UNIVERSITY OF GHANA
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CHARACTERISATION OF ANAEMIA AMONG DEFERRED BLOOD DONORS AT THE SOUTHERN AREA BLOOD CENTRE

BY

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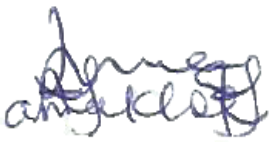
DECLARATION

I, Odelia Avu-Tamakloe of the Department of Haematology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, do hereby declare that with the exception of the references cited, this thesis is original and was duly carried out by me and results obtained therein are the true reflection of the work under the supervision and direction of my supervisors. This thesis has never been presented in part or whole to any institution for the award of any degree.



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ABSTRACT

Background: Anaemia is a state in which the amount of red blood cells (RBCs) is insufficient to meet the body's physiologic needs depending on age, gender and residential elevation. Blood transfusion may be indicated when there is anaemia. Blood for transfusion is obtained from a healthy person who gives consent for his/her blood to be drawn for the intention of transfusion. To ensure safe blood transfusion, adoption of stringent donor selection criteria is applied and potential blood donors who do not meet the standards are termed deferred donors. Globally, anaemia among deferred blood donors is a problem but it is widespread in developing countries (e.g. Ghana). Most of the anaemias recorded are often times treatable. There is paucity of data on the causes of anaemia among deferred blood donors which is required to help put in place measures to reduce anaemia and ultimately decrease donor deferral and as a result increase blood supply to patients.

Aim: The aim of this study was to characterise anaemia among deferred blood donors at the Southern Area Blood Centre.

Methods: A cross-sectional study was carried out in deferred blood donors at sites operated by the Southern Area Blood Centre. Ethylenediamine tetra acetic acid (EDTA) anticoagulated blood samples were used in performing full blood count (using the ABX Micros ES60 OT haematology autoanalyser), film comment, malarial tests (thin and thick films). Sera obtained from spun clotted blood from gel separator were aliquoted into Eppendorf tubes and stored at -20^{o}C and later used for biochemical analysis such as iron, ferritin, total iron binding capacity (TIBC) and C-reactive protein using the Urit - 8210 fully automated open system chemistry analyser. Data was entered into Microsoft Excel 2016 and transferred into Stata version 15 for statistical analysis.

Results: A total of 150 participants (69 (46.0%) males and 81 (54.0%) females) were recruited. The ages of the participants ranged from 17 - 55 years (mean age = 27.19 ± 11.41 years). The deferred anaemic blood donors had haemoglobin (Hb), RBCs, mean cell volume (MCV) and mean cell haemoglobin (MCH) mean values that were lower and statistically significant (all Ps = 0.000) when compared to the non-anaemic deferred blood donors. In contrast, means of red cell distribution width (RDW) and platelet (Plt) in the anaemic category were significantly high (p=0.000) when compared with those of the non-anaemic deferred group. The biochemical profile means such as iron, ferritin and TSAT of the deferred anaemic females were lower than that of their male partners [e.g. iron levels: females 9.52 ± 1.50µmol/l, males 10.15 ± 2.18 µmol/l)]. However, there were no significant differences (p>0.05) between their biochemical profiles. The prevalence of anaemia among the deferred blood donors was 48%. The morphological types of anaemia recorded were microcytic hypochromic (62.5%) and normocytic normochromic (37.5%) and some of the causes of anaemia established in this study were iron deficiency anaemia (63.9%), anaemia of inflammation (18.1%), sickle cell disease (4.2%) and hereditary elliptocytosis (2.8%). Factors such as occupation (social class), being a female, menstrual cycle and duration of menses (among females of reproductive age) and dietary intake were found to be risks determinants for anaemia development.

Conclusion: Prevalence of anaemia in potential healthy blood donors was found to be high (48%) and mostly due to iron deficiency anaemia (63.9%) which is a preventable cause of donor deferral. Dietary intake was found to be associated with anaemia and there is the need for nutrition counselling to help improve haemoglobin concentration. This will help prevent anaemia, reduce deferral rates and eventually increase blood supply.

DEDICATION

I dedicate this thesis to The Almighty God, Jehovah El Gibor for His grace and mercy towards me throughout the period. I also dedicate this work to the loving memory of my sweet super mother, the late Madam Happy Enyonam Hukporti for sacrificing many precious moments in her life so that I could have mine. Furthermore, this work is dedicated to my lovely father, WOI (Rtd) Johnson Avu-Tamakloe as well as my lovely siblings, Capt Napoleon Avu-Tamakloe, Miss Belinda Avu-Tamakloe and Mr. Mal Kon Royals-Newton whose support and guidance has earned me this success. All I have to say is that the God who sees in secret will reward you openly for all eyes to behold.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

%	Percentage
%TSAT	percentage transferrin saturation
5-MTHF	5-methyltetrahydrofolate
ACD	anaemia of chronic disease
BMP 6	bone morphogenetic protein 6
bpm	beats per minute
BTS	Blood Transfusion Service
BTT	beta thalassaemia trait
CD	cluster of differentiation
cm	centimetre
$CuSO_4$	copper sulphate
CV	coefficient of variation
DNA	deoxyribonucleic acid
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphate
e.g.	for example
EDTA	Ethylenediaminetetraacetic acid
EPB41	erythrocyte membrane protein band 4.1
EPO	erythropoietin
EPP	Erythrocyte protoporphyrin
et.al	and others
etc	and so on
FBC	Full Blood Count
Fe^{2+}	ferrous iron

Fe^{3+}	ferric iron
fl	fentolitre
g/dl	grammes per decilitre
GHS	Ghana Health Service
GHS	Ghana Health Service
GI	gastrointestinal
GNA	Ghana News Agency
GPC	glycophorin C
Hb	haemoglobin
HCS	haemoglobin colour scale
Hct	haematocrit
HE	Hereditary Elliptocytosis
HiCN	cyanmethhaemoglobin
HIV	human immunodeficiency virus
HS	Hereditary Spherocytosis
i.e.	that is
ICSH	International Council for Standardization in Haematology
ID	iron deficiency
IDA	iron deficiency anaemia
IDE	iron deficiency erythropoiesis
IFN-γ	interferon-γ
IFN-α	interferon- α
IFN-β	interferon- β
IL- 10	interleukin-10
IL- 12	interleukin-12

IL-1	interleukin-1
IL-6	interleukin-6
INACP	International Nutritional Anemia Consultative Group
IOM	Institute of Medicine
K_2EDTA	dipotassium ethylenediamine tetra acetic acid
KBTH	Korle-Bu Teaching Hospital
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean cell volume
mg/day	milligrams/day
MGUS	monoclonal gammopathy of undetermined significance
mins	minutes
ml	millilitre
mls	millilitres
MMA	megaloblastic macrocytic anaemias
MOH	Ministry of Health
mol/l	moles per litre
NBSG	National Blood Service, Ghana
NHLBI	National Heart, Lung and Blood Institute
^oC	Degree Celsius
PBF	Peripheral Blood Film
PCV	packed cell volume
pg	picogram
P	*Plasmodium*
Plt	platelets

RBC	red blood cell
RBCs	red blood cells
RDW	red cell distribution width
REDS-II	Retrovirus Epidemiology Donor Study-II
REDS-III	Recipient Epidemiology and Donor Evaluation Study-III
RES	reticuloendothelial system
RNA	ribonucleic acid
rpm	revolution per minute
SABC	Southern Area Blood Centre
SAM	S-adenosylmethionine
SPRING	Strengthening Partnerships, Results and Innovations in Nutrition Globally
SPTA1	α-spectrin erythrocytic 1
SPTB	β-spectrin erythrocytic
sTfR	Soluble transferrin receptor
TCI	transcobalamin I
TCII	transcobalamin II
TCIII	transcobalamin III
Tf	transferrin
TIBC	total iron binding capacity
TNF-α	tumour necrosis factor-α
TTIs	transfusion transmissible infections
VNRBD	voluntary non-remunerated blood donation
VNRBDs	voluntary non-remunerated blood donors
WBCs	white blood cells

WHO	World Health Organisation
ZPP	zinc protoporphyrin
µg/dl	microgram per decilitre
µls	microlitres

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Blood transfusion generally encompasses processes of receiving blood and blood products as part of medical intravenous treatment used as management for various medical ailments. Integral among such processes include a practice of blood donation which in itself comprises the voluntary offering of a healthy adult for blood to be drawn and used for transfusion and/or made into biopharmaceutical medications by a process called fractionation (Diamond, 1980; Jennings, 1883). Blood transfusion is indicated for these main reasons; when the volume of blood within the circulatory system is inadequate to sustain life and also when the RBCs are deficient either in quantity or quality. Additionally, it may be required in cases of functional or quantitative platelet defects as well as coagulation factor deficiencies (WHO, 2002b).

In order to guarantee that patients receive quality and safe blood, there is medical selection and screening of the blood donors so that recipients do not contract any infectious disease and donors do not develop iron deficiency (ID) and other untoward effects. Furthermore, it ensures the wellbeing of donors throughout the donation and afterwards. The screening can lead to either temporary or permanent deferral due to several reasons (Allen & Sayman, 1962; Diamond, 1980). Blood transfusion services face the challenges of ensuring that there is sufficient, quality and safe supply of blood and blood components for patients whose lives or wellbeing depend on blood transfusion. Blood supplies need to be constantly replenished since whole blood and blood components have a limited shelf life. Most countries battle to meet current requirements while at the same time responding to increasing clinical demands for

blood. Developed countries with well-structured health systems and blood transfusion services based on voluntary blood donation are generally able to meet the demand for blood and blood products. However, in developing countries, chronic blood shortages are common (WHO, 2010).

To ensure that the process of blood transfusion is safe, there is adoption of stringent donor selection criteria and this is aimed at evaluating the suitability or otherwise of a prospective blood donor. The donor selection criteria are made up of a set of questions and screening tests which are administered to every prospective blood donor before such a person is deemed fit to donate blood (WHO, 2012). The postponement of suitability of a person to donate blood or blood components ensuing from self-administered questionnaire, interview responses or medical assessment is known as deferral. Any prospective blood donor who does not pass these eligibility criteria is termed as deferred (Diamond, 1980; WHO, 2012).

Donor recruitment and retention are negatively affected by donor deferrals and this leads to the inability of blood banks to meet the demands of blood supply and blood reserves (Wevers, Wigboldus, De Kort, Van Baaren, & Veldhuizen, 2014). Potential blood donors are deferred either temporarily or permanently due to several reasons. Deferrals can be temporary or short term, long term, and permanent and a number of potential donors are deferred temporarily due to anaemia, which is most often a treatable cause. A potential donor may be deferred due to testing positive to incurable transfusion transmissible disease like hepatitis B infection or human immunodeficiency virus (WHO, 2012). The measurement of haemoglobin (Hb) level prior to blood donation is to ensure that the donor's health is not compromised and also to provide safe and quality

blood for recipients (Cable, 1995). According to a World Health Organisation (WHO) report, (WHO, 2017), global deferral rate differed largely between countries starting from less than 1% to greater than 37% and the average deferral rate was 12%.

The measurement of Hb level of potential blood donors is done by different methods (Perkins & Torg, 1962) and the most frequently used method is the copper sulphate (Phillips et al., 1950). The Hb screening is subsequently confirmed by a standard diagnostic method such as automated haematology analyser (Behrens, Brown, Gibson, & Detter, 1979).

Anaemia is a state in which the amount of red blood cells (RBCs) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a person's age, gender, residential elevation above sea level (altitude), smoking behaviour, and different stages of pregnancy (WHO, 2011). Males and reproductive females with Hb concentrations of <13.0 g/dl and <12.0 g/dl respectively are said to be anaemic (WHO, 2008). Furthermore, anaemia may be termed as mild, moderate or severe. Mild anaemia is defined as Hb ranging from 11-12.9 g/dl in adult males and 11-11.9 g/dl in adult females. Moderate anaemia is defined as Hb level of 8-10.9 g/dl and severe anaemia is defined as Hb level of less than 8 g/dl for all the age groups (WHO, 2008). Globally, 32.9% of the world's population is anaemic according to data at WHO (Kassebaum et al., 2014) and anaemia occurs at all stages of life, especially in pregnant women and children (WHO, 2008). In Ghana, anaemia was ranked as the fourth cause of hospital admissions (WHO, 2002c). Furthermore, anaemia was reported as the second contributor to death (WHO, 2002c) in a review report on disease profile and pathology results of selected hospitals.

1.1.1 Prevalence of anaemia

Anaemia is a condition in which the number of RBCs or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking and pregnancy status (WHO, 2011). Anaemia is not a diagnosis, but rather a feature of an underlying disease. Anaemia is a global public health problem (which affects both developed and developing countries) with major consequences for human health and socioeconomic development. A WHO report (Kassebaum et al., 2014), revealed that the global prevalence of anaemia was 32.9% (that is about two billion people were affected).

The prevalence of anaemia among reproductive women (15 - 49 years) in La Cote d'Ivoire, Nigeria, South Africa, India, United Kingdom and United States of America are 47.4%, 62%, 26.4%, 52%, 8.8% and 6.9% respectively (WHO, 2008). In Ghana, an organisation known as Strengthening Partnerships, Results and Innovations in Nutrition Globally (SPRING) and Ghana Health Service (GHS) in 2016 conducted a study and reported the prevalence of anaemia among reproductive women as 42.4% (SPRING & GHS, 2016) but unfortunately no work has been cited on the prevalence of anaemia among men nationally. However, a community-based study conducted at Otinibi (a peri-urban area in Accra, Ghana), recorded an 18.8% prevalence of anaemia among healthy men 18 years and above (Vuvor, Steiner-Asiedu, Saalia, & Owusu, 2016).

1.1.2 Prevalence of anaemia among deferred blood donors

The WHO has revealed that globally, a little below 1% and above 37% blood donors are deferred (WHO, 2017) and anaemia was responsible for 10% of the deferrals (Annen, Delaney, Leitch, & Mast, 2015; Mast et al., 2010). Such temporary deferrals are

discouraging (Custer, Chinn, Hirschler, Busch, & Murphy, 2007) and donors may never revisit to donate (Halperin, Baetens, & Newman, 1998).

The prevalence of anaemia among deferred blood donors in some developed and developing countries are as follows; United States of America -10% (Mast et al., 2010), United Arab Emirates - 9.29% (Alshaer, Sharma, & AbdulRahman, 2017), Turkey - 20.7% (Arslan, 2007), Brazil - 4.2% (Goncalez et al., 2013), 47% in Japan (Ngoma et al., 2014), Tanzania - 21.1% (Valerian et al., 2018), Ivory Coast - 42.5% (Kouao et al., 2012), Democratic Republic of Congo - 36.5% (Nzengu-Lukusa et al., 2016), Nigeria - 25.3% (Aneke, Ezeh, Nwosu, & Anumba, 2016), Namibia - 45% (Gonzo, Shuuveni, Mukesi, Chipare, & Moyo, 2016), Ghana 42.6% and India - 35.4% (Vasudev et al., 2016). The second most common reason for deferral among donor population in Ghana was hypertension (16.8%) (Acquaye, 1991). In Ivory Coast, the second most frequent reason for deferral was a reported change of a sexual partner (34.3%) (Kouao et al., 2012) and that of Nigeria was skin rashes (1.9%) (Aneke et al., 2016). India and Pakistan recorded underweight of 10.7% and 22.5% respectively as the second most common reason for deferral (Chauhan, Desai, Trivedi, & Agnihotri, 2015; Waheed & Zaheer, 2016).

Currently one in four persons who attempts to donate blood at the Southern Area Blood Centre (SABC) is deferred (SABC, 2016) and this negatively affects the capacity of blood banks to provide safe and adequate blood supply for patients. Most of these deferrals are due to anaemia which is often treatable (SABC, 2016).

1.2 Problem Statement

Worldwide, blood transfusion needs have witnessed an overwhelming increase over the years. For every 1000 inhabitants, 10 to 20 units of whole blood must be collected if the world is to meet growing demands of blood transfusion (WHO, 2014). Blood banks in Africa have been struggling to meet this demand and Ghana is no exception (Bates, Chapotera, McKew, & van den Broek, 2008; Bates & Hassall, 2010). Ghana's challenges and constraints in the provision of safe and sufficient blood are attributable to lack of infrastructure, support, finances and qualified personnel (Bates et al., 2008; WHO, 2007b, 2010). Furthermore, shortage of blood donors due to unrelenting and continuous donor deferrals, has led to an overwhelming deficit in blood supply (Bates et al., 2008; WHO, 2007b, 2010). In 2014, Ghana's minimum donation rate stood at 5.4% per 1000 members of the population (WHO, 2014) and this is below the WHO recommended minimum donation rate of 10-20% per population (WHO, 2014). This has proven inimical to smooth health care delivery in the areas of urgent demand for blood during surgeries, poorly managed health outcomes of bleeding mothers during labour, unfortunate medical treatment consequences for severely anaemic children and poor management of patients with malignant diseases who need frequent blood transfusions.

The estimated annual demands of the National Blood Service, Ghana (NBSG), is 250,000 units of blood (GNA, 2014) nevertheless just about 160,000 units of blood was collected in 2016 (MOH, 2016). Furthermore, out of the 22,000 units of whole blood and 16,220 units of concentrated red cells requests submitted by Korle-Bu Teaching Hospital in 2017, the SABC in Accra was barely able to make available 9,460 (43.0%) units and 9,440 (58.2%) units of whole blood and concentrated red cells respectively (SABC, 2017).

As reported earlier, factors such as inadequate infrastructure, support, funds and qualified human resources are responsible for the inability of blood banks to stock blood. Donor deferrals have been cited as the major reason for a massively reduced capability to stock blood banks in Ghana (Antwi-Baffour et al., 2015; SABC, 2016) and anaemia has been the chief cause of the deferrals (Antwi-Baffour et al., 2015; SABC, 2016). The relationship between characterisation of anaemia and deferred blood donation has not been sufficiently studied. The only work that has been cited was on the morphological types of anaemia among deferred blood donors and the findings showed 39 (42.4%) and 42 (46.7%) of deferred anaemic blood donors had microcytic hypochromic anaemia and normocytic normochromic anaemia respectively (Antwi-Baffour et al., 2015). Nonetheless, there is presently little empirical knowledge on the factors that contribute to the various causes of anaemia among deferred blood donors.

1.3 Justification

This study was performed to fill that gap by providing a better understanding of information regarding the characterisation of anaemia among deferred blood donors at the SABC. Additionally, this research was done to provide evidence that most of the causes of anaemia are modifiable and early discovery can aid stakeholders in the formulation of policy to offer timely intervention such as iron supplementation, food fortification and nutrition counselling. Subsequently, this will lead to the reduction of the rate of deferral of blood donors.

1.4 Aim and Specific Objectives

1.4.1 Aim

The aim of this study was to characterise anaemia among deferred blood donors at the Southern Area Blood Centre.

1.4.2 Specific Objectives

The specific objectives were:

1. To ascertain the prevalence of anaemia among deferred blood donors.
2. To ascertain the various types of anaemia (such as morphological and aetiological) as well as severity of anaemia among deferred blood donors.
3. To ascertain the association of factors such as socioeconomic status and nutritional (dietary) status with anaemia among deferred blood donors.
4. To ascertain the risk determinants of anaemia among deferred blood donors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Blood Donation and Transfusion

Blood donation occurs when a healthy person gives consent for his/her blood to be drawn for transfusion purposes. It involves the process of collecting, testing, preparing and storing blood and blood components. Blood transfusion is the process of infusing blood and blood components into a person's circulatory system intravenously to compensate for inadequate blood or blood components (Diamond, 1980; Jennings, 1883). Records from literature have revealed that Pope Innocentius VIII (Giovanni Battista Cibo) happens to be the first person to have been transfused with blood in 1492 (Gottlieb, 1991; Lindeboom, 1954). The 'blood' was obtained from three ten-year-old shepherd boys. However, there is paucity of information regarding the route of administration (intravenous or ingestion) of the blood to the pope. The three donors and the Pope died shortly afterwards (Gottlieb, 1991; Hajdu, 2003). There is also some level of indication that blood transfusion practices of some sort were undertaken by Egyptian physicians in prehistoric age (Hajdu, 2003). The circulatory system is known to have been first described by William Harvey (Harvey, l886) and this finding (human anatomy) and the circulation of blood led to further research into transfusion processes; animal-to-animal plus animal-to-human. This ultimately resulted in human-to-human transfusion.

It has also been reported that in 1666, Richard Lower from Oxford carried out an experimentation by transfusing blood from one animal to the other (Giangrande, 2000; Lower, 1665) and in Paris in 1667, Jean Baptiste Denis, also successfully transfused human beings with blood from animals but subsequent attempts failed (Giangrande,

2000). From then on, there was less development and activity with regards to blood transfusion till James Blundell in 1825 successfully transfused a women suffering from post-partum haemorrhage using her husband's blood (Waller, 1825). Blundell's subsequent attempts were all successful (Blundell, 1828). Karl Landsteiner, an Austrian physician, in 1901, discovered the ABO blood system and this offered a great opportunity to get rid of the primary cause of transfusion complication (transfusion reaction) at that point in time (Landsteiner, 1901). After Landsteiner's discovery, Reuben Otterberg in 1908, introduced the practice of cross-matching in transfusion practice (Ottenberg, 1908). In 1937, with the discovery of the next most essential blood group system, Rhesus (Rh) by Landsteiner and his associate, Wiener (Landsteiner & Wiener, 1940), blood transfusion has progressed steadily with a key interest in transfusion safety. Thus, the procedure of selecting blood donors and screening for infectious microorganisms of diseases has been introduced in contemporary blood transfusion practice and its aim is to eliminate donors at high risk of transmitting infections or diseases to recipients from donation. In addition, it stops donors who might be harmed as a result of the donation process from participating (Boulton, 2008).

2.2 Selection and Medical Assessment of Blood Donors

The provision of safe and adequate blood and blood products at the opportune time is the principal duty of a blood transfusion service (BTS). In order to carry out this task, the BTS makes sure that the process of blood donation is safe and also causes no harm to the donor as well as the recipient (Reiss, 2011; WHO, 2012). This is achieved by identifying and excluding prospective donors with high risk behaviours (Courtois et al., 1999). Therefore, every potential blood donor undergoes evaluation to ensure that he or she is suitable to donate blood at every instance and the rationale behind this is to guarantee

the health and safety of the donor. Furthermore, the recipient is also protected from unsafe blood because only apparently healthy donors are bled (Cable, 1995; WHO, 2012).

The procedure for selecting blood donors involves donors responding to questionnaire (consisting of a limited medical and social history), undergoing physical examination and being tested for haemoglobin levels and infectious agents of diseases based on scientific, informed medical views as well as standard regulations (WHO, 2012). Any person whose eligibility is adjourned after the selection process due to failure to meet the requirements is under deferral and consequently termed as a deferred donor. The deferral may just be for a temporary (specific period of time, i.e. temporary short term is 1-56 days and temporary long term is 57-365 days) period or permanent (indefinitely) period (WHO, 2012). Temporary deferral occurs when an individual is deferred based on a removable, time-bound factor such as low Hb whiles a permanent deferral happens when a person has non-removable, long lasting factors, such as positivity for any of the transfusion transmissible infections (TTIs) such as hepatitis B (WHO, 2012).

The prospective donors are interviewed through the standardised questionnaires presented to them during the history taking stage of donor selection and anyone can be deferred during this stage (Courtois et al., 1999). The donor questionnaire is based on questions pertaining to the history and current condition of their health as well as the provision of their demographics. Ultimately, donor questionnaire serves as a means to guarantee that the donor will be able to stand the donation process devoid of any risk to his/her health. Moreover, the recipient is also protected from any transfusion transmissible infection (Boulton, 2008).

In a study in Norway, 29,787 people presented for donation however 163 (3.9%) were deferred through questionnaire administered to them (Reikvam, Svendheim, Røsvik, & Hervig, 2012). In Germany, 27,460 (13.1%) was reported as the number of people who were excluded from donation via questionnaire out 209,617 people (Gillet & Neijens, 2018). Elsewhere at the North West General Hospital and Research Centre, Peshawar in Pakistan, 315 representing 8.7% accounted for deferrals via predonation screening interview out of 3,302 people who were pronounced fit to donate at that stage (Khan, Rehman, & Raziq, 2012) in a work carried out between May 2009 to May 2011. On the contrary in Turkey, a higher rate of 44.4% (610) was reported from work done at the children's hospital blood centre in Izmir where 610 people were deferred from a total of 2,207 (Gulen et al., 2006).

The period of physical check-up of potential blood donors is used to identify the presence of any medical or surgical situations that can cause damage to the health of the donor or recipient. Among other things, their ages are taken note of so as not to recruit any underage (<17years) nor over age persons (>60years). They are also evaluated for the right body weight (≥50kg). The blood pressure should also be ($\geq^{110}/_{70}$ mm/Hg $\leq^{160}/_{100}$ mm/Hg). In addition, the pulse (60 - 100 bpm and regular), body temperatures (not >37.5°C) are checked and the presence of signs of anaemia, jaundice, any skin rash is also noted (WHO, 2012). Pregnant and lactating mothers are not eligible for donation because of the high demands for iron for their physiological functions (WHO, 2012).

Globally, potential blood donors' haemoglobin levels are checked to prevent people with low haemoglobin levels from being bled. It also serves the purpose of identifying regular blood donors from getting ID due to blood donation (Eder, Goldman, Rossmann,

Waxman, & Bianco, 2009; Eder et al., 2008). Furthermore, it stops the situation where low quality (low haemoglobin) blood is collected for transfusion of patients which may compromise the efficiency of the blood (Goldman, 2005). Lastly, they are also screened for TTIs for instance human immunodeficiency virus (HIV), hepatitis B, hepatitis C and syphilis (Newman, 2001).

A research involving 261,016 potential blood donors from all blood centres in Germany, documented that 5,495 blood donors representing 3.8% were disqualified through medical examination (Houareau et al., 2017) and it is not consistent with findings by investigators in Saudi Arabia (Al Nouri, Maghrabi, Hamdi, Abd El-Ghany, & AlNouri, 2019). Al Nouri and associates in Jeddah, Saudi Arabia, also reported that 236 (47.20%) of deferrals among 500 prospective blood donors were due to medical examination done the by health professionals (Al Nouri et al., 2019).

A study by Tagny and partners to assess the blood donor selection process in 15 francophone countries in Africa, has brought to light that on an average, 13% of people who present to donate are disallowed based on medical examination (Tagny et al., 2012). However, this finding is at variance with an earlier work carried out in India, where a higher value (69.8%) was observed (Agnihotri, 2010).

2.3 Types of Blood Donation Systems

There are basically three types of blood donation systems namely; Voluntary non-remunerated blood donation (VNRBD), family/replacement directed donation and commercial/paid donation (WHO, 2002a). In the voluntary non-remunerated donation, a person gives blood, plasma or cellular constituents freely without receiving any

compensation in the form of money or in kind whiles a donation which aims at restoring or directly contributing to a transfusion required by a family relation or friend is known as family/replacement directed donation. Paid donation arises when a person is rewarded with cash or things that can be readily converted to cash and the reward could come from individuals, blood centres or sponsored institutions (WHO, 2002a).

The WHO has urged all member blood banks/services to exclusively collect blood from VNRBDs as one of the approaches to ensure the availability of safe blood for transfusion since they are unlikely to harbour agents of TTIs when compared to family/replacement directed donor or paid donor (WHO, 1975, 1984). This had been upheld by other researchers in their studies (Cunha et al., 2007; Mbanya & Tayou, 2005) but other findings have also shown that a first-time voluntary non-remunerated blood donor carries the same risk as a family replacement donor (Allain, 2011; Di Lorenzo Oliveira, Loureiro, De Bastos, Proietti, & Carneiro-Proietti, 2009) and that, it is only donation from both concepts done regularly that will guarantee the provision of safe and adequate blood (Tagny, 2012).

In Africa, due to limitations in economic and logistic resources, majority of countries are operating hospital-based family and/or replacement donation concept (Bates, Manyasi, & Medina Lara, 2007; Field & Allain, 2007; Tapko & Toure, 2013). A report from a WHO survey in Africa, reveals that generally, 74.8% of VNRD is practised in Africa with 100% in ten countries (e.g. Rwanda, South Africa, and Uganda), 0% in Equatorial Guinea and 27.1% in Ghana (Tapko & Toure, 2013). The family/replacement and paid arrangements are accountable for 24.7% and 0.4% of donation in Africa respectively (Tapko & Toure, 2013). Even as paid donation system is still practised in some

developing countries e.g. Democratic Republic of Congo (Tapko & Toure, 2013), advanced countries like United States of America (USA) had for several years, depended on donors who have been financially induced to guarantee sufficient supply of blood at their centres (Domen, 1995) but the paid donation system is now mostly for plasma collection since most of the paid donors have been successfully converted to VNRD in a considerable number of the blood banks in the USA (Domen, 1995).

In study undertaken at the blood bank of Ain Shams University Hospital, Egypt from August 2010 to January 2011, 17,118 prospective blood donors were recruited. Family/replacement donors were 15,017 (87.7%) and VNRD were 2,101 (12.3%). The prevalence of HBV among the family/replacement donors as well as the VNRD was 1.7% and 0.3% respectively with a statistically significant p-value of <0.001 (Abdel Messih, Ismail, Saad, & Azer, 2014). On the other hand, Allain and associates have also published that at the blood bank of Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana, there was no significant difference in the prevalence of viral indicators (anti-HIV and HBsAg) among first-time VNRD in addition to replacement donors (Allain, Sarkodie, Asenso-Mensah, & Owusu-Ofori, 2010). Moreover, at the Postgraduate Department of Immuno-haematology and Blood Transfusion Medicine, Government Medical College, Jammu, India, Meinia and Sawhney recruited 16,015 whole blood donors and 1,130 (7.1%) were exempted from donation. Among the disqualified group were 300 (26.5%) VNRDs and 830 (73.5%) were replacement donors (Meinia & Sawhney, 2016). With these records from literature, the practice of selecting blood donors is an essential process which serves as a useful and cost-effective means by which the safety of blood and blood products are ensured.

2.4 Methods Used in Predonation Haemoglobin Estimation (Anaemia Screening)

The assessment of haemoglobin (Hb) level is an essential aspect of screening of donors and this is to make sure that the donor is not anaemic and also do not become critically anaemic after the blood donation process. Furthermore, it ensures that the donated blood contains the ideal quantity of haemoglobin (Eder et al., 2009). The selection of techniques for the screening of potential blood donors for Hb prior to donation must be precise enough to prevent anaemic people from donating and not fail qualified donors needlessly. It must also be simple easy to use and must not cause any discomfort to the would-be donor (Goldman, Uzicanin, Yi, Acker, & Ramirez-Arcos, 2012).

