)g respectively.
- The mean nutrient intake of iron, folate and vitamin B_{12} were 9.10 (SD = 4.26; CI 8.54 -9.66) mg, 110.90 (SD = 56.36; CI 103.46-118.34) µg and 1.61 (SD = 1.26; CI 1.44-1.78) µg respectively.
- Girls who had had previous pregnancies had significantly higher intakes of iron compared to those who had not (11.0 mg verses 8.9 mg; $p = 0.041$).

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- Girls who practiced pica had significantly higher intakes of vitamin B_{12} compared to those who did not (2.11 µg verses 1.56 µg; $p = 0.003$).
- Significantly low proportion of girls had adequate intake of iron (1.3%; $p < 0.001$) and vitamin B_{12} (22.5%; $p < 0.001$) compared to those who had inadequate intakes.
- None of the girls had adequate intake of folate.
- Significantly higher proportion of the girls had inadequate intakes of iron, vitamin B_{12} and folate irrespective of their socioeconomic classification compared to those who had adequate intakes.
- A combination of folate, iron and vitamin B_{12} inadequate intakes was seen in 75.8% of the girls, folate and iron in 98.2%, folate and vitamin B_{12} in 76.7% and iron and vitamin B_{12} in 75.8% of the girls.
- Less than half of the girls (30.6%) reported frequent consumption (i.e 1-4 times per week) of green leafy vegetables.
- About 21.5%-29.1% of the girls reported that they consumed beef and beef products about 1 - 4 times a week.
- Sixty two percent (62%) of the girls consumed fish frequently.

4.6.2 Minimum dietary diversity (MDD-W) of the pregnant adolescent girls

- Consumption of foods from the grain, white roots, tubers and plantain food group was 100% among the pregnant adolescent girls.
- Consumption from the meat, fish and poultry group was 86.2%
- Consumption from the “other fruits” group was 3.1%.
- Consumption from the dark green leafy vegetables group was 9%.

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- Mean MDD-W score was 3.85 ± 0.95.
- The prevalence of good MDD-W was 24.6% and poor MDD-W was 75.4%.
- Comparison of the MDD-W against socioeconomic variables did not show any statistical difference.

4.6.3 Biochemical indices of pregnant adolescent girls

- Mean haemoglobin concentration was 10.32 (SD = 1.45) mg/dl.
- Mean and standard deviation of serum concentration of iron and TIBC were 20.62 (SD = 3.09) µg/L and 77.16 (SD = 15.24) µgmol/L respectively.
- Median and inter-quartile range of serum concentration for ferritin, folate and vitamin B_{12} were 14.10 (IQR = 16.76) ng/L, 3.72 (IQR = 9.15) µg/L and 552.09 (IQR=348.43) ng/L respectively.
- Prevalence of anaemia based on haemoglobin classification was 71.6%.
- Low serum concentration of iron, vitamin B_{12} and folate were 89.2%, 21.3% and 51.6% respectively.
- Girls who had low haemoglobin and deficient in iron, folate and vitamin B_{12} were 61.4%, 50.4% and 11.7% respectively.
- Multiple deficiencies of iron and folate were seen in 28% of the girls, iron and vitamin B_{12} deficiencies in 11.9%, folate and vitamin B_{12} deficiencies in 8.5% and 5.1% deficient in all three micronutrients.
- There were no significant differences between the proportion who had low and proportion who had normal serum folate levels within the socioeconomic variables.
- The significant predictors of anaemia based on haemoglobin were dietary iron (p = 0.039) and dietary vitamin B_{12} (p = 0.022).

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4.6.4 Relationship between dietary intakes and biochemical variables

- Significant positive correlation was seen between dietary folate and dietary iron (r = 0.509, $p < 0.001$).
- Significant positive relationship between dietary vitamin B_{12} and dietary iron (r = 0.156, $p = 0.021$).
- Significant positive relationship between serum vitamin B_{12} and dietary iron (r = 0.156, $p = 0.021$).
- Significant negative relationship between serum TIBC and serum ferritin (r = -0.243, $p = 0.004$).
- The most significant predictor of MDD-W was dietary folate ($p = 0.010$).
- The predictors explained 13.5% of the variance in predicting MDD-W.
- The predictors for anaemia based on haemoglobin explained 15.4% of the variance.
- The significant predictors of anaemia were dietary vitamin B_{12} ($p = 0.022$) and dietary iron ($p = 0.039$).

4.6.5 Nutrition knowledge, myths and misconceptions and sources of information regarding nutritional anaemia

- Fruits and vegetable consumption came up as the main dietary practice they had incorporated into their diets.
- Turkey berries (*Solanum torvum*), milk and malt drinks were regarded as foods that promote blood formation.
- Okro, mangoes, snails and energy drinks were regarded as foods to avoid during pregnancy.
- The pregnant girls expressed the main causes of anaemia to be excessive

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exposure to sunlight, inadequate food intakes, not drinking a lot of water and paradoxically drinking too much water.

- The girls identified the death of the mother and the baby as the main consequence of nutritional anaemia in pregnancy.
- Drinking a lot of water, herbal medications, exercising, consumption of fruits, vegetables and turkey berries were regarded as suitable treatments for anaemia.
- Grandmothers, nurses and the school were identified as the main sources of information on nutritional anaemia.

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CHAPTER 5

5.0 DISCUSSIONS

5.1 Discussions

Adolescent pregnancy is critical due to reported negative outcomes to both mother and baby (Ganchimeg et al., 2014). Thus, strategic measures such as prevention of adolescent pregnancy and improved and better healthcare when the pregnancy occurs are necessary. Good nutrition is one of the important determinants to achieving a good pregnancy outcome. In Ghana, about 14% of all pregnancies are carried by adolescents between 15-19 years as opposed to the global prevalence of 11% (WHO, 2011b; GSS, 2015). This study seeks to examine the nutritional anaemia situation of pregnant adolescents in selected communities in Accra, Ghana. Special emphasis has been placed on nutritional anaemia and dietary diversity to elicit comprehensive health care for the pregnant adolescent by adopting both quantitative and qualitative research techniques. This study is necessary as it addressed some current knowledge gaps in the nutrition situation of pregnant adolescents in Ghana.

5.2 Energy and nutrient adequacy with focus on iron, vitamin B_{12} and folate in pregnant adolescent girls

Optimal dietary intakes are crucial in meeting macro and micronutrient requirements during the period of adolescence and pregnancy. Analysis of the macronutrient intakes of the pregnant adolescent girls showed that energy and protein (1630 kcal and 40.25 g respectively) were low in comparison with recommendations of the Institute of Medicine (2820 kcal and 71 g respectively) (DRI, 2004). Again, these intakes were lower compared to mean intakes of adolescent girls elsewhere. Two different studies conducted

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in the US and UK, reported mean energy intakes among pregnant adolescent girls as 2273 kcal and similar protein intakes of 70.1 g and 72.1 g respectively (Derbyshire, 2009; Lee et al., 2014). In a study conducted in two health facilities in Western Kenya where about 66% of the pregnant girls were classified as having low incomes, energy and protein intakes were reported as 1983.6 kcal and 55.8 g respectively (Shipala, Wafula, Ettyang, and Were, 2012). Comparing the present study participants with similar socio-economic background as the Kenyan girls, depicts clearly that the Ghanaian girls had lower intakes of energy and proteins even though the pregnant Kenyan girls also had intakes below recommendations. It would have been expected that during the period of pregnancy, energy intakes among the girls in this present study would have been adequate but that was not the case. In fact energy (1622 kcal) and protein (40.25g) intakes were even lower when compared to the energy (1790 kcal) and protein (58.9g) intakes of non-pregnant UK adolescents reported by Derbyshire (2009).

These findings could be attributed to various interrelated factors that influenced their food choices hence their dietary intakes. It is possible that the financial means to afford adequate meals was not available because a large proportion of these girls were unemployed and those employed were engaged in very low income jobs such as petty trading that could not adequately cater for their needs. This agrees with findings that adolescent girls with low levels of education and income are more likely to become pregnant (Berglund, Liljestrand, Marin, & Salgado, 1997). Among Egyptian pregnant adolescent girls, Yassin, Sobhy, and Ebrahim (2004) reported that close to half of the girls (48.3%) had low incomes and 48% were illiterate and therefore these affected dietary intakes. In a recent review to identify factors that affected energy intakes among adolescents, the authors among many factors listed socio-economic status as an important

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factor in predicting dietary intakes among the adolescents, in addition to influence from parents and friends, educational levels, knowledge in nutrition, the media, body image and health status (Jodhun, Pem, & Jeewon, 2016). Less than half of the girls lived with their parents and the rest lived with their partners, lived alone or with a friend. It is plausible to expect that they would obtain additional support from parents, partners or even their friends. However given that the community as a whole is of low socio-economic status, it is unlikely that this additional help would be forthcoming.

The Ghanaian diet is built largely around carbohydrate based staples (Eli-Cophie, Agbenorhevi, & Annan, 2017), therefore it was not surprising that the mean carbohydrate intakes among the girls largely exceeded recommendations with as much as 94.2% attaining adequate intakes. Nti et al.,(2002) reported similar findings among expectant mothers in Ghana.

Low intakes of proteins were also observed. Proteins, particularly animal proteins are generally expensive and therefore high cost could be the reason why these low-income pregnant adolescent girls could not consume adequate quantities. This assertion is supported by the findings of Mayén, Marques-Vidal, Paccaud, Bovet, and Stringhini (2014) where higher socioeconomic status was seen to be significantly associated with higher intakes of protein. It is well known in Ghana that individuals with low income consume large amounts of carbohydrates, which are relatively cheaper, to compensate for low amounts of proteins, which are relatively more expensive.

Iron requirements during adolescence and pregnancy are high due to physiological demands. The mean iron intake (9.10 mg) for the girls in this study was well below the recommended intakes of 23 mg/d (DRI, 2004). About 99% of the girls did not have adequate intakes of iron. Meeting dietary iron requirements is generally a global problem

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even in affluent countries. In the UK, dietary iron intakes of 80% of a cohort of pregnant women between the ages of 18 to 45 years, were reported to be below the recommendations of the UK reference nutrient intakes (RNI) (Alwan et al., 2011). Among African American women between the ages of 18-36 years, dietary iron intakes below the EAR was reported in 89% of those studied (Groth et al., 2017). Similarly, in a systematic review, mean dietary iron intakes over the period of pregnancy in adolescent girls in some developed countries were reported to be between 11.2 mg to 20 mg (Marvin-Dowle, Burley, & Soltani, 2016). Bopape, Mbhenyane, and Alberts (2008), Oguntona and Akinyele (2002) and Shipala et al., (2012) reported mean intakes below recommendations among South African, Nigerian and Kenyan pregnant adolescent girls respectively. Iron intakes ranging between 9.7 mg and 11.8 mg were reported to have been consumed by the pregnant Nigerian adolescents across rural and urban areas while only 2% met recommended intakes among the South African girls. In an older cohort of pregnant Ghanaian females who were HIV positive and between the ages of 18-24 years, inadequate intakes were reported in about 73% of them (Laar et al., 2009). Contrary to findings of the current study, Alaofè, Zee, and Turgeon O'Brien (2007) observed 73% dietary iron intake adequacy among non-pregnant adolescent girls in Benin. However, the authors expressed concern over bioavailability of the iron in that population of girls because about 97% of the dietary iron consumed was non-haem.

Several reasons could be ascribed to the low dietary intakes of iron in this study. The first could be due to the low intakes of iron from animal source foods. One of the major sources of dietary iron is from animal source foods. Supplementation of the diet of Vietnamese women of reproductive age with animal source foods improved micronutrient intakes and the intake of iron (Hall et al., 2017). Results from the food

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frequency questionnaire in the present study, indicated that there was frequent consumption from animal source foods. Thus, the problem will be with the quantity of the animal source foods that were consumed by the pregnant adolescent girls. Animal source foods are generally expensive therefore the likelihood that these girls can afford enough animal source foods to meet their daily requirements will be low considering their low-income status and that of their helpers. This is consistent with findings from another study where higher socioeconomic status was associated with increased odds of consuming animal source foods (Herrador et al., 2015). Dietary iron from animal source foods are more bioavailable because it contains the haem type of iron (Hallberg, Björn-Rasmussen, Howard, & Rossander, 1979). Another source of dietary iron is from green leafy vegetables. This is less bioavailable because it carries the non-haem type of dietary iron. Consumption of green leafy vegetables was about 1- 4 times per week in about 30.6% of the girls, with close to 70% of them not consuming green leafy vegetables regularly and therefore not obtaining significant amounts of iron from this source.

Dietary folate intake among the pregnant girls was significantly low compared to the EAR (110.90 µg versus 520 µg; $p < 0.001$). The mean intakes could only meet 21.3% of the EAR. None of the girls met the requirements for folate indicating a 100% inadequacy. This is similar to a reported mean intakes of 155.3 µg and 98% inadequacy among South African pregnant adolescent girls (Bopape et al., 2008) and intakes between 158 µg and 183 µg among rural and urban pregnant Nigerian adolescents respectively (Oguntona & Akinyele, 2002). In Ghana, although other studies have reported folate intakes in adult women, there is lack of data on pregnant adolescent girls. However intakes in pregnant adult women could give an idea of what the pregnant adolescent girls could be consuming. This assertion is supported by Gutierrez & King (1993), that the entirety of

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nutritional needs of pregnant adolescents who have attained at least 2 years post menarche are comparable to that of pregnant adults. Akwetea (2015) showed in an unpublished work, the mean intakes of folate to be 123 µg among some women in rural Ghana. Also, Koryo-Dabrah, Nti, and Adanu (2012) in a work conducted in two hospitals in Accra, described dietary folate intakes among adult pregnant women to be inadequate. In yet another study that investigated the nutrient adequacy of the diets of pregnant adolescents in Guam located in the Oceania region, authors posited that the participants had intakes below the EAR in spite of the ostensibly high folate content of the Guamanian diet (Pobocik, Benavente, & Boudreau, 2003). Consistent with inadequate intakes of folate in this current study, Baker, Wheeler, Sanders & Thomas et al., (2009) reported inadequate intakes among UK pregnant adolescents in a study aimed at determining the relationship between some selected micronutrients and the delivery of small for gestational age babies as well as preterm deliveries. However, the intake of folate among this cohort of girls from the UK was about three times higher than the intake of participants of this current study. This disparity in the intakes can be explained by the addition of supplemental intakes of folate to the dietary intakes of the UK girls (about 44.2% were taking folate supplements during the pregnancy), which was not the case for these Ghanaian girls.

Under reporting of foods consumed could also have resulted in the recorded low intakes as has been reported in some studies to characterise dietary intake data (Subar et al., 2015). Akin to reports from other studies, the pregnant adolescents consumed vegetables but the frequency of consumption and amount consumed were low and therefore could not have met the required intakes. Additionally, poor preparation methods could all result in low intakes of folate (Bailey, Mahan, & Dimperio, 1980). Fortification of foods with

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folate will be necessary to mitigate the effect of poor dietary intakes as has been done elsewhere in Brazil and Tanzania (Barnabe et al., 2015; Noor et al., 2017). There is a current mandatory food fortification programme underway in the country where wheat flour is expected to be fortified with at least 20% of the requirements of EAR of folate in addition to other micronutrients (Nyumuah et al., 2012). This requirement is however woefully inadequate to meet the physiological needs of the pregnant adolescent and also in view of the fact that some of the flour manufacturing companies are not complying with the fortification requirements (Nyumuah et al., 2012). Effective policing and education is necessary to ensure food fortification compliance by manufactures. The requirements for folate increases to about 5 to 10 folds in pregnancy (Antony, 2007). Folic acid supplementation has also been useful in reducing the negative consequences of its deficiency in pregnancy (De-Regil, Pena-Rosas, Fernandez-Gaxiola, & Rayco-Solon, 2015). There are dire consequences for low folate intake in pregnancy. Established consequences of inadequate maternal intakes of folate include foetal neural tube defects and other congenital abnormalities (Czeizel & Dudás, 1992; Myers et al., 2001; Botto et al., 2002). In a prospective study involving pregnant adolescent population, low folate intakes below 187 µg/day as well as low red blood cell folate levels were found to increase the risk of delivery of small for gestational age (SGA) babies (Baker et al., 2009).

Low vitamin B_{12} intake in the pregnant woman poses a risk for developing anaemia and has been seen to be significantly associated with abnormal neuro-behavioural status of the infant (Neumann et al., 2013). This study has shown that even though low, vitamin B_{12} intakes were better in comparison with intakes of iron and folate among the pregnant adolescent girls studied. The mean intake of vitamin B_{12} (1.61 SD = 1.28 µg) was able to meet 73% of the EAR while that of iron and folate were able to meet 39.1% and 21.3%

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of the recommendations respectively. About 23% of the girls had adequate intake of vitamin B_{12} as compared to 1.3% for iron and 0% for folate. These findings agrees with other findings that folate and iron are the most common causes of anaemia in the sub-Saharan region of Africa (VanderJagt Brock, Melah, & El-Nafaty et al., 2007). The finding of the present study was contrary to other studies (Baker et al., 2009; Bopape et al., 2008; Lee et al., 2014). Among British, American and South African pregnant adolescents, mean intakes of vitamin B_{12} were reported to be 5.3 µg (Baker et al., 2009), 5.5 µg (Lee et al., 2014) and 2.3 µg (Bopape et al., 2008) respectively. Fifty six percent of the South Africa girls had inadequate intake compared to 77.5% in this study.

In comparison with the cited studies, it is apparent that the vitamin B_{12} intake among the girls in this current study was low. There is lack of published data of vitamin B_{12} intake among pregnant adolescent girls in Ghana, however in an unpublished work, mean intake of 2.2 µg was reported among non-pregnant adult females (Akwetea, 2015). To the best of the knowledge, this study bridges the knowledge gap of current information on the vitamin B_{12} intakes of pregnant adolescent girls in Ghana.

Low vitamin B_{12} dietary intakes have been reported in vegetarians elsewhere (Dhananjaya, Manjunatha, Manjunatha, & Kumar, 2015; Pawlak, Lester, & Babatunde, 2014; Thomas, Chandra, Sharma, Jain, & Pemde, 2015). Vegetarianism was not reported in the current study as evidenced by the food frequency questionnaire and the dietary diversity assessment. Therefore it would have been expected that the adequacy level of the vitamin would be high considering the frequency of consumption of animal products which are the main source of dietary vitamin B_{12}. It would thus seem that the effect of frequent intakes was blunted by a low amount consumed. In some suburban and rural areas in Ghana, Nti et al., (2002) reported daily but low consumption of animal source

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foods among expectant mothers. This, as has been argued previously, could be due to low income, or inadequate knowledge and/or cultural beliefs related to consumption of some animal products in the Ghanaian context (Arzoaquoi et al., 2015; Colecraft et al., 2006).

The adequacies of other nutrients were analysed and reported in Appendix G. However, it is important to discuss vitamin C because of its reported role in enhancing non-haem iron absorption. The mean dietary intake of vitamin C in this study was found to be 30.45 mg, meeting 38.1% of the EAR. It also fell short of the 50 mg recommended by Hallberg, Brune, and Rossander (1986) for achieving optimum absorption effect in a meal. Only 5% had adequate intakes of the vitamin C. Shipala et al., (2012) reported mean intakes of 63.03 mg among Kenyan pregnant adolescent girls. Even though mean intake was higher compared to the present study, it also fell short of the recommendations of EAR (66 - 70 mg/day) in adolescent pregnancy. Nonetheless, 90% of the requirement was met. In contrast to the findings of this study, Pena, Sanchez, Portillo, and Solano (2003) reported adequate vitamin C intakes among Venezuelan pregnant adolescent girls. The finding of the present study is worrying because of the likelihood effect on absorption of especially non-haem component of the dietary source of iron. Encouraging fruit intake among this physiological group is necessary to facilitate optimal non-haem iron absorption and also to avert the risk of other reported problems of iron deficiency such as preterm delivery and urinary tract infections (Ochoa-Brust et al., 2007; Siega-Riz, Promislow, Savitz, Thorp, & McDonald, 2003).

5.3 Minimum dietary diversity (MDD-W) in pregnant adolescent girls

Another focus of this study was to explore the quality of the diets of the pregnant adolescent girls using the newly developed minimum dietary diversity score indicator by FAO for women of reproductive age (MDD-W) (FAO & FHI360, 2016). The MDD-W

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is a dichotomous indicator and is used to categorize women of reproductive ages into whether they have had good dietary diversity if, they consumed food from at least five out of the ten defined food groups the day before the interview, or poor diversity if they consumed less. To the best of knowledge, this is the first study that has assessed the dietary diversity using the newly developed indicator among pregnant adolescent girls in Ghana. Earlier studies elsewhere have either looked at pregnant adult women only or together with adolescents or non-pregnant adolescent girls alone (Na et al., 2016; Shamim et al., 2016). One study that was conducted in Ghana was among adult women and conducted in the Northern Region part of the country (Saaka, Oladele, Larbi, & Hoeschle-Zeledon, 2017). This current study was however conducted in the Southern part of Ghana, specifically the Greater Accra Region and presents what the reality is at the Southern sector of the country.

Similar to what pertains in most developing countries, the grains, white roots, tubers and plantain group that forms the starchy staples were universally consumed. Similar findings were reported in a group of Kenyan pregnant women (99%) attending antenatal clinic (Kiboi, Kimiywe, & Chege, 2017). Contrary to our findings the starchy staples recorded the least consumption among Iranian non-pregnant adolescent girls (Mirmiran et al., 2004). This they attributed to cultural influences. However in the Ghanaian context, the starchy staples form the main component of the diet as earlier alluded to and therefore it was not surprising to find that all the girls consumed from this food group. In this present study, the meat/fish/poultry group was the second most consumed (86.2%). This is comparable to percentage (85.9%) consumption among adult Bangladeshi population of pregnant women (Shamim et al., 2016). On the contrary, only 24% of the pregnant women studied in Kenya consumed from this food group (Kiboi et al., 2017). In spite of

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the high percentage (86.2%) of girls consuming from this food group in the current study, this was not reflective on the mean protein intakes as the quantities consumed did not meet recommendations and the percentage of girls obtaining adequate intakes was low (28.3%). This shows that the adolescent girls may appreciate the need to consume adequate amounts of proteins but might be hindered from doing so because of the generally high cost of the animal proteins. In a study involving pre-schoolers in Ghana, among the factors identified as constraints to consuming animal source foods, low income in addition to inadequate knowledge and beliefs were identified (Colecraft et al., 2006).

The least consumed foods were from the vitamin A dark green vegetables (9.4%), other vitamin A fruits and vegetables (6.7%) and other fruit (3.1%) groups. The finding is in contrast to the findings of the study carried out in the Northern region of Ghana (Saaka et al., 2017). In that study, the percentage of pregnant women who consumed from the vitamin A dark green leafy vegetables, other vitamin A fruits and vegetables and other fruits were reported to be 72%, 46.5% and 7.8% respectively among the pregnant women studied (Saaka et al., 2017). Findings from another study in Kenya shows 58%, 32% and 51% of pregnant women consuming vitamin A dark green leafy vegetables, other vitamin A fruits and vegetables and other fruits respectively (Kiboi et al., 2017). It was worrying to observe that consumption from these three food groups among participants of the present study were extremely low. This implied that many of them will not be able to meet the requirement for a varied number of the micronutrients. This study was carried out among adolescents and consequently one of the reasons for not consuming enough from these food groups could be attributed to bad dietary practices that have been associated with this physiological age group (Banfield, Liu, Davis, Chang, & Frazier-Wood, 2016).

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Many adolescents resort to poor dietary choices and tend to go for flavoured drinks and sodas instead of opting for healthier options such as fruits and vegetables. Barros, Pereira, Gama, and Leal Mdo (2004) collaborates the findings of this study of low fruit and vegetable consumption among pregnant adolescents in Brazil. The upsurge of fast foods in the cities prevents many from eating the local foods that may contain more vegetables and fruits. Also the traditionally patronized food in the coast of Accra where this study was carried out is mainly *kenkey* (prepared from fermented maize dough) and fried fish with freshly grinded hot pepper sauce. This is mostly devoid of green leafy vegetables unlike the Northern region where green leafy vegetables contributes much to their meals and therefore could account for the low consumption of green leafy vegetables among participants of the present study compared to the study by Saaka et al., (2017). Accra, which is located in the south of Ghana, is more urbanized with a lot more people transiting into consumption of fast foods when compared to residents in the Northern Region of Ghana.

Rasmussen et al., (2006) reported of socio-economic status, parental intakes, individual preferences and accessibility/availability of home as factors that were positively associated with fruit and vegetable consumption among children and adolescents between 8-18 years. This observation could also be true of the pregnant adolescents in this present study. Findings from the present study indicated that majority of the girls were not living with their parents and therefore could not be influenced either positively or otherwise. The subjects of the current study may not have been socio-economically sound to afford these things daily because most of them were either unemployed or engaged in low income jobs that would not be able to support their upkeep.

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This study reports the overall mean minimum dietary diversity (MDD-W) to be 3.85, a figure indicating poor dietary diversity among this cohort of pregnant adolescent girls. A significant proportion of the girls (75.4%) were categorized as having poor diversity. Other studies have reported mean dietary diversity scores of 5.6 and 6.25 among Ethiopian and Iranian non- pregnant adolescent girls respectively (Mirmiran et al., 2004; Wassie et al., 2015). Similarly in Southwestern Nigeria, low dietary diversity score was recorded among adolescent girls and found to predispose the girls to iron deficiency anaemia (Olumakaiye, 2013). In adult women, a score ranging between 4.28-6.3 were reported in Bangladesh (Na et al., 2016; Shamim et al., 2016). In conformity with the findings of this study, the study conducted in the Northern Region of Ghana using the same MDD-W indicator, reported a mean score of 4.2 also indicating poor diversity among that cohort of adult pregnant women (Saaka et al., 2017).

One of the reasons that could account for the poor diversity among participants of this present study is the low level of education of the girls. Even though most of the girls in this study had some level of education, it should be noted that their educational attainment was at the basic level and this therefore could have had little or no impact on their nutrition knowledge. This agrees with the findings of Na et al., (2016) and Shamim et al., (2016) who both reported high dietary diversity scores among the more educated women in Bangladesh. In the present study, nutritional knowledge deficit could also explain the low dietary diversity scores. This is because the girls were interviewed on their first visit to the ante-natal care and therefore it is assumed that they might not have received education on good nutrition. In another study, frequent ante-natal care was reported to predict high dietary diversity scores (Saaka et al., 2017).

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5.4 Biochemical indices of nutritional anaemia (iron, folate and vitamin B_{12}) in pregnant adolescent girls.

Biochemical indicators have been useful in assessing anaemia status. Biochemical indicators of nutritional anaemia and the prevalence of deficiency based on the various indicators used were assessed. Anaemia reported as Hb < 11g/dl based on WHO classifications (WHO, 2011a) was prevalent in 71.6% of the girls. This was an indication that anaemia was very high in this population and this should be of public health significance. The prevalence of anaemia among the girls of this study was high in comparison with results from similar studies in other low and middle income countries, even though those studies also reported unacceptable prevalence ranging between 36-61% (Bopape et al., 2008; Mistry et al., 2017; Pessoa et al., 2015; Pinho-Pompeu, Surita, Pastore, Paulino, & Pinto e Silva, 2017; Shipala, Sowayi, Kagwiria, & Were, 2013). More disturbing about the findings of this present study is the fact that the prevalence was higher than the national prevalence (44.6%) among pregnant women (GSS, 2015).