The copper sulphate ($CuSO_4$) method has been used for several years using capillary blood or venous blood and it was originally described by Phillips and partners (Phillips et al., 1950). This method allows a drop of whole blood to fall into the $CuSO_4$ solution with specific gravities of 1.054 for men and 1.052 for women which are equivalent to 13.0g/dl of Hb and 12.0g/dl respectively (Ross, Gilpllan, Houston, & Heaton, 1986). The whole blood maintains its own density, for roughly 15 seconds and if the drop of blood is denser than the specific gravity of copper sulphate it will sink and this indicates that the donor's Hb level is acceptable. However, if the drop of blood floats on the copper sulphate solution, then the donor's Hb level is not enough to donate blood (Ross et al., 1986). The $CuSO_4$ technique which is the conventional method mostly used in the screening of anaemia among potential blood donors have been reported to erroneously include anaemic people (Mannarino & Macpherson, 1963; Newman, 1997) and exclude eligible persons (James, Jones, Turner, & Sokol, 2003; Keating, Gorman, & Moore, 1967; Tondon, Verma, Pandey, & Chaudhary, 2009). Therefore, haemoglobin screening is often verified using a standard diagnostic or reference technique e.g. automated

analyser (Chambers & McGuff, 1989) as recommended by the International Committee for Standardization in Haematology using the cyanmethhaemoglobin (HiCN) method (ICSH, 1995).

The HiCN method is based on the principle of converting haemoglobin to cyanmethhaemoglobin by adding potassium cyanide and ferricyanide and the absorbance is read at 540 nm in a spectrophotometer against a solution of known concentration (standard) (ICSH, 1995). HiCN technique has served as reference system where situations of false pass and false fail were rectified with successful outcomes. For instance, in India, Akhtar and partners screened 3000 voluntary blood donors using $CuSO_4$ method and 120 (4%) were disqualified. Upon retesting with Hemocue device, 39 (32.5%) were successful. The remaining 81 (67.5) were further tested via haematology autoanalyser and finally 53 (65.4%) were permitted to donate and 28 (34.6%) were precluded (Akhtar, Ara, & Sherwani, 2014). In another study at the University Malaya Medical Centre, Malaysia; Nadarajan and Eow also investigated the prevalence of anaemia and ID among 187 blood donors who had passed the $CuSO_4$ test. Upon re-estimation of the Hb levels using the reference method (automated analyser), 40 (21.4%) were found to have been inappropriately accepted. It was discovered that 23 (12.3%) people had ID and the disturbing situation was that 10 (43.5%) of the iron deficient group truly had iron deficiency anaemia (Nadarajan & Eow, 2002).

In the United States of America, Patel and partners in their work conducted at the Department of Transfusion Medicine at the National Institutes of Health (NIH), Bethesda, Maryland, sought to establish the accuracy as well as agreement between Hb concentrations obtained via Hemocue method and those gotten from the autoanalyser

among blood donors who were excluded from donation due to their failure at the Hemocue test. They recorded 9 (6.0%) and 5 (3.3%) as false pass and false fail rates correspondingly after re-measurement of Hb samples using the reference technique, Cell-dyn automated haematology analyser (Patel, Wesley, Leitman, & Bryant, 2013).

Hemocue is another method for estimation of Hb and it works by the principle of photometry (Neufeld et al., 2002). A drop of blood is placed on a cuvette, which is patented to Hemocue and quantitative Hb values are shown in less than 15 seconds. Sodium deoxycholate haemolyses erythrocytes and Hb is released. Sodium nitrite converts Hb to methaemoglobin which, together with sodium azide, gives azidemethaemoglobin. The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample (Neufeld et al., 2002). There are varied views concerning the use of Hemocue in estimating Hb. Underestimation of Hb concentration has been observed in some studies (Cohen & Seidl-Friedman, 1988; Neville, 1987) and this may lead to disqualifying individuals without anaemia (wrongful deferment) (Gomez-Simon et al., 2007) and thereby reducing the blood stock. In contrast, Bahadur and co-workers as well as Gupta and partners reported Hb levels that were over estimated by Hemocue method and suggested its rejection in blood donation as to prevent the bleeding of anaemic persons (Bahadur, Jain, & Jain, 2010; Gupta, Mehra, Jain, & Maheshwari, 2015). Also, similar results had been previously documented by other investigators (Deb, Chhaya, & Bharucha, 2002). Other investigators had also suggested the use of the Hemocue method for retesting blood donors deferred earlier by the $CuSO_4$ method (James et al., 2003; Sawant, Bharucha, & Rajadhyaksha, 2007).

In the year 1995, the WHO initiated the usage of haemoglobin colour scale (HCS), an easy, dependable and reasonably priced device for detecting the presence of anaemia (Lewis, Stott, & Wynn, 1998; Stott & Lewis, 1995). With this semi-qualitative technique, a drop of blood is put on a test paper strip and the colour of the blood on the test strip is compared with six colour shades of red on the scale corresponding to Hb concentrations of 2, 6, 8, 10, 12 and 14g/dl (Lewis et al., 1998; Stott & Lewis, 1995). From records, HCS, can be used for field work (Montresor et al., 2000; WHO, 2001a) and thus for screening blood donors (Lewis & Emmanuel, 2001). The utilisation of HCS to screen potential donors have been supported by other studies (Sawant et al., 2007; Timan, Tatsumi, Aulia, & Wangsasaputra, 2004) although other researchers have called for it to be abolished (Darshana & Uluwaduge, 2014; Paddle, 2002; Tondon et al., 2009).

Other investigators have also employed an additional method, microhaematocrit for the detection of anaemia and it is a simple and cost-effective means (Pirofsky & Nelson, 1964). In this system, a capillary tube is filled with 0.5ml of blood, sealed at one end and spun using a diminutive centrifuge to obtain haematocrit (packed cell volume, PCV); the proportion of volume occupied by RBCs when compared to the entire volume of blood expressed as a fraction or a percentage by means of the haematocrit reader (Pirofsky & Nelson, 1964). This method has been recommended for use on the field (INACP, 1985; WHO, 2007a) and at the blood bank for screening purposes (Cable, 1995) in addition to validating the eligibility of would-be blood donors previously rejected by the $CuSO_4$ test (Avoy, Canuel, Otton, & Mileski, 1977; Keating et al., 1967; Kliman, 1967). It has been documented that this method can give unsuitable Hb levels

due to trapped plasma (Solomon & Grindon, 1986), differences in the tube's diameter or the anticoagulant (Gotch et al., 1991).

2.5 Donor Deferral Rates

A number of people who present themselves for donation are not able to accomplish their mission due to their inability to meet the requirements of the standardised guidelines put in place for recruiting and selecting donors. This consequently leads to temporary or permanent deferrals, which are either the initiative of the blood centre based on the disclosure made by the prospective donor or via self deferral (Eder et al., 2009). A retrospective study undertaken at the blood bank of Government Medical College, Patiala, India from August 2014 to August 2015, revealed that 4,125 (17.1%) people were deferred for various reasons out of 24,062 (Taneja, Bhardwaj, Arora, & Agarwal, 2015) that were in attendance. Among the study population, 86.1% were temporarily deferred whiles permanent deferrals were 13.9% (Taneja et al., 2015).

There are variations in the rates of deferral across countries and blood centres; thus prevalence rate of deferred blood donors is estimated to be <1% - 37% globally (WHO, 2017) whiles that of the African region is 11.7% with the lowest and highest deferral rates reported in Burundi as well as Eritrea as 1.8% and 64.3% in that order (Tapko & Toure, 2013). Furthermore, in some advanced countries like France (Lawson-Ayayi & Salmi, 1999), Germany (Müller-Steinhardt et al., 2012) and the United States of America (Zou et al., 2008) deferral rates of 10.8%, 6.2% and 12.8% were documented correspondingly. Additionally, Lim and colleagues reported that in Singapore, 14.4% prevalence rate of deferral existed (Lim, Tien, & Ong, 1993) whiles in Japan 14% was reported (Ngoma et al., 2013).

In a number of developing nations, the records were as follows; Ivory Coast - 10.8% (Kouao et al., 2012), Nigeria - 16% (Ekwere, Ino-Ekanem, & Motilewa, 2014) and Sudan - 6% (Abbas, Khalil, Yasir, Fadlallah, & Huwaida, 2015). In addition, Namibia documented 8.6% as deferral rate (Gonzo et al., 2016) whiles Tanzania (Valerian et al., 2018) and Zimbabwe (Mafirakureva, Khoza, Van Hulst, Postma, & Mvere, 2013) recorded 12.7% and 7% respectively. Ghana's SABC documented 25% as its deferral rate (SABC, 2016). Furthermore, the 13% of deferred blood donors were voluntary blood donors while their replacement counterparts accounted for 20% in Ghana (Owusu-Ofori et al., 2005).

The disparities in deferral rates may be linked to nonexistence of donor selection standards especially in some developing countries or proper donor selection measures, or differences in the ways donors are recruited (WHO, 2017).

2.5.1 Deferral due to anaemia (low haemoglobin level or haematocrit)

Blood transfusion services usually expect prospective blood donors to have minimum haemoglobin (Hb) or haematocrit (Hct) levels before they are allowed to donate blood and this is to guarantee that the donor is protected from becoming anaemic and also the recipient get optimum quality of blood or blood components (Eder et al., 2009; Eder et al., 2008). However, these values are restricted for donation in diverse geographical locations. For example, in the United States of America and Canada, it is set at Hb (≥12.5g/dl) and Hct (≥38%) for both sexes (Newman, 2008; Price, 2008). Females in Europe (e.g. United Kingdom, Germany, Spain) must have a minimum Hb of 12.5g/dl whiles their male counterpart must have minimum Hb of 13.5g/dl (Council of Europe, 2009).

According to Salvin and partners in Australia, Hb eligibility levels have been set at ≥12g/dl and ≥13g/dl for females and males in that order (Salvin, Pasricha, Marks, & Speedy, 2014). In Brazil, it is ≥12.5g/dl and ≥38% as well as ≥13.0g/dl and ≥39% for males and females respectively (Stern, O'Meara, Infanti, Sigle, & Buser, 2012). Lee and co-workers have reported that in China, it is 11.5 g/dl for female as well as 13.0 g/dl for male donors (Lee et al., 2013). In Japan, the cut-off for Hb has been set at 12·0 g/dl for 200 mls donation and 12·5 g/dl for 400 mls donation regardless of one's sex (Shimizu, 1998). The SABC of Ghana, uses the WHO Hb cut-off of ≥12.0g/dl and ≥13.0g/dl for males and females respectively (SABC, 2016; WHO, 2008).

Donor deferral on account of anaemia is one of the main factors contributing to blood donors being deferred temporarily (Madrona, Herrera, Jiménez, Giraldo, & Campos, 2014; Sultan, Irfan, Baig, Usman, & Shirazi, 2017) with 10% (Annen et al., 2015; Mast et al., 2010) and 1.4% (Tapko & Toure, 2013) reported as anaemia prevalence among donors globally and in Africa respectively. This deferral on account of low level of haemoglobin is principally in the interest of the donor and its aim is to permit the deferred blood donors ample time to undergo additional medical investigations to detect the aetiology of the anaemia and also sufficient time for the restoration of their iron reserves (Cable, 1995). In the United States of America, Mast et al. (2010), in their work discovered that 10% of blood donors exempted from donation was due to anaemia. Additionally, some other developed countries like United Arab Emirates (Alshaer et al., 2017), Turkey (Arslan, 2007), Brazil (Goncalez et al., 2013) as well as Japan (Ngoma et al., 2014) recorded 9.29%, 20.7%, 4.2% and 47% as prevalence of anaemia among deferred blood donors respectively. Reports from studies conducted in blood centres of some developing African countries are as follows; Tanzania - 21.1% (Valerian et al.,

2018), Ivory Coast - 42.5% (Kouao et al., 2012), Democratic Republic of Congo - 36.5% (Nzengu-Lukusa et al., 2016), Nigeria - 25.3% (Aneke et al., 2016), Namibia - 45% (Gonzo et al., 2016), Ghana 39% (SABC, 2016), and India - 35.4% (Vasudev et al., 2016).

The disparities observed in the deferral rates may be as a result of factors like differences in the criteria used to select donors in the various countries such as Hb levels, age, weight in addition to blood donation interval. Furthermore, it may also be because of diverse methods and/or the differences in Hb cut-off values that are used (Cable, 1995).

2.5.2 Deferral due to other reasons

Apart from anaemia, there are other bases for deferring blood donors and this action also serves as a protection from harm for the donor and the recipient. Some of these reasons are high blood pressure, seropositivity for any transfusion transmissible infection, underweight, vaccination history, high pulse rate, respiratory infection, medication usage (WHO, 2012). The deferral rate due to other reasons among blood donors has been reported to be 10.3% in the African region (Tapko & Toure, 2013). Maleki and co-workers in their retrospective study at the Ilam Blood Transfusion Organization, Iran, to establish the reasons for disqualification of blood donors from donation observed 23.7% ($^{557}/_{2349}$) of blood donors were excluded because of medication usage and that was the highest reason (Maleki, Mohammadi, Taghinejad, Shoohani, & Amraei, 2015). In Trinidad and Tobago, the deferral reason with the most frequency was high blood pressure 25.8% ($^{126}/_{488}$) (Vuma, Mayers, Legall, & Justiz Vaillant, 2015). Other

researchers in Saudi Arabia documented high pulse rate of 19% ($^{764}/_{4035}$), as the most common reason among deferred blood donors (Abdelaal & Anwar, 2016).

2.6 Anaemia Prevalence

Anaemia is a state in which the amount of RBCs (and consequently their oxygen-carrying capacity) is inadequate to carry out the physiological needs of the body. The physiological needs vary within age groups, gender, residential elevation above sea level, smoking behaviour and different gestational stages (WHO, 2011). Clinically a male is said to be anaemic when his Hb level is <13.0 g/dl whiles a female is anaemic when the level of her Hb is <12.0 g/dl (WHO, 2008). The WHO in its report on anaemia burden in the world from 1990 to 2010 pointed out that 32.9% (that is about two billion) of people suffered from anaemia (Kassebaum et al., 2014). The prevalence of anaemia among men globally is 12.7% while that of reproductive women is 30.2% and in Africa, the WHO estimated anaemia prevalence rate was 64.6% and It further added that 44.4% of reproductive women were anaemic (WHO, 2008). In Ghana, anaemia among women of reproductive age stood at 42.4% (SPRING & GHS, 2016) and a peri-urban study revealed that 18.8% of men had anaemia (Vuvor et al., 2016). According to the SABC, anaemia was responsible for 39% of deferrals among blood donors at its blood centres (SABC, 2016).

2.7 Anaemia Classification

Anaemia is routinely categorised on the basis of morphology using the size of the RBCs and this was originated by Dr. Maxwell Myer Wintrobe (Wintrobe, 1930). With his technique, values for haemoglobin, haematocrit (packed cell volume) and RBCs were manually determined and those results were subsequently used to calculate red cell

indices (Wintrobe's indices) namely MCV (mean cell volume), MCH (mean cell haemoglobin) and the MCHC (mean cell haemoglobin concentration) (Wintrobe, 1931, 1932). MCV describes the mean size of the RBC; the normal range is 76 - 96fl and values above 100 fl show macrocytosis and microcytosis is indicated by values usually > 76 fl. The Hb content of RBC is defined by MCH. The reference range is 27-30pg. Values below 27pg indicate hypochromia. The MCHC indicates the concentration of haemoglobin in the general cell population; the normal range is 32-36g/dl. Low MCHC is seen in microcytic, hypochromic anaemias but is usually normal in macrocytic anaemias. Consequently, the morphological types of anaemia are microcytic, normocytic and macrocytic (Wintrobe, 1930, 1934).

The introduction of automated haematology analysers has led to improvement in accuracy as well as precision and reduction in subjective errors in the determination of red cell indices and besides added information like RDW (red cell distribution width) which depicts how homogenous or heterogeneous (anisocytosis) the RBC population is, has been incorporated (Lemelson-MIT Program, 2000). The normal range of RDW as coefficient of variation (CV) is 11.6-13.0% (Park & Kim, 1987).

The aetiological classification of anaemia depends on the primary cause of the anaemia (Carmel & Cassileth, 1999) and thus, it may be as a result of accelerated destruction of RBCs (e.g. thalassaemia, malaria), increased loss of RBCs (chronic blood loss e.g. peptic ulcer disease as well as acute blood loss e.g. trauma) and insufficient production of RBCs (e.g. ID, folate deficiency). There is an association involving the morphology of RBCs as well as the aetiology of anaemia, for this reason the red cell indices play

essential roles in diagnosing, classifying and treatment monitoring in any anaemia situation (Brugnara & Mohandas, 2013).

In 1983, the improved classification of anaemias using MCV and RDW was put forward by Bessman and collaborators (Bessman, Gilmer, & Gardner, 1983). With this grouping, the differential diagnosis for microcytosis (low MCV) with normal RDW (homogenous) are thalassaemia trait, anaemia of chronic disease (ACD) and other haemoglobinopathies whiles that of microcytosis with high RDW (heterogenous) are ID, haemoglobin H disease, thalassaemia trait and fragmentation syndromes. In the case of normocytosis with normal RDW, the differential diagnoses are ACD, non-anaemic haemoglobinopathies and normocytosis in the presence of high RDW may indicate incompletely treated iron or vitamin deficiency and anaemic haemoglobinopathy e.g. sickle cell disease. Macrocytosis accompanied by normal RDW point to aplastic anaemia and myelodysplasias whiles macrocytosis amid high RDW may suggest vitamin B_{12} or folate deficiency and liver disease.

Three years after that proposal, a study by Flynn and workmates revealed considerable inadequacies with it because they could not categorise their microcytic cases using the RDW as the main variable (Flynn, Reppun, & Bhagavan, 1986) and this observation was corroborated in the following year, by another researcher (Simel, 1987).

2.7.1 Morphological

Anaemia among blood donors is also evaluated morphologically using red cell indices as initiated by Dr. Wintrobe (Wintrobe, 1934). A dimorphic picture (dual population of

RBCs on examination) may be encountered when the anaemia is caused by two or more factors (Gjorup, Bugge, Hendriksen, & Jensen, 1986).

A study conducted in India from February 2015 to June 2015 in Sardar Patel Medical College, Department of Transfusion Medicine with 11,635 prospective blood donors, 866 (7.5%) were found to be unqualified to donate on diverse grounds and among them were 114 (57%) and 45 (22.5%) having anaemia of microcytic hypochromic and normocytic normochromic origins respectively. Macrocytic anaemia was found in 7 (3.5%) and the rest 34 (17%) had dimorphic anaemia (Kumari et al., 2016). Afterwards in Pakistan, Sultan and associates reported on their work done at the Blood Bank of Liaquat National Hospital (LNH) and Medical College, Karachi which took place from January 2014 to December 2015 involving 36,954 prospective blood donors (Sultan et al., 2017). According to the report, 3,101 (8.39%) were deferred and 2445 (91.8%) had anaemia. Out of that, 1431 (58.5%) and 958 (39.0%) of them had microcytic hypochromic anaemia and normocytic normochromic anaemia respectively. Macrocytic anaemia was found to be 61 (2.5%) among the deferred blood donors (Sultan et al., 2017). The findings of this study are similar to an earlier work performed in India (Kumari et al., 2016).

In Nigerian State of Kano, Ahmed and Kagu screened 1,502 first-time blood donors for anaemia and 182 (12.1%) were not successful at donation. Among the deferral group on account of anaemia, 103 (56.6%) were found to be microcytic hypochromic anaemic while 12 (6.6%) and 81 (19.2%) were normocytic normochromic anaemic and macrocytic anaemic in that order. Dimorphic anaemia was also found among 32 (17.6%) of the deferred donors (Ahmed & Kagu, 2011). Ahmed and Kagu's work did not

compare with the subsequent findings of Antwi-Baffour and team in their study at the Accra Area Blood Bank of SABC (Antwi-Baffour et al., 2015). In that investigation, 1120 prospective blood donors were involved and 39 (42.4%) and 42 (46.7%) of the deferred blood donors had microcytic hypochromic anaemia and normocytic normochromic anaemia in that order (Antwi-Baffour et al., 2015).

2.7.2 Aetiological

Any of the different causes of anaemia among the general population can equally lead to anaemia among blood donors. The diverse causes may contribute to anaemia in different ways; thus, a person may suffer from anaemia arising from one or more sources (Milman, 2011).

2.7.2.1 Iron deficiency anaemia (IDA)

Accessible data at the WHO, point to the fact IDA is the most widespread microcytic hypochromic anaemia as well as micronutrient deficiency anaemia in the world; 50% of anaemias are traced to ID (WHO, 2011). This is based on the analysis of reports from 1990 - 2010 of 187 countries by the World Health Organisation (Kassebaum et al., 2014; WHO, 2011).

Iron is an essential mineral that is found in diet and it acts as oxygen transport in the blood. IDA is as a result of deficient Hb synthesis that leads to microcytic and hypochromic RBCs. By reason of the insufficient Hb, there is inadequate supply of oxygen to the cells and tissues (Conrad, Umbreit, & Moore, 1999). Additionally, iron as a vital element is needed for the body to function properly and therefore it is crucial to keep it in balance (Anderson, Frazer, & McLaren, 2009; Bothwell, Charlton, Cook, &

Finch, 1979). As a result, iron balance is sustained by controlling the amount of iron in plasma (transferrin bound iron) and this is achieved by four harmonised activities; how iron is absorbed in the duodenum, how iron is recycled by the macrophages in the spleen, how iron is stored in the hepatocytes of the liver and the use of iron in the process of erythropoiesis (Bothwell et al., 1979; Hentze, Muckenthaler, Galy, & Camaschella, 2010).

Dietary iron is in the form of haem iron (organic; about 10% and from animal protein) as well as non-haem iron (inorganic; about 90% animal products and plants) and their absorption happens primarily in the duodenum and proximal jejunum (Andrews, 1999; Bothwell et al., 1979). At physiological pH (i.e. non-acidic pH), iron is present in the oxidised, ferric (Fe^{3+}) form however, for the absorption of iron to take place, it has to be in the ferrous (Fe^{2+}) form or bound to a protein e.g. haem. In the stomach, the high acidic content provides low pH and this causes the dissociation of haem iron from haemoproteins whiles non-haem iron is kept stable in its reduced state (Fe^{2+}) (Andrews, 1999).

Iron in the diet is absorbed via the enterocytes as haem as well as non-haem (Fe^{2+} and Fe^{3+}). The haem iron is extremely bioavailable (readily absorbed) but non-haem is not. The haem in diet is liberated from the haemoproteins (Hb and myoglobin) via proteolysis by the gastric and intestinal proteases (Conrad, Benjamin, Williams, & Foy, 1967). The haem iron is absorbed as an unbroken metalloporphyrin (iron-protoporphyrin) complex (Wyllie & Kaufman, 1982) into the cells of the mucosa (enterocytes) with the aid of haem carrier protein 1 (HCP-1) through endocytosis and it then undergoes degradation by the action of haem oxygenase-1 leading to the liberation

of non-haem (Dunn, Rahmanto, & Richardson, 2007; Laftah et al., 2009). Afterwards, the iron is exported through the iron exporter ferroportin present in the enterocytes (Shayeghi et al., 2005).

Conversely, non-haem is less bioavailable, it goes through solubilisation by the action of the acidic secretions in the gastric and duodenal lumens. The ferrireductase; duodenal cytochrome B (DcytB) reduces Fe^{3+} to Fe^{2+} with the help of low pH of gastric acid as well as duodenal acid and dietary ascorbic acid and this enhances the absorption of iron (McKie et al., 2001).

The entire Fe^{2+} iron is transferred into the enterocytes by the carrier protein divalent metal transporter-1 (DMT-1) following the reduction process by DcytB (Andrews, Fleming, & Gunshin, 1999). Inside the enterocytes of the duodenum, the iron supplied by both haem and non-haem go into a general pool of iron (McKie et al., 2001). The quantity of iron in stores is monitored by the iron absorption process and the absorption of iron is sustained by ID plus increased erythropoiesis as well as deficiency (Forth & Rummel, 1973), and down-regulation of iron absorption is influenced by inflammation as well as iron repletion (Finch, 1994).

Iron absorption process is controlled by hepcidin (Nicolas et al., 2002), which adjusts the amount of iron that is absorbed into the enterocytes and also obstructs the liberation of iron from the enterocytes in addition to macrophages (Moore, Arrowsmith, Welch, & Minnich, 1939). Research has proven that the absorption of non-haem iron is enhanced by the consumption of foods rich in ascorbic acid (vitamin C) and foods rich in haem iron (Bjorn-Rasmussen, 1983). On the contrary, the non-haem iron absorption is

suppressed by tannins (tea, coffee) plus phytates (Zijp, Korver, & Tijburg, 2000) and polyphenols (Hurrell, Reddy, & Cook, 1999). Hallberg and co-workers as well as Roughead and partners have identified that calcium is the distinct factor that inhibits the absorption of both haem and non-haem iron (Hallberg, Brune, Erlandsson, Sandberga, & Rossander-Hultén, 1991; Roughead, Zito, & Hunt, 2005).

Based on iron requirements, the iron that has been absorbed will be stored in the enterocytes in the form of ferritin (will be lost when the enterocytes are sloughed), used for the production of haem or sent into circulation with the aid of ferroportin 1 (Sharp & Srai, 2007). The ferrous iron transferred out of the enterocytes is instantly oxidised to ferric iron by membrane bound ferroxidases; hephaestin as well as ceruloplasmin and subsequently binds to transferrin (Tf) (Vulpe et al., 1999). The erythrocytes possess transferrin receptors which take delivery of the iron-transferrin complexes. These complexes are then endocytosed and finally iron is incorporated into Hb (Mackenzie, Iwasaki, & Tsuji, 2008).

In the developing countries, ID and IDA normally result from inadequate dietary intake which is secondary to a low protein diet due to poverty and ignorance (Kotecha, 2011; WHO, 2008). Additionally, the loss of blood due to worm infestation, malaria or both also result in ID and IDA (Menendez, Fleming, & Alonso, 2000; WHO, 2008), whiles in the developed countries, certain dietary habits (e.g. a vegetarian diet or no intake of red meat) (Srilakshmi, 2005) and pathologic conditions (e.g. chronic blood loss or malabsorption) (Franceschi, Zuccalà, Roccarina, & Gasbarrini, 2014) are the most regular causes. The body maintains iron balance in a homeostatic fashion and in cases of inadequate iron intake, there is mobilisation of iron from body stores to compensate for

it and there is constant provision of iron to the tissues as and when required (Skikne & Cook, 1992).

It has been established that ID occurs in three stages of progression and these stages also determine the severity of the ID (Heinrich, 1968; Wu, Lesperance, & Bernstein, 2002). In the first stage, iron depletion occurs devoid of limitation of iron supply to the tissues and thus Hb production remains unaltered and a donor at stage one ID will be successful at the predonation Hb screening. Nevertheless, this stage one can be identified by using biochemical indicators (Bainton & Finch, 1964; Cook, Boy, Flowers, & Daroca Mdel, 2005). During the second stage, there is considerable reduction in the amount of iron that is incorporated into Hb so there is an impact on the production of Hb, transport and functional sections. However, the Hb and red cell indices in the peripheral blood remain within the reference range. Results of both biochemical and haematological indicators will point to the inability of the iron getting to the erythron. At this point a donor may or may not be successful at the predonation Hb screening (Brugnara, 2002, 2003; Cook et al., 2005). The last stage is the most severe stage; there is complete development of IDA because there are inadequate iron stores to sustain the production of Hb and finally the IDA is manifested through low Hb, symptoms and signs. The donor at this point will most likely fail the predonation Hb screening. The anaemia is discovered via the use of haematological and biochemical indicators of ID (Bainton & Finch, 1964; Brugnara, 2003).

2.7.2.1.1. Iron deficiency and iron deficiency anaemia among blood donors

Blood donation has been identified by a number of researchers as a hazard for the development of ID and IDA among blood donors because of the significant loss of iron

(200 - 250 mg) following donation of 450ml of whole blood (Finch, Cook, Labbe, & Culala, 1977; Lieden, 1973; Milman & Kirchhoff, 1991). Anytime a person is bled, his/her system mobilises iron from body stores (Finch et al., 1977) and with decreased in iron stores, there is increased absorption of iron from the gastric plus duodenal lumens (Jacob, Sandstead, Klevay, & Johnson, 1980). With the continuous loss of iron stores, the person will reach a state of balance at lower level of iron stores or the iron stores will be exhausted and results in ID and finally IDA if there is no compensation for the lost iron (Cable et al., 2012; Lipschitz, Cook, & Finch, 1974). However, the probability of donating blood devoid of the development IDA depends on factors like differences in dietary iron intake, the frequency of ID in that particular populace, iron loss via menstruation in reproductive women, how often the person donates, iron supplementation and also the frequency of blood donation, the use of supplemental iron, as well as how much iron is absorbed (Milman & Sondergard, 1984; Simon, Garry, & Cooper, 1981).

Surveys performed among blood donors have revealed that there is association between prevalence of IDA and the frequency of donation (Garry, Koehler, & Simon, 1995; Simon et al., 1981). Furthermore, other works done show a high frequency of ID among the blood donors with females as well as repeat donors having greater risk of being iron deficient even when they pass the eligibility criteria (Bianco et al., 2002; Newman, 2006). In the United States of America, the National Heart, Lung and Blood Institute's (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II) programme carried out a prospective study known as the REDS-II Donor Iron Status Evaluation (RISE) on the iron status among blood donors and reported that among the first-time female blood donors, 51% had ID whiles the corresponding portion for males was 20%. In repeat

donors, females with ID were 62% and males with ID were 47% (Cable et al., 2012). These findings were not in agreement with an earlier research in Germany. Alvarez-Ossorio and colleagues in their study in Germany, revealed that ID was found among 6% of first-time female blood donors with no record of ID among their male counterparts (Alvarez-Ossorio, Kirchner, Kluter, & Schlenke, 2000).

In contrast to Alvarez-Ossorio et al. (2000), a study performed in Brazil, reported the prevalence rate of ID among blood donors as 11% with 5.5% ($^{13}/_{237}$) and 31.7% ($^{20}/_{63}$) occurring in male and female blood donors respectively (Cancado, Chiattone, Alonso, Langhi Júnior, & Alves, 2001). Investigators in Hong Kong, observed higher prevalence of ID among deferred blood donors when compared with Cable et al. (2012) and established that 35.1% and 65.3% of males and females respectively were all iron deficient (Lee et al., 2013).

A study conducted in Australia observed ID among 14.7% of blood donors (Salvin et al., 2014) and this result is comparable to researches in Brazil (Cancado et al., 2001) and South Africa (Van den Berg et al., 2018) but inconsistent with those in Hong Kong (Lee et al., 2013). Besides, Salvin and colleagues obtained 3.9% as IDA prevalence in blood donors and it is similar to what was documented later on in Pakistan (Waheed et al., 2018) but not in Nigeria (Jeremiah & Koate, 2010). Jeremiah and Koate, documented a prevalence rate of 20.6% and 12.0% as ID and IDA in that order among blood donors at Braithwaite Memorial Specialist Hospital, Port Harcourt in Nigeria (Jeremiah & Koate, 2010) and it agrees with the outcomes obtained by later researchers in Pakistan (Waheed et al., 2018). Waheed and partners in their investigations at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU),

Islamabad, Pakistan, discovered that the prevalence of ID and IDA among 528 blood donors were 20.2% and 9.7% respectively (Waheed et al., 2018).

2.7.2.2 Anaemia of chronic disease / Anaemia of inflammation

Anaemia of Chronic Disease is the second most common cause of anaemia after ID worldwide (Weiss, 2002; Weiss & Goodnough, 2005) and it is the most common anaemia among patients having chronic illnesses (Dallman, Yip, & Johnson, 1984). ACD was initially discovered in 1962 after studies on anaemia connected with infection (Cartwright & Wintrobe, 1962). Even though ACD is frequently associated with infection, inflammatory conditions, autoimmune diseases (e.g., inflammatory bowel disease, connective tissue disease) and malignancy, anaemia may also accompany other acute and chronic disorders with an inflammatory component (e.g., diabetes, heart failure) (Cartwright, 1966). The ACD is caused by the mechanisms which lead to impaired iron mobilisation and utilisation, reduced erythropoietin (EPO) production, impaired marrow erythropoietic response (reduced erythropoiesis) as well as shortened RBC survival (Cartwright & Lee, 1971). In the early stages of ACD, the RBCs are normocytic and normochromic, but in the latter stage, with increases in cytokine production, the cells may become microcytic and hypochromic on account of poor iron utilisation (Cartwright, 1966).

During inflammation, bone morphogenetic protein (BMP) 6 (D. H. Wu & Hatzopoulos, 2019) and cytokines e.g. IL-1, IL-6, TNF-α, INF-α INF-β, INF-γ signal the production of hepcidin (by the hepatocytes) causing modification in iron homeostasis leading to a build-up of iron in the storage locations in the bone marrow and reticuloendothelial systems (RES) (Weiss, 2002); consequently the depriving invading microbes and the

bone marrow erythroblast of the much needed iron . Thus, the bone marrow erythroblasts are unable to proliferate and differentiate (Gangat & Wolanskyj, 2013). In addition, hepcidin hinders the release of iron from macrophages and as a result create hypoferraemia (Nemeth et al., 2004). Additional work done by others has revealed that the activities of the cytokines promote erythrophagocytosis by the macrophages resulting in reduced survival rate of the RBCs (Milner et al., 2010).

2.7.2.2.1 Anaemia of chronic disease and blood donors

Studies have shown that newly discovered anaemia may be an indication of an unknown ailment mostly in men and menopausal women (Knight, Wade, & Balducci, 2004; Weiss & Goodnough, 2005). Few of such works done in the United States of America have been described.

Annen et al. in their investigations into the health repercussions of low Hb concentrations among deferred irregular blood donors who underwent further consultations and investigations observed that, most of the blood donors had anaemias that were ascribed to colon polyps, gastric ulcers, diabetes, arthritis, prostate cancer, multiple myeloma, mantle cell lymphoma, monoclonal gammopathy of undetermined significance (MGUS), lupus as well as myelodysplastic syndrome (Annen et al., 2015). Earlier researchers established medical conditions such as gastrointestinal (GI) bleeding, hyperthyroidism with thyrotoxicosis and uncontrolled diabetes among apparently healthy blood donors barred from donation due to low Hb concentrations (Bryant et al., 2012).

In 2011, Delaney and partners studied the assessment of care given to deferred blood donors who were excused for having low levels of Hct and referred for further medical attention. It was found that two of them were diagnosed with acute lymphocytic leukaemia and Stage IV lung cancer (Delaney et al., 2011).

2.7.2.3 Thalassaemias

2.7.2.3.1 Introduction

The thalassaemias are a different group of inherited disorders caused by genetic mutations that decrease or prevent the synthesis of one or more of the globin chains of the haemoglobin (Hb) tetramer. The characteristics of thalassaemia such as anaemia, splenomegaly, mild hepatomegaly, and mongoloid facies were first described in 1925 among four children (Cooley & Lee, 1925). Based on previous studies, the thalassaemias are classified according to the affected gene (s) e.g. α, β, γ, or δβ etc, or whether the reduction in the rate of synthesis of the affected globin is partial e.g. β^+, or absolute e.g. β^o. and finally the genotype, e.g. homozygous β^o (Bank, 1978; Weatherall & Clegg, 1981). Mutations affecting the α- or β-globin gene are most clinically significant since Hb A ($\alpha_2\beta_2$) is the major (95-98%) adult Hb. Hb A_2 ($\alpha_2\delta_2$) and Hb F ($\alpha_2\gamma_2$) are the remainder of Hb in adults constituting <3.3% and ≤ 1% respectively (Nienhuis & Benz, 1977).

2.7.2.3.2 Alpha (α) - thalassaemias

Generally, α globin chain is made by four genes, two on each strand of chromosome 16 (Forget, 1979). Therefore, alpha thalassaemia comes about when there is reduction in the synthesis of alpha globin chains due to deletion or mutation in the α-chain cluster. The length of the deletion may affect one or both of the α-genes (Orkin & Nathan, 1976;

Weatherall & Clegg, 1981). In α-thalassaemia, build-up of non-α chains (γ-chains and β-chains) has varied outcomes. The α chains are shared by foetal Hbs and adult Hbs, so the reduction in the production of α chains leads to surplus production of γ chains in the foetus whiles surplus production of β chains occurs from 6 months to adult life (Altay, Gurgey, & Tuncbilek, 1980). In the foetus, there is accumulation of excess γ chains even if a single α gene is deleted or non-functional. These surplus γ chains then become γ4 tetramers of Hb (Hb Bart), the Hb H as well as Hb Barts are unstable but soluble, so they do not precipitate to any considerable measure in the developing erythrocytes in the bone marrow and thus do not cause severe ineffective erythropoiesis (Altay et al., 1980; Nathan, Strossel, Gunn, Zarkowsky, & Laforet, 1969).

However, as the mature erythrocytes age in circulation, the tetramers undergo precipitation and produce inclusion bodies. The spleen then gets rid of these inclusions via pitting leading to extravascular haemolysis (Nathan & Gunn, 1966). The decline in Hb synthesis results in microcytic hypochromic RBCs and the anaemia is due to ineffective erythropoiesis and reduced life span because the precipitation of the α-chain causes harm to their membrane (Nathan et al., 1969). Research has shown that clinically, there are four syndrome presentations in α-thalassaemia based on the degree of impairment in the production of α-globin chains (Kan, Schwartz, & Nathan, 1968; Wasi, Na-Nakorn, & Pootrakul, 1974).

Silent carrier state is one of the clinical syndromes of α-thalassaemia; it is caused by the deletion of one α-globin gene (Embury et al., 1980). The α/β chain ratio is nearly normal because the absence of one α-globin gene causes a slight decrease in α- chain production and there are no clinical nor haematologic abnormalities (Wasi et al., 1974). Therefore,

people under this category may qualify to donate blood when they are subjected to standard procedures for donor selection because they are asymptomatic and usually have normal haematological findings (Hb 12.0-14.0g/dl, MCH < 27pg, MCV 80-85fl) (Hedge, White, Hart, & Marsh, 1977; Higgs et al., 1980).

The next one is α-thalassaemia minor/α-thalassaemia trait; these individuals have two of their α-globin genes deleted. It exists in two forms: homozygous α^{+} (– α/– α) or heterozygous α^{0} (– –/αα) and people with homozygous α^{+} thalassaemias are often less affected when their red cell indices are compared (Wasi et al., 1974). This syndrome is asymptomatic and characterised by a mild anaemia with microcytic hypochromic RBCs. The RBC count may be elevated 5.1-6.1×10^{12}/l whiles Hb and red cell indices slightly reduced (MCV 60-70fl, MCH<19-25 pg, Hb 10.0-13.0g/dl) (Hedge et al., 1977; Higgs et al., 1980). Persons with α-thalassaemia minor/α-thalassaemia trait may present for blood donation and with the above haematological outcomes they may suffer deferment.

When there is deletion of three α-globin genes, it results in Hb H disease where only one α-globin gene remains to produce α chains (– –/– a) and such individuals experience moderate to severe microcytic hypochromic haemolytic anaemia with mild jaundice and moderate hepatosplenomegaly (Wasi et al., 1974). These persons will not present at blood donation due to the nature of their conditions and haematological results (Hb 8.0-9.0g/dl, MCV 60-69fl, MCH 17-20pg) (Hedge et al., 1977; Higgs et al., 1980). α-Thalassaemia Major/ Hb Bart hydrops foetalis syndrome is a homozygous α^{0}-thalassaemia (– –/– –) and results when there is absence production of all α- chains and this state is not compatible with life so usually results in death in utero or shortly after birth (Kan, Allen, & Lowenstein, 1967; Wasi et al., 1974).

2.7.2.3.3 Beta (β) - thalassaemias

The production of β - globin chains is regulated by two genes and hence β-thalassaemias comprise of all the disorders of reduced β- globin chain production arising from the mutation of the β-globin cluster on chromosome 11 (Forget, 1979). In β - thalassaemia, the unmatched up surplus α- chains are extremely not soluble so they precipitate much faster (when compared with unpaired surplus β- chains in α-thalassaemia) at the cell surface of the developing RBCs, causing oxidative membrane damage leading to untimely death of the erythrocytes in the bone marrow and this is known as ineffective erythropoiesis (Fessas & Loukopoulos, 1973; Nathan et al., 1969). In this circumstance, the bone marrow tries to produce erythrocytes except that it is unable to release viable RBCs into the blood stream. A number of these erythrocytes with damaged membranes depart from the bone marrow into the blood stream and are prone to haemolysis and pitting by the spleen (Fessas & Loukopoulos, 1973; Nathan et al., 1969). Hence the anaemia of β-thalassaemia is as a result of ineffective production in addition to increased destruction of erythrocytes. In general, persons with β-thalassaemia do not have symptoms throughout foetal life till 4-6 months of age since Hb F $\alpha_2\gamma_2$ is the major circulating Hb at this period; the symptoms manifest just after the switching of γ-chains to β-chains take place (Pootrakul et al., 2000).

From thorough investigations by researchers, β-thalassaemias have been divided into four categories based on clinical manifestations (Weatherall & Clegg, 1981). The clinical manifestations of the various mutations as reported by researchers depend on whether one or both of the β-globin genes are affected and the extent to which the affected gene or genes are expressed (Olivieri & Weatherall, 2001).

Silent carrier state of β-Thalassaemia has been observed; the designation β^{silent} includes the various heterogeneous β-globin gene mutations that lead to the production of just a minute decrease in the amount of β- chains that are produced (Schwartz, 1969). The silent carrier state (β^{silent}/β) results in almost a normal ratio of α-β chains and there are no abnormal clinical and haematological results and they may qualify for blood donation after undergoing the selection process (Aicardi, Naselli, Sciaratta, & Sansone, 1979).

β-thalassaemia minor/ β-thalassaemia trait (β^{+}/β; β^{0}/β) results when one β-globin gene is affected by a mutation that decreases or stops its expression, whereas the other β-globin gene is normal (heterozygous state) (Malamos, Fessas, & Stamatoyannopoulos, 1962; Rowley, 1976). Literature has shown that this group of people usually presents with mild microcytic hypochromic anaemia and the haematological results are usually low (MCV 65-75 fl, MCH 18-22, Hb 9.0-10.0g/dl) except erythrocytes count which are elevated $5.52\text{-}6.48\times10^{12}$/l (Malamos et al., 1962). The β-thalassaemia minor / β-thalassaemia trait individuals may be encountered at donation but will be exempted from donating due to their abnormal haematological report (Brecher, 2005).

β-Thalassaemia Intermedia ($\beta^{silent}/\beta^{silent}$; $\delta\beta^{0}/\delta\beta^{0}$; $\beta^{0}/\delta\beta^{0}$) is an anaemic condition that is more severe than thalassaemia minor but is not transfusion-dependent as in the case of thalassaemia major from studies done (Aksoy, Dincol, & Erdem, 1978). For instance, individuals who are homozygous for silent thalassaemia gene mutation ($\beta^{silent}/\beta^{silent}$) typically have moderate microcytic, hypochromic anaemia; MCV 50-70fl, MCH16-21pg, Hb 7.0-9.0g/dl and elevated RBC count (Millard, Mason, Serjeant, & Serjeant, 1977). As a result, anybody from this group ($\beta^{silent}/\beta^{silent}$) will be deferred at donation should there be an attempt to donate blood (Brecher, 2005).

β-Thalassaemia major (Cooley's anaemia) happens when none (β^0/β^0) or just a small quantity (β^+/β^+ or β^0/β^+) of β- chains are produced due to mutation and is characterised by a severe anaemia that requires regular transfusion therapy (Weatherall & Clegg, 1981). People with this condition will not present at blood donation due to the severity of the anaemia (MCV 50-60fl, MCH <20pg, Hb <7.0g/dl) (Millard et al., 1977).

2.7.2.3.4 Thalassaemias and blood donors

A number of studies have been cited regarding the prevalence of thalassaemia among the blood donor population. In Malaysia, Lim and partners reported that the prevalence rate of thalassaemia among 242 blood donors at Pusat Darah Negara, Kuala Lumpur was 3.3% (W. C. Lim et al., 2005). A year later, Rosline and associates recorded a higher prevalence rate of 16.3% but among 80 blood donors at Hospital Universiti Sains Malaysia (Rosline et al., 2006). Tiwari and Chandola investigated the prevalence of beta thalassaemia trait (BTT) among 925 microcytic and non-microcytic blood donors and made the following observations; BTT was 36% and 3.9% microcytic group and non-microcytic group respectively (Tiwari & Chandola, 2009). Indonesia recorded 5.8% as prevalence rate for thalassaemia among 138 blood donors at the Red Cross Blood Centre Unit in Jakarta (Maharani, Soedarmono, & Nainggolan, 2014). In Africa, researchers observed 6% prevalence rate of BTT among 200 blood donors at the National Blood Transfusion Centre, Cairo, Egypt (Soliman, Kamal, Elsalakawy Walaa, & Sallam Mohamed, 2014).

2.7.2.4 Hereditary disorders of red blood cell membranes

2.7.2.4.1 Introduction

Red cell membrane disorders are associated with modifications in the shape of the cell and are categorised according to the shape of the abnormal RBCs (Bossi & Russo, 1996). Tse and Lux noted that these types of haemolytic anaemias have various clinical presentations as well as laboratory results (Tse & Lux, 1999).

2.7.2.4.2 Hereditary spherocytosis (HS)

Hereditary Spherocytosis is an inborn haemolytic disorder characterised by an inherited defect in the proteins entailed in the vertical interactions involving the skeletal membrane as well as the lipid bilayer of the RBC and this leads to the formation of spherocytic RBCs as examined on a peripheral blood film (PBF) (Weed, 1975). HS is the most frequently reported chronic haemolytic disease in Northern Europe as well as North America and it has a prevalence rate of 1 in 5000 (Morton, Mackinney, Kosower, Schilling, & Gray, 1962). It has also been found in Japan (Yawata et al., 2000). Work done by Godal and Heisto revealed that there are more mild types of HS in the population (Godal & Heisto, 1981) and this was later affirmed by other investigators (Eber, Pekrun, Neufeldt, & Schroter, 1992). A report from a study has shown that genetic defects leading to protein defect in ankyrin is the most common cause of HS, followed by α-spectrin as well as β-spectrin. The rest are protein band 3 and protein 4.2 (pallidin) in the order of decreasing prevalence (Palek, 1993). HS results from defective genes like ANK1, EPB3 and ELB42 genes, which encode for ankyrin, band 3 and protein 4.2, in that order, and also in the SPTA1 gene for α- chains and SPTB gene for β-chains (Delaunay et al., 1996).

As acknowledged previously, in HS, defect in of any of the membrane constituents leads to weakening and destabilisation of the skeleton of the RBC. Consequently, it leads to alteration in shape, the ability of the cell to deform and how elastic the RBC is. The collective effect is the formation of abnormal morphological red cell with decreased rate of survival, arising from the trapping of spherocytes during microcirculation within the spleen and eventually phagocytosed by macrophages (Delaunay et al., 1996). The clinical features of HS as stated in previous studies differ largely - comprising mainly haemolytic anaemia (of varying degree) which can be compensated or a severe anaemia which may require exchange transfusion and/ or repeated blood transfusions, irregular jaundice, splenomegaly and gallstones (Eber, Armbrust, & Schroter, 1990; Hassoun & Palek, 1996). With regards to laboratory findings, studies have shown that the main marker is spherocytes on blood film on examination accompanied by reticulocytosis in the presence or absence of anaemia (Da Costa et al., 2001). The silent carriers do not show clinical symptoms and possess normal laboratory findings and are mostly diagnosed with positive family history. Individuals with moderate to severe anaemia will have levels of unconjugated bilirubin increased (Wong, Powars, Abdalla, & Wu, 1990) with high RBC count, increased osmotic fragility, low MCV (Cynober, Mohandas, & Tchernia, 1996) and high MCHC (Clark, 1989) with a negative Coomb's test (Eber et al., 1990; Hassoun & Palek, 1996). Bolton-Maggs and partners have made known in their investigations that a normal osmotic fragility test does not rule out the diagnosis of HS and may arise in 10-20% of individuals with HS (Bolton-Maggs et al., 2004).

2.7.2.4.2.1 Blood donation and hereditary spherocytosis (HS)

Among blood donors in Germany, Eber and colleagues investigated 1,464 apparently healthy people and 34 known individuals with hereditary spherocytosis at the blood bank

of University Hospital of Gottingen for increased osmotic fragility of RBCs using the acidified glycerol lysis test. In all, 16 (1.1%) of the blood donors were classified as pathogenic (Eber et al., 1992). A similar outcome (1.0%) had earlier been observed among Norwegian blood donors (Godal & Heisto, 1981).

2.7.2.4.3 Hereditary elliptocytosis (HE)

Hereditary elliptocytosis is a heterogeneous group of congenital haemolytic anaemias that is caused by defects in proteins that interrupt the horizontal cytoskeleton of the red cell membrane. It is differentiated by the presence of elliptical shaped RBCs on PBF (Hunter & Adams, 1929, 1932). HE is widely distributed across the globe however, it is commonly found in areas endemic with malaria and among people of African and Mediterranean origin. From literature, the prevalence rate of HE in the United States has been found to be one in 2,000-4,000 people (McCarty, 1934; Wyandt, Bancroft, & Winship, 1941). In West Africa, report from a study has shown that the prevalence of HE ranges between 0.6% and 1.6% (Dhermy, Schrevel, & Lecomte, 2007). Researchers have shown that, there are four genes that can mutate and be implicated in HE, namely: α-spectrin erythrocytic 1 (SPTA1), β-spectrin erythrocytic (SPTB), erythrocyte membrane protein band 4.1 (EPB41) in addition to glycophorin C (Gerbich blood group) (GPC C) and these genes code for spectrin α and β chains, protein 4.1 and GPC C correspondingly (Anstee, Ridgwell, Tanner, Daniels, & Parsons, 1984; Delaunay et al., 1996). The main defect as discovered, is in the proteins in charge of the horizontal cytoskeletal interactions especially spectrin or those of the junction complex protein 4.1, GPC C; the firmness of the cell's skeleton is reduced and this renders the skeleton weak (Kakkar, Singh, & Dhanoa, 2004; Tse & Lux, 1999).

A study by Tse and Lux has shown that an estimated 80% of all HE is considered to be due to α- spectrin, 15% β- spectrin and the remaining 5% is due to protein 4.1 (Tse & Lux, 1999). Studies on red cell membrane, has revealed that the main defect (qualitative or quantitative) is the production of abnormal proteins that account for the horizontal cytoskeletal interactions (in particular spectrin) or those in charge of the complex at the intersection (protein 4.1, glycophorins) (Coetzer & Zail, 1982; Tse & Lux, 1999), which decreases the ability of the cytoskeleton to remain compact, resulting in its weakening (Conboy et al., 1993; Delaunay & Dhermy, 1993). Earlier studies have indicated that the precursors of HE RBCs are round and steadily become elliptical as they age in circulation (Florman & Wintrobe, 1938; Rebuck & Van Slyck, 1968; Wyandt et al., 1941). This is because RBCs of HE have membranes that do not the capacity to endure the shear stress during circulation and they consequently suffer unending distortion (Liu, Derick, & Palek, 1993; Mohandas & Chasis, 1993).

In later studies, it was indicated that the defect in the cytoskeleton of red cells in HE aids the shear stress-provoked reorganisation of the protein framework after persistent or recurring distortion of the blood cells and this prevents the recovery of the regular biconcave form (Gallagher, 2004; Lux & Palek, 1995). As a result, the typical protein bonds are broken during circulation due to the high shear forces and are replaced by fresh bonds leading to elongation of the cells (elliptocytes) (Gallagher, 2004; Lux & Palek, 1995). In severe cases, the membranes of RBCs are fragmented with decreased surface area, producing round RBCs and afterwards they undergo sequestration and are phagocytosed in the spleen (An & Mohandas, 2008; Gallagher, 2004; Mohandas & Chasis, 1993).

HE is manifested clinically, in diverse forms ranging from severe transfusion-dependent haemolytic anaemia to asymptomatic type (no anaemia but haemolysis), compensated haemolytic disease and lastly, HE with no haemolysis (benign) due to the varied molecular characteristics (Dacie, 1985; Lux & Palek, 1995; Palek, 1993). The laboratory diagnosis of HE is the distinctive finding of elliptical or cigar-shaped normocytic and normochromic RBCs on the PBF in numbers that can vary from a few to 100%. However, the presence of at least >25% of elliptocytes on PBF has been recommended as criterion for the diagnosis of HE (Bain, 2006). In patients with haemolytic type of HE the PBF shows poikilocytosis and in severe types, RBCs fragments are observed. The haemolytic HE individuals may have Hb levels between 9-10g/dl, normal MCV and increased MCHC. The osmotic fragility test of an individual with a typical HE is normal with a negative Coomb's test however, it is raised in severe HE (Kakkar et al., 2004).

2.7.2.4.3.1 Hereditary elliptocytosis (HE) among blood donors

Lecomte and colleagues in their work in France, established that approximately, 75% of individuals with HE do not exhibit any symptom and do not have anaemia (Lecomte, Garbartz, & Gautero, 1993). An additional study by Silveira and partners, has also affirmed the earlier outcome by Lecomte et al. (1993) (Silveira, Cynober, Dhermy, Mohandas, & Tchernia, 1997). Research has shown that these persons without symptoms and anaemia may qualify to donate blood when they are subjected to regular donor selection procedures (DeGruchy, Loder, & Hennessey, 1962; Quaife, Gregorius, & Kelly, 1978). This outcome was documented by Kruskall and partners in their work in a population of blood donors (Kruskall, Messier, Doherty, Pacini, & Popovsky, 1987).

A latest study in the United States of America has attested to the findings of the previous investigators where an individual with HE successfully donated blood and these elliptical RBCs were discovered during cross-matching via Coombs test (DeSimone et al., 2019). These donated red cells have been proven to have reduced life span when transfused into recipients (DeGruchy et al., 1962; Kruskall et al., 1987; Quaife et al., 1978). In another study in Bahrain involving 2,000 blood donors, 42 (2.1%) of them were found to have HE (Dash, Nadkarni, & Banerjee, 1995) and it has been suggested that with such low prevalence rate of HE, it is not necessary to screen blood donors for HE (Dash et al., 1995; Kruskall et al., 1987).

2.7.2.5 Anaemia of malaria

Malaria is an acute febrile illness and it is caused by five *Plasmodium* (*P*) species: *P. vivax*, *P. ovale*, *P. falciparum*, *P. malariae* and *P. knowlesi* (Moxham, 1994). *Plasmodium vivax*, *P. ovale* and *P. malariae* are linked to morbidity but not major mortality while *P. falciparum* is linked to both morbidity and mortality. *Plasmodium knowlesi* is a primate malaria parasite and it is found in some areas in Southeast Asia (Graves & Gelband, 2006). Anaemia is a frequent complication in both acute as well as chronic malaria (Wickramasinghe & Abdalla, 2000). The anaemia of malaria is due to diverse pathophysiological mechanisms involving accelerated destruction of RBCs as well as diminished production of RBCs (Ghosh & Ghosh, 2007).

Intravascular haemolysis of parasitized RBCs is one of the mechanisms that lead to accelerated destruction of RBCs invaded by the malarial parasites in circulation (R. E. Phillips & Pasvol, 1992) as well as also those that have been sequestered in deep vasculature of subcutaneous tissues (Nakazawa et al., 1995). In addition, malaria

infection leads to increased production of macrophages in the reticuloendothelial system (RES) for the phagocytosis of both parasitized and unparasitized RBCs (Jakobsen, Bate, Taverne, & Playfair, 1995). Jakeman and co-workers in their work revealed that for every one invaded RBC, ten non-infected RBCs are cleared from the circulation (Jakeman, Saul, Hogarth, & Collins, 1999). Hypersplenism is one of the features of malaria infection (Looareesuwan et al., 1987); unparasitized RBCs are significantly haemolysed (Woodruff, Ansdell, & Pettitt, 1979) and pitting out of the parasites from RBCs by the spleen leads to the formation of spherocytes which reduces their survival rate (Angus, Chotivanich, Udomsangpetch, & White, 1997).

The immune system triggers extravascular haemolysis via the identification of parasite antigens by the host's immunoglobulins leading to phagocytosis (Adam et al., 1981). Additionally, due to the loss of complement regulatory factors (complement receptor type 1; CR1, CD55 as well as CD59) on the surfaces of parasitized red blood cells and that of non-invaded RBCs (Stoute et al., 2003), they are marked by complements and so are prone to phagocytosis by macrophages and haemolysis (Waitumbi, Opollo, Muga, Misore, & Stoute, 2000). An earlier published work indicated that, blackwater fever may come about due to massive intravascular haemolysis of RBCs in the blood stream leading to haemoglobinaemia followed by haemoglobinuria and this sort of anaemia can be associated with renal failure (Delacollette, Taelman, & Wery, 1995). Blackwater fever mostly occurs in *P. falciparum* malaria, but it has also been found in *P. vivax*, *P. malaria,* in cases of mixed infection and G6PD deficiency as per Delacollette and associates (Delacollette et al., 1995).

As stated earlier, decreased RBCs production is one of the causes of anaemia in malaria; erythroid hypoplasia stimulated by inflammation occurs because regular response to erythropoietin (EPO) by precursors of RBCs is hindered by inflammatory cytokines leading to suppression of growth of erythroids (Phillips et al., 1986) and further delays the rate at which reticulocytes are released into circulation (Jootar et al., 1993). Individuals with malaria infection were found to have suppressed synthesis of erythropoietin and this reduced the production rate of their erythrocytes resulting in anaemia (El Hassan, Saeed, Fandrey, & Jelkmann, 1997). Moreover, Wickramasinghe and Abdalla documented in their study that in malarial infection, there is disturbance in the process of erythropoiesis, depicted by erythropoietic inhibition with resultant dyserythropoeisis; this lessens parasitaemia to some extent except that it worsens anaemia (Wickramasinghe & Abdalla, 2000). It has also be found that the occurrence of haemozoin; a metabolic product obtained from the catabolism of haemoglobin by malaria parasites also provoke the production of cytokines which subsequently hinder erythropoiesis by macrophages found in the bone marrow (Casals-Pascual et al., 2006; Lamikanra, Theron, Kooij, & Roberts, 2009).

Finally, malarial infection causes a state of cytokine dysregulation because there is an imbalance between the production of pro-inflammatory cytokines and anti-inflammatory cytokines (Roberts, Casals-Pascul, & Weatherall, 2005). Toxins of malaria parasites induce the production of pro-inflammatory cytokines by macrophages to cause anaemia (Jakobsen et al., 1995; Schofield & Hackett, 1993). IFN-γ, IL- 12 and TNF-α provoke increased apoptosis in nucleated precursors of RBC (Dai & Krantz, 1999) and also impede the process of erythroid differentiation as well as the production of erythropoietin (Jelkmann, 1998) and delay the proliferation, differentiation as well as

maturation of erythroid progenitors (Chang & Stevenson, 2004). Jakosen and colleagues have shown that elevated concentrations of anti-inflammatory cytokines e.g. IL-10 and IL-12 (Jakobsen et al., 1995) resist the activities of the pro-inflammatory cytokines; this accordingly averts the development of severe anaemia of malaria (Othoro et al., 1999).

2.7.2.5.1 Malaria parasitaemia among blood donors

The prevalence of malaria parasitaemia in blood donors has been found to vary from 0.6% to above 50.0% in sub-Saharan Africa (Owusu-Ofori, Parry, & Bates, 2010) and it was 0.7% (low endemic area) and 8.6% (high endemicity area) in Kenya (Rajab, Waithaka, Orinda, & Scott, 2005). Benin (Kinde-Gazard, Gnahoui, & Massougbodji, 2000), Nigeria (Epidi, Nwani, & Ugorji, 2008) and Cameroon (Noubouossie, Tagny, Same-Ekobo, & Mbaya, 2012) recorded 33.5%, 51.6% and 6.5% in that order. In the United States of America, 72% of deferred blood donors were disqualified for travelling to malaria-endemic areas of Mexico (Spencer et al., 2011). Furthermore, the rate of malaria parasitaemia in blood donors declared 'fit' for donation at the then National Blood Transfusion Service (NBTS), Korle-Bu Teaching Hospital, Accra-Ghana, was 7% (Degenu, 2003) and this rate was lower than the 12% reported among 'fit' blood donors at NBTS in 1994 (Amarti, 1994). On the contrary, studies carried out afterwards at other blood centres recorded no malarial case among blood donors. For instance, in Saudi Arabia, Elyamany and partners screened 180,000 blood donors at the Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia for malaria infection, but the prevalence rate was 0% (Elyamany, Al Gharawi, Alrasheed, & Alsuhaibani, 2016). Similar results were obtained at two different blood centres in Egypt; Blood Bank Unit of Mansoura University Hospital (Salem & Baiomy,

2016) and Blood Transfusion Centre of Fayoum University Hospital (Bakr, Edris, Fattah, Ibrahim, & El-Khadragy, 2017).