There is lack of reported data on the prevalence of anaemia among pregnant adolescents in Ghana. Nonetheless, two studies in adult pregnant women reported prevalence of 34% (Engmann, Adanu, Lu, Bose, & Lozoff, 2008) and 70% (Dei-Adomakoh, Acquaye, Ekem, & Segbefia, 2014) suggesting that anaemia is still unacceptably high in some populations in Ghana. This brings to the fore the enormity of the problem of anaemia in pregnancy. According to a report in the Lancet, only 0.2-0.3 percentage point advancement has been realized per year in the last two decades in the fight against anaemia (Stevens et al., 2013). In fact, it has been estimated that it will take more than 150 years to achieve a prevalence of 15% of anaemia among women in the West Africa Region (Mason, Martorell, Saldanha, & Shrimpton, 2013). Therefore, the need to step

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up efforts to improve the anaemia situation among adolescent girls in this population is imperative.

The observed high prevalence of anaemia in this study could be attributed to multiple interrelated factors such as nutrition, infections and socioeconomic determinants (Balarajan, Ramakrishnan, Özaltin, Shankar, & Subramanian, 2011). Poor dietary intakes, poor dietary diversity, low levels of education and other socioeconomic variables alone or in various combinations could all account for the high anaemia prevalence in this population of girls.

Iron deficiency still remains the most common micronutrient deficiency in the world and has negative consequences for both the mother and child during pregnancy (Acosta et al., 2014; Kassebaum, 2016). This study showed high prevalence of depleted iron stores in the pregnant adolescent girls. Recent studies have reported low serum ferritin concentrations in both adolescent and adult pregnant women, an indication of iron deficiency (Bopape et al., 2008; Senadheera, Goonewardene, & Mampitiya, 2017). In two separate studies conducted among Ghanaian women, low serum ferritin were reported in 21.3% and 16% of pregnant women (Dei-Adomakoh et al., 2014; Engmann et al., 2008).

The present study has demonstrated that, iron deficiency is still a major cause of anaemia in pregnancy. In this study the mean intake of dietary iron was very poor (9.10 mg) as against the recommendation of 23 mg. In addition, the girls were not on any supplements as at their first antenatal visit. It can be seen from the data obtained from both the dietary diversity scoring and the food frequency that most of their sources of iron came from animal source foods. Iron from animal source foods is more bioavailable and therefore it was good to find that this was their main source. However, their low socioeconomic

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status as well as low levels of education resulted in small quantities being consumed, which blunted this advantage. Consumption from green leafy vegetables, which is less bioavailable was also low and therefore could not have contributed much to the dietary iron intake.

In some other studies that have been carried out to determine the iron deficiency status of pregnant women, participants were on some form of iron supplementation and therefore could explain the low prevalence of iron deficiency in those studies as compared to the present study (Pinho-Pompeu et al., 2017). In this study, the pregnant girls were not on any supplements at the time of recruitment into the study even though iron supplementation in pregnancy is mandatory in Ghana. This underscores the need to present early for ante-natal sessions to correct any nutritional deficiencies.

Another reason that could account for the high prevalence of iron deficiency in the present study could be the cut-off for serum ferritin (<30µg/ml) used to categorize those who had low serum concentrations of ferritin. Varied cut-off points have been used in categorizing iron deficiency based on serum ferritin levels. Cut-offs ranged between <12 µg/ml to 40 µg/ml (Bopape et al., 2008; Dei-Adomakoh et al., 2014; Engmann et al., 2008). In this study, the cut-off 30µg/ml was chosen because of its wide usage in pregnant populations and also taking into account inflammation (WHO, 2007). Some studies have used cut-offs < 16 µg/ml and have resulted in reporting low prevalence of iron deficiency. Raising the cut-off to <30µg/ml was considered appropriate to account for inflammatory conditions. Serum ferritin levels are raised under inflammatory conditions (Kell & Pretorius, 2014).

This study reported folate deficiency in 50.4% of the girls studied. The folate deficiency result in this study was in agreement with the findings of Htet et al. (2016) among non-

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pregnant adolescent girls (54%) in Myanmar. Lower prevalence (9%) were however reported in pregnant South African adolescents living in Limpopo (Bopape et al., 2008). Studies in Ghana mainly focussing on adults have shown folate deficiency of 16.7% and 30% respectively in urban and rural populations (Akwetea, 2015; Dei-Adomakoh et al., 2014).

Contrary to the findings of this present study, an earlier study in Northern Nigeria recorded very low prevalence of folate deficiency (2%) in non-pregnant adolescent girls (VanderJagt et al., 2000). The authors attributed this low prevalence to diet rich in sources of folate when compared to vitamin B_{12} sources. They further indicated groundnut (peanuts) as being the greatest contributor to the folate intake of the girls. In this current study, dietary intakes of folate were woefully inadequate with the girls achieving mean intakes of 110.9 µg as against the recommendation of 520 µg providing a strong explanation for the high serum folate deficiency in these pregnant girls. Intakes from the good sources of folate; dark green leafy vegetables (9.4%), nuts and seeds (15.2%), legumes (31.3%) and fruits (3.1%) groups as assessed by dietary diversity all showed very low intakes. Furthermore, substantial amount of folate could have been lost during food preparation as has been demonstrated by some studies, culminating in the low serum levels (Bassett & Samman, 2010; McKillop et al., 2002).

The findings of this study are of great concern given the critical role folate plays in the expression of genes, reproduction and cell division. The need to intensify food fortification efforts to address folate deficiency is a critical step that needs to be enforced by government and all stakeholders. Food fortification is considered one of the less expensive strategies to use in the prevention of folate deficiency (Noor et al., 2017). Countries such as Indonesia, US and Tanzania have demonstrated the positive effects of

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food fortification with folic acid on blood concentrations of women in their reproductive ages (Hardinsyah, 2004; Noor et al., 2017; Pfeiffer et al., 2012). In Ghana, the need for regulatory bodies to enforce the mandate to fortify by industry players is imperative because not all industries are fortifying as required, citing reasons of high production cost (Nyumuah et al., 2012). Adequate intakes of folate will aid in reducing prevalence of neural tube defects which have been linked to low intakes of folate. Available data suggests a high prevalence of 11.5 per 10,000 births of neural tube defects in Ghana; an estimate that is higher than 6.0 per 10,000 births, the achievable rate through adequate intake of folic acid during the peri-conceptional period (Zaganjor et al., 2016). In Ghana, it is a national nutrition policy to supplement pregnant women with 5 mg of folic acid daily (MOH, 2013). However, many of these girls would have missed the critical period in pregnancy when folic acid is needed most because they report after the first trimester. Thus, increasing the risk of neural tube defects in infants delivered by the girls.

Vitamin B_{12} is essential in the synthesis of homocysteine to methionine. It is also needed in maintaining and repairing neural axons (Pawlak et al., 2014). To our knowledge, this study is the first to report on the vitamin B_{12} deficiency status of pregnant adolescent girls in Ghana.

Contrary to two other studies in Ghana conducted in adult populations where no deficiencies were found (Akwetea, 2015; Dei-Adomakoh et al., 2014), 11.7% of the pregnant girls in this present study were deficient in vitamin B_{12}. This is quite low compared to iron and folic acid deficiencies. This could be due to vitamin B_{12} being stored in substantial amounts in the body mainly in the liver which takes about 5 years for deficiency symptoms to be observed. Thus it is possible that some of these girls were drawing on their body stores hence the lower prevalence of deficiency. Htet et al., (2016)

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reported less than 1% prevalence deficiency among adolescent girls in Myanmar. In other studies in South Africa, Nigeria, Kenya, Pakistan and Colombia, prevalence between 4-18.6% were reported (Bopape et al., 2008; Khan, Fatima, Imran, & Khan, 2010; Ramírez-Vélez, Correa-Bautista, Martínez-Torres, Meneses-Echávez, & Lobelo, 2016; VanderJagt et al., 2000).

Higher prevalence ranging between 18%-51.1% have also been reported in populations where the practice of vegetarianism is common (Jeruszka-Bielak et al., 2017; Samuel, 2013; Schroder et al., 2017). This is because the main dietary source of vitamin B_{12} are animal source foods/products and therefore diets restricted in animal food sources/products are potential risk factors for developing vitamin B_{12} deficiency in these populations. For example, among South Indian women who were mainly vegetarians, reasons given for the high prevalence of vitamin B_{12} deficiency were inadequate dietary intakes from animal source foods and not taking supplements (Samuel, 2013). In a study carried out in children in Accra and Central regions of Ghana, the results demonstrated that children who were vegetarians had zero intakes of vitamin B_{12} when compared to those who were not vegetarians (Osei-Boadi, Lartey, Marquis, & Colecraft, 2012). Other reasons that have been attributed to high levels of deficiency have included hemodilution in pregnancy (Visentin et al., 2016). The presence of deficiency in vitamin B_{12} could be attributed to the low intakes of animal source foods as exhibited by the low amount of protein consumption among the girls in spite of its frequent consumption as shown by the food frequency and dietary diversity.

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5.5 Relationship between socio- demographic factors and nutritional anaemia in pregnant adolescent girls

As has earlier been alluded to, the causes of anaemia in developing countries including Ghana are multifaceted (Nguyen et al., 2015). Socio-demographic characteristics are important factors that need to be considered in dealing with anaemia especially in reproductive age women. This is however often overlooked (Ndukwu & Dienye, 2012).

In the present study, education, marital status, religion, who the girls lived with and whether they practiced pica or not were found to have an impact on some of the variables studied. Dietary iron intake was found to be higher in those who had had previous pregnancies when compared to those who were experiencing pregnancy for the first time (11.0 mg verses 8.9 mg, $p = 0.04$). This may mean that in spite of the overall low iron intakes in this cohort of pregnant adolescents, those who had been pregnant before and had attended antenatal clinics remembered some nutritional advice and tried to apply it. Vitamin B_{12} and vitamin C intakes were higher among those who practice pica as against those who did not engage in pica. It is difficult to find a reason that clearly explains this.

Formal education is a key sociodemographic variable that can impact on the nutritional status of an individual. According to Nwizu et al., (2011), pregnant women with no education were two times at risk of anaemia. Similarly Noronha et al., (2010) also reported low educational status among South Indian pregnant women studied. This was associated with increased prevalence of anaemia. The characteristics of the girls in the current study according to haemoglobin levels revealed that anaemia was significantly high whether the girls had formal education or not, suggesting that the low level of basic

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education that most of them had made no difference between those who had any formal education compared to those who did not. That is to say that, low level of basic education was as bad as no education at all in this regard. This calls for the need for stakeholders to incorporate adequate nutrition education into the education curriculum during the basic education period.

No significant differences were seen between the proportions that were married and anaemic among those that had normal haemoglobin. Studies have reported conflicting results on the relationship between anaemia and some sociodemographic variables. For example, in one Nigerian and one Ethiopian study, married women were less likely to be anaemic (Nwizu, Iliyasu, Ibrahim & Galadanci, 2011; Gebre & Mulugeta, 2015). However in another Ethiopian study, marital status did not significantly affect anaemia status (Abay, Yalew, Tariku & Gebeye, 2017). Ndukwu & Dienye (2012), on the other hand, reported anaemia to be more prevalent among married women. In the present study, anaemia was very prevalent whether the girls were married or not. Significant differences were not observed in the proportions probably due to the small sample size of girls that were married. Marriage was expected to have provided some financial security therefore enabling the girls to properly care for themselves in terms of their dietary intakes and other healthcare needs.

5.6 Dietary practices/modifications by pregnant adolescent girls

The dietary practices/modification that the pregnant adolescent girls had made to their diet since they became pregnant was also evaluated. Modifications made by the girls centred on consumption of oily or fatty foods, quantities of food consumed, consumption

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of fruits and vegetables and the practice of pica. The girls of this present study generally abhorred consumption of foods high in fat. They also indicated that they had increased the quantities of foods they consumed and particularly increased their fruit and vegetable intakes. Consistent with the findings of the present study, Nti et al., (2002) reported among 73% of expectant Ghanaian mothers studied, that their food intakes had increased since they became pregnant. Likewise, in a systematic review of studies across Europe and America, pregnant women reported increases in the quantities of foods they consumed as well as increased consumption of fruit and vegetables since they became pregnant (Hillier & Olander, 2017). It was encouraging to find that girls in this present study had started some positive dietary changes. However these changes were unlikely to meet dietary recommendations when compared to the results obtained when the intakes were quantified. Gharaibeh, Al-Ma'aitah, and Al Jada (2005) made similar observations among Jordanian pregnant women and saw that women's dietary modifications during pregnancy did not comply with dietary recommendations. Also their modifications focussed more on the quantity of the food but not of the quality. This, the authors explained, could be attributed to low socioeconomic status which made it difficult for the women to meet their nutritional recommendations. Similar explanations could be given for the pregnant adolescent girls in the present study considering their socioeconomic levels. The need to ensure good dietary practices and modifications in pregnancy is paramount especially among these pregnant adolescents considering their high risk of nutritional vulnerability. This will necessitate nutritional education as this has been effective in preventing some complications in pregnancy (O'Brien, Grivell, & Dodd, 2016).

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5.7 Myths and misconceptions that may affect nutrient intakes of pregnant adolescent girls

Promotion of blood formation to prevent anaemia in pregnancy is of concern to both healthcare professionals and pregnant women during the period of pregnancy. The common foods the girls attributed to promoting blood formation were Turkey berries (*Solanum torvum*), malt drink and milk. There is a popular belief among Ghanaians that Turkey berries promote blood formation (Akoto, Borquaye, Howard, & Konwuruk, 2015). Additionally traditional healing practitioners have been reported to prescribe the juice of Turkey berries for the treatment of anaemia in some parts of Ghana (Asiedu-Darko, 2010). Therefore it was not surprising to obtain this information from the pregnant girls. de-Graft Aikins (2014) similarly reported on pregnant women believing in the blood promotion properties of turkey berries in another cohort of Ghanaian pregnant women. Similar findings were also reported among pregnant women living in a rural community in Kenya (Riang'a, Broerse, & Nangulu, 2017). Akoto et al., (2015) have documented that turkey berries, are high in iron, therefore their consumption by the girls will be beneficial in promoting blood formation. The concern that arises is the bioavailability of the iron in turkey berries. Iron from plant food sources is mainly non-haem and is known to have low bioavailability (Hurrell & Egli, 2010). In this study, the reported mode of consumption of blending the turkey berries and mixing it with milk (a good source of calcium) may hinder the absorption of iron. Calcium has been reported to hinder iron absorption by 50-60% (Hallberg, Brune, Erlandsson, Sandberg, & Rossander-Hulten, 1991). This in effect calls for education of the girls on the proper way to prepare the juice for consumption to maximise the availability of non-haem iron present in turkey berries. Since vitamin C is known to enhance the absorption of non-haem, education should be

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focussed on encouraging them to mix the juice with citrus fruits instead of milk and malt drinks (Hurrell & Egli, 2010). However, one also needs to be cautious about this kind of mixture since the toxicity levels of this mixture has not be proven and therefore safety in frequent consumption should be of concern.