2.7.2.6 Megaloblastic macrocytic anaemias

2.7.2.6.1 Introduction

Megaloblastic macrocytic anaemias (MMA) are distinctive kind of anaemias characterised by macrocytic red blood cells with unique changes in the morphology of RBC precursors. In the bone marrow, the precursors of RBCs in MMA are larger than the normal mature erythrocytes and show disproportion in nuclear-cytoplasmic maturation as well as abnormal megakaryocytes (Savage, Ogundipe, Allen, Stabler, & Lindenbaum, 2000). As noted by earlier researchers, the characteristic features are due to impairment in the synthesis of deoxyribonucleic acid (DNA) and these classical findings on PBF examination are macro-ovalocytes plus hypersegmented neutrophils (Lindenbaum & Nath, 1980). MMA is mostly caused by vitamin B_{12} (cobalamin) deficiency or folate deficiency or deficiencies of both vitamins nevertheless it can also occur subsequent to chemotherapy and in myelodysplasia (Colon-Otero, Menke, & Hook, 1992).

2.7.2.6.2 Folate deficiency anaemia

Folate is a naturally occurring water soluble vitamin B_9 which is found in food; the artificial form is known as folic acid (Gregory, 1997; Kamen, 1997). It is mainly found in dark green leafy vegetables, meat, liver, legumes, some fruits (Scott & Weir, 1994), fortified cereals as well as nutritional supplements (Berry, Bailey, Mulinare, Bower, & Dary, 2010; IOM, 2000). It has been established that folic acid is more bioavailable than folate (Gregory, 1995, 1997; McKinley et al., 2001).

Studies have reported that folate plays a critical role in the synthesis of nucleotides (purines and pyrimidines) which are required for the synthesis of DNA and ribonucleic acid (RNA) (Choi & Mason, 2002). Folate mediates the reaction for the de novo synthesis of thymidine, through the process of converting deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) via the transfer of a methyl group (Bailey & Gregory, 1999). Crinder and associates in their work also published that being methyl group donors, folates are engaged in methylation reactions in addition to DNA methylation, a key epigenetic procedure which has the capability of affecting how genes are expressed (Crider, Yang, Berry, & Bailey, 2012). Folate is also used in converting homocysteine to methionine which is needed for the formation of S-adenosylmethionine (SAM) (Chiang et al., 1996). This step is accomplished through a one-carbon shift to vitamin B_{12}, which will be utilised in the process of the methylation of homocysteine to methionine. Through the addition of adenine to methionine, SAM is produced and this is the foremost methyl donor for DNA (Choi, Friso, Keyes, & Mason, 2005).

In food, folate is present as pteroylmonoglutamates and pteroylpolyglutamate varieties (Butterworth, Baugh, & Krumdieck, 1969). Through the process of digestion, the pteroylpolyglutamate form is then converted to monoglutamate by the action of the enzyme, pteroylpolyglutamate hydrolase at a pH of 5.5 with zinc at hand (Chandler, Wang, & Halsted, 1986). The folates (pteroylmonoglutamate and pteroylpolyglutamate) are primarily absorbed in the duodenum and jejunum (Qiu et al., 2006). Thereafter, the conversion of monoglutamates to 5-methyltetrahydrofolate (5-MTHF) monoglutamate by the intestinal epithelial cells takes place prior to entering the portal blood circulation (Devlin et al., 2000). There is uptake of a large amount of this type of folate by the liver

and after that; the metabolism of it to polyglutamate derivatives occurs and they are stored or freed into the blood stream or bile (Carmel, 2005).

Folate is transported in plasma with just about $^{1}/_{3}$ loosely bound to albumin and in the entire body fluids; the main type is 5-MTHF monoglutamates. The transport of folate into cells is mediated via membrane carriers or folate-binding protein-mediated systems (Kamen, 1997) and these transport components are unsaturated by folate under physiological circumstances. However, there is high level of inflow into tissues when the plasma folate concentration goes up (Baker, Bordley, & Longcope, 1932). The surplus folate within the intracellular goes into circulation for filtration by glomeruli and then finally gotten rid of via the urine (O'Brien, 1960). Also, folate may be excreted through the skin as well as bile and so there has to be restock of the body's requirements to meet physiological activities (Whitney & Rolfes, 1999).

Folate deficiency is the most common vitamin deficiency all over the globe (WHO, 2004); it can happen at any age (Hamid et al., 2007) and the deficiency of folate may possibly modify the process of gene expression as well as intensify DNA damage (Choi & Mason, 2000). Folate deficiency may be due to poor or insufficient dietary intake of folate (Said et al., 2000) and this is common among alcoholics (Allen, 2008), the aged and persons having psychiatric conditions (Edeh & Toone, 2007). Malabsorption of folate is another cause of folate deficiency as documented by researchers (Tapan & Donaldson, 2003; Weir, McGing, & Scott, 1985) and this may be due to chronic alcoholism (Allen, 2008), celiac disease (Calvani, Parisi, Guaitolini, Parisi, & Paolone, 2001; Yarali et al., 2007), tropical sprue (Tapan & Donaldson, 2003) and the use of medications like anticonvulsant drugs and oral contraceptives. Furthermore, conditions

like pregnancy as well as neonatal growth that demand increased folate requirement has also been reported to cause folate deficiency (Milman, Byg, Hvas, Bergholt, & Eriksen, 2006). A deficiency in folate results in low cell division activity especially in RBCs and eventually macrocytic anaemia develops (Fedosov, Petersen, & Nexø, 1996).

2.7.2.6.2.1 Folate deficiency and blood donors

There is scanty information on the subject of folate deficiency and blood donation when compared with that of ID. A study was undertaken to determine the effect of blood donation on water soluble vitamins among blood donors in Germany by Kalus and colleagues at the blood donor centre of the Charite´- University Medicine, Berlin. In all, blood donors on supplements were 73 (33.6%) and those not on supplements were also 144 (66.4%). It was established that none of the donors on supplements had folate deficiency whiles among donors on supplementation, 70 of them were first-time donors and 2 (2.9%) were found to be folate deficient while among the repeat blood donors, folate deficiency was discovered in one person (0.9%) (Kalus et al., 2008). Another work done in faraway Australia recorded 6.8% as the section of first-time blood donors with folate deficiency (Booth, Clark, & Fenn, 1998). In 2000, Italy documented that 9.9% of persons donating blood for the first-time were found to be folate deficient and later in 2013, as much as 77.5% blood donors were also established as folate deficient (Zappacosta et al., 2013). In nearby Nigeria, Olowoselu and associates worked on 263 prospective blood donors and 153 (58.7%) were excluded from donation due to low Hb. Subsequently, 3 (4.8%) of the anaemic deferred blood donors were established as iron deficient (Olowoselu, Akanmu, Osunkalu, Olowoselu, & Ayanshina, 2017). Here in Ghana, no literature has been cited on folate levels among blood donors, however, a work on pregnant women, indicates that 4% of second trimester pregnant women at

Mamprobi Polyclinic, Accra were folate deficient (Dei-Adomakoh, Acquaye, Ekem, & Segbefia, 2014).

2.7.2.6.3 Vitamin B_{12} deficiency anaemia

Vitamin B_{12} or cobalamin is a water soluble vitamin that is required for normal neurological function, production of RBCs and the synthesis of DNA (Gruber, Puffer, & Krautler, 2011; Scalabrino, 2009).When it attaches to a cyano, adenosyl or methyl, it is known as cyanocobalamin, adenosylcobalamin or methylcobalamin respectively (Gruber et al., 2011). Vitamin B_{12} is solely found in animal products (e.g. meat, fish, liver, kidney, poultry, shellfish, milk, butter, cheese and eggs) (Heyssel, Bozian, Darby, & Bell, 1966; Scott & Weir, 1994) and not in plant sources (Herbert, 1988). However, foods like cereals can also be fortified with vitamin B_{12} (Watanabe, 2007). Vitamin B_{12} is importantly required to convert homocysteine to methionine; methylmalonic acid to succinyl coenzyme A and to convert 5-methyltetrahydrofolate to tetrahydrofolate; a procedure essential for DNA synthesis and RBC production (Herbert & Zalusky, 1962; Watanabe & Nakano, 1991).

Vitamin B_{12} is found in foods attached to R-proteins or R-binders through peptide; food proteins (Herbert, 1988). During digestion, the stomach secretes pepsinogen and by the action of hydrochloric acid it is stimulated to pepsin which subsequently digests the food to release vitamin B_{12} from R-binders in the food (Nielsen, Rasmussen, Andersen, Nexø, & Moestrup, 2012). The liberated vitamin B_{12} then binds to haptocorrins; another R-binder produced by salivary and oesophageal glands. The vitamin B_{12}-haptocorrins union journeys into the small intestine where pancreatic proteases digest the

haptocorrins to enable gastric intrinsic factor (IF) to bind to the freed vitamin B_{12} to form IF-vitamin B_{12} compound (Dawson, Gozzard, & Lewis, 1988).

Studies have shown that vitamin B_{12} is absorbed into the blood circulation by two unique means; active and passive (Castle & Hale, 1998). The active system is the regular means that is employed, where the IF-vitamin B_{12} compound is carried to absorptive enterocytes of the ileum, (where IF-Vitamin B_{12} receptor (cubulin) is exclusively expressed) (Castle & Hale, 1998). In the enterocytes, vitamin B_{12} liberated from the IF then binds to the transport (carrier) proteins; transcobalamin I (TCI), transcobalamin II (TCII) and transcobalamin III (TCIII); synthesised in the villi of the ileum. The TCII is the least among them; however, it transports vitamin B_{12} to every cell of the body (Quadros, Regec, Khan, Quadros, & Rpthenberg, 2003). From the ileum, vitamin B_{12} is carried into portal circulation systematically via the process of endocytosis (Seetharam, 1999). Inside the cells, the vitamin B_{12} is transformed into its enzymatic active varieties (methylcobalamin plus adenosylcobalamin) (Swain, 1995).

With respect to the passive means, the absorption takes place in the duodenum as well as ileum without the involvement of intrinsic factor and intrinsic factor-vitamin B_{12} receptor. Vitamin $B_{12,}$ when ingested uses the passive means (Castle & Hale, 1998). It is a quick process except that it is an ineffective one, since it is merely $\leq$ 1% of liberated vitamin B_{12} that is taken up via diffusion across the ileal epithelia (Castle & Hale, 1998). Following the uptake of vitamin B_{12} into the cells (a large portion of it is stored in the liver) and any extra vitamin B_{12} then goes into the bloodstream, and it is filtered via the glomeruli and is removed through the urine (Birn, 2006).

Exhaustion of vitamin B_{12} from stores is uncommon because of enterohepatic circulation (Herbert, 1988), therefore the occurrence of vitamin B_{12} deficiency requires longer years to happen as compared to folate deficiency and it is normally common among the aged (mostly due to pernicious anaemia) (Green, 1996) but it may also be a frequent occurrence among expectant mothers and children mostly in developing countries (Allen, 2009). It has been documented by researchers that vitamin B_{12} deficiency may occur as a result of dietary lack (e.g. strict vegetarianism, lacto-ovo vegetarianism) (Hermann, Schorr, Purschwitz, Rassoul, & Richter, 2001; Majchrzak et al., 2006), malabsorption (e.g. Pernicious anaemia, gastrectomy and pancreatic insufficiency) (Lahner & Annibale, 2009; Oh & Brown, 2003), biological competition for dietary vitamin B_{12} (e.g. bacterial overgrowth syndromes) and impaired utilisation (e.g. congenital tanscobalamin II deficiency) (Savage et al., 2000). Furthermore, other works have also established that prolonged usage of medications (e.g. oral contraceptives) (Sutterlin, Bussen, Rieger, Dietl, & Steck, 2003) and metformin (De Jager et al., 2010) can lead to vitamin B_{12} deficiency.

2.7.2.6.3.1 Vitamin B_{12} deficiency and blood donors

The prevalence of vitamin B_{12} deficiency has been reported among apparently healthy people and not much work has been carried out among blood donor population in contrast to ID (Kant, 2000; Schulze, Linseisen, Kroke, & Boeing, 2001). A study in Germany discovered that there was no vitamin B_{12} deficiency among first-time and repeat blood donors on supplements. Conversely, vitamin B_{12} deficiency was reported among first-time donors (n=6, 8.6%) and repeat donors (n=12, 11.8%) (Kalus et al., 2008). Booth and co-workers documented a similar finding (13%) among first-time donors in Australia (Booth et al., 1998). In Italy, a prevalence rate of 83.7% vitamin B_{12}

deficiency was reported among blood donors (Zappacosta et al., 2013). With regards to Ghana, available records indicate that there is no citation on the levels of vitamin B_{12} among blood donors and vitamin B_{12} deficiency seems to be rare (Dei-Adomakoh et al., 2014).

2.8 Socioeconomic Status (SES) / Social Class (SC)

Social class refers to the categorisation of people or an assembly based on rank in the society or culture. These characteristics normally depend on the economic or political position of the individual or group. Alternatively, SC is the measure of honour, power, privilege or prestige that is attached to someone's rank in the society (Warner, Meeker, & Ells, 1949). Social status can be achieved status (via e.g. education, marriage or occupation) or ascribed status (via inheritance). By and large the higher the rank of an individual, the better and the more power an individual has over resources and in the society (Abramson, Gofin, Habib, Pridan, & Gofin, 1982; Weber, 1978).

Years later, two major forms of social stratification were put forward namely single item index and multiple items index (Barber, 1957). In the single item index, a single factor (e.g. occupation, level of education, annual income or rental value of the house in which the person is living and lifestyle) is considered before placement in a SC is made whiles in the multiple items index, all the above listed items are combined so as to increase the validity of the SC placement (Barber, 1957). The total score of the outcome of such combination exercise is termed Index of Social Characteristics (ISC) (Barber, 1957).

Warner's class model was founded using index of occupation and this produced three main classes; upper, middle plus lower with each having subdivisions upper upper class,

lower upper class, upper middle class, lower middle class, upper lower class and lower lower class (Warner et al., 1949). The SC and social status have been merged by sociologists to generate SES; which is the collective evaluation of the economic position together with social position of a person or family in relation to others using income, education or occupation (Wright & Shin, 1988). Therefore SES (definite resources and status) is the position a person occupies within the social make-up which indicates the kind of resources available to him/her (Lynch & Kaplan, 2000). The definite resources are what the person already possesses e.g. education, material wealth plus social support and the status (privilege or grade-dependent markers) is the possibility of those resources being available when needed (Oakes & Rossi, 2003). Thus, SC and SES can be substituted with each other. When is SES is decided on by occupation, to some extent, it signifies the person's level of education, financial prospect and also serves as an indication of his/her social rank (Krieger, Williams, & Moss, 1997).

The SES classifications using occupational indices gave six SCs and these classifications were employed. Additionally, the single item index (prospective blood donor's occupation) was employed for this study since there is a general tendency among people to ask first, for the job/occupation of an individual than his/her income in order to evaluate his or her social significance. Furthermore, it is easier to ascertain a person's occupation/job than salary which may be considered private.

The classifications are illustrated as follows (note-a housewife is classified using her husband's occupation) (Akanmu, Abudu, & Akinsete, 1998; Warner et al., 1949);

Table 2.1: Social Class and occupations

Social Class	Attributes or Type of occupation within the class
Class 1 / Upper upper	Professional in large business concerns, e.g. bankers, manufacturers, owners of malls, managers, lawyers, doctors, dentists, professionals and engineers.
Class 2 / Lower upper	Proprietors of small business concerns, wholesale and retail dealers including some market women with big shops, contractors, spare parts sellers, accountants, junior doctors and lawyers.
Class 3 / Upper middle	White collar and semi-professional workers, nurses, salesmen, technicians, teachers, furniture makers, traders with shops, fashion designers and caterers.
Class4 / Lower middle	Skilled workers and foremen-carpenters, artist, machinists, plumbers, printers, mason/bricklayer, cooks, barbers/hairdressers, painters and photographers.
Class 5 / Upper lower	Semi-skilled workers like truck drivers, farm tenants, machine operators, telephone operators, service station attendants, waiters/waitresses and street hawkers.
Class 6 / Lower lower	Unskilled workers - garage labourers, sweepers, porters, street cleaners, construction labourers, farm attendants, vendors and security agents.

2.8.1 Association of socioeconomic status, deferral rate and anaemia

Socioeconomic Status (SES) is a key determinant of the standard of living and health status of an individual since it has influence on the incidence and prevalence of diverse health situations based on researches done in the past years (Adler & Rehkopf, 2008; Curtiss & Grahn, 1980). Additionally, it has been proven by other researchers that a person's health is better when he possesses an equivalent higher SES (Adler & Newman, 2002; Evans & Kantrowitz, 2002). The impact of SES indicators (e.g. occupation, level of education, income) on anaemia among sample populations has been determined. Reports from studies that have been carried out, indicates that there is definite

association between SES and anaemia (Balarajan, Ramakrishnan, Özaltin, Shankar, & Subramanian, 2011; Kim et al., 2014; WHO, 2008). Haverkate and colleagues obtained similar results when data of twenty-one African countries' demographic and health surveys undertaken between the period of 2003 and 2010 were analysed (Haverkate, Smits, Meijerink, & Van Der Ven, 2014). Report from work done in Pakistan (Hassan, Salim, & Humayun, 2017) was also comparable except that other researchers in Nigeria (Ugwuja, Ogbonnaya, Obuna, Awelegbe, & Uro-Chukwu, 2015) and India (Chinchole & Najan, 2017) found no association between SES and anaemia.

In another study in India, Bahadur and partners reported a 9% incidence of deferment among blood donors and anaemia was the most common reason (32.8%) for that (Bahadur, Jain, Goel, Pahuja, & Jain, 2009). Furthermore, the impact of low socioeconomic status on the health of blood donors (ages 18-40 years) was evident with as much as 89.7% been deferred due to anaemia (Bahadur et al., 2009). It has also been reported that people with low SES tend to have monetary limitations (Chandrakumari, Sinha, Singaravelu, & Jaikumar, 2019) and are more likely to consume food with low contents of micronutrients, animal proteins as well as vitamins but high in carbohydrates plus phytates which may hinder the of absorption iron and other nutrients like zinc which could lead to poor nutritional status and eventually bring about anaemia (Bukar, Audu, Sadauki, Elnafaty, & Mairiga, 2009; Vander Jagt et al., 2007). A low level of SES most often has unfavourable consequence on the health of an individual.

2.9 Laboratory Diagnosis of Iron Deficiency Anaemia

Iron homeostasis is a closely regulated process that be depicted in a collection of markers of iron status since there is no distinct "best" test for the diagnosis of ID with or

without anaemia (Hastka, Lasserre, Schwarzbeck, Reiter, & Hehlmann, 1996). The "gold standard" test for the diagnosis of ID and IDA is bone marrow biopsy with Prussian blue staining for iron stores but bone marrow aspiration is an invasive as well as expensive process and so is not routinely used (Ali, Luxton, & Walter, 1978; Bezwoda et al., 1979). Based on work done, there are also divergent views on the use of bone marrow test. Barron and co-workers reported that the absence of haemosiderin may be incorrect >30% of cases and when really correct may not always correspond to ID (Barron, Hoyer, & Tefferi, 2001).

In addition, other researchers have also established that about 35% of bone marrow aspirate may not be sufficient to be interpreted (Krause & Stolc, 1979). A study by Ervarsti and colleagues has also shown that patients with stainable iron in their bone marrow aspirate actually had functional ID (Ervasti, Kotisaari, Romppanen, & Punnonen, 2004). The BM test is only recommended when the diagnosis of ID is still inconclusive after the use of biochemical tests (Rockey, 2005). Therefore, the combination of indirect measurements is usually used; haematological and biochemical (Hastka et al., 1996). The haematological tests are readily accessible and less costly as compared with the biochemical tests. Iron status adequacy is determined via various iron compartments of the body; transport iron (iron to meet cellular requirements), and functional iron (iron available to tissues) (Hastka et al., 1996).

The level of Hb is used to screen for the presence of anaemia or and also used for as a screening tool for ID (Finch, 1982). However, it has low specificity and sensitivity (Cook, 2006). Hct is the ratio of the volume of packed red cells to the total blood volume. It indicates the concentration of RBCs in the blood and impaired Hb formation.

Therefore, it is low when anaemia is present (Finch, 1982). However, its use is limited because it can be affected by white cell count (Lynch, 2004). By and large, Hb is a more sensitive measure of anaemia given that Hb level is likely s to drop before decreased Hct can be identified (Lynch, 2004).

However, other biochemical markers are needed to evaluate ID distinctively. The red cell indices possibly offer clues about the presence of IDE, at a much earlier stage. It has been reported that persons may have normal levels of Hb although the MCV and MCH may be slightly reduced or at the lower end of the reference range but with increased RDW (England, Ward, & Down, 1976). RDW levels can be distinctly elevated in IDA (England et al., 1976). The iron status of a person can be evaluated through the measurement of plasma or serum ferritin (Leggett et al., 1990). Ferritin is found in cells as stored iron but it can be found in circulation as an acute phase reactant produced by the liver (Leggett et al., 1990). It compares well (sensitive) with the total body iron stores in healthy individuals and when it is low it indicates low or depleted iron stores (Waiters, Miller, & Worwood, 1973). Serum ferritin can be determined by using immunoradiometric assay or an enzyme linked immunosorbance assay (Worwood, 1980). The levels of ferritin are affected by infection and so its usage is limited and so the interpretation of ferritin in such instance is done in collaboration with C-reactive protein, another acute phase protein (Marnell, Mold, & Du Clos, 2005). The presence of low ferritin levels depicts iron deficiency and so when it is combined with Hb, IDA but not iron deficiency erythropoiesis (IDE) can be evaluated (Cook, Skikne, Lynch, & Reusser, 1986). Transferrin is the carrier protein for nearly all iron circulating in plasma and levels of transferrin goes up in ID, during pregnancy, parenchymal liver damage and

oral contraceptive usage. Serum iron is decreased while and TIBC is increased during IDE (Borch-Iohnsen, 1995).

Transferrin saturation (TS) is the quantification of the ratio of serum iron to total iron binding capacity (TIBC). TS is used in the assessment of ID (low ferritin) and IDE (Bainton & Finch, 1964). As a result, very low transferrin saturation (TS) is a sign of nutritional ID and it has an effect on tissue iron supply (Bainton & Finch, 1964). When TS and iron levels are low but TIBC is normal, it indicates the presence of an infection (Bainton & Finch, 1964). TIBC is a measure of total transferrin concentration serum iron and TIBC are estimated using the colorimetric technique (ICSH, 1990). Erythrocyte protoporphyrin (EPP) also determines IDE and specifically signifies reduced iron supply for the synthesis of RBCs. EPP levels increases when there is inadequate concentration of iron for the production of haem for erythrocyte protoporphyrin (Beard, 2007). The use of EPP is limited since it is falsely increased during infection, lead poisoning and haemolytic anaemia (WHO, 2001b). EPP can be determined in a research setting via the use of fluorescence or haematofluorometer (WHO, 2001b).

Serum transferrin receptor (sTfR) is an additional biomarker that can be used to evaluate IDE (Kohgo et al., 1987). During severe ID, when and iron supply to the tissues are hindered, sTfR concentrations in the blood increase, so as to reflect the up-regulation of transferrin receptors on the cells to bind more iron in the tissues (Kohgo et al., 1987) and so increased levels of sTfR indicate tissue ID (Kohgo et al., 1987). sTfR is valuable since it is not affected by infection. The ratio of sTfR to serum ferritin can be used to differentiate low iron due to nutritional ID from the one arising from infection and inflammation (Punnonen, Irjala, & Rajamaki, 1997).

CHAPTER THREE

3.0 METHODS

3.1 Study Design

The study was cross-sectional and it was carried out from May - July, 2018.

3.2 Study Site Description

The study was conducted at sites operated by the Southern Area Blood Centre (SABC). The SABC is located at the premises of the National Blood Service, Ghana (NBSG) a multi purpose-built blood facility at Korle-Bu. The NBSG has three coordinating blood services namely Northern Area Blood Centre (NABC), Central Area Blood Centre (CABC) and SABC. The SABC is made up of health facilities of catchment areas within Eastern, Central, Volta, Greater Accra and parts of Western regions of Ghana. The SABC in 2016 screened 44,275 prospective blood donors out of which 33,677 passed. An average of 2,806 units of blood was collected per month (SABC, 2016). Some of the services offered at the SABC include haemoglobin estimation, blood screening for infectious agents (e.g. HIV, hepatitis B, hepatitis C and syphilis), blood grouping and cross-matching, antibody and antenatal screening, antibody identification, diagnostic as well as therapeutic venesections.

3.3 Study Population

All consenting potential blood donors who failed to meet the requirement for donation for varied reasons.

3.3.1 Inclusion criteria

All deferred blood donors from age 17 - 60 years with low haemoglobin levels of <12.0 g/dl for females and <13.0 g/dl for males were included. Deferred blood donors with body weight <50kg, blood pressure $<^{110}/_{70}$ mm/Hg or $>^{160}/_{100}$ mm/Hg) were also included. Additionally, any deferred blood donor with the pulse < 60bpm or >100 bpm or body temperature >37.5°C was also included.

3.3.2 Exclusion criteria

All individuals who were less than 17 and more than 60 years of age were excluded. Any individual, who had donated blood in the past four months, had been transfused or undergone surgery in the last six months was also excluded. Females who were pregnant, lactating or menstruating were excluded. All individuals who were on supplements such iron, folic acids were excluded. Furthermore, deferred blood donors who sought for incentives were excluded.

3.4 Sample Size Calculation

The sample size was based on the information on the prevalence of deferral rate from an earlier study and determined by the formula (Kish, 1965):

$$n = \frac{\not{Z}^2(Pq)}{d^2}$$

Where

n =sample size

$\not{Z}$ = 1.96, the normal value corresponding to the 95% confidence interval

P = Proportion variance available from previous data

q = 1-P

d= the desired or required size of standard error allowed

P= 42.6% or 0.426 deferral rate among blood donors (Antwi-Baffour et al., 2015).

q=1-P

=1-0.426

=0.574

d=0.09 or 9%

Z = 1.96

$$n = \frac{(1.96)^2\ (0.426 \text{ x } 0.574)}{(0.09)^2}$$

$$n = \frac{(3.842)(0.245)}{0.008}$$

$$n = \frac{0.941}{0.008}$$

=117.625

~118

Accounting for contingencies such as incomplete data, the sample size was increased by 20% (23.6, ~24) and the minimum sample size was ~142. However, a total of 150 subjects were recruited.

3.5 Samples and Data Collection

3.5.1 Chemicals, equipment, reagents and materials

Items and equipment used for various procedures and tests have been listed in Appendix I.

3.5.2 Questionnaire survey

All potential blood donors present as at the time of data collection were sampled for the study. Preceding the collection of sample, a structured questionnaire (Appendix II) was

administered to each consenting participant to obtain relevant information: demographic data of age, sex, SES (e.g. occupation), medical history (current and past), ascertain donors' nutritional status (using a standard nutritional assessment tool for dietary and food frequency), history of blood loss and drugs (e.g. oral contraceptive pills, NSAIDS). A validated non-quantified nutrient food frequency questionnaire [(FFQ) (Appendix II)] (Akwetea, 2015) was administered to the study participants to evaluate their normal dietary intakes. The FFQ comprised of selected food items contained in the specific food groups (Cereals and Grains, Starchy Root and Plantain, Legumes/oilseeds/nuts, Fruits, Vegetables, Animal and animal Products, Beverages, Processed food items, Deep Fried Foods and Pastries) with 70 questions and responses "Never, Seldom, Once a month, Twice a month, 1-2x/ week, 3-4x/ week, 5- 6x/ week" with a score ranging from 1-8. An index variable was generated for all domains with an overall score ranging from 153-387. Raw scores for each domain and overall was used as continuous variables.

3.5.3 Blood collection and processing

Whole blood (5 mls) was collected into BD (Becton Dickinson) Vacutainer® SST ™ II Advance tubes (gel separator tubes; the tubes contain spray-coated silica to aid in clotting and a polymer gel for serum separation). Furthermore, 4 mls of whole blood specimen were collected into BD Vacutainer® dipotassium ethylenediamine tetra acetic acid (K_2EDTA) anticoagulated tubes [(the tubes contain spray-dried K_2EDTA yielding a ratio of 1.5-2.2mg/ml (3.7-5.4umol) of blood when the evacuated tube is filled correctly to its fill volume)] (ICSH, 1993).

The EDTA anticoagulated blood was used in performing full blood count (FBC), preparing thin film for comment as well as preparing thin film and thick film for malaria parasites on each day of sample collection. The gel separator tubes were spun at 3000

rpm for 5 minutes after the blood had clotted and the sera were aliquoted into labelled Eppendorf tubes and stored at -20^{o}C prior to determination of serum iron, TIBC, serum ferritin, percentage transferrin saturation C-reactive protein (CRP), serum vitamin B_{12} and serum folate.

3.5.3.1 Procedure for venepuncture technique with evacuated container (BD Vacutainer® system)

Each participant was made to sit comfortably and well positioned in a chair, whiles the BD Vacutainer® SST ™ II Advance tube and BD Vacutainer® K_2EDTA tube were labelled with the participant's unique identification code. The arm of the participant was hyper extended to locate a suitable vein preferably antecubital fossa using the index finger and palpation. The BD Vacutainer® Eclipse™ Needle was screwed into a BD Vacutainer® One Use Holder to assemble them. A tourniquet was wrapped around the upper arm above the venepuncture site (about 7.5 -10 cm) to apply a little pressure and limit blood flow through the vein. Afterwards, the site for venepuncture was disinfected with 70% isopropyl alcohol pad in a circular fashion and allowed to air dry and the disinfected site was not retouched.