The misconception that malt drinks promote blood formation was also common among the girls. This is a general misconception among the wider populace, due in part to product advertisements. Obuzor and Ajaezi (2010) analysed the nutrient content of popular malt drinks on the Nigerian market and concluded that the content of iron was negligible. Three of the malt drinks mentioned by Obuzor and Ajaezi (2010) are popular on the Ghanaian market and could be assumed to have similar nutritional compositions. The misconception of malt drinks promoting blood formation should be dispelled through much needed education.

Studies have reported on various myths and misconceptions and taboos surrounding foods during pregnancy (de-Graft Aikins, 2014; Riang'a et al., 2017; Yeasmin & Regmi, 2013). In this present study, the girls reported myths and misconceptions surrounding the consumption of okro (*Abelmoschus esculentus*), snails and mangoes (*Mangifera sp*). Myths and misconceptions surrounding snails have also been reported among Nigerian, Bangladeshi and Kenyan pregnant women (Ekwochi et al., 2016; Riang'a et al., 2017; Yeasmin & Regmi, 2013). Other studies in Ghana have reported similar findings (de-Graft Aikins, 2014; Otoo et al, 2015; Gadegbeku et al., 2013). The common reason given by the girls for not consuming snails and okro was that their babies will drool if they consume them because of their slimy nature. Similar reasons that have been given elsewhere in Nigeria include baby will become sluggish and spit saliva (Ekwochi et al., 2016).

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The avoidance of mangoes (*Mangnifera indica*) and oranges (*Citrus* × *sinensis*) in pregnancy were new discoveries. Consumption of mangoes was believed to be associated with diarrhoea, fever and the development of a "yellow face" by the child, while orange consumption was associated with the baby developing stomach ulcer. Mangoes are estimated to contain 60-138 µg/100g of folate, whiles oranges contain about 20-43 µg of folate making them good sources of folate (Akilanathan, Vishnumohan, Arcot, Uthira, & Ramachandran, 2010). Mangoes and oranges are cheap and easy to come by when in season. This necessitates nutritional counselling and education addressing such misconceptions which are likely to deter pregnant women from consuming mangoes and oranges thereby forestalling the adverse consequences of folate deficiency.

5.8 Nutritional knowledge and sources of information of pregnant adolescent girls in relation to nutritional anaemia.

The anaemia - related nutrition knowledge of the pregnant girls were investigated and found to be inadequate among the pregnant girls studied. The causes of nutritional anaemia outlined by the pregnant girls were mainly too much exposure to sunlight and drinking of too much water. Even though some indicated inadequate food intakes, they could not link it to inadequate intakes of iron or food sources of iron. In other studies, causes of anaemia that were outlined by women of the reproductive ages included malnutrition, heavy workload, household responsibilities and lack of hygiene (El Ati et al., 2008). Among the reproductive women studied, Kabir, Shahjalal, Saleh, and Obaid (2010) indicated that 68% did not know what anaemia was. In the present study, the inadequate knowledge on anaemia could be attributed to low levels of nutritional knowledge and formal education. The girls had just reported to ante-natal care and therefore they might not have received any form of nutrition education.

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It is imperative to devise measures to get the adolescent girls to be educated on anaemia in view of the importance of the nutrients under consideration. Nutrition education has been found to increase knowledge in the prevention of anaemia in other areas (Moreshwar, Naik, & Chrostina, 2014). Adolescent girls have been empowered through educational interventions to improve anaemia related knowledge and hence reducing the incidence of anaemia (Jalambo, Sharif, Naser, & Karim, 2017; Seyed, Roshan, Navipor, & Alhani, 2014). Nutrition education should be included in basic education to ensure that good nutritional habits are developed from childhood and sustained throughout life.

In treating anaemia, the pregnant girls mainly reported remedies such as drinking a lot of water, turkey berry juice, herbal medications and consumption of fruits. A few also mentioned taking medications from the doctor. Similar studies also reported that women in their reproductive ages resorted to various remedies to treat anaemia. Among the treatments that they had resorted to were sugar and salt water solutions, coconut water, milk and red soil/stones (Chatterjee & Fernandes, 2014). Among Bangladeshi adolescent girls, participants in the study who had some knowledge on anaemia indicated it could be treated with iron tablets and foods rich in iron such as eggs, liver and green leafy vegetables (Kabir et al., 2010). The findings of this study re-enforces the need to provide nutrition education that will teach on the dietary sources of iron and the most bioavailable forms to consume to provide maximum benefit. Maximum advantage should be taken when the girls report for ante-natal health care to provide the needed dietary counselling to improve maternal and child health. These measures have been found to improve maternal knowledge and lower the risk of maternal anaemia by 30% (Darnton-Hill, 2013).

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The main sources of information on nutritional anaemia were from school, grandmothers and nurses. These findings are consistent with findings among Nigerian adolescents who reported obtaining their nutrition related information from their teachers (63.4%), health professionals (11.8%) and mothers (64.4%) (Ene-Obong & Akosa, 1993). Elsewhere in the United States of America, 30.8% of pregnant women interviewed reported receiving their nutrition information from healthcare professionals. However, the majority (88.5%) resorted to the use of the internet search engine GOOGLE to obtain their nutrition information (Blondin & LoGiudice, 2018). The use of the internet to seek for nutrition information was not mentioned in the present study. This can be attributed to the low level of education of participants of this present study as compared to the study by Blondin & LoGiudice (2018) who reported that most of the participants had at least a bachelor's degree. In another study that aimed at examining the health information seeking behaviours of pregnant adolescent girls in the Ejisu-Juaben Municipality of Ghana, authors presented similar findings of the sources of health information among pregnant adolescent girls. Consistent with girls of this present study, the pregnant girls in Ejisu- Juaben also reported family members and neighbours as their main sources of health information. The researchers recommended that the establishment of an adolescent only antenatal care programme will be effective in meeting the information needs of pregnant adolescent girls (Owusu-Addo, Owusu-Addo, & Morhe, 2016). To ensure adolescents receive proper nutrition education in the Ghanaian setting, it is imperative to empower all stakeholders with the right nutrition information so they can provide accurate information geared at improving nutrition of the pregnant adolescent. Adequate nutrition education should also be incorporated into the school curriculum right from the basic school to ensure that adolescent girls who might have spent some time in

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school at least up to the Junior High School level would have gained some understanding of the importance of good nutrition during child bearing.

Strengths of the study

1. This study employed both quantitative and qualitative methodological approaches. This allows readers to have a comprehensive view of the problem.
2. Laboratory tests and dietary recalls employed trained individuals to avoid disparities in reporting.
3. This study is probably the first of its kind in Ghana to explore the nutritional anaemia situation in pregnant adolescents whiles adopting both qualitative and quantitative methodologies.

Limitations of the study

1. Findings may not necessarily reflect what pertains in the country as a whole because the study population used was drawn mainly from low socioeconomic areas in Accra, an urban area, which may not be representative. However, it brings to the fore what is likely to be happening in the peri-urban and rural settings.
2. Seasonal variations in collecting dietary diversity data was not factored in the data collection because only one round of data was collected across the seasons. However seasonal variations affecting food security in the home is becoming less popular because of improved farming and postharvest preservation methods.
3. Lack of current Ghanaian food composition data base could affect the estimation of dietary intakes.

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CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings of this research underscore the problem of anaemia in pregnant adolescent girls as still a problem of public health concern. The prevalence of anaemia in pregnant adolescent girls was estimated to be 71.6%. Iron and folate deficiencies, and to a lesser extent B_{12}, are of major public health concern and need to be addressed among this physiological age group.

The mean nutrient intakes of the major micronutrients (iron, folate, vitamin B_{12}) of concern in relation to nutritional anaemia in this study, were found to be inadequate compared to recommendations. Majority of the girls were seen to have inadequate intakes of not only one of the micronutrients but two or more. This is an indication for the need to effectively roll out implementable policies and programmes that can ensure girls in this reproductive age category get adequate intakes of the nutrients of concern.

Overall, poor MDD-W was observed among the girls. Just about 25% were able to achieve good MDD-W. This is an indication of the likelihood that many of the girls would not be able to achieve their micronutrient intakes.

This study found significant relationship existing between serum haemoglobin and dietary vitamin B_{12} and serum haemoglobin and serum vitamin B_{12}. MDD-W was also found to correlate significantly with dietary iron and dietary folate. Dietary folate was the most significant predictor of MDD-W. These findings call for the need for all stakeholders through collaborative efforts to improve nutritional intakes through

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promoting and enforcing food fortification and supplementation and encouraging dietary diversification by enacting and implementing policies and educational interventions that particularly address the deficiencies of iron, folate and vitamin B_{12}.

In this study, socio-demographic variables did not significantly impact on dietary intakes, MDD-W and the serum concentrations of iron, folate and vitamin B_{12} mainly due to the not so wide variations in the socio-demographic characteristics of the adolescent girls.

The pregnant adolescent girls' knowledge on nutritional anaemia were found to be inadequate. This, eventually can be seen in the poor outcomes of the indicators of anaemia seen throughout the study. Some of the responses expressed as the causes of anaemia were inaccurate and could have contributed to the development of anaemia. Many raised dietary misconceptions about milk and malt drinks as the main foods needed for blood promotion. Myths regarding okro, oranges, mangoes and snails were also raised and therefore avoided in pregnancy. These misconceptions and myths can be seen to have contributed to the poor micronutrient intakes thereby raising the anaemia prevalence among this physiologic age group.

6.2 Recommendations

In reference to the findings of this study, the following recommendations are made to aid in decision making in improving the health of pregnant adolescent girls;

1. Early identification of pregnant adolescents at risk of nutrient deficiencies through adequate and thorough routine dietary assessment at their first antenatal visit should be undertaken. Dietitians/Nutritionists should be engaged to conduct thorough dietary assessment that will provide relevant information to aid in treating nutritional deficiencies.

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2. Nutrition education centred at the community level to educate adolescent girls on nutritional needs even before they get pregnant is necessary to avert the early consequences of these nutritional deficiencies in pregnancy.
3. Nutrition education programmes at antenatal clinics should be prioritized and strengthened by conscientiously incorporating the promotion of dietary diversity. Nutrition education programmes should be geared towards behavioural change to ensure that an impact is made in the health of these pregnant adolescent girls and subsequently their infants.
4. Folic acid supplementation should be universally carried out on all adolescent girls in the communities studied since one is not sure which of these girls may become pregnant. This is in view of the fact that many of these girls do not turn up early for antenatal clinics when they are pregnant by which time the harm of the deficiency on the developing foetus would have already been caused.
5. Enforcement of the regulation on food fortification is necessary and should be vigorous.
6. With the advent of free Senior High School education which incorporates free feeding and free school feeding programmes in the basic levels of education, food fortification can be used to address these micronutrient deficiencies. Some specific foods can be fortified and the schools mandated to cook with these ingredients.
7. Nutrition for pregnant women should be demystified and misconceptions corrected through further education by dieticians and nutritionists during clinic attendance and at the community level.

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6.3 Future Research Directions

1. The cross-sectional nature of this study does not allow for the establishment of causality even though it provides evidence for what may be happening to pregnant adolescents. Therefore future studies can be designed longitudinally to allow for following up of participants and to see the outcomes of their pregnancies.

2. Similar researches should be carried out in other parts of the country particularly rural communities to provide information on what pertains in the country as a whole since adolescents are among the least studied groups in the country and also to provide hard facts to drive policy interventions.

6.4 Contribution to knowledge

1. This study fills in the knowledge gap in the micronutrient status of iron, folate and vitamin B_{12} in Ghana among young populations such as adolescents and in pregnancy. The adolescent population is one of the least studied in Ghana. It is probably the first to have reported on the vitamin B_{12} deficiency prevalence in pregnant adolescent girls.

2. This is the first study to use the new MDD-W tool to assess the micronutrient adequacy of diets in pregnant adolescent girls in Ghana.

3. The avoidance of mangoes and oranges in pregnancy because of the belief of causing diarrhoea, fever, stomach ulcers and “yellow face” in babies are new discoveries that has been documented.

4. The study has reinforced some knowledge already known about nutritional anaemias in pregnant women in Ghana, and in pregnant adolescents in particular.

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6.5 Contributions of research candidate

The candidate contributed to the development of this research thesis by engaging in the following activities;

- Conceptualization of thesis.
- Development of questionnaires.
- Data collection with the help of research assistants
- Assisted with laboratory analysis.
- Data entry and cleaning.
- Data analysis with assistance from statistician.
- Writing – up of entire thesis.

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APPENDICES

APPENDIX A: QUESTIONNAIRE

ID NO.....

UNIVERSITY OF GHANA

College of Health Sciences, School of Biomedical and Allied Health Sciences

NUTRITIONAL ANAEMIA IN PREGNANT ADOLESCENT GIRLS

Introduction

You are invited to take part in the research on nutritional anaemia in pregnant adolescent girls. This questionnaire seeks to obtain information on nutritional anaemia in pregnant adolescent girls. You are assured that any information given is solely for academic purposes and will be kept confidential. You are requested to answer all the questions as accurately and honestly as possible. Thank you

DEMOGRAPHIC INFORMATION		**CODE**
1. Name		
2. Age		AGE
3. TELEPHONE NO.		
4. Religion	(1)Christian (2)Moslem (3) Traditonalist (4)Other.....	RELI
5. Community	(1)James town (2) La (3) Mamprobi	COMM
6. What is your level of education?	(1) No education (2)Primary (3) JHS (4) SHS (5) Tertiary	EDULEV
7. What is your occupation?	(1) Unemployed (2) Artisan (3) Trader (4) other	OCCU
8. What is your marital status?	(1) Married (2) Single (3) Co habitation (4) Divorced	MSTAT
9. Who do you live with?	(1)Parents (2) Husband/Partner (3) Alone (4) other relative (5) Other	LIVWIT

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INFORMATION ON PREGNANCY		
10. How many months pregnant are you?	(1) (2) (3) (4) (5) (6) (7) (8) (9)	MPREG
11. When did you have your last period?	Day.....Month..... year.....	LPRED
12. Have you had any previous pregnancy	(1) Yes (2) No	PPREG
13. If yes how many	(1) (2) (3) (4) (5)	HPREG
14. Have you attempted to terminate this pregnancy?	(1) Yes (2) No	TPREG

15. Where you on any nutritional supplements before getting pregnant?	(1) Yes (2) No	NSUPP
16. If yes what supplement was it?		WSUPP
17. Do you take any of these substances?	(1)white clay (2) Chalk (3) Mud (4)starch (5) wood/paper (6) Other.....	PICA
18. When was the last time you took a de-wormer?	(1)less than one month ago (2) two months ago (3)three months ago (4) four months ago (5) more than four months (6) OTHER	DWORM
19. Do you sleep under a mosquito net?	(1) Yes (2) No	MNET
20. When was the last time you took a malaria treatment course?	(1)less than one month ago (2) two months ago (3)three months ago (4) four months ago (5) more than four months (6) OTHER	MCOU
21. What is your sickling status?	(1) Positive (2) Negative	SSTATU

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DIETARY ASSESSMENT

24-hour recall dietary assessment **DAY 1**

	Handy measures estimation	Grams
Breakfast		
Mid-Morning snack		
Lunch		
Afternoon snack		
Supper		
Bedtime snack		

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DAY 2

	Handy measures estimation	Grams
Breakfast		
Mid-Morning snack		
Lunch		
Afternoon snack		
Supper		
Bedtime snack		

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DAY 3

	Handy measures estimation	Grams
Breakfast		
Mid-Morning snack		
Lunch		
Afternoon snack		
Supper		
Bedtime snack		

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APPENDIX B: FOOD FREQUENCY QUESTIONNAIRE

Can you please answer the following questions?

How often do you usually eat the following foods? (Please tick one)

Food/dish	**Once a day**	**More than once a day**	**5-6x a Wk**	**3-4x a week**	**1-2x a Wk**	**1-2x a month**	**Rarely**	**Never**
Beverages								
Tea Leaf								
Chocolate drink Bournvita /Milo/ Cocoa powder etc.								
Horlicks								
Coffee								
Porridges								
Corn porridge (koko)								
Millet porridge(hausa)								
Rice porridge								
Oats porridge								
Wheat porridge								
Weanimix								
Breakfast cereal e.g. corn flakes, weetabix								
Milk and milk products								
Carnation								
Semi-skimmed milk								
Skimmed milk								
Condensed milk								
Yoghurt								
Ideal milk								

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Food/dish	Once a day	More than once a day	5-6x a Wk	3-4x a week	1-2x a Wk	1-2x a month	Rarely	Never
Ice cream								
Soymilk								
Cheese								
Spreads								
Butter								
Margarine								
Polyunsaturated margarine								
Peanut butter								
Marmalade/Jam								
Bread								
Wholemeal (brown b								
White bread								
Sugar bread								
Butter bread								
Deep fried foods								
Fried yams								
Fried plantain								
French fries								
Potato chips								
Oils								
Palm oil								
White oil e.g. sunflower, frytol/ soybean								
Coconut oil								

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Food/dish	Once a day	More than once a day	5-6x a Wk	3-4x a week	1-2x a Wk	1-2x a month	Rarely	Never
Palm kernel oil								
Groundnut oil								
Vegetables								
Turkey berries/Pebble garden egg (abeduru)								
Cabbage								
Kontomire								
Mixed vegetables (e.g. Coleslaw								
Carrots								
Garden eggs								
Cauliflower								
Okro								
Green beans (runner beans etc)								
Brussels sprouts								
Cucumber								
Sweet peppers								
Lettuce								
Fish and seafood								
Fish								
Canned fish								
Shrimps								
Lobster								
Crabs								

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Food/dish	Once a day	More than once a day	5-6x a Wk	3-4x a week	1-2x a Wk	1-2x a month	Rarely	Never
Meat and meat products								
Beef								
Salted beef								
Chicken								
Turkey								
Pork								
Goat meat								
Pig feet								
Pig feet (unsalted)								
Snails								
Offals (gizzard, Liver, tripe, etc)								
Bacon								
Sausages								
Cow's skin								
Canned meat (corned beef)								
Game (Bush meat)								
Cow foot								
Eggs								
Starches								
Banku								
Kenkey								
Fufu flour								
Food/dish	**Once**	**More than**	**5-6x**	**3-4x**	**1-2x**	**1-2x**	**Rarely**	**Never**

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	a day	once a day	a Wk	a week	a Wk	a month		
Fufu (pounded)								
Plantain								
Yam								
Gari								
Cocoyam								
Rice								
Pasta, macaroni, spaghetti								
Soups								
Palm soup								
Ground-nut soup								
Light soup								
Okro soup								
Stews								
Kontomire stew (no agushie)								
Kontomire stew (plus agushie)								
Aubergine or garden egg stew								
Agushie stew								
Tomato stew								

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Food/dish	Once a day	More than once a day	5-6x a Wk	3-4x a week	1-2x a Wk	1-2x a month	Rarely	Never
Fruits								
Pawpaw								
Pineapple								
Mango								
Citrus fruits								
Banana								
Pear								
Melon								
Apples								
Avocado pear								
Grapes								
Guava								
Coconut								
Sugarcane								
Fruit juices(eg. apple								
Soft drinks								
Fanta, coke etc.								
Supermalt								
Malta guinness								
Diet drinks								

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Food/dish	Once a day	More than once a day	5-6x a Wk	3-4x a week	1-2x a Wk	1-2x a month	Rarely	Never
Alcoholic beverages								
Beers								
Wine								
Hard liquor								
Snacks								
Crisps and savoury snacks								
Pastries								
Plantain chips								
Biscuits								
Roasted groundnuts								
Sweets								
Toffees, chocolates etc.								
Legumes								
Black/red-eyed beans								
Baked beans								
Soy flour								
Bambara beans								
Fast Foods								
Hamburgers, pizza, takeaway etc.								

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APPENDIX C: DESCRIPTION OF FOOD GROUPS

The food groups are described as pertained in the FAO manual (FAO & FHI360, 2016).

Grains, tubers and white roots and plantains

This group is usually referred to as the starchy staples. They are very good sources of energy and sources of some of the B vitamins. Plantains are included in this group because they provide the energy similar to the grains, tubers and white roots even though they are not roots/tubers. All non-coloured food items that mainly provide energy are placed in this group. Common examples of Ghanaian foods in this group are Maize, Millet, Oats, Wheat, Rice, Sorghum, plantain, banana, yam, potatoes (all skin colours), sweet potatoes (white/pale yellow).

Pulses (lentils, peas and beans)

Plants in this group are high in protein and some B vitamins. Groundnuts and plants harvested immature or green and eaten in their pods are not included in this group. Common examples in Ghana are cowpeas, soyabeans, Bambara beans and kidney beans.

Nuts and seeds

This group is made up of mainly tree nuts and also includes groundnuts. They are rich sources of proteins, minerals, fibre, unsaturated fatty acids and tocopherols. Common examples in Ghana are groundnuts, cashew nuts, melon seeds, shea butter seeds,

Dairy

This is the milk and milk product group. It includes milk and milk products from goats, sheep, cow, camels or buffalo. They are high in proteins, calcium, potassium and vitamin

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B_{12}. Examples include fresh whole milk, skim and low fat milk, powdered milk, cheese and yoghurt.

Meat, poultry and fish

All meat, poultry and fish processed and unprocessed are categorized in this group. This group are important source of high quality proteins. Examples are poultry, meat, organ meat, birds, fresh and dried fish and shell fish.

Eggs

This group comprises of eggs from any kind of bird. They are high sources of proteins and vitamin B_{12}. Chicken eggs, duck eggs and guinea fowls are all examples in this group.

Dark green vegetables

This group comprises of mainly medium to dark coloured leafy vegetables. They are rich sources of vitamin A, folate and other micronutrients. Common examples in Ghana are cocoyam leaves, cassava leaves, dandelion, lettuce, *corchorus sp*. (ayoyo), water leaf "(Bokoboko)" (Talinum triangulare), "(Aleefu)" (Amaranth cruentus) and Moringa oleifera.

Other vitamin A rich fruits, vegetables roots and tubers

These are vitamin A rich foods that are not obtained from green leafy vegetables. They may also be rich sources of folate and vitamin C. Common examples are mangoes, ripe pawpaw, carrots, sweet potatoes (orange-dark yellow flesh) and palm fruit.

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Other vegetables

These are all other vegetables that are not included in the vitamin A rich group. It also includes legumes that are consumed with the pod for example green beans. Other common examples include in this group include tomato, cucumber and okra.

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APPENDIX D LABORATORY DETERMINATIONS

Serum sample preparation

Five ml (5ml) of venous blood was drawn into a serum separator tube and centrifuged for 5 minutes at 10000 g. The serum was collected and stored at -20^{o}C until analysis was carried out.

Folate determination

Principle of assay

The assay is an ELISA technique involving the use of microtiter plate wells coated with the microorganism *Lactobacillus rhamnosus* which metabolizes folic acid. When the serum sample is introduced to the plate, there is a folic acid-dependent growth response until the vitamin is finally consumed. The plate is incubated at 37^{o}C for 48 hour after which the growth of the *lactobacillus rhamnosus*, which is proportional to the amount of folic acid present, is measured at 610 nm (ID-Vit® Folate Manual, 2015).

Procedure for determination

Serum folate was determined following manufacturer's instructions;

Materials supplied in the kit

1. One *Lactobacillus rhamnosus*-precoated microtiter plate, ready to use (PLATE)
2. Sample treatment solution 5 ml, ready to use (SOL)
3. Water 30 ml, ready to use (DIL)
4. Folic acid-Assay-Medium (ASYMED)
5. Folic acid-Assay-Medium (STD)
6. Cover plastic foil (FOL)

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7. Replace holder for 96-well plates (FRA)
8. Folic acid medium treatment buffer 1.5ml (ASYBUF)
9. Control folic acid (CTRL)

Materials required but not supplied in the kit

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90°C - 100°C)
- ELISA-Reader 610 nm
- Micropipette 20 - 200 µl
- Micropipette 100 -1000 µl
- Micropipette tips to deliver 20 - 200 µl and 100 -1000 µl, sterile
- Pipettes of 5 and 10 ml
- 1,5 - 2 ml reaction vials, sterile
- 0,2 µm sterile polyethersulfone filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 x g)

Preparation of sample and dilution

Hundred microliters (100 µl) of serum sample was added to 400 µl sample preparation solution (SOL), heated for 95ºC for 30 minutes, quickly cooled and then centrifuged for 5 minutes at 10000 xg. Seven hundred microliters (700 µl) of water (DIL) was added to 50 µl from the supernatant of the treated serum sample resulting in a final sample dilution of 1:75.

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Preparation of Assay medium [ASYMED]

The medium was freshly prepared. One milliliter (1 ml) of the medium treatment buffer [ASYBUF] and 10 ml of water [DIL] were added to the assay medium [ASYMED], securely closed in a bottle. The bottle was shaken very well and heated in a water-bath at 90 - 100 °C for 5 min, while being shaken well at least twice. It was ensured that the bottle was closed tightly at all times. The solution was quickly cooled to under 30°C. Ten milliter (10 ml) of the ASYMED was filtered sterilely with a 0.2 µm sterile polyethersulfone filter into a 15 ml Falcon tube.

Preparation of Standard [STD]

The standard curve solutions were freshly prepared before the test. Two thousand, two hundred microlitres (2.2 ml) water [DIL] from the test kit was added to the standard bottle [STD], closed and the bottle shaken (= standard concentrate).

The distilled water [DIL] was aliquoted into 6 sterile reaction vials (capacity 1.5 – 2.0 ml) and then pipetted into the standard concentrate. A standard curve was prepared using the following scheme (Table 3.1);

Table 3.1: Standard curve parameter for folate determination

	Folic acid [µg / l]	Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank	0	450	+	0	=	450
Standard 1:	0.04	450	+	50	=	500
Standard 2:	0.08	400	+	100	=	500
Standard 3:	0.16	300	+	200	=	500
Standard 4:	0.24	200	+	300	=	500
Standard 5:	0.32	100	+	400	=	500

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Preparation of Control [CTRL]

The control was freshly prepared before each test run.

An amount of 0.125 ml of water [DIL] from the test kit was added to the control bottle [CTRL]. The bottle was closed and contents dissolved by vortexing the bottle (= control). The control was then treated as a sample afterwards.

One hundred and fifty microliters (150 µl) of the pretreated sample or diluted control [CTRL] were then pipetted into each well as required.

Initiation of test and measurement

An amount of 150 µl Folic acid assay medium [ASYMED] was pipetted into the plate cavities. One hundred and fifty microliters (150 µl) standard [STD] and 150 ul of sample were pipetted respectively into the cavities and the cavities carefully sealed with plastic foil (FOL) pressing down the foil with the hand to ensure that the cavities were airtight. The plate was then incubated at 37^{o}C for 48 hours.

Reading

The foil [FOL] was securely pressed down with the hand, the plate turned upside down onto a tabletop and the germination well shaken. The plate was then turned over and the foil carefully removed by pulling it diagonally at an angle of 180^{O}. All air bubbles were removed with the aid of a pipette. The turbidity was then read in an ELISA-Reader at 610 nm.