Subsequently, the BD Vacutainer® SST ™ II Advance tube was then introduced into the holder and the tourniquet was released as soon as blood began to flow into the tube. The tube was withdrawn when it was filled to the 5ml mark and gently inverted 5 to 6 times. The BD Vacutainer® K_2EDTA tube was also introduced (in same fashion as the earlier tube) and withdrawn when it was filled to the 4ml mark and then inverted 5 to 6 times to ensure adequate mixing. Shortly, the needle was gently removed from the venepuncture site on the arm and the puncture site was covered with dry cotton wool and the

participant made to apply sufficient pressure to avoid undue bleeding as well as formation of a haematoma. The needle was carefully recapped on a table using the green needle shield. The needle and the holder were disposed of into the sharps container. The venepuncture site was checked to ensure that bleeding had stopped and the area was covered with a dressing of dry cotton wool and adhesive tape.

3.6 Laboratory Analysis

Samples were transported to the laboratories (Research Laboratory of the Haematology department, University of Ghana School of Medicine and Dentistry, University of Ghana and the Cardio Laboratory Services of the National Cardiothoracic Centre), where FBC, blood film for comment, thin and thick films (for malaria parasites) were done on each day of sample collection. Sickling and haemoglobin electrophoresis were performed on samples of participants with peripheral blood smear features suggestive of a haemoglobinopathy. Samples in gel separator tubes were spun at 3000 rpm for 5 minutes after the blood had clotted and the sera were aliquoted into Eppendorf tubes and stored at -20^{o}C prior to determination of serum iron, TIBC, serum ferritin, CRP, serum vitamin B_{12} and serum folate. All samples were brought to room temperature before processing and performance of each test.

3.6.1 Performance of full blood count using ABX Micros ES60 OT haematology analyser

The ABX Micros ES60 OT is a full three-part automated haematology analyser (Horiba Medical, France) used for the performance of blood cell count. The automated machine works on the principles of impedance measurement (Coulter principle) and spectrophotometry for the haemoglobin estimation.

3.6.1.1 Test principles

Principle of all the haematological and biochemical tests have been provided in Appendix III

3.6.1.2 Performance of start-up analysis

When the analyser is switched on, a self-check and automatic start-up occurs. This requires three minutes to take place and this operation guarantees that the background is blank and so there are no particles to hamper with the measurement of the real sample. The results comprising of haemoglobin, RBCs, haematocrit, white blood cells and platelets are displayed on the screen following the completion of background check and these are normally zero. This signifies that the start-up has been successful. If the start-up fails, the machine, repeats the procedure automatically until it passes. However, if the inscription "start-up fails" appears then troubleshooting will be done and if it is also not successful then technical assistance is sought.

3.6.1.3 Full blood count (FBC) quality control analysis

Quality control analysis was carried out daily on normal control blood material for red cells, white cells plus platelets) to authenticate that the machine is performing within the precise ranges of the quality control materials prior to any test being done following manufacturer's instructions.

3.6.1.4 Performance of full blood count (FBC)

The BD Vacutainer® K_2EDTA tubes containing the blood were serially arranged on the roller mixer according to identification codes and were thoroughly mixed between 3-5 minutes. The mode for sampling was chosen from the main menu of the analyser and the

identification code of the sample was keyed into the analyser. The cap of the tube was removed and placed under the nozzle whiles lifting the tube to allow the sampling needle to lower into the blood with the simultaneous pressing of the manual sample bar. Aspiration of the blood then occurred for analysis after which the full blood count results were displayed on the LCD screen of the analyser and then printed out automatically.

3.6.2 Film comment for blood cells

3.6.2.1 Procedure for preparation of thin blood film for comment

A clean dry grease-free frosted end microscope glass slide was labelled with the corresponding identification code of the sample. The EDTA anticoagulated whole blood was thoroughly mixed by inverting the tube ten times and with the help of a capillary tube a drop (5 µl) of the blood was transferred at about 1cm from the frosted end. With the aid of a clean spreader at an angle of 45^{o} and in contact with the blood (the spreader was held in that position until the blood spread across the width of the slide), a swift forward movement was applied to produce a smooth feathered end (wedge shaped film). Subsequently, the slide was air-dried and then arranged on a staining rack.

3.6.2.2 Staining procedure for blood film comment using Leishman's stain

Ten slides were arranged side up on the staining rods at a time. It was ensured there was no contact between slides and none of the slides were tilted throughout the staining period. Leishman's stain (Appendix I) (ten counted drops) was used to flood the film completely for about three minutes to allow for fixing and staining of the blood cells. The stain covering the film was later diluted with double portion (twenty drops) of phosphate buffer (Appendix I) of pH6.8 and well mixed. This was done by drawing in the stain at one point and discharging it at the other end with no spillage. Next, staining

was done for about ten minutes uninterrupted after which the slide was drained of the stain and the buffer was used to wash off the stain. However, some buffer was left on the slide for about two minutes to let differentiation take place. After the two minutes, the slide was drained of the buffer; the back of the slide was wiped clean with cotton and was set upright in a draining rack to air dry.

3.6.2.3 Microscopic examination of Leishman's stained blood film for comment

The microscopic examination of all the blood films for comment was done using binocular light microscope (Leica DM500 Binocular Microscope; Leica Microsystems, Heerbrugg, Switzerland) by the Principal Investigator and subsequently reviewed by a Consultant Haematologist.

The participant's identification was checked and confirmed and the microscope slide matched with the corresponding FBC report. The Leishman's stained air-dried film was mounted onto the microscope stage with the label to the left and the microscope was switched on. Scanning was carried out using the 40x objective lens with the condenser iris sufficiently closed to provide satisfactory contrast. An optimal (suitable) area was selected towards the tail end of the film where red cells were just touching and generally distributed in a uniform manner. The microscope stage was moved down, the 100x objective lens was brought into place and a drop of oil immersion was placed on the film after which the stage was moved up. A sharp focus was achieved by using both the coarse and fine adjustments.

Soon after, evaluation of the erythrocyte (RBC) morphology was done by assessing the size, shape, haemoglobinisation in addition to presence of red cell inclusions. The

grading of morphological characteristics of the RBCs (Table 3.1) was based on ICSH recommendations (Palmer et al., 2015).

Table 3.1: Morphology grading table (Palmer et al., 2015)

	Grading System		
Cell Name	**Few/1+**	**Moderate/2+, %**	**Many/3+, %**
Red Blood Cells (RBCs)			
Anisocytosis	N/A	11-20	>20
Macrocytes	N/A	11-20	>20
Oval macrocytes	N/A	2-5	>5
Microcytes	N/A	11-20	>20
Hypochromic cells	N/A	11-20	>20
Polychromasia	N/A	5-20	>20
Acanthocytes	N/A	5-20	>20
Bite cells	N/A	1-2	>2
Blister cells	N/A	1-2	>2
Echinocytes	N/A	5-20	>20
Elliptocytes	N/A	5-20	>20
Irregularly contracted cell	N/A	1-2	>2
Ovalocytes	N/A	5-20	>20
Schistocytes	<1%	1-2	>2
Sickle cells	N/A	1-2	>2
Spherocytes	N/A	5-20	>20
Stomatocytes	N/A	5-20	>20
Target cells	N/A	5-20	>20
Teardrop cells	N/A	5-20	>20
Basophilic stippling	N/A	5-20	>20
Howell-Jolly bodies	N/A	2-3	>3
Pappenheimer bodies	N/A	2-3	>3
White Blood Cells (WBCs)			
Dohle bodies	N/A	2-4	>4
Vacuolation (neutrophil)	N/A	4-8	>8
Hypogranulation (neutrophil)	N/A	4-8	>8
Hypergranulation (neutrophil)	N/A	4-8	>8
Platelets (Plts)			
Giant Platelets	N/A	11-20	>20

Assessment of white cell morphology was made based on: presence of any changes in leucocytes (e.g. left shift of neutrophils and toxic granulation), immature cells,

hypersegmented neutrophils as well as reactive lymphocytes and various leucocyte numbers e.g. normal, increased or reduced. Platelets were evaluated based on their sizes (platelet anisocytosis, large platelets and giant platelets) and numbers (increases and decreases) and any abnormality like platelet clumps (aggregation) as well as platelet satellitism. The morphological findings (normal/abnormal) of RBCs, WBCs and platelets of each slide were recorded on the laboratory report form.

3.6.3 Malaria parasitaemia

3.6.3.1 Preparation of thick and thin blood films on the same slide for malarial parasites (WHO, 2015).

The EDTA anticoagulated whole blood was thoroughly mixed and with the aid of a capillary tube a drop of blood (5 µl) was placed near the frosted end and another drop on the middle part of a clean dry grease-free microscope labelled slide. With the help of a second slide, the drop of blood near the frosted end was spread in a circular movement to a size of 1 cm in diameter and the thickness was such that a print was just barely readable through it. A spreader was held at an angle of 45^{o} and drawn back against the drop of blood on the middle part of the slide until the blood spread along the entire width of the spreader. However, it ensured that the spreader did not have contact with the thick film. With the spreader at the same angle, it was swiftly pushed forward to create a smooth feathered end. The thick and thin films were allowed to dry completely before staining (the thin film was fixed with three drops of absolute methanol for one minute and allowed to air dry before staining. To permit de-haemoglobinisation, the thick film was neither exposed to methanol nor methanol vapour).

3.6.3.2 Staining of thick and thin blood films for malarial parasites using Giemsa stain

Ten slides including a positive film (for malaria parasite) were stained at an instance. The slides were carefully arranged side up on the staining rods making sure that they did not have contact with each other. Furthermore, tilting of the side during the time of staining was avoided. The two films on each slide were flooded with the working Giemsa stain solution (Appendix I) for twenty minutes. The stain was gently flushed off the slide with the phosphate buffer of pH7.2 (Appendix I) at the end of the staining period and was drained of; the back of the slide was wiped clean with cotton and the slide was positioned upright in the draining rack to air dry.

3.6.3.3 Microscopic examination of blood films for malaria parasites

The microscopic examination for every blood film for malarial parasites was carried out by means of binocular light microscope (Leica DM500 Binocular Microscope; Leica Microsystems, Heerbrugg, Switzerland) by the Principal Investigator and later reviewed by a Consultant Haematologist.

The Giemsa-stained air-dried film was mounted onto the microscope stage with the label to the left and the thick film was positioned in line with the 10x objective lens. The microscope was switched on, the light source optimally adjusted and scanning for malaria parasites and blood elements was done by selecting a portion that was well-stained with evenly distributed white blood cells. Then the microscope stage was moved down, the 100x objective lens was switched to and a drop of oil immersion was placed on the film after which the stage was moved up. A sharp focus was achieved by using both the coarse and fine adjustments.

The slide was examined for the absence or presence of malaria parasites [(e.g. trophozoites - deep red chromatin and blue or pale purplish-blue cytoplasm), (gametocytes - banana or sickle shaped)] in a systematic manner by starting at the periphery of the top left of the film and then moving horizontally to the right, field by field. When the other end of the film was reached, the slide was slightly moved downwards, then to the left, field by field. The grading was based on the Plus system (WHO, 2003). The presence or absence of malaria parasites of each slide was recorded on the laboratory report form.

The plus system for malaria parasite grading system was used (WHO, 2003):

No malaria parasite	= zero parasite per 200 thick film fields
+	= 1-10 parasites per 100 thick film fields
++	= 11-100 parasites per 100 thick film fields
+++	= 1-11 parasites per single thick film field
++++	= more than 10 parasites per single thick film field

3.6.3.4 Microscopic examination of Giemsa-stained thin film for malaria parasites

The protocol for microscopic examination of Giemsa-stained thin film for malaria parasites is to examine corresponding thin film of previously reported thick film having the presence of malaria parasites (WHO, 2015). However, in this study the speciation of malaria parasites for the thin films could not be done on account of the ‘no malaria parasite’ findings of the microscopic examined thick films.

3.6.3.5 Storage of slides

For the purpose of storage, the oil immersion was removed by placing the slides face down on soft absorbent paper for overnight. The slides were arranged according to the identification codes and stored in the slide box and their positions were registered. The slide boxes were kept away from moist and dusty conditions as well as insects.

3.6.4 Procedure for sickling test / sickle slide test

With the help of a capillary tube, a drop (5 µl) of known positive control EDTA anticoagulated whole blood was delivered onto the middle portion of a labelled clean dry grease-free microscope slide. A drop (5 µl) of freshly prepared 2% Sodium metabisulphite solution was added to the blood on the slide and with the aid of a corner of a clean dry grease-free slide and carefully mixed in an evenly manner. A cover slip was cautiously placed on the mixture devoid of bubbles and the slide was placed in the slide file for incubation for one hour. The above procedures were repeated using negative control plus the test samples and they were labelled appropriately as negative control and with identification codes.

Microscopic examination of the positive control was done first, followed by the negative control and test (participants). The slide was mounted on the stage with the label to the left and the RBCs were foremost brought into focus using the 10x objective lens and examined thoroughly in several parts of the field including the edges. The positive control slide demonstrated sickle shaped or crescent cells and it was reported as sickling positive whiles the negative control slide displayed no sickle shaped nor crescent cells and it was declared as sickling negative. The result of each test slide was documented on the laboratory report form as positive or negative.

3.6.5 Haemoglobin electrophoresis

3.6.5.1 Standardization of haemolysates (Dacie, 1985)

Three fresh blood samples (controls; AC, SS and F cord blood) were obtained and one millilitre (1.0 ml) of each was put into labelled test tube and filled with physiological saline (0.85% w/v). The test tubes containing the mixture were spun at 3000 rpm for 5 mins and the supernatants were decanted using Pasteur pipettes. The cells were washed four more times until the supernatants were clear. One volume of the saline washed packed red cells of each control was haemolysed by the addition of two volumes of distilled water with subsequent mixing. Carbon tetrachloride (one volume) was added to each mixture and vortexed for 1 minute and finally centrifuged for 10 minutes at 3000rpm. The clear supernatants were pipetted into test tubes by means of Pasteur pipettes, labelled and 2-5 drops of 0.3mol/l KCN (2g/dl) were added to the haemolysates to stabilise the haemoglobin as cyanmethaemoglobin (HiCN). The haemoglobin content of each haemolysate was adjusted to 10g/dl using distilled water and equal volumes of the three haemolysates were mixed and ready to be used as control for electrophoresis. The preceding processes were done for each test sample except that the test samples were left as individual haemolysates.

3.6.5.2 Test procedure

With the power supply disconnected, the electrophoresis tank was prepared by pouring equal volumes of the Tris-EDTA borate buffer into each of the outer buffer compartments of the tank to about half its capacity. The cellulose acetate membranes were soaked for 5 minutes by immersing slowly (to avoid bubbles) into the reservoir of buffer. The applicator (sample) well plates were filled with 10µl of control haemolysates (mixture of Hb A, S, F and C) and test haemolysates. The cellulose acetate membranes

were removed from the Tris-EDTA borate buffer and blotted twice between two layers of clean blotting paper without drying and this was to reduce excess buffer. The applicator was then filled by depressing the combs into the applicator wells and the haemolysates smeared on each finger of the comb (representing each sample). The initial load was applied onto a clean blotting paper. The applicator was reloaded and the control and test haemolysates were applied onto the previously soaked and blotted cellulose acetate membrane subsequently. The loaded cellulose acetate membrane was placed across the two bridges of the electrophoresis tank with the cellulose acetate portion down such that the origins were closer to the cathode (the negatively charged electrode) while the blank portion was closer to the anode (the positively charged electrode) and the membranes were in direct contact with the buffer. Soon after, the electrophoresis tank was connected to a power pack which had been set to 250-350 volts, 50 mA of electric power and allowed to run for 30 minutes.

After clear separation of the various haemoglobin molecules (bands) had been observed, the current was stopped and the entire cellulose acetate membrane was transferred into the staining well containing Ponceau S and stained for 5 minutes. The excess stain was then washed off by rinsing of the membrane three times in 2% acetic acid solution and blotted with a clean blotting paper, left to dry and finally labelled. With reference to the migration distance of the controls (Hb A, S, F and C), the various haemoglobins of the test haemolysates were interpreted and identified when compared. The interpretations (Fig. 3.1) were based on recommendations from Center for Disease Control, Atlanta (Schmidt & Brosious, 1976). The results of the haemoglobin bands were then recorded as e.g. AA, AS, AC or SS as appropriate.

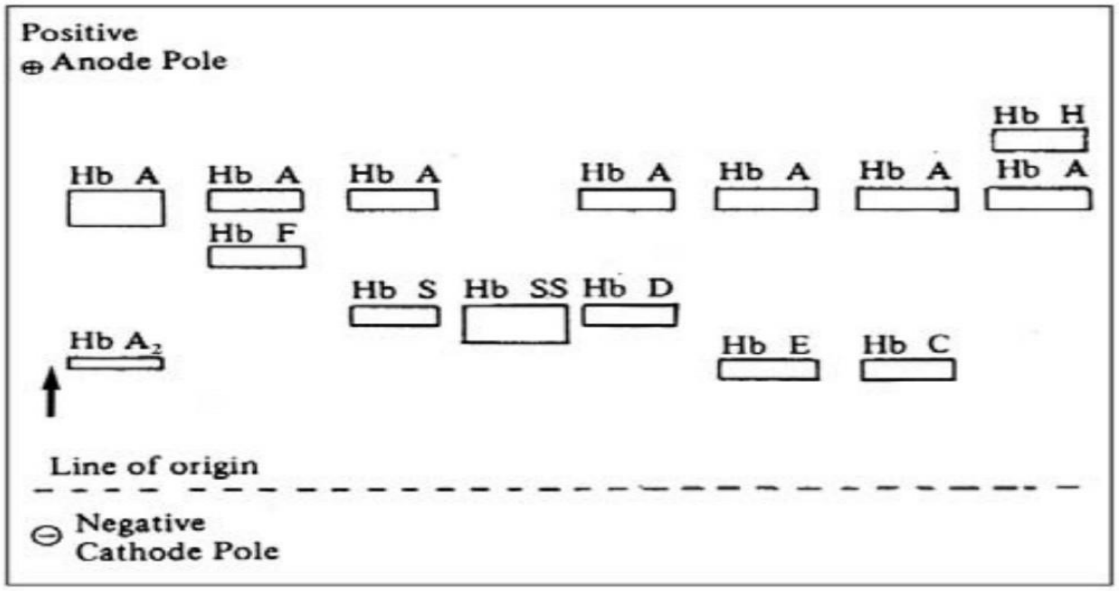


Figure 3.1: Relative mobilities of some haemoglobins by electrophoresis cellulose acetate membrane at alkaline pH (Schmidt & Brosious, 1976).

3.6.6 Biochemical tests

Analysis of serum iron, TIBC, serum ferritin and CRP were done for all samples with haemoglobin concentration of <12.0 g/dl and <13.0 g/dl for females and males respectively. Investigation of macrocytosis is often required when MCV >100fl but for the purpose of this study, macrocytosis was defined as MCV > 90fl (Kaferle & Strzoda, 2009; Veda, 2013). Determination of serum vitamin B_{12} and serum folate could not be done as planned due to the fact that all the MCV results of all the anaemic samples were MCV < 90fl. Control samples supplied by the manufacturer were analysed prior to sample analysis.

3.6.6.1 Determination of serum iron, TIBC, serum ferritin and CRP

The Urit - 8210 (Urit Medical Electronic Co., Ltd, China), a fully automated open system chemistry analyser was used to determine the serum iron, TIBC, serum ferritin and CRP. Interpretation of results was carried out as indicated in Table 3.2.

Table 3.2: Interpreting laboratory blood test results to assess iron status (Pasricha et al., 2010; WHO, 2008)

Diagnosis	**Reference ranges[#]**							
	Hb (g/dl) [1]M 13.0-18.0 [2]F 12.0-16.0	**MCV (fl)** 76- 96	**MCH (pg)** 27-30	**SF (µg/l)** 15-300	**Iron (µmol/l)** [1]M 12.5-32.2 [2]F 10.5-30.2	**TIBC (µmol/l)** 36-72	**%TSAT** [1]M 20-50 [2]F 15-50	**CRP (mg/l)** <10
IDA	Low	Low (or normal in early IDA)	Low (or normal in early IDA)	Low	Low	High	Low	Normal
ACD/AI	Low	Normal (may be mildly low)	Normal (may be mildly low)	Normal or elevated	Low	Normal	Low	High
IDA + ACD/AI	Low	Low	Low	Low or normal	Low	Normal	Normal or high	High

[#]IDA= iron deficiency anaemia, ACD= anaemia of chronic disease, AI= anaemia of inflammation, Hb= haemoglobin, MCV= mean cell volume, MCH= mean cell haemoglobin, SF= serum ferritin, TIBC= total iron binding capacity, %TSAT= percentage transferrin saturation, CRP= C-reactive protein, [1]M = Male, [2]F = Female.

3.7 Data Management

Confidentiality of data acquired from all participants that took part in the study was guaranteed. Hard copies have been gathered under lock and key in cabinets with restricted access to the investigators. Distinctive identification numbers were allocated to each participant in place of names. The entire data were coded and doubly entered into password-protected database Microsoft Excel 2016.

3.8 Statistical Analysis

The entire data was transferred to Stata version 15 for data cleaning, recoding, reclassification and analysis. Categorical variables like sex, occupation (social class),

educational level, number of children, reason for deferral, type of anaemia, drugs etc. were shown as frequencies and percentages in a tabular form and descriptive diagrams such as pie charts and bar graphs. For the purpose of clarity, two approaches of data analysis were carried which involved descriptive and inferential analysis. Descriptive involved cross tabulations which were presented in frequencies and percentages. Inferential analysis involved performing chi-square test to assess covariate factors associated with dependent variable and student t-test (unpaired) with equal variance was used to compare mean of continuous variables among anaemic and non-anaemic participants. Furthermore, inferential analysis involved logistic regression with univariate and multivariate models was employed to ascertain risk determinants of anaemia among deferred blood donors by reporting crude and adjusted odd ratio's at 95% confidence interval. A p-value ≤ 0.05 was deemed as statistically significant.

3.9 Ethical Issues

Ethical approval was obtained from the Ethical and Protocol Review Committee of College of Health Sciences, University of Ghana (Appendix IV) and The National Blood Service, Ghana (Appendix V). An informed consent form (Appendix VI) was administered to prospective participants subsequent to explaining the purpose of the research, potential risk or discomfort to the participants and potential benefits to the participants.

CHAPTER FOUR

4.0 RESULTS

4.1 General Profile of Prospective Blood Donors

A total number of 1,565 prospective blood donors consisting of 1,328 (84.9%) males and 237 (15.1%) females presented to donate blood during the study period (Table 4.1). The total number of successful donors was 1140 (88.4%). Out of total number of prospective blood donors, 27.2% (425/1565) individuals comprising of 74.6% (317/425) males and 25.4% (108/425) females were disqualified (deferred) (Table 4.2) from donation for various reasons. From the 425 deferred donors, 150 people consented to participate in this study.

Table 4.1: General profile of prospective blood donors

Prospective Donors (N = 1565)	**n (%)**
Total number of male donors	1328 (84.9)
Total number of female donors	237 (15.1)
Successful Donors (N = 1140)	
Total number of male donors	942 (73.1)
Total number of female donors	198 (15.3)

Table 4.2: General profile of deferred blood donors

Deferred Donors (N = 425)	**n (%)**
Total number of deferred male donors	317 (74.6)
Total number of deferred female donors	108 (25.4)

4.2 Deferred Blood Donors

4.2.1 Methods of haemoglobin estimation

Figure 4.1 shows the error bar chart of deferral rates obtained from two different methods of estimating predonation haemoglobin (copper sulphate and automated haematology analyser). Among the 150 deferred blood donors, the incidence of deferral was established by using the haematology analyser following the copper sulphate method.

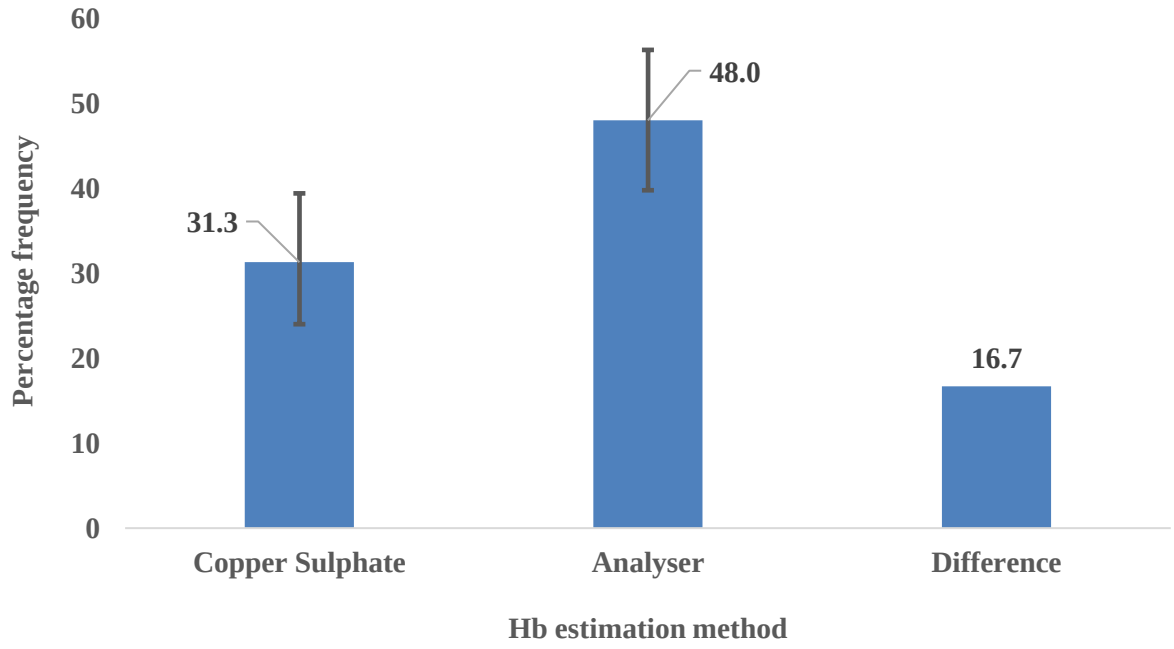


Figure 4.1: Methods of haemoglobin estimation

The deferral rate by the copper sulphate method was 31.3% (47/150) whiles that of the haematology analyser was 48.0% (72/150). Therefore, the false pass rate was 16.7%.

4.2.2 Reasons for deferrals

Figure 4.2 shows the various reasons for deferral. The most reported reasons for deferral were high blood pressure (44.67%, [67/150]), anaemia (low haemoglobin concentration)

(31.33%, [47/150]) and low weight (10.0%, [15/150]). Candidiasis (0.7%, [1/150]) was the least reason for a prospective donor to be deferred. Furthermore, low Hb (anaemia) was reported as the most frequent reason (48.15%, [39/81]) responsible for deferment among the female blood donors whiles among their male counterpart it was high blood pressure (66.67%, [46/69]) (Table 4.3).

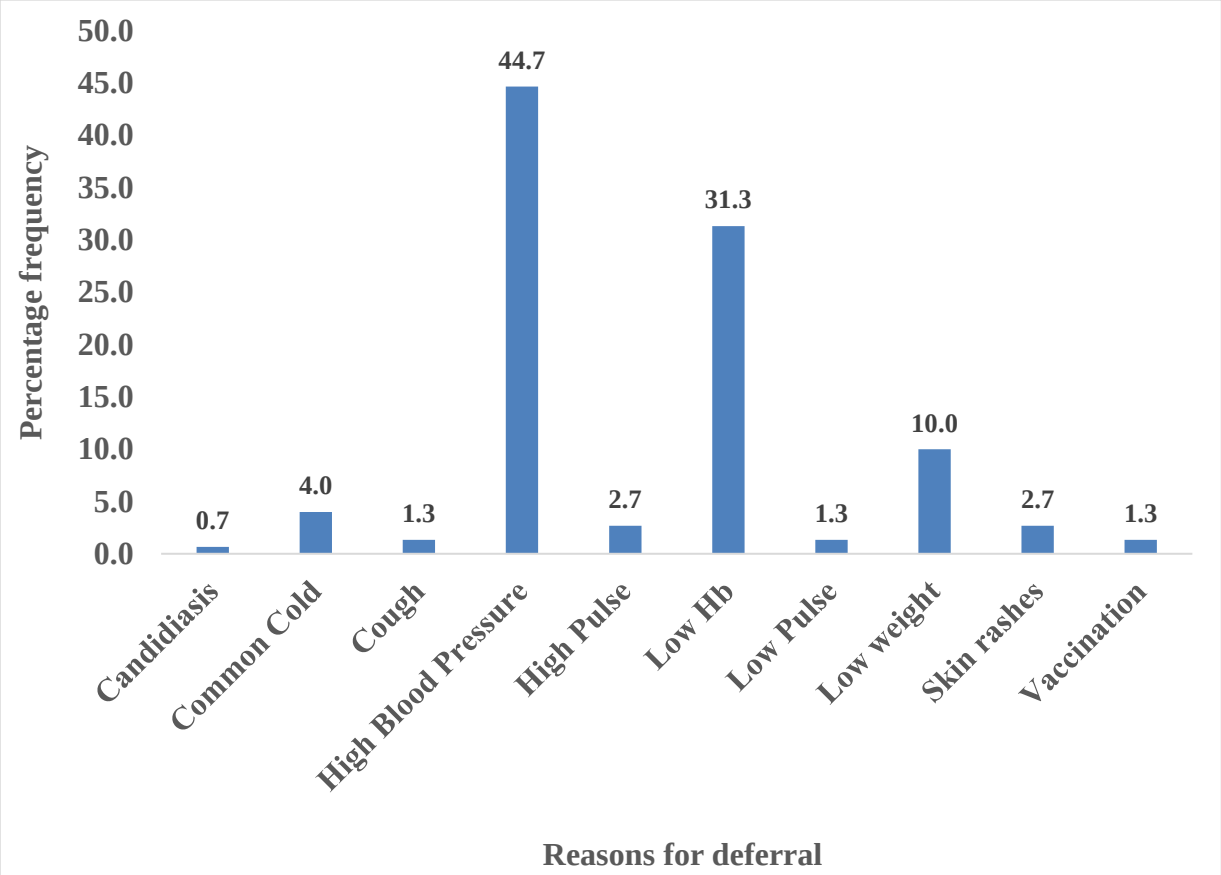


Figure 4.2- Reasons for deferrals among the deferred blood donors.