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Evaluation of results

A four parameter algorithm was used to calculate the concentrations from a graph. This was done by incorporating the sample dilution factor of 1:75. Reference range for the assay was 3.8 - 23.2 µg/L.

Vitamin B_{12} determination

Principle of assay

The plates of the microtiter wells were coated with *Lactobacillus delbrueckii subsp. lactis*. The vitamin B_{12} in the sample or the standard when dropped into the well allows the *Lactobacillus delbrueckii subsp. lactis* to feed on it, stimulating its growth. After incubation at 37^{o}C for 48 hours, the growth of the *Lactobacillus delbrueckii subsp. lactis* is measured at 610 nm in an ELISA reader and a standard curve generated from the dilution. The concentration of vitamin B_{12} is directly proportional to the turbidity (ID-vit B_{12} Manual, 2015).

Procedure for determination

Serum vitamin B_{12} was determined using manufactures instructions as follows;

Materials supplied in the kit

1. *Lactobacillus delbrueckii subsp. lactis*-precoated microtiter plate, ready to use (PLATE)
2. Sample treatment solution 5 ml, ready to use (SOL)
3. Stabilizer (STAB)
4. Water 30 ml, ready to use (DIL)

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5. Vitamin B_{12}-Assay-Medium (ASYMED)
6. Vitamin B_{12}-Assay-Medium (STD)
7. Cover plastic foil (FOL)
8. Replace holder for 96-well plates (FRA)
9. Control Vitamin B_{12} (CTRL)

Materials required but not supplied in the kit

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90°C - 100°C)
- ELISA-Reader 610 nm
- Micropipette 20 - 200 µl
- Micropipette 100 -1000 µl
- Micropipette tips to deliver 20 - 200 µl and 100 -1000 µl, sterile
- Pipettes of 5 and 10 ml
- 1,5 - 2 ml reaction vials, sterile
- 0,2 µm sterile polyethersulfone filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 g)

Sample preparation

Four millilitres (4 ml) of the sample preparation buffer labelled as SOL was pipetted into the stabilizer (STAB) and mixed well. An amount of 75 µl of the serum sample or control were pipetted into 300µl of the prepared solution. The mixture was heated to 95^{O}C for 30 minutes and quickly cooled and then centrifuged at 10000 g for 10 minutes. Hundred

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microliters (100 µl) of the supernatant of the sample or the control was added to 400 µl of water (DIL) and then mixed together resulting in a 1:25 dilution.

Preparation of assay medium

The assay medium was freshly prepared by adding 10 ml of water (DIL) to the bottle labelled ASYMED. The bottle was heated in a water bath at 90-100^{o}C for 5 minutes while shaking it for at least twice. The medium bottle was quickly cooled to under 30^{o}C and filtered into a 15 ml Falcon tube using a sterile 0.2 µm polyethersulfone filter.

Preparation of Standard [STD]

The standards were freshly prepared before the test. An amount of 2.0 ml of water (DIL) was added to the bottle labelled STD. The standard curve was drawn following these volumes on the table;

Table 3.2: Standard curve parameters for vitamin B_{12} determination

Vitamin B_{12} [ng / l]	Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank: 0	700	+	0	=	700
Standard 1: 6	700	+	50	=	750
Standard 2: 18	400	+	100	=	500
Standard 3: 27	350	+	150	=	500
Standard 4: 36	300	+	200	=	500
Standard 5: 54	200	+	300	=	500

Initiation of test measurement

One hundred and fifty microliters (150 µl) vitamin B_{12} assay medium [ASYMED] was pipetted into each cavity. One hundred and fifty microliters (150 µl) of the standard was added to create the standard curve. For the rest of the cavities, the controls and the

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samples were added. The plate was then carefully sealed with a plastic foil ensuring that the seal was airtight. The plate was then incubated at 37°C for 48 hours.

Reading

The foil was securely pressed down with the hand and the plate was turned upside down, the culture well was shaken, the plate then turned up again and the foil carefully removed. Air bubbles in the cavities were removed and the plate placed in an ELISA Reader. Turbidity was read at 610 nm.

Evaluation of results

The four Parameter-algorithm was used to obtain the concentrations from a graph. The sample dilution factor of 1:25 was considered in data evaluation.

Serum ferritin determination

Serum ferritin was determined using the AccuBind ELISA microwells test kit (Monobind Inc. USA) with the product code 2825-3000. The procedure is based on an Immunoenzymometric assay technique. Manufacturer's instructions were followed in the ferritin determination.

Principle of assay

The principle behind the assay is as follows;

The procedure is an immunoenzynometric assay, requiring reagents with high affinity and specific antibodies (enzymes and immobilized), with distinct epitope recognition in excess and native antigen. During the assay procedure, the immobilization takes place at the surface of the microplate well through the interaction of streptavidin which is coated

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on the well and exogenous addition of biotinylated monoclonal anti-ferritin antibody. When monoclonal biotinylated antibody is mixed with the serum which contains the native antigen, there is a reaction with native antigen and the antibody forming antibody-antigen complex. The biotin bound to the antibody simultaneously binds to the streptavidin coated in the micro-wells. This results in the immobilization of the complex. The complex is illustrated with the equation below;

$$Ag_{(ferritin)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftarrows}} Ag_{(ferritin)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

$AgI_{(ferritin)}$ = Native Antigen (Variable Quantity)

$Ag_{(ferritin)}$ -${}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quan.))

ka = Rate Constant of Association

k-a = Rate Constant of Disassociation

$Ag_{(ferritin)}$ -${}^{Btn}Ab_{(m)}$ + Streptavidin $_{C.W.}$ ⇒ immobilized complex $_{(IC)}$

Streptavidin $_{C.W.}$ = Streptavidin immobilized on well

Immobilized complex (IC) = Ag-Ab bound to the well

After the desirable period of incubation, the antibody-antigen fraction is separated from the unbound antigen by decantation. Another antibody which is directed at a different epitope labeled with an enzyme is added. The reaction forms antibody-antigen-biotinyladed-antibody complex in the wells. The excess enzyme was washed away. With the addition of a suitable substrate, a colour was produced which is measured using a spectrophotometer. A dose response curve is generated by using different serum

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references of known antigen concentration. From this curve, an unknown antigen concentration can be determined.

$$_{(IC)} + {}^{Enz}Ab_{(ferritin)} \underset{-kb}{\overset{kb}{\leftrightarrows}} {}^{Enz}Ab_{(ferritin)\,-\,IC}$$

$^{Enz}Ab_{(ferritin)}$ = Enzyme labeled Antibody (Excess Quantity)

$^{Enz}Ab_{(ferritin)\,-\,IC}$ = Antigen-Antibodies Complex

kb = Rate Constant of Association

k-b = Rate Constant of Dissociation

Reagents and materials provided in kit

1. **Ferritin Calibrators – 1ml / vial**

   Six (6) vials of Ferritin calibrators at levels of 0(**A**), 10(**B**), 50(**C**), 150(**D**), 400(**E**) and 800(**F**) ng/ml.

2. **Ferritin Biotin Reagent – 13ml/vial -**

   One (1) vial containing biotinylated monoclonal mouse IgG in buffer, dye, and preservative.

3. **Ferritin Enzyme Reagent – 13 ml/vial**

   One (1) vial containing Horseradish Peroxidase (HRP) labeled anti-ferritin IgG in buffer, dye and preservatives.

4. **Streptavidin Coated Plate – 96 wells -**

   One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent.

5. **Wash Solution Concentrate – 20 ml -**

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One (1) vial containing a surfactant in buffered saline. A preservative has been added.

6. **Substrate A – 7ml/vial -**

   One (1) bottle containing tetramethylbenzidine (TMB) in buffer.

7. **Substrate B – 7ml/vial -**

   One (1) bottle containing hydrogen peroxide (H2O2) in buffer.

8. **Stop Solution – 8m/vial - Icon STOP**

   One (1) bottle containing a strong acid (1N HCl).

Required But Not Provided in kit:

1. Pipette capable of delivering 25, & 50 µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 ml and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional). Microplate Reader with 450 nm and 620 nm wavelength absorbance capability.
4. Absorbent Paper for blotting the microplate wells.
5. Plastic wrap or microplate cover for incubation steps.
6. Vacuum aspirator (optional) for wash steps.
7. Timer.
8. Quality control materials.

Preparation of reagent

Wash Buffer

The content of the wash solution was diluted with distilled water to make 1000 ml.

Working Substrate Solution

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The contents of the amber vial labeled Solution 'A' was added to the clear vial labeled Solution 'B'. The contents were mixed and labeled accordingly.

Test procedure

1. An amount of 25 µl of the appropriate serum reference, control or specimen were pipetted into their assigned well.
2. This was followed by 100 µl of the Ferritin Biotin Reagent, pipetted into each of the wells.
3. The microplate was swirled gently for 20-30 seconds to mix and cover.
4. The plate was incubated for 30 minutes at room temperature.
5. The contents of the microplate were discarded by decantation
6. An aliquot of 350 µl of wash buffer was pipetted into each of the wells and then decanted. This was repeated two times making a total of three washings.
7. One hundred microliters (100 µl) of the Ferritin Enzyme Conjugate was then added to each well.
8. Incubation was done for 30 minutes at room temperature.
9. The contents of the microplate were discarded by decantation.
10. An amount of 300 µl of wash buffer was added and decanted. This was repeated two times
11. One hundred microliters (100 µl) of working substrate solution was pipetted into all the wells.
12. Another incubation was done at room temperature for fifteen (15) minutes.
14. A stop solution of 50 µl was added to each well and mixed gently for 15-20 seconds.
15. The absorbance in each well was read at 450 nm.

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Serum iron and TIBC determination

Serum iron was determined using the VITROS Chemistry Fe Slides method (Ortho-Clinical Diagnostics, UK). Manufacturer's instructions were followed. This method relies on the use of VITROS Fe slides and the VITROS Chemistry Products Calibrator 4 on VITROS Chemistry systems.

Principle of procedure

The VITROS Fe slide is an analytical element developed based on the dry slide technology, coated and supported on polyester (Figure 3.2). The slide is multi-layered. When a sample of blood is dropped on the slide, it gets evenly distributed to the underlying layers. Iron in the form of ferric is removed from transferrin at a reduced pH (acidic) and moved to the reducing layer. On this layer, ascorbic acid reduces iron bound to the dye and a coloured complex is formed in the reagent layer. After the sample has been dropped on the slide, it is incubated and the density of reflection emitted is read after the first and fifth minutes. The difference between the two readings is proportional to the iron concentration in the sample.

Reaction

$$\text{Transferrin-Fe}^{3+} \xrightarrow{\text{pH 4.0}} \text{Transferrin} + \text{Fe}^{3+}$$

$$\text{Fe}^{3+} + \text{Ascorbic acid} \longrightarrow \text{Fe}^{2+}$$

$$\text{Fe}^{2+} + \text{Dye} \longrightarrow \text{Fe}^{2+}\text{-dye (Coloured complex)}$$

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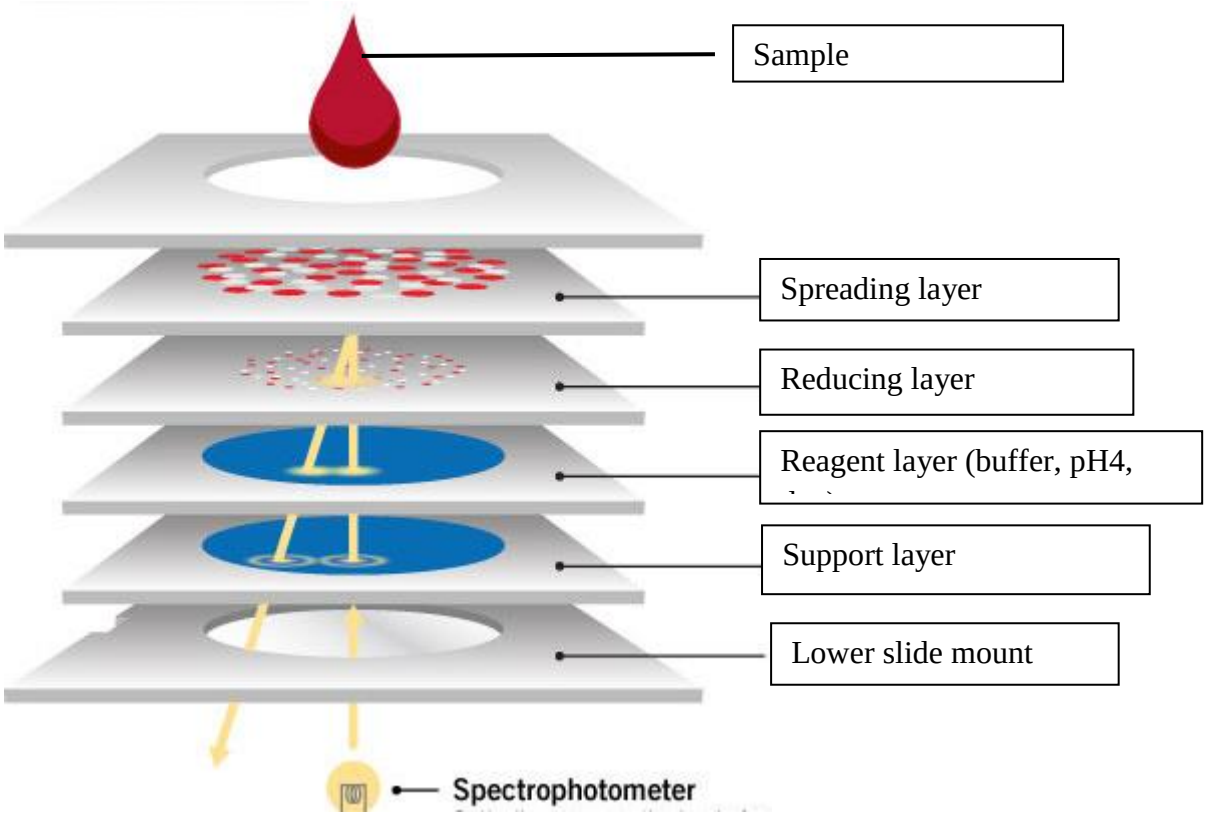

Figure 3.1: Diagram of VITROS Fe Slide

TIBC determination

TIBC was determined using the VITROS chemistry products TIBC kit, VITROS Fe slides (Ortho-Clinical Diagnostics, UK). Test was done following manufacturer's instructions.