Table 4.3: Deferral reasons among female and male deferred blood donors

Reason for deferral	Deferred blood donors[1]		Total[1]
	Female	Male	
Candidiasis	1 (1.23)	0 (0.00)	1 (0.67)
Common Cold	2 (2.47)	4 (5.80)	6 (4.00)
Cough	1 (1.23)	1 (1.45)	2 (1.33)
High Blood Pressure	21 (25.93)	46 (66.67)	67 (44.67)
High Pulse	3 (3.70)	1 (1.45)	4 (2.67)
Low Hb (anaemia)	39 (48.15)	8 (11.59)	47 (31.33)
Low Pulse	0 (0.00)	2 (2.90)	2 (1.33)
Low weight	12 (14.81)	3 (4.35)	15 (10.00)
Skin rashes	1 (1.23)	3 (4.35)	4 (2.67)
Vaccination	1 (1.23)	1 (1.45)	2 (1.33)
Total	**81 (54.0)**	**69 (46.0)**	**150**

[1]All values are n (%)

Table 4.4 shows the reasons for deferral among the age groups. Majority of the participants, 24/60 (40%), in the 17-19 years age group were deferred due to low haemoglobin level and high blood pressure was the most frequent deferral reason among the rest of the age groups.

Table 4.5 shows the reasons for deferral among the various social classes. The highest number, 75.86% (22/29), of deferred blood donors had high blood pressure and were from the lower - class social class category. Additionally, most of the participants deferred due to low Hb (anaemia) were students 35 (39.33%).

4.2.3 Socio-demographic characteristics and anaemic status

Table 4.6 shows the socio-demographic characteristics and anaemic status of the deferred blood donors. Among the 150 deferred blood donors were 69 (46.0%) males and 81 (54.0%) females. The mean age of the deferred blood donors was 27.19 ± 11.41 (range 17 - 55 years). Among the females, the average age was 24.02 ± 9.32 years (range

- 17-50 years) and that of the males was 30.89 ± 12.53 years (range 17-55 years). The age group that had the highest number of people participating in the study was 17-19 years age group with 60 (40%) and whiles the age group with the least deferred was the 50-55 years; consisting of 7 (6.7%) people. With respect to social class, students were the most represented grouping with 37 (41.6%) of them being non-anaemic and 52 (58.4%) anaemic. In all 7 (4.7%) people from the upper class were deferred with 3 (42.9%) having anaemia. The educational level of most of the participants were secondary school 89 (62%), followed by tertiary 29 (19.3%) and among the deferred anaemic group; one person (1.4%) did not have any form of formal education whiles no participant with primary as educational level had anaemia.

Table 4.4: Reasons for deferral among the deferred blood donors by age groups

	Age group (years)[1]					
Reasons	**17-19**	**20-29**	**30-39**	**40-49**	**50-55**	**Total**
Candidiasis	1 (1.67)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.67)
Common Cold	2 (3.33)	3 (8.11)	1 (4.17)	0 (0.00)	0 (0.00)	6 (4.00)
Cough	1 (1.67)	0 (0.00)	0 (0.00)	0 (0.00)	1 (10.00)	2 (1.33)
High Blood Pressure	14 (23.33)	16 (43.24)	13 (54.17)	17 (89.47)	7 (70.00)	67 (44.67)
High Pulse	3 (5.00)	1 (2.70)	0 (0.00)	0 (0.00)	0 (0.00)	4 (2.67)
Low Hb (anaemia)	24 (40.00)	13 (35.14)	8 (33.33)	0 (0.00)	2 (20.00)	47 (31.33)
Low Pulse	1 (1.67)	1 (2.70)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.33)
Low weight	13 (21.67)	2 (5.41)	0 (0.00)	0 (0.00)	0 (0.00)	15 (10.00)
Skin rashes	1 (1.67)	1 (2.70)	1 (4.17)	1 (5.26)	0 (0.00)	4 (2.67)
Vaccination	0 (0.00)	0 (0.00)	1 (4.17)	1 (5.26)	0 (0.00)	2 (1.33)
Total	**60 (40.0)**	**37 (24.7)**	**24 (16.0)**	**19 (12.7)**	**10 (6.7)**	**150 (100)**

[1]All values are n (%)

Table 4.5: Reason for deferral in the various social classes among the deferred blood donors

	Social class[1]				
Deferral Reasons	**Lower class**	**Middle class**	**Upper class**	**Student**	**Total**
Candidiasis	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.12)	1 (0.67)
Common Cold	0 (0.00)	1 (4.00)	0 (0.00)	5 (5.62)	6 (4.00)
Cough	1 (3.45)	0 (0.00)	0 (0.00)	1 (1.12)	2 (1.33)
High Blood Pressure	22 (75.86)	16 (64.00)	3 (42.86)	26 (29.21)	67 (44.67)
High Pulse	0 (0.00)	1 (4.00)	0 (0.00)	3 (3.37)	4 (2.67)
Low Hb (anaemia)	4 (13.79)	5 (20.00)	3 (42.86)	35 (39.33)	47 (31.33)
Low Pulse	0 (0.00)	0 (0.00)	0 (0.00)	2 (2.25)	2 (1.33)
Low weight	0 (0.00)	0 (0.00)	0 (0.00)	15 (16.85)	15 (10.00)
Skin rashes	2 (6.90)	1 (4.00)	0 (0.00)	1 (1.12)	4 (2.67)
Vaccination	0 (0.00)	1 (4.00)	1 (14.29)	0 (0.00)	2 (1.33)
Total	**29 (19.3)**	**25 (16.7)**	**7 (4.7)**	**89 (59.3)**	**150 (100)**

[1]All values are n (%)

Table 4.6: Socio-demographic characteristics of deferred blood donors

Socio-demographic variable	Anaemic status			p-value
	Total	**Non-anaemic[1]**	**Anaemic[1]**	
		78 (52.0)	**72 (48.0)**	
Sex				35.30 (**0.000**)
Female	81	24 (29.6)	57 (70.4)	
Male	69	54 (78.3)	15 (21.7)	
Age group (years)				12.86 (**0.012**)
17-19	60	22 (36.7)	38 (63.3)	
20-29	37	21 (56.8)	16 (43.2)	
30-39	24	13 (54.2)	11 (45.8)	
40-49	19	15 (78.9)	4 (21.1)	
50-55	10	7 (70.0)	3 (30.0)	
Occupation (Social class)				12.29 (**0.015**)
Lower class	25	18 (72.0)	7 (28.0)	
Middle class	25	15 (60.0)	10 (40.0)	
Student	89	37 (41.6)	52 (58.4)	
Unemployed	4	4 (100)	0 (0)	
Upper class	7	4 (57.1)	3 (42.9)	
Level of Education				6.98 (0.137)
JHS	20	13 (65.0)	7 (35.0)	
Nil	4	3 (75.0)	1 (25.0)	
Primary	4	4 (100)	0 (0)	
Secondary	93	45 (48.4)	48 (51.6)	
Tertiary	29	13 (44.8)	16 (55.2)	
Marital Status				3.88 (0.144)
Divorced	9	6 (66.7)	3 (33.3)	
Married	29	19 (65.5)	10 (34.5)	
Single	112	53 (47.3)	59 (52.7)	
No. of children				9.24 (**0.010**)
None	102	45 (44.1)	57 (55.9)	
1-2	29	18 (62.1)	11 (37.9)	
3+	19	15 (78.9)	4 (21.1)	
First-time donor				7.93 (**0.005**)
Yes	109	49 (45.0)	60 (55.0)	
No	41	29 (70.7)	12 (29.3)	

[1]All values are n (%)

Under marital status, the singles were the dominant category, 112 (74.7%) whiles the divorced group were the least, 9 (6%). Most of the deferred blood donors 109 (72.7%) turned out to be first timers at donation and majority of them 60 (55.0%) were anaemic. Sixty (forty percent) females of reproductive age among the deferred blood donors had normal (28 days) menstrual cycle and forty-two (seventy percent) of them were exempted from donation because of anaemia. Also, within the longer (35 days) menstrual cycle group, 4 (2.7%) people were deferred from donation and all of them (100%) were attributable to anaemia.

4.2.4 Anaemia and its associated factors

The prevalence of anaemia among the deferred blood donors was 48% (72/150). Factors such as age, occupation (social class), number of children and being a first-time donor, all had association with anaemia among the participants with p-values of ≤0.05 (Table 4.6). Among the females of reproductive age, factors such menstrual cycle, duration of menses and intensity of menstrual flow (heavy menses) were associated with anaemia (p≤0.05) (Table 4.7). There was no association (p>0.05) between factors such as marital status, level of education (Table 4.6) and number of meals eaten per day (Table 4.7) with anaemia.

4.2.4.1 Risk determinants

Among the variables that may possibly determine low levels of haemoglobin (anaemia), sex, occupation (social class), level of education, menstrual cycle and duration of menses demonstrated statistical significance (p≤0.05) (Table 4.8a). Univariate data analysis showed that females were 8.55 times likely to be anaemic compared to males [cOR (95% CI) p-value =8.55 (4.05-18.01) 0.000] (Table 4.8a).

Table 4.7: Factors associated with anaemia

Demographic variable	Anaemic status			Chi (p-value)
	Total	**Non-anaemic[1] n=78 (52.0)**	**Anaemic[1] n=72 (48.0)**	
Meals/day				0.618 (0.432)
Twice	42	24 (57.1)	18 (42.9)	
Thrice	108	54 (50.0)	54 (50.0)	
Meals eaten away from home				2.12 (0.547)
None	12	7 (58.3)	5 (41.7)	
Once	38	19 (50.0)	19 (50.0)	
Twice	55	32 (58.2)	23 (41.8)	
Thrice	45	20 (44.4)	25 (55.6)	
Malarial Treatment				1.41 (0.235)
No	132	71 (53.8)	61 (46.2)	
Yes	18	7 (38.9)	11 (61.1)	
Bleeding Piles				3.32 (0.069)
No	147	78 (53.1)	69 (46.9)	
Yes	3	0 (0)	3 (100)	
Gastric ulcer				4.45 **(0.035)**
No	146	78 (53.4)	68 (46.6)	
Yes	4	0 (0)	4 (100)	
Menstrual cycle (days)				38.04 **(0.000)**
None	74	57 (77.0)	17 (23.0)	
Short (21)	12	3 (25.0)	9 (75.0)	
Normal (28)	60	18 (30.0)	42 (70.0)	
Longer (35)	4	0 (0)	4 (100)	
Duration of menses (days)				40.06 **(0.000)**
None	74	57 (77.0)	17 (23.0)	
2-4	26	11 (42.3)	15 (57.7)	
5-7	50	10 (20.0)	40 (80.0)	
Heavy menses				30.95 **(0.000)**
No	126	78 (61.9)	48 (38.1)	
Yes	24	0 (0)	24 (100)	
Oral contraceptive usage				0.003 (0.955)
No	148	77(52.0)	71 (48.0)	
Yes	2	1 (50.0)	1 (50.0)	

[1]All values are n (%)

Table 4.8a: Logistic regression showing crude odds ratio (cOR) and adjusted odds ratio (aOR) determining risk of anaemia at 95% confidence interval among deferred blood donors.

Risk determinants	cOR [95%CI] p-value	aOR [95%CI] p-value
Sex		
Male	**Ref***	**Ref**
Female	8.55 [4.05-18.01] **0.000**	5.06 [0.23-113.4] 0.306
Age group		
50-55	**Ref**	
17-19	4.03 [0.94-17.19] 0.060	
20-29	1.78 [0.39-7.97] 0.452	
30-39	1.97 [0.41-9.51] 0.397	
40-49	0.62 [0.11-3.56] 0.594	
Occupation (social class)		
Lower class	**Ref**	**Ref**
Middle class	2.09 [0.65-6.74] 0.214	1.19 [0.21-6.65] 0.840
Student	4.42 [1.71-11.42] **0.002**	0.21 [0.02-2.64] 0.229
Upper class	2.36 [0.42-13.19] 0.329	4.63 [0.12-183.7] 0.414
Level of Education		
Nill/Primary	**Ref**	
JHS	3.76 [0.38-37.14] 0.256	
Secondary	7.47 [0.88-63.11] 0.065	
Tertiary	8.61 [0.94-79.27] 0.057	
Marital Status		
Married	**Ref**	
Divorced	0.95 [0.19-4.62] 0.949	
Single	2.11 [0.90-495] 0.084	
No. of children		
3+	**Ref**	**Ref**
None	4.75 [1.47-15.31] **0.009**	3.55 [0.24-51.4] 0.354
1-2	2.29 [0.60-8.69] 0.223	6.38 [0.12-68.2] 0.125

*Ref= Reference; Bold figures indicate statistical significance

Furthermore, females with 5-7 days as duration of menses were 13.41 times as likely to have anaemia [cOR (95% CI) p-value =13.41 (5.56-32.3) 0.000] as compared to the reference group (none i.e. men and menopausal women).

In addition, all the food groups except cereals and grains showed association with anaemia within the univariate data analysis (Table 4.8b). In the multivariate data analysis, beverages were found to be a risk determinant [aOR (95% CI) p-value =1.60 (1.10-2.34) 0.014] (Table 4.8b).

4.2.5 Haematological profile

The results of haematological profile of the deferred blood donors (anaemic and non-anaemic) are shown in Table 4.9. The deferred anaemic group had Hb, RBC, MCV as well as MCH mean values that were lower and statistically significant when compared to the non-anaemic group (p=0.000). On the contrary, means of RDW and Plt in the anaemic category were significantly elevated (p=0.000) when judged against those of the non-anaemic deferred group.

Table 4.10 presents information on the haematological profile of deferred blood donors (males and females). The deferred females had significantly lower mean Hb, RBC, MCV plus MCH levels than their male counterparts (p=0.000). In contrast, a significantly higher mean RDW and Plt values were recorded among the deferred females, compared to the deferred males ($p \leq 0.05$).

Table 4.8b: Logistic regression showing crude odds ratio (cOR) and adjusted odd ratios (aOR) determining risk of anaemia at 95% confidence interval among deferred blood donors

Risk determinants	cOR [95%CI] p-value	aOR [95%CI] p-value
First time donor		
No	**Ref***	**Ref**
Yes	2.96 [1.36-6.40] **0.006**	5.11 [0.56-47.1] 0.150
Meals/day		
Twice	**Ref**	
Thrice	1.33 [0.65-2.73] 0.432	
Meals eaten away from home		
None	**Ref**	
Once	1.40 [0.38-5.19] 0.615	
Twice	1.01 [0.28-3.57] 0.992	
Thrice	1.75 [0.49-6.35] 0.395	
Malarial treatment		
No	**Ref**	
Yes	1.83 [0.67-5.01] 0.240	
Menstrual cycle		
None	**Ref**	**Ref**
	14.53 [3.70-57.02]	
Short/Longer	**0.000**	12.60 [0.21-769.5] 0.227
Normal	7.82 [3.61-16.96] **0.000**	6.94 [0031-154.3] 0.221
Duration of menses		
None	**Ref**	**Ref**
2-4	4.57 [1.77-11.79] **0.002**	0.15 [0.01-3.09] 0.220
5-7	13.41 [5.56-32.3] **0.000**	
Dietary intakes (Food group)		
Cereals and Grains	1.02 [0.96-1.08] 0.559	
Starchy root and plantain	0.92 [0.86-0.99] **0.033**	1.20 [0.76-1.90] 0.430
Legumes/oilseeds/nuts	0.69 [0.61-0.78] **0.000**	0.75 [0.52-1.06] 0.098
Fruits	0.75 [0.68-0.82] **0.000**	0.77 [0.53-1.12] 0.180
Vegetables	0.85 [0.80-0.89] **0.000**	0.95 [0.77-1.18] 0.668
Animal and animal products	0.87 [0.83-0.91] **0.000**	0.97 [0.78-1.21] 0.794
Beverages	1.11 [1.02-1.19] **0.011**	1.60 [1.10-2.34] **0.014**
Processed food items	0.82 [0.70-0.96] **0.013**	0.81 [0.50-1.33] 0.416
Deep fried foods and pastries	0.88 [0.81-0.96] **0.003**	1.14 [0.77-1.69] 0.516
Overall	0.94 [0.93-0.96] **0.000**	0.98 [0.76-1.28] 0.902

*Ref= Reference; Bold figures indicate statistical significance

Table 4.9: Haematological profile of deferred non-anaemic and anaemic blood donors

	Deferred blood donors[1]			
Haematological Parameter	**Non-anaemic**	**Anaemic**	**DoM[2]**	**p-value**
Hb (g/dl)	14.17 (1.17)	10.52 (1.40)	3.65	**0.000**
RBC ($x10^{12}$/l)	5.01 (0.45)	4.31 (0.59)	0.70	**0.000**
MCV (fl)	85.67 (3.14)	75.28 (8.70)	10.39	**0.000**
MCH (pg)	28.83 (1.43)	24.36 (3.95)	4.48	**0.000**
RDW (%)	13.51 (0.63)	15.58 (2.25)	-2.07	**0.000**
WBC ($x10^9$/l)	5.48 (1.21)	5.86 (1.21)	-0.38	0.060
GRA ($x10^9$/l)	2.86 (0.88)	3.05 (0.91)	-0.19	0.190
MON ($x10^9$/l)	0.29 (0.13)	0.40 (0.68)	-0.10	0.188
LYM ($x10^9$/l)	2.33 (0.67)	2.52 (0.63)	-0.19	0.074
Plt ($x10^9$/l)	246.19 (53.16)	322.82 (137.14)	-76.63	**0.000**

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.10: Haematological profile of deferred male and female blood donors

	Deferred blood donors[1]			
Haematological Parameter	**Female**	**Male**	**DoM[2]**	**p-value**
Hb (g/dl)	11.12 (1.65)	13.94 (1.84)	-2.82	**0.000**
RBC ($x10^{12}$/l)	4.34 (0.53)	5.07 (0.50)	-0.73	**0.000**
MCV (fl)	78.17 (8.36)	83.62 (7.15)	-7.98	**0.000**
MCH (pg)	25.52 (3.71)	28.68 (3.16)	-2.52	**0.000**
RDW (%)	14.95 (2.07)	13.98 (1.60)	0.97	**0.002**
WBC ($x10^9$/l)	5.89 (1.27)	5.40 (1.08)	0.49	0.013
GRA ($x10^9$/l)	3.10 (0.94)	2.78 (0.89)	0.31	0.032
MON ($x10^9$/l)	0.34 (0.57)	0.35 (0.36)	-0.01	0.918
LYM ($x10^9$/l)	2.52 (0.62)	2.31 (0.68)	0.21	**0.050**
Plt ($x10^9$/l)	315 (117.17)	245.09 (84.99)	70.16	**0.000**

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

4.2.6 Dietary intakes: Food frequency consumption

4.2.6.1 Cereals and grains, starchy roots and plantain

More than three-quarter (86, 57.3%) of the deferred blood donors consumed maize on daily basis. Polished rice and spaghetti (macaroni) were eaten daily by 73 (48.7%) and 33 (22.0%) the participants respectively and 104 (69.3%) had never consume fortified cereal mix (Appendix VIIa). In addition, cassava as well as plantain were consumed daily by 62 (41.3%) and 21 (14.0%) of the deferred blood donors correspondingly. In the starchy root and plantain category, 34 (22.7%) of the deferred blood donors had never eaten potato (Appendix VIIa).

4.2.6.2 Legumes/oilseeds/nuts

Under this category, beans and groundnut were the most regularly consumed food with frequencies of 55.7% and 42.7% in that order. Agushie was consumed among 32 (21.3%) of the deferred blood donors (Appendix VIIb).

4.2.6.3 Fruits

The frequency of consumption of fruits on daily basis was generally low among the deferred blood donors. Citrus, pawpaw and mangoes were consumed as follows 57 (38%), 23 (15.3%) and 19 (12.7%) correspondingly (Appendix VIIc).

4.2.6.4 Vegetables

Among the deferred blood donors, 122 (81.3%) of them consumed tomatoes on daily basis and kontomire consumption was 18.7% on bimonthly basis (Appendix VIId).

4.2.6.5 Animal and animal products

Less than half of the participants consumed animal and animal products on daily basis with the exception of fish which recorded 91 (60.7%) (Appendix VIIe). Chicken consumption was 68 (45.3%) among the deferred blood donors. Furthermore, 46 (30.7%) of the deferred blood donor population, consumed eggs and milk daily.

4.2.6.6 Beverages

Fresh juice was consumed by just 37 (24.7%) of deferred blood donors on daily basis. Coffee and tea were drank daily by 18 (12%) and 24 (16%) respectively (Appendix VIIf)

4.2.6.7 Processed food items, deep fried foods and pastries

On each day, 14 (9.3%) of the deferred blood donors consumed pastries and 139 (92.7%) of them had never eaten dried fruits. Koose and Maasa were consumed by 71 (47.3%) and 54 (36%) of the deferred blood donors who participated in this research in that order (Appendix VIIg).

4.2.6.8 Mean comparison of food consumption

There were significant differences in the consumption of starchy roots and plantain, legumes/oilseeds/nuts, fruits and vegetables, animal and animal products as well as beverages of non-anaemic and anaemic deferred blood donors (all ps$\leq$0.05) (Tables 4.11a through to 4.11h). Generally, there were no significant differences ($p>0.05$) in the consumption of cereals and grains among the non-anaemic and anaemic deferred blood donors (Tables 4.10a). However, the consumption of food items like polished rice and brown bread under the cereals and grains food group witnessed differences statistically (Tables 4.10a).

Table 4.11a: Dietary intakes of cereals and grains among deferred blood donors

Food Group	Deferred blood donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Cereals and Grains	37.28 (5.53)	37.82 (5.77)	-0.54	-0.58 (0.562)
Polished Rice	6.50 (1.65)	7.24 (1.32)	-0.74	-3.01 (0.003)
Local Rice	2.37 (1.56)	2.63 (1.36)	-0.25	-1.06 (0.293)
Wheat	4.68 (1.51)	3.93 (1.55)	0.75	3.00 (0.003)
Maize	7.09 (1.46)	7.26 (0.96)	-0.17	-0.85 (0.394)
Brown Bread	5.31 (1.58)	4.36 (1.45)	0.95	3.81 (0.000)
Spaghetti/Macaroni	3.42 (2.14)	5.86 (2.58)	-2.44	-6.53 (0.000)
Millet	5.86 (1.53)	5.13 (1.66)	0.74	2.82 (0.006)
Fortified cereal mix	2.05 (1.93)	1.42 (1.00)	0.63	2.49 (0.014)

[1]All values are n (%); [2]DoM is Difference of means

Table 4.11b: Dietary intakes of starchy roots and plantain among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Starchy Root and Plantain	26.09 (25.27)	24.61 (4.54)	1.64	2.19 **(0.000)**
Yam	5.72 (1.25)	5.15 (1.45)	0.57	2.41 (0.017)
Cocoyam	5.29 (1.45)	4.82 (1.47)	0.48	1.99 (0.048)
Cassava	6.68 (1.46)	6.78 (1.31)	-0.10	-0.43 (0.667)
Plantain	5.78 (1.58)	4.94 (1.71)	0.84	3.11 (0.002)
Potato	2.78 (1.80)	2.92 (1.69)	-0.13	-0.47 (0.638)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11c: Dietary intakes of legumes/oilseeds/nuts among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Legumes/oilseeds/nuts	26.09 (3.64)	19.62 (18.51)	6.46	9.42 **(0.000)**
Beans	7.14 (1.18)	6.14 (1.61)	1.00	4.37 (0.000)
Groundnut	7.59 (0.89)	6.01 (1.85)	1.58	6.73 (0.000)
Agushie	6.22 (1.61)	3.81 (1.62)	2.41	9.13 (0.000)
Palm nut	5.14 (1.86)	3.75 (1.50)	1.39	5.02 (0.000)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11d: Dietary intakes of fruits among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Fruits	47.31 (6.20)	31.59 (8.49)	15.71	13.00 **(0.000)**
Citrus	7.45 (1.03)	4.72 (1.58)	2.73	12.64 (0.000)
Mangoes	6.51 (1.17)	4.29 (1.50)	2.22	10.17 (0.000)
Banana	6.12 (1.22)	4.25 (1.69)	1.92	8.00 (0.000)
Water melon	5.60 (1.91)	3.34 (1.52)	2.26	7.94 (0.000)
Pawpaw	5.77 (1.81)	3.58 (1.66)	2.19	7.69 (0.000)
Pineapple	6.17 (1.84)	3.79 (1.82)	2.39	7.99 (0.000)
Coconut	6.58 (1.71)	4.69 (2.18)	1.88	5.91 (0.000)
Avocado (Pear)	3.05 (1.86)	2.92 (1.84)	0.13	0.44 (0.658)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11e: Dietary intakes of vegetables among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Vegetables	68.61 (11.98)	46.67 (9.80)	21.94	12.22 **(0.000)**
Okro	6.50 (1.78)	5.65 (1.71)	0.85	2.97 (0.004)
Aleefu	2.49 (2.25)	2.00 (1.83)	0.49	1.45 (0.151)
Garden eggs	6.04 (1.57)	4.72 (2.08)	1.32	4.40 (0.000)
Turkey berries (bedru)	4.51 (2.27)	2.18 (1.68)	2.33	7.11 (0.000)
Tomato	7.78 (0.86)	7.61 (0.85)	0.17	1.22 (0.224)
Kontomire	5.62 (1.68)	3.17 (1.30)	2.45	9.91 (0.000)
Bokoboko	3.77 (2.35)	1.79 (1.06)	1.98	6.55 (0.000)
Bitter leaf	2.58 (2.04)	1.57 (0.85)	1.01	3.90 (0.000)
Ayoyo	2.23 (1.91)	1.42 (0.78)	0.81	3.37 (0.001)
Cassava leaves	3.10 (2.40)	2.31 (1.92)	0.80	2.24 (0.027)
Cabbage	5.19 (1.79)	3.25 (1.57)	1.94	7.04 (0.000)
Lettuce	5.36 (1.73)	3.03 (1.28)	2.33	9.33 (0.000)
Carrot	5.47 (1.78)	3.07 (1.39)	2.40	9.18 (0.000)
Green pepper	5.50 (1.70)	3.44 (1.64)	2.06	7.51 (0.000)
Dandelion	2.47 (2.11)	1.46 (0.99)	1.01	3.72 (0.000)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11f: Dietary intakes of animal and animal products among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Animal and animal products	80.14 (14.18)	56.39 (53.53)	23.75	10.9 6 **(0.000)**
Egg	6.42 (1.51)	6.00 (5.58)	0.42	1.57 (0.119)
Milk	6.51 (1.82)	4.74 (1.72)	1.78	6.13 (0.000)
Cheese/Waagashie	4.28 (2.81)	2.97 (2.12)	1.31	3.19 (0.002)
Yogurt	4.58 (1.83)	3.07 (1.24)	1.51	5.87 (0.000)
Burkina	3.23 (2.63)	2.14 (1.68)	1.09	3.00 (0.003)
Mutton	2.42 (2.20)	2.04 (1.74)	0.38	1.17 (0.244)
Chicken	7.44 (1.09)	5.58 (1.98)	1.85	7.16 (0.000)
Beef (Cow meat)	7.03 (1.51)	4.35 (1.67)	2.69	10.37 (0.000)
Fish	7.69 (1.11)	6.28 (1.81)	1.41	5.81 (0.000)
Pork	2.23 (2.04)	1.40 (0.96)	0.83	3.14 (0.002)
Chevon (Goat meat)	3.38 (1.87)	2.18 (1.00)	1.20	4.87 (0.000)
Turkey	2.55 (1.80)	1.75 (1.15)	0.80	3.22 (0.002)
Guinea fowl	1.88 (1.55)	1.43 (0.80)	0.45	2.22 (0.028)
Game	1.94 (1.61)	1.43 (0.82)	0.51	2.39 (0.018)
Crab	3.87 (1.98)	2.36 (1.24)	1.51	5.56 (0.000)
Lobster	3.97 (2.02)	2.22 (1.11)	1.75	6.50 (0.000)
Oyster	3.72 (2.11)	2.24 (1.20)	1.48	5.22 (0.000)
Snail	3.13 (2.10)	1.99 (1.19)	1.14	4.05 (0.000)
Shrimp	3.85 (2.01)	2.22 (1.88)	1.62	5.84 (0.000)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11g: Dietary intakes of beverages among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Beverages	20.55 (4.47)	22.56 (4.68)	-2.00	-2.68 **(0.008)**
Coffee	2.56 (1.58)	5.63 (2.24)	-3.06	-9.73 (0.000)
Tea	2.35 (1.20)	5.97 (2.17)	-3.62	-12.79 (0.000)
Milo	4.97 (1.88)	3.15 (1.35)	1.82	2.35 (0.000)
Fresh juice	6.05 (2.37)	3.50 (1.78)	2.55	7.42 (0.000)
Guinness	1.14 (0.44)	1.01 (0.12)	0.13	2.33 (0.021)
Wine	1.10 (0.44)	1.11 (0.62)	-0.01	-0.10 (0.922)
Beer	1.28 (0.84)	1.14 (0.68)	0.14	1.15 (0.254)
Hard liquor	1.09 (0.61)	1.04 (0.47)	0.05	0.62 (0.536)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

Table 4.11h: Dietary intakes of processed food items, deep fried foods and pastries among deferred blood donors

Food Group	Deferred Blood Donors[1]		DoM[2]	t-test (p-value)
	Non-anaemic	Anaemic		
Processed food items	6.12 (2.29)	5.22 (1.99)	0.91	2.57 **(0.011)**
Pastries	4.97 (2.20)	4.15 (1.84)	0.82	2.47 (0.015)
Dried fruits	1.15 (0.63)	1.07 (0.39)	0.10	0.98 (0.327)
Deep fried foods and pastries	10.92 (4.12)	8.90 (3.87)	2.02	3.09 **(0.002)**
Koose	6.88 (1.95)	5.97 (2.10)	0.91	2.76 (0.007)
Maasa	4.04 (2.92)	2.93 (2.42)	1.11	2.52 (0.013)

[1]All values are n (%); [2]DoM is Difference of means; Bold figures indicate statistical significance

4.3 Deferred Anaemic Blood Donors

4.3.1 Haematological profile according to gender

From Table 4.12, the deferred anaemic females had significantly lower mean Hb and RBC levels than their male anaemic group ($p \leq 0.05$). Furthermore, there were no significant differences in the means of the other parameters when both groups were compared ($p > 0.05$).