Principle of procedure

The sample is preheated. Reagent prepared from excess iron citrate is added to the serum sample. This saturates all the available apotransferrin sites. The mixture is incubated for five minutes after which the sample is applied to an alumina column. The iron that is not bound to transferrin is adsorbed while the iron that is bond to transferrin is the total iron binding capacity in the sample. The TIBC is determined using the VITROS Fe slides.

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The blood sample is deposited on the slide and incubated at 37^{O}C. The reflection density is read at 600 nm at the first and fifth minutes. The difference in readings is proportional to the concentration of iron in the sample (VITROS Chemistry products, 2005).

Reaction

Apotransferrin + free Fe^{3+} ⟶ Fe-transferrin + free Fe^{3+}

Fe-transferrin +Fe^{3+}+ Alumina ⟶ Fe-transferrin + alumina-Fe

Determination of haemoglobin

Haemoglobin levels were determined using the Sysmex Automated Haematology analyser (Sysmex XT 2000i, Japan). The haematology analyser operates using the Sodium Lauryl sulphate (SLS) method as the underlying principle. The SLS reagent causes lyses of the red and white blood cells in the blood. A stable coloured complex of SLS-hgb is formed when the hydrophilic part of SLS binds to the haem part. The haemoglobin concentration is then measured.

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APPENDIX E: F0CUS GROUP DISCUSSION GUIDE

1. How did you feel when you found out that you were expecting?
2. Since you became pregnant, what changes have you made in your eating habits?
3. Since you became pregnant, what concerns do you have about your dietary intake?
4. What changes should a pregnant woman make to her diet?
5. Has your eating pattern changed since you got pregnant and why?
6. Are there any food beliefs you have heard concerning nutrition for pregnant women/ girls and how has it changed your dietary habits?
7. What foods should pregnant women be taking and why?
8. What do you know about anemia in pregnancy?
9. How do you get anemia in pregnancy?
10. How do you prevent anemia in pregnancy?
11. What are the symptoms of anemia?
12. What do you know about Iron/ Folic acid?
13. What have you heard about Iron/ Folic acid in pregnancy?
14. What are some of the good sources of Iron/ Folic acid in the Ghanaian diet?
15. If 'No', why? If 'Yes', why?
16. Have you received any education or information on anaemia?
17. If yes, when did you receive this information?
18. Who gave you the information?

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APPENDIX F: ETHICAL CLEARANCE LETTER

SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
Academic Affairs

Phone: +233-21-687974/5
Fax: +233-21-688291

My Ref. No. SAHS/10328184

Your Ref. No.

P O Box KB 143
Korle-Bu
Accra
GHANA

11th April, 2011

Mrs. Freda Dzifa Intiful,
Dept. of Dietetics,
SAHS.

Dear Mrs. Intiful,

ETHICAL CLEARANCE

Ethical Identification Number: SAHS – ET./10328184/AA/26A/2011-2012

The Ethical Review Committee of the School of Allied Health Sciences, at its meeting held on **Thursday, 14th March, 2011** unanimously approved your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: **"Nutritional Anaemia in pregnant adolescent girls in selected communities in the Greater Accra region".**

PRINCIPAL SUPERVISOR: **Prof. E. K. Wiredu**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical Review Committee at the completion of the study. The Committee may observe or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethical identification number in all future correspondence in relation to this protocol.

Thank you.

Yours sincerely,

M. M. Addae
(Chairperson, Ethical Review Committee)

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APPENDIX G: INFORMATION AND INFORMED CONSENT

UNIVERSITY OF GHANA

College of Health Sciences, School of Biomedical and Allied Health Sciences

NUTRITIONAL ANAEMIA IN PREGNANT ADOLESCENT GIRLS

We would like to invite you to participate in this research. Please take time to read through the consent form carefully before you choose to participate in this study. If you decide not to participate, you will not be at any disadvantage. You can ask questions to clarify any information you do not understand.

There is enough evidence to suggest that there is high rate of anaemia among pregnant women. However, there is little information about pregnant adolescent girls when they are singled out of pregnant women. This study aims at determining the prevalence of nutritional anaemia among pregnant adolescent girls. If you decide to partake in this study, you will be asked questions about yourself and your diet. 5ml of blood would be drawn from your arm. This will be of little discomfort to you.

If you decide to participate in this study, you will receive dietary counselling that will enable you make good choices in the selection of your food. The information you give will not benefit you directly, however it will be used in providing good healthcare to all pregnant women.

All information collected from you will be kept confidential and only be seen by investigators. You are free to withdraw from this research any time without reason and it will not be held against you.

DECLARATION

I .....which to state that the procedures involved in this research has been fully explained to my understanding and agree to be part of this study.

Signed
.....Date.....

Investigators statement

I confirm that I have carefully explained the nature, demands and foreseeable risks of the proposed study to the volunteer.

Signed.....Date.....

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Contact

Freda Intiful. School of Biomedical and Allied Health Sciences. University of Ghana, P.O.Box KB 143 Korle-bu. The Chairman SAHS Ethical Review Board. P.O.Box KB 143 Korle-bu

Tel: 0243439389

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APPENDIX H: PERCENTAGE OF GIRLS WITH ADEQUATE AND INADEQUATE INTAKES OF SELECTED MICRO AND MACRONUTRIENTS

Percentage of girls with adequate and inadequate intakes of carbohydrate according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake N (%)	Not Adequate N (%)	P-value
Educational level			
No formal Education	59 (93.7)	4 (6.3)	<0.001
Formal Education	151 (94.4)	9 (5.6)	<0.001
Marital status			
Married	8 (88.9)	1 (11.1)	0.02
Single	202 (94.4)	12 (5.9)	<0.001
Religion			
Christian	155 (93.9)	10 (6.1)	<0.001
Islam	53 (94.6)	3 (5.4)	<0.001
Previous Pregnancy			
Yes	22 (95.7)	1 (4.3)	<0.001
No	188 (89.5)	12 (6.0)	<0.001
Lives with			
Parents	80 (94.1)	5 (5.9)	<0.001
Husband/Partner	73 (94.8)	4 (5.2)	<0.001
Alone	12 (92.3)	1 (7.7)	0.002
Friend/relative	45 (93.8)	3 (6.2)	<0.001
Pica Practice			
Yes	18 (100)	0 (0.0)	-
No	192 (79.7)	13(6.3)	<0.001
Occupation			
Unemployed	96 (93.2)	7 (6.8)	<0.001
Artisan	38 (95.0)	2 (5.0)	<0.001
Trader	76 (95.0)	4 (5.0)	<0.001

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Percentage of girls with adequate and inadequate intakes of calcium according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No formal Education	6 (9.5)	57 (90.5)	<0.001
Formal education	15 (9.4)	145 (90.6)	<0.001
Marital status			
Married	0 (0.0)	9 (100)	-
Single	21 (9.8)	193 (90.2)	<0.001
Religion			
Christian	15 (9.5)	150 (90.9)	<0.001
Islam	6 (10.7)	50 (89.3)	<0.001
Previous Pregnancy			
Yes	3 (13.0)	20 (87.0)	<0.001
No	18 (9.0)	182 (91.0)	<0.001
Lives with			
Parents	11 (12.9)	74 (87.1)	<0.001
Husband/Partner	7 (9.1)	70 (90.9)	<0.001
Alone	0 (0.0)	13 (100.0)	-
Friend/relative	3 (6.3)	45 (93.8)	<0.001
Pica Practice			
Yes	1 (5.6)	17 (94.4)	<0.001
No	20 (9.8)	185 (90.2)	<0.001
Occupation			
Unemployed	8 (7.8)	95 (92.2)	<0.001
Artisan	4 (10.0)	36 (90.0)	<0.001
Trader	9 (11.3)	71 (88.8)	<0.001

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Percentage of girls with adequate and inadequate intakes of zinc according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No formal education	1 (1.6)	62 (98.4)	<0.001
Formal education	3 (1.9)	157 (98.1)	<0.001
Marital status			
Married	0 (0.0)	9 (100)	-
Single	4 (1.9)	210 (98.1)	<0.001
Religion			
Christian	1 (0.6)	164 (99.4)	<0.001
Islam	3 (5.4)	53 (94.6)	<0.001
Previous Pregnancy			
Yes	0 (0.0)	23 (100)	-
No	4 (2.0)	196 (98.0)	<0.001
Lives with			
Parents	1 (1.2)	84 (98.8)	<0.001
Husband/Partner	2 (2.6)	75 (97.4)	<0.001
Alone	0 (0.0)	13 (100)	-
Friend/relative	1 (2.1)	47 (97.9)	<0.001
Pica Practice			
Yes	0 (0.0)	18 (100)	-
No	4 (2.0)	201 (98.0)	<0.001
Occupation			
Unemployed	3 (2.9)	100 (97.1)	<0.001
Artisan	1 (2.5)	39 (97.5)	<0.001
Trader	0 (0.0)	80 (100)	-

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Percentage of girls with adequate and inadequate intakes of copper according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No Formal Education	0 (0.0)	44 (100)	-
Formal Education	0 (0.0)	109 (100)	-
Marital status			
Married	0 (0.0)	5 (100)	-
Single	0 (0.0)	148 (100)	-
Religion			
Christian	0 (0.0)	110 (100)	-
Islam	0 (0.0)	41 (100)	-
Previous Pregnancy			
Yes	0 (0.0)	17 (100)	-
No	0 (0.0)	136 (100)	-
Lives with			
Parents	0 (0.0)	61 (100)	
Husband/Partner	0 (0.0)	46 (100)	-
Alone	0 (0.0)	8 (100)	-
Friend/relative	0 (0.0)	38 (100)	-
Pica Practice			
Yes	0 (0.0)	13 (100)	-
No	0 (0.0)	140 (100)	-
Occupation			
Unemployed	0 (0.0)	76 (100)	-
Artisan	0 (0.0)	27 (100)	-
Trader	0 (0.0)	50 (56.2)	-

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Percentage of girls with adequate and inadequate intakes of selenium according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No formal Education	10 (15.9)	53 (84.1)	<0.001
Formal education	27 (16.9)	133 (83.1)	<0.001
Marital status			
Married	0 (0.0)	9 (100)	-
Single	37 (17.3)	177 (82.7)	<0.001
Religion			
Christian	26 (15.8)	139 (84.2)	<0.001
Islam	10 (17.9)	46 (82.1)	<0.001
Previous Pregnancy			
Yes	3 (13.0)	20 (87.0)	<0.001
No	34 (17.0)	166 (83.0)	<0.001
Lives with			
Parents	11 (12.9)	74 (87.1)	<0.001
Husband/Partner	12 (15.6)	65 (84.4)	<0.001
Alone	2 (15.4)	11 (84.6)	0.013
Friend/relative	12 (25.0)	36 (75.0)	0.001
Pica Practice			
Yes	3 (16.7)	15 (83.3)	0.005
No	34 (16.6)	171 (83.4)	<0.001
Occupation			
Unemployed	13 (12.6)	90 (87.4)	<0.001
Artisan	8 (20.6)	32 (80.0)	<0.001
Trader	16 (20.0)	64 (80.0)	<0.001

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Percentage of girls with adequate and inadequate intakes of iodine according to socioeconomic status (N=223)

Socioeconomic characteristics	Adequate intake n (%)	Not Adequate n (%)	P-value
Educational level			
No formal Education	0 (0.0)	63 (100)	<0.001
Formal education	6 (2.7)	154 (97.3)	<0.001
Marital status			
Married	0 (0.0)	9 (100)	-
Single	6 (2.8)	208 (97.2)	<0.001
Religion			
Christian	5 (3.0)	160 (97.0)	<0.001
Islam	1 (1.8)	55 (98.2)	<0.001
Previous Pregnancy			
Yes	2 (8.7)	21 (91.3)	<0.001
No	4 (2.0)	196 (98.0)	<0.001
Lives with			
Parents	2 (2.4)	83 (97.6)	<0.001
Husband/Partner	2 (2.6)	75 (97.4)	<0.001
Alone	1 (7.7)	12 (92.3)	<0.002
Friend/relative	1 (2.1)	47 (97.9)	<0.001
Pica Practice			
Yes	0 (0.0)	18 (100)	-
No	6 (2.9)	199 (97.1)	<0.001
Occupation			
Unemployed	1 (1.0)	102 (99.0)	<0.001
Artisan	3 (7.5)	37 (92.5)	<0.001
Trader	2 (2.5)	78 (97.7)	<0.001

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APPENDIX I: FREQUENCY OF CONSUMPTION OF FOODS

Frequency of consumption of foods (N=265)

Food groups	More frequent (≥5x/wk)	Frequent (1-4x/wk)	Sometimes (1-2x/mth)	Rarely	Never
Beverages					
Tea leaf	6 (2.3)	172 (64.9)	51 (19.2)	33 (12.5)	3 (1.1)
Chocolate	9 (3.4)	184 (69.5)	54 (20.4)	15 (5.7)	3 (1.1)
Horlicks	0 (0.0)	5 (1.9)	11 (4.2)	46 (17.4)	203 (76.6)
Coffee	5 (1.9)	8 (3.0)	47 (17.7)	72 (27.2)	131 (49.4)
Porridges					
Corn porridge (koko)	7 (2.6)	43 (16.2)	53 (20.0)	85 (32.1)	77 (29.1)
Millet porridge (hausa koko)	6 (2.3)	174 (65.7)	56 (21.1)	20 (7.6)	9 (3.4)
Rice porridge	6 (2.3)	44 (16.6)	71 (26.8)	102 (38.5)	42 (15.8)
Oats porridge	7 (2.6)	34 (12.8)	87 (32.8)	84 (32.1)	52 (19.6)
Wheat porridge	6 (2.3)	4 (1.5)	17 (6.4)	63 (23.8)	175 (66.0)
Weanimix	1 (0.4)	2 (0.8)	10 (3.8)	28 (10.6)	224 (84.5)
Breakfast cereals	2 (0.8)	2 (0.8)	8 (3.0)	36 (13.6)	217 (81.9)
Milk and milk products					
Carnation milk	6 (2.3)	23 (8.7)	69 (26.0)	38 (14.3)	129 (48.7)
Semi skimmed	5 (1.9)	6 (2.3)	12 (4.5)	42 (15.8)	200 (75.5)
Skimmed milk	2 (0.8)	8 (3.0)	19 (7.2)	44 (16.6)	192 (72.5)
Condensed milk	1 (0.4)	10 (3.8)	13 (4.9)	70 (26.4)	171 (64.5)
Yoghurt	3 (1.1)	20 (7.5)	53 (20.0)	49 (18.5)	140 (52.8)
Ideal milk	6 (2.3)	137 (51.7)	95 (35.8)	12 (4.5)	15 (5.7)
Ice cream	6 (2.3)	42 (15.8)	116 (43.8)	62 (23.4)	39 (14.7)
Soymilk	4 (1.5)	7 (2.6)	35 (13.2)	91 (34.3)	128 (48.3)
Cheese	5 (1.9)	9 (3.4)	33 (12.5)	104 (39.2)	114 (43.0)
Spreads					
Butter	2 (0.8)	12 (4.5)	28 (10.6)	63 (23.8)	160 (60.4)
Margarine	2 (0.8)	9 (3.4)	21 (7.9)	91 (34.3)	141 (53.2)
Polyunsaturated margarine	1 (0.4)	6 (2.3)	3 (1.1)	39 (14.7)	214 (80.8)
Peanut butter	2 (0.8)	9 (3.4)	34 (12.8)	93 (35.1)	127 (47.9)
Marmalade/jam	2 (0.8)	5 (1.9)	23 (8.7)	96 (36.2)	139 (52.5)

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Frequency of consumption of foods CONT'D

Food groups	More frequent (≥5x/wk)	Frequent (1-4x/wk)	Sometimes (1-2x/mth)	Rarely	Never
Breads					
Wheat bread	3 (1.1)	55 (20.8)	88 (33.2)	66 (24.9)	53 (20.0)
Tea bread	8 (3.0)	120 (45.3)	90 (34.0)	36 (13.6)	11 (4.2)
Sugar bread	8 (3.0)	129 (48.7)	94 (35.5)	26 (9.8)	8 (3.0)
Butter bread	8 (3.0)	119 (44.9)	96 (36.2)	32 (12.1)	10 (3.8)
Deep fried foods					
Fried yam	6 (2.3)	62 (23.4)	111 (41.9)	38 (14.3)	48 (18.1)
Fried plantain	8 (3.0)	57 (21.5)	96 (36.2)	41 (15.5)	63 (23.8)
French fries	1 (0.4)	5 (1.9)	11 (4.2)	26 (9.8)	222 (83.8)
Plantain chips	1 (0.4)	18 (6.8)	22 (8.3)	47 (17.7)	177 (66.8)
Oils					
Palm oil	13 (4.9)	195 (73.6)	40 (15.1)	8 (3.0)	9 (3.4)
White oil/sunflower/frytol	12 (4.5)	203 (76.6)	36 (13.6)	4 (1.5)	10 (3.8)
Coconut oil	3 (1.1)	42 (15.8)	52 (19.6)	42 (15.8)	126 (47.5)
Palm kernel	5 (1.9)	4 (1.5)	4 (1.5)	13 (4.9)	239 (90.2)
Groundnut oil	5 (1.9)	4 (1.5)	2 (0.8)	12 (4.5)	242 (91.3)
Vegetables					
Pebble garden(abedru))	9 (3.4)	30 (11.3)	88 (33.2)	69 (26.6)	69 (26.0)
Cabbage	7 (2.6)	48 (18.1)	111 (41.9)	65 (24.5)	34 (12.8)
Kontomire/other green leaves	4 (1.5)	81 (30.6)	111 (41.9)	48 (18.1)	21 (7.9)
Mixed vegetables	1 (0.4)	52 (19.6)	108 (40.8)	76 (28.7)	28 (10.5)
Carrots	7 (2.7)	39 (14.7)	99 (37.4)	78 (29.4)	42 (15.8)
Cauliflower	3 (1.1)	9 (3.4)	15 (5.7)	25 (9.4)	213 (80.4)
Okro	7 (2.6)	81 (30.6)	83 (31.3)	38 (14.3)	56 (21.1)
Green beans	5 (1.9)	11 (4.2)	46 (15.1)	55 (20.8)	154 (58.1)
Brussel sprout	0 (0.0)	6 (2.3)	30 (11.3)	28 (10.6)	201 (75.8)
Cucumber	6 (2.3)	36 (13.6)	72 (27.2)	68 (25.7)	83 (31.3)
Sweet pepper	5 (1.9)	40 (15.1)	83 (31.3)	68 (25.7)	67 (25.3)
lettuce	8 (3.0)	58 (21.9)	57 (21.5)	67 (25.3)	74 (27.9)

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Frequency of consumption of foods CONT'D

Food groups	More frequent (≥5x/wk)	Frequent (1-4x/wk)	Sometimes (1-2x/mth)	Rarely	Never
Fruits					
Pawpaw	14 (5.2)	49 (18.5)	92 (3.5)	77 (2.9)	32 (12.1)
Pineapple	14 (5.2)	39 (14.7)	96 (36.2)	91 (34.3)	25 (9.4)
Mango	12 (4.5)	35 (13.2)	85 (32.1)	108 (40.8)	25 (9.4)
Citrus fruits	21 (7.9)	48 (18.1)	136 (51.3)	52 (19.6)	8 (3.0)
Banana	20 (7.5)	62 (23.4)	126 (47.5)	41 (15.5)	16 (6.0)
Pear	12 (4.5)	16 (6.0)	58 (21.9)	103 (38.9)	76 (28.7)
Melon	16 (6.0)	23 (8.7)	81 (30.6)	70 (26.4)	76 (28.7)
Apples	8 (3.0)	8 (3.0)	18 (6.8)	64 (24.2)	167 (63.0)
Avocado	8 (3.0)	8 (3.0)	25 (9.4)	44 (16.6)	180 (67.9)
Grapes	5 (1.9)	4 (1.5)	8 (3.0)	37 (14.0)	211 (79.6)
Guava	1 (0.4)	10 (3.8)	6 (2.3)	37 (14.0)	211 (79.6)
Coconut	9 (3.4)	27 (10.2)	88 (33.2)	72 (27.2)	69 (26.0)
Sugar cane	9 (3.4)	13 (4.9)	66 (24.9)	100 (37.7)	77 (29.0)
Soft Drinks/beverages					
Fruit juice	0 (0.0)	20 (7.5)	41 (15.5)	178 (67.2)	26 (9.8)
Minerals (coke,fanta, sprite etc)	0 (0.0)	61 (23.2)	106 (40.3)	76 (28.7)	22 (8.3)
Super malt	0 (0.0)	35 (13.2)	95 (35.8)	94 (35.5)	41 (15.5)
Malta guiness	2 (0.8)	27 (10.2)	87 (32.8)	78 (29.4)	71 (26.8)
Diet drink	2 (0.8)	10 (3.8)	14 (5.3)	39 (14.7)	200 (75.4)
Beer	1 (0.4)	2 (0.8)	4 (1.5)	15 (5.7)	243 (91.7)
Wine	0 (0.0)	0 (0.0)	4 (1.5)	13 (4.9)	248 (93.5)
Hard liquor	0 (0.0)	2 (0.8)	5 (1.9)	25 (9.4)	233 (87.9)
Snacks					
Crisps and savoury snacks(e.g princles)	3 (1.1)	7 (2.6)	28 (10.6)	98 (37.0)	129 (48.6)
Pastries	3 (1.1)	16 (6.0)	59 (22.3)	110 (41.5)	77 (29.0)
Plantain chips	3 (1.1)	37 (14.0)	87 (3.8)	95 (35.8)	43 (16.2)
Biscuits	5 (1.9)	40 (15.1)	108 (40.8)	94 (35.5)	18 (6.8)
Sweets					
Toffee/ chocolates	4 (1.5)	36 (13.6)	90 (34.0)	104 (39.2)	31 (11.7)

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Frequency of consumption of foods CONT'D

Food groups	More frequent (≥5x/wk)	Frequent (1-4x/wk)	Sometimes (1-2x/mth)	Rarely	Never
Legumes					
Black eye beans	1 (0.4)	67 (25.3)	78 (29.4)	33 (12.5)	86 (32.5)
Baked beans	1 (0.4)	12 (4.5)	24 (9.1)	59 (22.3)	169 (63.8)
Soyflour	2 (0.8)	3 (1.1)	11 (4.2)	33 (12.5)	216 (81.5)
Bambara beans	1 (0.4)	28 (10.6)	61 (23.0)	57 (21.5)	118 (44.5)
Fast foods					
Pizza/hamburger, takeaway etc.	0 (0.0)	121 (45.7)	92 (34.7)	20 (7.5)	32 (12.1)
Meat and meat products					
Beef	6 (2.3)	77 (29.1)	108 (40.8)	50 (18.9)	24 (9.1)
Salted beef	9 (3.4)	57 (21.5)	110 (41.5)	63 (23.8)	26 (9.8)
Chicken	11 (4.2)	69 (26.0)	111 (41.9)	32 (12.1)	42 (15.8)
Turkey	7 (2.6)	9 (3.4)	20 (7.5)	68 (25.7)	161 (60.8)
Pork	3 (1.1)	11 (4.2)	34 (12.8)	72 (27.2)	145 (54.7)
Goat	5 (1.9)	20 (7.5)	62 (23.4)	60 (22.6)	118 (44.6)
Pig feet	6 (2.3)	10 (3.8)	29 (10.9)	58 (21.9)	162 (62.3)
Unsalted pig feet	5 (1.9)	9 (3.4)	25 (9.4)	53 (20.0)	173 (65.3)
Snails	6 (1.7)	3 (0.9)	11 (3.0)	54 (20.4)	191 (72.1)
Offals	10 (3.8)	7 (2.6)	26 (9.8)	67 (25.3)	155 (58.5)
Bacon	3 (1.1)	13 (4.9)	31 (11.7)	19 (7.2)	199 (75.1)
Sausage	2 (0.8)	46 (17.4)	100 (37.7)	27 (10.2)	90 (33.9)
Cow skin	3 (1.1)	20 (7.5)	98 (37.0)	75 (28.3)	70 (26.4)
Corned beef	2 (0.8)	14 (5.3)	60 (22.8)	78 (29.7)	111 (41.9)
Game	5 (1.9)	11 (4.2)	23 (8.7)	75 (28.3)	151 (57.0)
Cow foot	6 (2.3)	61 (23.0)	61 (23.0)	62 (23.4)	75 (28.5)
Eggs	18 (6.8)	178 (67.2)	61 (23.0)	2 (0.8)	6 (2.2)
Fish and sea foods					
Fish	15 (5.7)	165 (62.3)	37 (14.0)	8 (3.0)	40 (15.1)
Canned fish	2 (0.8)	62 (23.4)	77 (29.1)	49 (18.5)	75 (28.3)
Shrimps	10 (3.8)	6 (2.3)	30 (11.3)	57 (21.5)	162 (61.1)
Lobster	9 (3.4)	12 (4.5)	21 (7.9)	20 (7.5)	203 (76.6)
Crabs	11 (4.2)	28 (10.6)	74 (27.9)	59 (22.3)	93 (35.1)

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Frequency of consumption of foods CONT'D

Food groups	More frequent (≥5x/wk)	Frequent (1-4x/wk)	Sometimes (1-2x/mth)	Rarely	Never
Starches					
Banku	21 (7.9)	219 (82.6)	20 (7.5)	2 (0.8)	3 (1.2)
Kenkey	19 (7.2)	185 (69.8)	27 (10.2)	6 (2.3)	28 (10.6)
Fufu flour	5 (1.9)	27 (10.2)	23 (8.7)	39 (14.7)	171 (64.4)
Fufu (pounded)	9 (3.4)	162 (61.1)	73 (27.5)	14 (5.3)	7 (2.6)
Plantain	8 (3.0)	150 (56.6)	81 (30.6)	22 (8.3)	4 (1.5)
Yam	12 (4.5)	127 (47.9)	82 (30.9)	35 (13.2)	9 (3.4)
Gari	6 (2.3)	79 (29.8)	92 (34.7)	47 (17.7)	41 (15.4)
Cocoyam	9 (3.4)	54 (20.4)	37 (14.0)	98 (37.0)	67 (25.3)
Rice	30 (11.3)	181 (68.3)	33 (12.5)	13 (4.9)	8 (3.0)
Pasta, macaroni, spaghetti	2 (0.8)	81 (30.6)	80 (30.2)	77 (29.1)	25 (9.4)
Soups					
Palm soup	12 (4.5)	136 (51.3)	88 (33.2)	23 (8.7)	3 (1.1)
Groundnut soup	13 (4.9)	168 (63.4)	68 (25.7)	10 (3.8)	6 (2.2)
Light soup	15 (5.7)	155 (58.5)	70 (26.4)	12 (4.5)	13 (4.9)
Okro soup	14 (5.3)	69 (26.1)	69 (26.0)	79 (29.8)	34 (12.8)
Stews					
Kontomire stew (no agushie)	13 (4.9)	109 (41.1)	98 (37.0)	30 (11.3)	15 (5.6)
Kontomire (plus agushie)	11 (4.2)	90 (34.0)	94 (35.5)	53 (20.0)	17 (6.4)
Garden egg stew	6 (2.3)	29 (10.9)	53 (20.0)	104 (39.2)	73 (26.5)
Agushie stew	3 (1.1)	50 (18.9)	32 (12.1)	101 (38.1)	79 (29.8)
Tomato stew	13 (4.9)	199 (75.1)	5 (1.9)	6 (2.3)	42 (15.9)