Table 4.12: Haematological profile of deferred anaemic male and anaemic female blood donors

Haematological Parameter	Deferred Anaemic Blood Donors[1]		DoM[2]	p-value
	Female	Male		
Hb (g/dl)	10.35 ± 1.30	11.16 ± 1.61	-0.81	**0.046**
RBC ($x10^{12}$/l)	4.21 ± 0.54	4.68 ± 0.64	-0.47	**0.005**
MCV (fl)	75.33 ± 8.30	75.07 ± 10.41	0.27	0.917
MCH (pg)	24.36 ± 3.80	24.35 ± 4.63	0.00	0.100
RDW (%)	15.50 ± 2.21	15.89 ± 2.45	-0.40	0.550
WBC ($x10^9$/l)	5.97 ± 1.22	5.45 ± 0.95	0.52	0.131
GRA ($x10^9$/l)	3.12 ± 0.93	2.78 ± 0.80	0.34	0.405
MON ($x10^9$/l)	0.36 ± 0.67	0.53 ± 0.71	-0.17	0.918
LYM ($x10^9$/l)	2.58 ± 0.64	2.32 ± 0.53	0.26	0.162
Plt ($x10^9$/l)	330 ± 130.27	295.07 ± 162.66	35.06	0.383

[1]All values are mean (SD); [2]DoM = Difference of means; Bold figures indicate statistical significance

4.3.2 Anaemia distribution according to morphology and severity

Table 4.13 shows anaemia distribution according to morphology in deferred blood donors. The predominant morphological type of anaemia among deferred blood donors was microcytic hypochromic 45 (62.5%) and it was common in both sexes [females 36 (63.2%) and males 9 (60.0%)]. Majority of the deferred blood donors in the 17-19 years age group had microcytic hypochromic anaemia (68.4%). In the social class grouping, students were leading [36 (69.2%)] with microcytic hypochromic anaemia. Deferred blood donors with highest educational level as secondary school were in the majority with both microcytic hypochromic anaemia [30 (62.5%)] and normocytic normochromic anaemia [18 (37.5%)]. There was equal dominance (50%) between microcytic hypochromic anaemia and normocytic normochromic anaemia within the married group.

Additionally, 75% of the deferred blood donors with moderate anaemia were due to microcytic hypochromic and there was no incidence of severe anaemia among the normocytic normochromic group (Table 4.14).

4.3.3 Features of haemoglobinopathy

4.2.3.1 Sickling profile

Following the microscopic examination of the thin films for comment, further tests were required to be performed on 12 (16.7%) samples of the deferred anaemic blood donors. Sickling test was done and 7 (58%) were declared sickling positive as shown in figure 4.3.

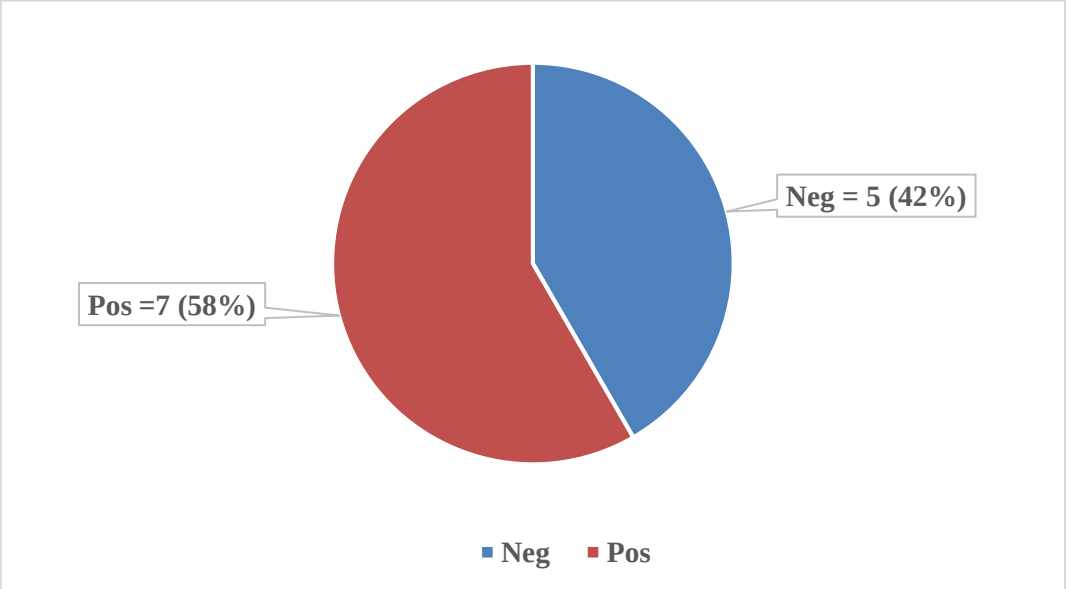
Table 4.13: Anaemia distribution according to morphology in deferred blood donors

Demographic variable	Total	Morphology[1]	
		MH[2] 45 (62.5)	NN[3] 27 (37.5)
Sex			
Female	57	36 (63.2)	21 (36.8)
Male	15	9 (60.0)	6 (40.0)
Age group (years)			
17-19	38	26 (68.4)	12 (31.6)
20-29	16	10 (62.5)	6 (37.5)
30-39	11	6 (54.5)	5 (45.5)
40-49	4	0 (0)	4 (100)
50-55	3	3 (100)	0 (0)
Occupation (Social class)			
Lower class	7	2 (28.6)	5 (71.4)
Middle class	10	5 (50.0)	5 (50.0)
Student	52	36 (69.2)	16 (30.8)
Upper class	3	2 (66.7)	1 (33.3)
Level of Education			
JHS	7	3 (42.9)	4 (57.1)
Nil	1	1 (100)	0 (0)
Primary	0	0 (0)	0 (0)
Secondary	48	30 (62.5)	18 (37.5)
Tertiary	16	11 (68.8)	5 (31.3)
Marital Status			
Divorced	3	1 (33.3)	2 (66.7)
Married	10	5 (50.0)	5 (50.0)
Single	59	39 (66.1)	20 (33.9)

[1]All values are n (%); [2]MH=Microcytic hypochromic; [3]NN=Normocytic normochromic.

Table 4.14: Severity of anaemia and morphological types of anaemia

Morphology	Severity of anaemia[1]			Total
	Mild (44.4%)	Moderate (50.0%)	Severe (5.6%)	
Microcytic hypochromic	14 (43.75)	27 (75.00)	4 (62.50)	45 (62.50)
Normocytic normochromic	18 (56.25)	9 (25.00)	0 (0.00)	27 (37.50)
Total	32(100.00)	36(100.00)	4(100.00)	72(100.00)



NOTE: Pos = positive, Neg = negative

Figure 4.3: Sickling profile of some selected deferred anaemic blood donors

4.2.3.2 Haemoglobin electrophoresis

Subsequent to the performance of sickling test, haemoglobin electrophoresis was done to ascertain the presence or absence of some common haemoglobinopathies. From the results haemoglobin molecule (genotype) AC was the most dominant (42%) as expressed in figure 4.4.

4.3.4 Biochemical profile

The biochemical profile means such as iron, ferritin and TSAT of the deferred anaemic females were lower than that of their male partners [e.g. iron levels females 9.52 ± 1.50µmol/l, males 10.15 ± 2.18 µmol/l)]. However, there were no significant differences ($p>0.05$) as demonstrated in Table 4.15.

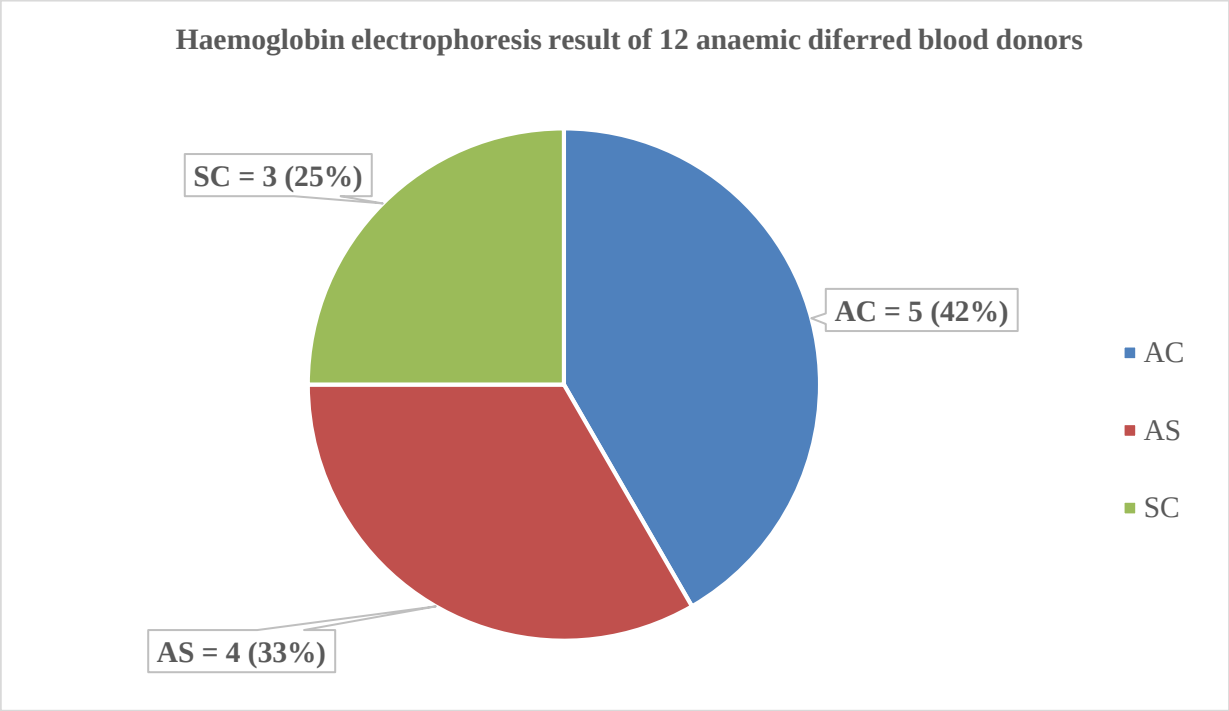


Figure 4.4: Haemoglobin electrophoresis profile of selected anaemic differed blood donors

Table 4.15: Biochemical profile of deferred anaemic female and anaemic male blood donors

	Deferred anaemic blood donors[1]			
Biochemical Test	**Female**	**Male**	**DoM[2]**	**p-value**
Iron (µmol/l)	9.52 (1.50)	10.15 (2.18)	-0.64	0.192
Ferritin (µg/l)	26.85 (29.02)	31.30 (38.48)	-4.45	0.624
TIBC (µmol/l)	64.61 (17.60)	55 (13.35)	9.61	0.053
TSAT (%)	16.21 (6.18)	18.27 (5.68)	-2.06	0.248
CRP (<10mg/l)	9.18 (12.09)	9.29 (14.66)	-0.01	0.975

[1]All values are n (%); [2]DoM = Difference of means

From Table 4.16, majority 62 (86.1%) of the deferred anaemic blood donors had low iron concentrations when compared with the reference range (men 12.5-32.2 µmol/l, women 10.5-30.2 µmol/l). Additionally, 37 (51.4%) and 17 (23.6%) of the deferred anaemic blood donors had low ferritin levels and normal levels of CRP respectively.

Table 4.16: Biochemical profile among deferred anaemic blood donors

	Test Results[1]						
Biochemical Test	**Below Ref**	**Within Ref**	**Above Ref**	**Total**	**Min**	**Max**	**Mean (SD)**
Iron (µmol/l)	62 (86.1)	10 (13.9)	-	72	4.5	13.2	9.65 (1.67)
Ferritin (µg/l)	37 (51.4)	35 (48.6)	-	72	6.6	154.1	27.78 (30.97)
TIBC (µmol/l)	2 (2.8)	47 (65.3)	23 (31.9)	72	30.0	110.0	62.61 (17.17)
TSAT (%)	39 (54.2)	33 (45.8)	-	72	6.0	37.0	16.64 (6.10)
CRP (<10mg/l)	-	55 (76.4)	17 (23.6)	72	0.5	64.1	9.20 (12.56)

Ref = reference range

4.3.5 Severity and aetiological types of anaemia

The causes of anaemia among the deferred blood donors who were excluded from donation because of low haemoglobin levels (anaemia) are listed in Table 4.16. The most common type of anaemia, IDA, was found among 46 (63.9%) deferred blood donors consisting of 38 (66.7%) females and 8 (53.3%) males. Anaemia of chronic disease (ACD) was the second highest with a prevalence rate of 18.1%. There were two cases of HE among the deferred blood donors; one deferred blood donor (1.4%) exclusively had HE whiles the other had IDA and HE with sickle cell trait (AS) (1.4%). There were three (4.2%) cases of sickle cell disease; two with IDA (2.78%) and one with ACD (1.39). However, there was no record of malaria parasitaemia (anaemia of malaria). The incidence of mild IDA was 62.5% (Table 4.17).

Table 4.17: Aetiological types of anaemia among deferred blood donors

	Deferred Anaemic Blood Donors[1]		
Aetiology of Anaemia[#]	**Female**	**Male**	**Total**
ACD	11 (19.3)	2 (13.3)	13 (18.1)
ACD + Sickle Cell Disease (SC)	0 (0)	1 (6.7)	1 (1.4)
HE	1 (1.8)	0 (0.0)	1 (1.4)
IDA	38 (66.7)	8 (53.3)	46 (63.9)
IDA + Hb C Trait (AC)	2 (3.5)	3 (20.0)	5 (6.9)
IDA + Sickle Cell Disease (SC)	2 (3.5)	0 (0)	2 (2.8)
IDA + Sickle Cell Trait (AS)	2 (3.5)	1 (6.7)	3 (4.2)
IDA + HE + Sickle Cell Trait (AS)	1 (1.8)	0 (0)	1 (1.4)
Anaemia of malaria	0 (0)	0 (0)	0 (0)
Total	**57**	**15**	**72**

[#]ACD=Anaemia of Chronic Disease, HE =Hereditary Elliptocytosis, IDA =Iron Deficiency Anaemia, Hb C = Haemoglobin C.

[1]All values are n (%)

Table 4.18: Distribution of Severity of anaemia aetiological types of anaemia

	Severity of anaemia[1]			
Cause of Anaemia	**Mild**	**Moderate**	**Severe**	**Total**
ACD	6 (18.75)	7 (19.44)	0 (0.00)	3 (18.10)
ACD + Sickle Cell Disease (SC)	0 (0.00)	1 (2.78)	0 (0.00)	1 (1.39)
HE	1 (3.13)	0 (0.00)	0 (0.00)	1 (1.39)
IDA	20 (62.50)	23 (63.89)	3 (75.00)	46 (63.89)
IDA + Hb C Trait (AC)	4 (12.50)	1 (2.78)	0 (0.00)	5 (6.74)
IDA + Sickle Cell Disease (SC)	0 (0.00)	2 (5.56)	0 (0.00)	2 (2.78)
IDA + Sickle Cell Trait (AS)	1 (3.13)	2 (5.56)	0 (0.00)	3 (4.17)
IDA + HE + Sickle Cell Trait (AS)	0 (0.00)	0 (0.00)	1 (25.00)	1 (1.39)
Anaemia of malaria	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**32 (100.00)**	**36 (100.00)**	**4 (100.00)**	**72 (100.00)**

[1]All values are n (%)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Globally, the requirements for blood supply and reserves have increased greatly and the WHO recommends 10-20% per 1000 population as the lowest donation (WHO, 2014). However, Ghana's minimum rate for donation is only 5.4% (WHO, 2014) and this is disadvantageous to the smooth running of health care delivery in situations of urgent surgeries, severely anaemic children and post-partum haemorrhage. In spite of the soaring demand for blood and blood products by the blood banks, a lot of prospective blood donors are exempted from donation for diverse reasons which are temporary deferrals in most cases although majority of these blood donors do not return to the blood centre again (Eder et al., 2009). This study sought to characterise anaemia among deferred blood donors at the SABC.

5.1.1 Socio-demographic characteristics

The findings of this study showed that a significantly higher number of males (1328, 84.9%) presented for donation than females (237, 15.1%) and this finding is in line with an earlier research at the Accra Area Blood Bank of the SABC (males 1120, 88.7% and females 143, 11.3%) (Antwi-Baffour et al., 2015). Additionally, this outcome is similar to the study by other researchers in Nigeria (male - 1853, 98.2%; female - 33, 1.8%) (Okoroiwu & Asemota, 2019) and India (male - 7402 82%; female - 1634, 18%) (Faheem et al., 2019). Earlier researchers in Brazil also made comparable observations (Cancado et al., 2001). This may be as a result of the occurrence of males dominating blood donation systems over the years because of possible cultural belief that females should refrain from donating blood due to the loss of blood through menstruation or

misconceptions that women cannot partake in blood donation. The male preponderance in blood donation may also be linked to the general perception that males are generally in good health as against females and so are not fit to donate blood. The female donor population can be increased through effective and proficient counselling prior to donation. However, a study in Spain does not corroborate with the findings of this current study in that, majority of the prospective donors (380) were females (213; 56%) as compared to males (167, 44%) (Gomez-Simon et al., 2007).

The mean age of the deferred blood donors was 27.19 ± 11.41 years with majority of the participants (40%) in the 17-19 years age group. These outcomes are similar to that of James and co-workers who observed mean age of 27.3 ± 12.4 years (A. B. James, Josephson, Castillejo, Schreiber, & Roback, 2012). Furthermore, the earlier work at SABC, recorded a mean age of 29.8 ± 8.0 years (Antwi-Baffour et al., 2015). The mean age of this work is not in line with that of a Nigerian study which recorded 39 ± 21 years (Erhabor et al., 2014).

Works done in United States of America (Shaz, James, Hillyer, Schreiber, & Hillyer, 2010), Brazil (Goncalez et al., 2013) and Japan (Ngoma et al., 2013) likewise recorded higher incidence of deferment among this age group, 17%, 31% and 24% respectively. This demography could be due to the fact that people present themselves for donation when they have a sense of wellbeing and therefore the youth often get inspired and they tend to partake more in blood donations as compared to the older ones. Furthermore, the present study highlights the need to retain blood donors and also increase counselling because the youth are more receptive and may be readily retained. A greater percentage of the deferred blood donors in this present study were first-time donors (109, 72.7%) as

compared to repeat donors (41, 27.3%) and this is in agreement with the outcomes of an Ethiopian report (first timers =293, 69.4% and repeat donors =129, 30.6%) (Guracha, Tsegaye, & Negash, 2019) and Indian report (first timers =406, 96.9% and repeat donors =13, 13.1%) (Chauhan, Chavda, & Patel, 2018). On the other hand, this finding is not in line with those of other investigators in Ivory Coast, first-time donors (591, 22.6%) and repeat donors (2027, 77.4%) (Kouao et al., 2012) and Iran, first-time donors (68, 376, 48.2%) and repeat donors (73, 444, 51.8%) (Kasraian & Negarestani, 2015). This may have happened because repeat blood donors have education on criteria for blood donor selection and also take preventive steps ahead of donation. Within the occupation (social class) category, students were in the predominate group (89, 59.3%) of deferrals and this is consistent with a previous study in India where the majority group were students (2440, 28.01%) (Unnikrishnan et al., 2011). This is probably due to the fact that they are more often than not the target grouping of most blood services when blood donation exercises are been organised so as to get them to become non-remunerated repeat donors (Kumari, 2015).

5.1.2 Deferral rate, reasons for deferment and prevalence of anaemia

In this study, a deferral rate of 27.2% was observed among the prospective blood donors and it is higher than what was established in France (10.8%) (Lawson-Ayayi & Salmi, 1999), Ivory Coast (10.74%) (Kouao et al., 2012), and Tanzania (12.7%) (Valerian et al., 2018) and South Africa (18.3%) (Van den Berg et al., 2018). Lower rates of deferral were documented in Zimbabwe (7%) (Mafirakureva et al., 2013), Namibia (8.6%) (Gonzo et al., 2016) as well as Sudan (6%) (Abbas et al., 2015) and higher results inconsistent with the present study were obtained in SABC, Ghana (42.6%) (Antwi-Baffour et al., 2015), Brazil (22.5%) (Goncalez et al., 2013) as well as Trinidad and

Tobago (35.6%) (Charles, Hughes, Gadd, Bodkyn, & Rodriguez, 2010). The variations in deferral rates may have occurred due to diverse blood donor selection criteria (e.g. age, Hb/Hct levels and blood donation interval), interpretation of guidelines, local medical views as well as prospective donors deficient in information concerning blood donor selection.

In this current research, the deferral due to anaemia was 31.3% using the copper sulphate ($CuSO_{4)}$ method and following confirmation with the cyanmethhaemoglobin method (ABX Micros haematology analyser as reference method), the rate of deferral was 48% and the false pass rate was 16.7%. This finding is inconsistent with what was obtained in an earlier study at the Accra Area Blood Bank of SABC where the deferral rate for $CuSO_4$ and autoanalyser methods were 17.1% and 21.2% respectively and the false pass rate was 4.1% (Antwi-Baffour et al., 2015). Gomez-Simon and partners made similar observations as compared with this study; 83.5% and 59.4% were rates of deferment using $CuSO_4$ technique plus Coulter Max-M analyser respectively with 21.4% as false pass rate (Gomez-Simon et al., 2007). Furthermore, this current study confirms a Malaysian report where 14.6% of blood donors were inappropriately passed after using the Sysmex automated analyser (Riahi, Mei, Idris, George, & Noor, 2015). Alternatively, an earlier research undertaken at the Northern Regional Blood Transfusion Centre and the Department of Haematology, Royal Victoria Infirmary, Newcastle, England, established 1.4% deferral rate with the $CuSO_4$ method and 1.3% using the Coulter automated haematology analyser. The false pass rate was 0.1% (Lloyd, Collins, Fail, & Hamilton, 1988). Other researchers in India (Gupta et al., 2015), United States of America (Patel et al., 2013) and Ethiopia (Guracha et al., 2019) also

documented a lower false rate of 3.8%, 6% and 9.2% correspondingly in comparison to this work.

The major cause of deferral in this research was high blood pressure (44.7%) and this confirms the findings of other researchers in India (40.08%) (Kokani & Menapara, 2019) but contradicts an earlier study here in Ghana where anaemia (low Hb) was reported as the main cause of deferral (Acquaye, 1991). The other identified reasons for deferment in this current study were anaemia (low haemoglobin concentration) (31.3%) low weight (10.0%) and common cold 4.0%. Among the deferred females, anaemia was the commonest deferral reason and in the male group, it was high blood pressure and it is in accordance with the findings in a previous study (Birjandi, Gharehbaghian, Delavari, Rezaie, & Maghsudlu, 2013). The high incidences of high blood pressure may be caused by fright of phlebotomy and white coat hypertension, sight of blood or anxiety about first-time donation or due to an already existing hypertension. The prevalence of hypertension in Ghana was found to be 25-48% (Bosu, 2010). Ngoma and associates also reported a higher figure for anaemia (68%) as the major reason for deferment among females in their study and it is comparable with this present study (Ngoma et al., 2013). This is because females are at a higher risk of becoming anaemic due to menstruation because of unsuccessful compensation of the lost blood (Cable et al., 2012).

Students were in the majority (89, 59.3%) of the deferred blood donors and most of the deferrals were as a result of anaemia and this may have been caused by poor nutrition and this agrees with a report from Cameroon (Kwenti & Kwenti, 2016). Most of the deferred anaemic participants 38 (63.3%) were in the 17-19 years age group may have

occurred because these individuals are usually active and experience fast growth and eventually require corresponding dietary needs which may not have been met.

The prevalence of anaemia was 48.0% in this study and it was slightly lower than an earlier finding of 52.9% in 1991 at the Blood Transfusion Service, Korle-Bu (Acquaye, 1991). However, the outcome of this present research is higher than the 21.2% reported by Antwi-Baffour and colleagues (Antwi-Baffour et al., 2015). The results of this study is akin to other researches in Japan - 47% (Ngoma et al., 2014), Ivory Coast - 42.5% (Kouao et al., 2012) and Namibia - 45% (Gonzo et al., 2016) but different from those from United States of America - 10% (Mast et al., 2010), United Arab Emirates - 9.29% (Alshaer et al., 2017) and Brazil 4.2% - (Goncalez et al., 2013). The divergence in rates of anaemia deferral may be due to variations in the SES of each locality and country as well as differences in guidelines in selecting blood donors.

5.1.3 Haematological profiles of deferred blood donors

In this present study, the deferred anaemic group had Hb, RBC, MCV as well as MCH mean values that were lower than those of the non-anaemic deferred blood donors whiles the means of RDW and Plt in the anaemic category were higher when judged against those of the non-anaemic deferred group. Furthermore, there were significant associations between the mean findings of the aforementioned haematological profiles and anaemic deferred blood donors (p=0.000). Additionally, the mean Hb, RBC, MCV plus MCH levels of the deferred females were lower than their male counterparts. Contrastingly, means of RDW and Plt values obtained in the deferred females were lower as compared to the deferred anaemic males. Moreover, there was strong association between the mean outcomes of abovementioned haematological parameters

and the females ($p \leq 0.05$). Also, the means of the Hb and RBC of the anaemic males were higher than that of the females and there was association between the Hb and RBC means of the anaemic deferred females and anaemic deferred males.

The present findings of this study are consistent with an earlier report in Nigeria which revealed significantly lower haemoglobin levels among deferred blood donors as against non-anaemic group (Olowoselu et al., 2017). An earlier work by Boulton and partners in the United Kingdom had also made similar observations (Boulton, Collis, Inskip, Paes, & Garlick, 2000). However, the findings of this current study are in disagreement with a study done in India that established no statistically significant association between groups of blood donors and their haematological parameters (Deepa, Arumugam, Hamsavardhini, & Radhiga, 2017). This present study resonates the findings of a previous study in Ethiopia in which the means of haematological measures like Hb, RBC count etc. of male participants were significantly different from that of their corresponding females (Eshete & Weldemariam, 2016). Similar observations were made by other investigators in Nigeria where there was a significantly higher mean concentration of haemoglobin among male donor population as compared to their female counterparts (Buhari et al., 2015; Erhabor et al., 2014).

5.1.4 Morphological typing of anaemia

In order to categorise the morphological type of anaemia among the participants, the MCV as well as MCH were used. From this research, the major morphological type of anaemia is microcytic hypochromic (62.5%) and normocytic normochromic constituted (37.5%). There was no record of macrocytic anaemia among the participants. The figures obtained from this study are not in agreement with the results of a similar study

conducted at the Accra Area Blood Bank of SABC, which reported 42.39% and 46.74% of microcytic hypochromic anaemia and normocytic normochromic anaemia in that order (Antwi-Baffour et al., 2015). Results of an earlier study in England, established a 38.5% prevalence of microcytic hypochromic anaemia and 61.3% of normocytic normochromic anaemia among deferred blood donors and these findings were at variance with this present study (Lloyd et al., 1988). In contrast to this current study, other researches in Nigeria (Ahmed & Kagu, 2011), Pakistan (Sultan et al., 2017) and India (Kumari et al., 2016) recorded lower rates of microcytic anaemia.

This present study recorded a preponderance of microcytic hypochromic anaemia among both sexes and this does not match with the findings of other researchers who documented a dominance of microcytic hypochromic anaemia in females against their male partners (Agravat, Padia, Dhruva, Bhojani, & Pujara, 2016).

5.1.5 Biochemical profile of deferred female and male anaemic blood donors

The means of the biochemical profile like iron, ferritin and TSAT of the deferred anaemic females were lower than that of their male counterparts. However, there were no significant differences ($p>0.05$) between the mean findings of the abovementioned haematological profiles and anaemic deferred blood donors. In addition, majority 62 (86.1%) of the anaemic deferred blood donors had low iron concentrations when judged against sex specific reference range (men 12.5-32.2 µmol/l, women 10.5-30.2 µmol/l). Furthermore, 37 (51.4%), 555 (76.4%) as well as 17 (23.6%) were recorded as proportion of participants with low ferritin levels, normal levels of CRP and high CRP levels respectively.

The findings of Imoru and team in Nigeria, which recorded higher values of iron as well as ferritin and lower figures for TIBC are at variance with outcomes of this current study (Imoru, Abdulkadir, Yahaya, & Osaro, 2018). The CRP results from this study are inconsistent with the results of a Finnish survey which documented 11.7% and 1.2% as blood donors with normal and high levels of CRP correspondingly (Lobier et al., 2018). These discoveries reaffirm the earlier reports which established that iron stores in females are naturally lower as compared to their corresponding males due to menstruation and childbearing (Milman, Rosdahl, Lyhne, Jørgensen, & Graudal, 1993).

5.1.6 Aetiology of anaemia

The haematological parameters MCV, MCH, PBF, haemoglobin electrophoresis as well as biochemical parameters were very valuable in identifying the various aetiological types of anaemia among the deferred blood donors. Furthermore, the Mentzer index (the ratio of MCV over RBC where a value >13 favours IDA and a value <13 favours thalassaemia) was used to exclude any incidence of thalassaemia. The major type of anaemia recorded in this study was IDA (63.9%), followed by anaemia of inflammation (AI) 18.1%. Other causes of anaemia included HE and sickle cell disease but there was no record of anaemia of malaria. The high figure of 63.9% as prevalence rate for IDA from this study is incomparable with the low figures established by other authors in Nigeria (12%) (Jeremiah & Koate, 2010), Germany (26%) (Alvarez-Ossorio et al., 2000) Australia (3.9%) (Salvin et al., 2014) and Pakistan (9.7%) (Waheed et al., 2018). Iron deficiency anaemia was predominant among the female participants (66.7%) and it is consistent with an earlier work in Germany (Alvarez-Ossorio et al., 2000). The high numbers of IDA could have been caused by poor intake or absorption of iron, increased

requirement during adolescence, and in case of reproductive females, duration and heaviness of menstrual flow.

Malaria has often been associated with anaemia in sub-Saharan Africa in often times (Stoltzfus et al., 2000) but surprisingly we did not record any case of malaria in this work. This corroborates earlier findings of Salem and Baiomy (2016) as well as Bakr et al. (2017) in Egypt and Elyamany and colleagues in Saudi Arabia (Elyamany et al., 2016). However, the findings were contradictory to previous works done in Benin (Kinde-Gazard et al., 2000), Nigeria (Epidi et al., 2008) and at National Blood Transfusion Service (NBTS), Korle-Bu (Degenu, 2003).

The extensive campaign by the national malaria control programme on the use of preventive measures such as insecticide treated nets, mosquito repellent creams and sprays and the fact that this study was done in urban Ghana may be responsible for the no incidence of malarial infection among the participants.

5.1.7 Severity of anaemia and factors associated with anaemia

The anaemia severity was done using the WHO classification of anaemia based on the concentrations of haemoglobin where mild is (11-12.9g/dl for males and 11.0-11.9g/dl for females), moderate is (8-10.9g/dl for both sexes) and severe is (<8g/dl for both sexes) (WHO, 2008). In all, 150 blood donors were deferred; 72 (48%) of them had anaemia and prevalence of mild, moderate and severe anaemia were 44.4%, 50.4% and 5.6% respectively. The outcome of this current study contradicts the higher and lower figures of mild and moderate anaemias respectively established by other researchers in India (mild=79.5%, moderate=18.0%, severe=2.5%) (Bahadur, Pujani, & Jain, 2011)

and Pakistan (mild=78.2%, moderate=20.5%, severe=1.2%) (Sultan et al., 2017) for the various stages of anaemia severity. Variables like sex, occupation (social class), being a first-time donor, having gastric ulcer, type of menstrual cycle, duration of menses and heaviness of menses (among females of reproductive age) were strongly associated with anaemia. From literature, anaemia is associated with repeat donors (Boulton et al., 2000; Okpokam, Osim, Usanga, Emeribe, & Emeribe, 2018; Sarakul, Sommart, Tolahan, & Poophapun, 2017) and this is inconsistent with the outcome of this research where being a first-timer at donation is associated with anaemia. This could have happened because most of the participants in this study were young and also first-timers.

5.1.8 Dietary determinants of anaemia

The development of ID and consequent development of IDA is influenced by dietary factors. In developed countries most people consume foods rich in bioavailable iron whiles a lot of foods containing phytates which inhibit iron absorption are consumed more in less developed nations (Gibson, 2005).

There was significant difference ($p \leq 0.05$) in the food frequency consumption of animal and animal products between non-anaemic and anaemic deferred blood donors. This report is in line with the work by previous investigators (Rangan, Aitkin, Blight, & Binns, 1997). In addition, reproductive females in the United States who consumed red meat were found to have higher levels of ferritin and haemoglobin and lower TIBC as against those who were lacto-ovo vegetarians or consumed mainly fish or poultry (Worthington-Roberts, Breskin, & Monsen, 1988). A Japanese report did not find association between intake of meat or haem iron (Asakura et al., 2009). A study

established a no association between iron status and the intake of eggs (Blanco-Rojo et al., 2014).

With regards to the consumption of fruits and vegetables, a strong association existed between the non-anaemic and anaemic blood donors and this is in line with a preceding work (Cade et al., 2005). A significant difference in terms of consumption of beverages (e.g. coffee, tea) existed among the two deferred blood donors based on this present work and this is similar to the views of other investigators (Belgnaoui & Belahsen, 2007; Munoz, Lonnerdal, Keen, & Dewey, 1988). However, this is in disagreement with what was observed in a different research (Mennen et al., 2007).

On the other hand, there were no significant differences in the intake of cereals and grains ($p>0.05$). This observation from the present study confirms what other authors have published in which there was found no association between iron status (such ferritin, Hb, soluble transferrin) and consumption cereals and grains (Leonard, Chalmers, Collins, & Patterson, 2014).

5.1.9 Risk determinants of anaemia

A number of factors put a person at risk of being anaemic and being a female is the main independent risk factor associated with anaemia and hormonal differences has been reported to be responsible for that (Kennedy & Gilbertsen, 1957). Also, women have lower concentration of Hb as compared to their male counterparts regardless of their iron status (Murphy, 2014). Cable and associates in their work observed that females of reproductive age are most likely to have depleted iron stores due to menstruation and childbirth (Cable et al., 2012) and as a result women are most likely to be deferred at

donation due to anaemia as established by other authors (Almeida et al., 2013; Baart, De Kort, Moons, & Vergouwe, 2011; Custer et al., 2014).

This study has also revealed that females are at a higher risk of becoming anaemic (cOR=8.55, p=0.000) as compared to their male counterparts and this concurs with the previous reports (Kwenti & Kwenti, 2016; Madrona et al., 2014). A similar report was also established earlier in Japan (Ngoma et al., 2014).

Furthermore, the menstrual cycle and duration of menses were found to risk determinants of anaemia among female deferred anaemic blood donors of reproductive ages and these observations are in agreement with literature (Aghamohammadi, Maghsoodlu, Naghadeh, & Shirin, 2017; Eloísa Tedeschi Dauar et al., 2015). Additionally, dietary intake or dietary status was established to be a risk determinant of anaemia. However, level of education was found not to be a determinant of risk for the development of anaemia in this study and it is in disagreement with an earlier research that was undertaken in India (Jaiswal & Pandey, 2017).

5.1.10 Limitation of the study

This study could not investigate other possible causes of IDA due to blood loss from worm infestation through the examination of stool samples of participants for the presence of intestinal worms. Serum ferritin was not done for all the study participants and thus iron deficient participants were missed. Moreover, the various conditions responsible for the ACD or anaemia of inflammation recorded in the present study could not be investigated due to human resource challenges and time constraints.

5.2 Conclusion

Prevalence of anaemia in potential healthy blood donors was found to be high (48%) and mostly due to IDA (63.9%) which is a preventable cause of donor deferral. Knowledge of donors' iron status provides evidence for the blood centre to provide appropriate and timely care for the donors. This study has also shown the importance of using a more sensitive test such as an automated haematology analyser to confirm anaemia in prospective blood donors this may improve the quality of blood donated. Factors such as dietary intake and being a female were found to be associated with anaemia and also risk determinants of anaemia.

Based on the findings of this study, it is recommended that:

1. There should be intensive public education on dietary requirements, nutrition counselling and also formulation of policy to implement supplementation.
2. There should be avenues for the review of deferred blood donors to get medical attention when necessary.
3. A more sensitive method such as use of the automated haematology analyser should be used to screen for anaemia.

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APPENDICES

APPENDIX I - CHEMICALS, EQUIPMENT, REAGENTS AND MATERIALS

Venepuncture Technique with evacuated container

- 4ml BD Vacutainer® K_2EDTA tubes
- 5ml BD Vacutainer® SST ™ II Advance tubes
- 70% isopropyl alcohol pad
- BD Vacutainer® PrecisionGlide™ multiple-sample needle
- Gloves
- Adhesive tapes
- Tourniquet
- Laboratory coat
- Dry cotton wool
- Biohazard waste bin
- Sharps container
- Marker for labelling

Equipment and Materials for the performance of Full blood count using ABX Micros ES60 OT haematology analyser

- ABX Micros ES60 OT Haematology Analyser (Horiba Medical, France)
- Reagents (Horiba Medical, France) - Minidil, Minilyse, Miniclean
- Quality Control samples (Horiba Medical, France) - low, normal and high
- Roller mixer, Swelab Instrument, Sewden (Type 810)
- Printer

Equipment and Materials for preparation of blood film for comment, thick and thin blood films for malarial parasites

- Absolute Methanol for fixation
- Beakers
- Capillary tube
- Cotton wool
- Frosted end microscope glass slides - clean, dry and grease-free
- Giemsa stain
- Leishman's stain
- Measuring cylinders
- Pasteur Pipette
- Pencil for labelling
- Phosphate buffer - pH6.8
- Phosphate buffer - pH7.2
- Slide box
- Spreader
- Staining rods
- Timer
- Slide drying rack

Materials and equipment for the microscopic examination of Leishman's stained blood film and Giemsa-stained blood films.

- Laboratory report form
- Microscope Lens cleaner

- Leica DM500 Binocular Microscope (Leica Microsystems, Heerbrugg, Switzerland)
- Oil Immersion
- Soft absorbent paper
- Slide box

Equipment and reagents for haemolysate preparation

- Pasteur pipettes
- Carbon tetrachloride (CCl_4)
- Distilled water
- Physiological saline (0.85% w/v)
- Centrifuge
- Marker for labelling
- Vortexer
- Test tubes (75 x 12 mm)
- Test Tube rack
- Distilled water
- Potassium cyanide (KCN)
- Cuvettes
- A blunt-nosed thumb forceps
- Photometer

Materials and reagents for Haemoglobin electrophoresis

- Electrophoresis Tank
- Power Pack

- Cellulose Acetate Membranes
- Applicator
- Staining Equipment
- Tris-EDTA borate (TEB) buffer, pH 8.5
- Applicator Well
- Test Haemolysates
- Control Haemolysates
- Staining solution (Ponceau S)
- Destaining solution (2% v/v acetic acid)
- Blotting paper
- Staining well

Leishman's Stain Preparation

Leishman's powdered dye, 0.6g was weighed and transferred into a capacity conical flask of 500ml capacity. Absolute methanol of volume 400ml was added and placed in a water bath at a temperature of $37^{O}C$ with intermittent mixing for about 30 minutes. The mixture was filtered into a container with a tight fitting lid after it had cooled and stored away from sunlight following labelling as follows: reagent name, preparation and expiry dates and the initials of the personnel who prepared it.

Preparation of Solutions A and B for phosphate buffer

Solution A

Potassium dihydrogen phosphate (anhydrous) KH_2PO_4 (9.1g) was weighed and dissolved in one litre of distilled water in a beaker.

Solution B

Dipotassium hydrogen phosphate (anhydrous) Na_2HPO_4 (9.5g) was weighed and dissolved in one litre of distilled water in a beaker.

Preparation of Phosphate Buffer (pH 6.8)

To prepare the working solution for staining 50.8ml of stock solution A was mixed with 49.2mls of stock solution B in addition to 900ml of distilled water to make a litre. The pH of the buffer was ascertained using a pH meter to establish that the pH was 6.8. It was then transferred into a container that was leak-proof and labelled as follows: reagent name, preparation and expiry dates and initial of the personnel who prepared it.

Preparation of Phosphate Buffer (pH 7.2)

Phosphate buffer of pH 7.2 was prepared by mixing 28ml of stock solution A with 72ml of stock solution B as well as 900ml of distilled water to make a litre. A pH meter was used to verify that the pH of the working solution was 7.2 after which it was transferred into a leak-proof bottle and then labelled with the relevant details such as reagent name, preparation and expiry dates and initials of the person who prepared it.

Stock Giemsa stain

Absolute methanol……250 ml

Giemsa stain powder……3.8 gm

Glycerol……250 ml

Preparation of Stock Giemsa stain

Glass beads were put into 500 ml clean and dry dark brown bottle and 3.8gm Giemsa powder added. 250ml of methanol was added, cap tightly covered and solution shaken. The solution was put in a water bath at a temperature of 56ºC with half hourly shaking for 2 hours.

250 ml of glycerol was added whiles still in the water bath and the Giemsa stain was kept tightly stoppered and also free from moisture and labelled appropriately. The solution was allowed to ripen in the sun for 2-3 days and afterwards the stock Giemsa stain was stored at room temperature. To use the Giemsa stock solution, the bottle is shaken and small amount of the stock stain is filtered through Whatman #1 filter paper into a test tube. Subsequently a 1 in 10 dilution was prepared as working Giemsa stain per day.

Preparation of Giemsa stain (working solution)

The working solution was prepared by diluting one part of the Giemsa stock solution with nineteen parts of phosphate buffer (1 in 20 dilution).

Sodium metabisulphite (2% w/v) freshly made

Sodium metabisulphite ($Na_2S_2O_5$).....0.2g

Distilled water.....10ml

0.2g of Sodium metabisulphite was weighed and transferred into a leak-proof bottle of 15mls capacity and 10mls of distilled water was added and mixed well until completely dissolved.

The solution was labelled and used whiles still fresh (i.e. on the day of preparation).

Preparation of Tris-EDTA Borate (TED) buffer (pH 8.5) Electrophoresis buffer.

Tris - EDTA borate buffer, pH 8.5

To make 1 litre:

Tris – (hydroxymethyl) aminomethane.....10.2 g

Ethylenediamine tetra acetic acid (EDTA).....0.6 g

Boric acid.....3.2 g

Distilled water.....to1 litre

Preparation

The chemicals were weighed and transferred them into a 1 litre flask. The flask was about half filled with distilled water, mixed to dissolve the chemicals and made up to the 1 litre mark with more distilled water. The content of the flask was then transferred into a storage container, labelled and stored at 2-8^{o}C.

Fixative / staining solution.

Ponceau S……..5 g

Trichloroacetic acid…… 7.5 g

Distilled water……..to 1 litre

Destaining solution

3% (v/v) acetic acid…….. 30 ml

Distilled water …… to 1 litre

Physiological saline, 8.5 g/l (0.85% w/v)

Sodium chloride . . . . . 8.5 g

Distilled water . . . . to 1 litre

Preparation

The sodium chloride was weighed and transferred into a leak-proof bottle premarked to hold 1 litre. Distilled water was added to the 1 litre mark and mixed until the salt was fully dissolved.

Labelling was done and the solution was stored at room temperature.

Determination of serum iron

Reagent

Fortress Iron Ferrozine. Colorimetric (BXC0235).

Source of reagent: Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom).

Reagent composition

R1 Buffer

R2 (Ferrozine)

R4 (Standard)

Reagent preparation

R1: Ready to use

R2 : Ready to use

R4 : Ready to use

Determination of TIBC

Reagent

Fortress Iron TIBC DIRECT. Colorimetric (BXC0237).

Source of reagent: Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom).

Reagent composition

R1 (Acidic Reagent)

R2 (Neutral Buffer)

R3 (Standard)

Reagent preparation

R1: Ready to use

R2 : Ready to use

Standard preparation

R3 : The TIBC calibrator was reconstituted with 1.0 ml of distilled water, mixed gently and incubated for 10 minutes at room temperature.

Determination of Ferritin

Reagent

Fortress Iron TIBC DIRECT. Turbidimetric (BXC0441).

Source of reagent: Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom).

Reagent composition

R1 (Assay Buffer)

R2 (Latex Reagent)

R4 (Ferritin Calibrator)

Reagent preparation

R1: Ready to use

R2 : Ready to use

R4 : Ready to use

Determination of C-reactive protein (CRP)

Reagent

Fortress Iron CRP Turbidimetric (BXC0382).

Source of reagent: Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom).

Reagent composition

R1 (Assay Buffer)

R2 (Antibody Reagent)

R4 (Calibrator)

Reagent preparation

R1: Ready to use

R2 : Ready to use

R4 : Ready to use

APPENDIX II - QUESTIONNAIRE

Characterisation of anaemia among deferred blood donors at the Southern Area Blood Centre.

1. ID No..... Sex..... Age.....
2. Nationality……
3. Occupation:

   Student…… Unemployed……..Other (specify)……
4. Level of education:

   Nil….. Primary …… JHS/Middle School …… Secondary…….

   Tertiary…….
5. Marital Status:

   Single…….. Married……. Divorced……. Widowed……
6. Number of Children……..
7. Are you a first-time donor? Yes…….. No……
8. When did you last donate blood? ……..
9. How many times have you donated in the past one year? .....
10. Medical history:

    Diabetes..... Hypertension..... Other (specify).....
11. Past medical history:

    Diabetes..... Hypertension..... Other (specify).....

Dietary Questionnaire

12. How many times do you eat in a day? ..... time/times
13. Do you take any special foods like moringa, dandelion for your health?

    Yes….. No……

14. Do you take any nutritional supplements like folic acid? Yes….. No……

15. How many times in a week do you usually eat away from home? ……..

16. a. Are you currently a vegetarian (no meat, poultry or fish)? Yes…… No…….

b. If yes, since what age? At birth..... or ..... years of age

17. Food Frequency Questionnaire

Food Groups	**Daily**	**5- 6x/ week**	**3-4x/ week**	**1-2x/ week**	**Twice a month**	**Once a month**	**Seldom**	**Never**
Cereals and Grains								
Polished Rice								
Local rice								
Wheat								
Maize								
Brown bread								
Spaghetti/macaroni								
Millet								
Fortified cereal mix								
Starchy Root and Plantain								
Yam								
Cocoyam								
Cassava								
Plantain								
Potato								
Legumes/oilseeds/nuts								
Beans								
Groundnut								
Agushie								
Palm nut								
Fruits								
Citrus								
Mangoes								
Banana								

Water melon								
Pawpaw								
Pineapple								
Coconut								
Avocado (Pear)								
Vegetables								
Okro								
Aleefu								
Garden eggs								
Turkey berries *(bedru*)								
Tomato								
Kontomire								
Bokoboko								
Bitter leaf								
Ayoyo								
Cassava leaves								
Cabbage								
Lettuce								
Carrot								
Green pepper								
Dandelion								
Animal and Animal Products								
Egg								
Milk								
Cheese/Waagashie								
Yogurt								
Burkina								
Mutton								
Chicken								
Beef (Cow meat)								
Fish								
Pork								
Chevon (Goat meat)								
Turkey								
Guinea fowl								

Game								
Crab								
Lobster								
Oyster								
Snail								
Shrimp								
Beverages								
Coffee								
Tea								
Milo								
Fresh juice								
Guinness								
Wine								
Beer								
Hard liquor								
Processed food items								
Pastries								
Dried fruits								
Deep Fried Foods and Pastries								
Koose								
Maasa								

History of blood loss:

18. When was your last treatment for malaria? …….

19. Do you have bleeding piles? Yes…….. No…….

20. Do you have stomach ulcer? Yes…….. No…….

21. Have you recently bled from injury? Yes…….. No…….

22. If yes, what was the cause? .....

23. Have you recently had surgery? Yes…….. No…….

24. Are you on any medication like Non-steroidal anti-inflammatory drugs, NSAIDS (e.g.diclofenac, brufen)? Yes…….. No……

25. Is there any case of repeated anaemia among your sibling/siblings?

    Yes…….. No…….

STRICTLY FOR FEMALE PARTICIPANTS OF REPRODUCTIVE AGE

26. How often do you have your period?

    Every 3weeks / 21days…….

    Every 4weeks / 28days…….

    Every 5weeks / 35days……..

27. How long do your periods last?.....

28. Are your periods heavy? Yes…….. No…….

29. How often do you change in a day during your period?

    Once……. 2 Times……. 3 Times……. More than 3 times……..

30. Are you on any oral contraceptive pills? Yes…….. No…….

APPENDIX III - PRINCIPLES OF TESTS

Principle of operation (ABX Micros ES60 OT Automated Haematology Analyser)

The ABX Micros ES60 OT is a full a three-part automated haematology analyser (Horiba Medical, France) used for the performance of blood cell count. The automated machine works on the principle of impedance measurement (Coulter principle) and spectrophotometry. The measurement of the blood cells is based on the generation of an electronic field about the calibrated micro-aperture within which the blood cells travel after dilution in an electrolytic diluent. The lysing reagent converts all haemoglobin derivatives (Hb (haemoglobin), Hi (methaemoglobin) and HbCO (carboxyhaemoglobin) apart from SHb (sulhaemoglbin) to HiCN (cyanmethhaemoglobin) and the absorbance is measured at 540nm.

Within the detector, the nozzle (the sampling needle) was located ahead of the aperture and aligned with the centre. First of all, the samples were gently but thoroughly mixed by means of a roller mixer for 3-5 minutes. The cap of BD Vacutainer® K_2EDTA tube blood was removed and the tube placed under the nozzle. The tube was then lifted up to allow the sampling needle to lowers into the blood and the manual sample bar is concurrently pressed and 10µl was aspirated via the aperture centre into a conical chamber. There was creation of impedence (resistance) by the blood cells in the electronic field amid the pair electrodes as the blood cells travelled through the calibrated micro-aperture.

Given that the current is constant and stays unadjusted, the bigger the blood cell, the greater the resistance it has and vice versa. The cell is measured by the voltage and it is proportional to the size of the cell. Furthermore, the larger the cell, the higher its voltage

and vice versa and this results in the generation of electronic pulse which is then magnified and measured and subsequently calculated mathematically to produce a numerical value. After the completion of the analysis, the "Sample analysis" dialogue box is closed and the results are automatically displayed in the "Result display" screen for about a minute and it is then printed automatically. The results were validated but rerun if a repeat was required.

Principle of Colorimetric method of serum Iron Test

Iron is dissociated from the transferrin-iron complex under weak acidic condition/medium. Ascorbate (ascorbic acid) then reduces the released (liberated) ferric ions (trivalent form) to ferrous ions (bivalent form). The ferrous ions react with ferrozine to form a violet coloured complex which is then measured spectrophotometrically at a wavelength of 570 nm.

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

$$Fe^{2+} + \text{Ferrozine} \longrightarrow \text{Ferrous Ferrozine (Coloured complex)}$$

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

Principle of the Direct TIBC test using Colorimetric method

An acidic reagent containing an iron binding dye and ferric chloride is added to the serum. The low pH of the reagent causes the releases of iron from transferrin and this iron forms a complex with the dye. (This coloured complex is the combination of the serum iron excess iron already present in the acidic reagent). A neutral buffer is then

added and this shifts the pH, resulting in large increase in affinity of transferring for iron. The serum transferrin rapidly binds the iron by abstracting it from the dye-iron complex. The observed decrease in absorbance of the coloured dye-iron complex measured at a wavelength of 660 nm was directly proportional to the TIBC of the serum sample.

Principle of the Ferritin Test (Particle-Enhanced Immunoturbidimetric Assay method) Ferritin present in the serum sample reacts with the latex particles coated with anti-human ferritin antibodies and this produces agglutination. The turbidity caused by the agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of ferritin in the sample. The actual concentration is then determined by a calibration curve prepared from calibrators of known ferritin concentration. The absorbance was measured at wavelength of 550 nm.

Percentage Transferrin Saturation

This test measures the percentage of transferrin that is attached to iron. It was calculated using the formula;

$$\%\text{Transferrin Saturation} = \frac{\text{serum iron (umol/L)}}{\text{TIBC (umol/L)}} \text{ x } 100$$

Principle of CRP Immunoturbidimetric Test

Latex particles coated with specific human anti-CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, which depends on the CRP contents of the test sample that can be quantified by comparison from a calibrator of known CRP concentration. The absorbance was read at 540 nm.

APPENDIX IV - ETHICAL APPROVAL FROM THE ETHICAL AND PROTOCOL REVIEW COMMITTEE OF COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/MAY/2018 May 23, 2018

Odelia Avu-Tamakloe
Department of Haematology
School of Biomedical and Applied Health Sciences
Korle- Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.8 – P1.7/2017-2018***

The College of Health Sciences Ethical and Protocol Review Committee at its meeting on April 26, 2018 reviewed and unanimously approved your research proposal.

Title of Protocol: **"The Aetiology of Anaemia among Deferred Blood Donors at the Southern Area Blood Centre"**

Principal Investigator: **Odelia Avu-Tamakloe**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol 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 and Protocol 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.

This ethical clearance is valid till May 23, 2019.

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

Signed: .....
Rev. Dr. Thomas A. Ndanu
For: Chair, Ethical and Protocol Review Committee

Cc: Provost, CHS
Dean, SBAHS
Head, Dept. of Haematology

• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
• Email: admin.chs@ug.edu.gh / provost.chs@ug.edu.gh • Website: www.chs.ug.edu.gh

APPENDIX V - ETHICAL APPROVAL FROM THE NATIONAL BLOOD SERVICE, GHANA

NATIONAL BLOOD SERVICE, GHANA

Our Ref: NBTS/RES-76/RDAL-02

Your Ref:.....

Post Office Box KB 78
Korle-Bu, Accra

25 May 2018

Ms. Odelia Avu-Tamakloe
University of Ghana Medical School
Department of Haematology
College of Health Sciences
School of Biomedical & Allied Health Science

Dear Ms. Odelia Avu-Tamakloe,

Re: Research Protocol (NBSGRD/180518/2) "Characterization of Anaemia Among Deferred Blood Donors at the Southern Area Blood Center"

Thank you for your letter seeking approval to conduct the above research at the Southern Area Blood Centre. You provided the following documents for consideration:

- Project Proposal
- Introduction Letter
- Permission Letter
- Completed application form (online)
- Ethical Clearance
- Consent Form
- Data Collection Sheet

These documents have been considered and the project has been approved. Approval is conditional upon:

- Continued adherence to NBSG approved operating procedures.
- Adherence to all ethical requirements.
- Provision of notification of when the data collection commences and ends.

You are to collect blood samples and data from 150 consenting blood donors as per your proposal from the donor clinics of the Southern Area Blood Centre by 30th October, 2018.

You are required to submit a copy of the final report once the study is completed.

Yours sincerely,

Dr. Lucy Asamoah-Akuoko
Head, Research & Development
E-mail: lucyasamoah@yahoo.com

Cc: Ag. Donor Services Manger SABC
In-Charge, SABC Donor Care
Head, Laboratory SABC
Research Officers, R&D

Tel: 0302 – 663701, 663702 | Website: www.nbsghana.org | Facebook: National Blood Service Ghana

APPENDIX VI - PARTICIPANT'S INFORMATION FORM AND CONSENT FORM

Title: Characterisation of anaemia among deferred blood donors at the Southern Area Blood Centre.

Principal Investigator: Odelia Avu-Tamakloe, Department of Haematology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Legon.

Dear volunteer,

This form contains information about a research titled **"Characterisation of anaemia among deferred blood donors at the Southern Area Blood Centre."**

In order to be sure that you are duly informed about your participation in this research, you are being asked to read (or have read to you) this consent form. You will also be asked to sign (or thumbprint before a witness) and be given a copy of this form. You have every opportunity to ask as many questions about this research and are assured to receive satisfactory answers to them.

Why this study is planned

You are being asked to participate in the above study, in order to provide knowledge on the cause of anaemia among deferred blood donors at the Southern Area Blood Centre. Anaemia is a condition which results in low haemoglobin (blood) level for your age or sex. This study seeks to decide the causes of anaemia among deferred blood donors so that the data that will be generated can help to put in place possible solution.

Possible Benefits

You will not derive any direct benefits but help to provide a better understanding of causes of anaemia among deferred blood donors and their management. You will also not incur any cost for participating in this study.

Possible Risks

There are no risks involved in participating in this study. However, venous blood will be taken from you and this may cause slight pain and discomfort. Please be assured that this procedure will be done carefully and with much expertise.

Withdrawal from study

Your participation in this study is strictly voluntary. You are also free to terminate your participation at any point in time should you decide not to continue. Any such decision will be respected without any further discussion.

Confidentiality

All information gathered would be treated with strict confidentiality. When results of this study are to be published, your identity would not be shown.

Contacts

If you have any questions about this study or any other related problems, you may contact me, the principal investigator, **Odelia Avu-Tamakloe**, Department of Haematology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Legon. Tel: **0243140351**. E-mail: audiatjoy@yahoo.co.uk

You may also contact my supervisors:

Prof J. K. Acquaye, Department, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Legon. Tel: **0244374025**,

Dr Y. Dei-Adomakoh, Department, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Legon. Tel: **0243550980**.

You are free to ask any questions.

Thank You.

CONSENT FORM

I have read and understood the information given me regarding the benefits, risks and procedures for the research titled: **Characterisation of anaemia among deferred blood donors at the Southern Area Blood Centre.**

I have been given the opportunity to ask questions and have had them answered to my satisfaction. I hereby agree to participate as a volunteer.

Date: ---------- Name of volunteer: ----------

Signature or Thumbprint of volunteer----------

If volunteer cannot read the form, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

Date: ---------- Name of witness ----------

Signature or Thumbprint of witness----------

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Date: ---------- Name of investigator: ----------

Signature of investigator----------

APPENDIX VII - FOOD FREQUENCY CONSUMPTION TABLES

Appendix VIIa: Food frequency consumption of cereals and grains, starchy root and plantain

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Cereals and Grains									
Polished Rice	-	5 (3.3)	3 (2.0)	6 (4.0)	8 (5.3)	24 (16.0)	31 (20.7)	73 (48.7)	150
Local Rice	39 (26.0)	56 (37.3)	22 (14.7)	21 (14.0)	5 (3.3)	2 (1.3)	4 (2.7)	1 (0.7)	150
Wheat	3 (2.0)	19 (12.7)	27 (18.0)	31 (20.7)	30 (20.0)	29 (19.3)	10 (6.7)	1 (0.7)	150
Maize	-	1 (0.7)	1 (0.7)	6 (4.0)	11 (7.3)	11 (7.3)	34 (22.7)	86 (57.3)	150
Brown Bread	2 (1.3)	9 (6.0)	25 (16.7)	19 (12.7)	39 (26.0)	36 (24.0)	14 (9.3)	6 (4.0)	150
Spaghetti/Macaroni	21 (14.0)	27 (18.0)	13 (8.7)	13 (8.7)	16 (10.7)	10 (6.7)	17 (11.3)	33 (22.0)	150
Millet	2 (1.3)	8 (5.3)	10 (6.7)	15 (10.0)	25 (16.7)	50 (33.3)	27 (18.0)	13 (8.7)	150
Fortified cereal mix	104 (69.3)	24 (16.0)	7 (4.7)	5 (3.3)	2 (1.3)	1 (0.7)	3 (2.0)	4 (2.7)	150
Starchy Root and Plantain									
Yam	-	5 (3.3)	13 (8.7)	19 (12.7)	27 (18.0)	55 (36.7)	21 (14.0)	10 (6.7)	150
Cocoyam	-	7 (4.7)	18 (12.0)	24 (16.0)	40 (26.7)	38 (25.3)	16 (10.7)	7 (4.7)	150
Cassava	-	1 (0.7)	5 (3.3)	5 (3.3)	12 (8.0)	39 (26.0)	26 (17.3)	62 (41.3)	150
Plantain	1 (0.7)	8 (5.3)	11 (7.3)	26 (17.3)	29 (19.3)	38 (25.3)	16 (10.7)	21 (14.0)	150
Potato	34 (22.7)	49 (32.7)	24 (16.0)	17 (11.3)	11 (7.3)	8 (5.3)	4 (2.7)	3 (2.0)	150

Appendix VIIb: Food frequency consumption of legumes/oilseeds/nuts

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Legumes/oilseeds/nuts									
Beans	-	2 (1.3)	6 (4.0)	6 (4.0)	10 (6.7)	43 (28.7)	19 (12.7)	64 (42.7)	150
Groundnut	-	3 (2.0)	4 (2.7)	10 (6.7)	19 (12.8)	8 (5.4)	22 (14.8)	83 (55.7)	150
Agushie	2 (1.3)	19 (12.7)	17 (11.3)	24 (16.0)	19 (12.7)	32 (21.3)	11 (7.3)	26 (17.3)	150
Palm nut	2 (1.3)	21 (14.0)	32 (21.3)	18 (12.0)	38 (25.3)	18 (12.0)	7 (4.7)	14 (9.3)	150

Appendix VIIc: Food frequency consumption of fruits

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Fruits									
Citrus	-	5 (3.3)	16 (10.7)	14 (9.3)	14 (9.3)	27 (18.0)	17 (11.3)	57 (38.0)	150
Mangoes	-	12 (8.0)	11 (7.3)	19 (12.7)	27 (18.0)	37 (24.7)	25 (16.7)	19 (12.7)	150
Banana	-	14 (9.3)	15 (10.0)	18 (12.0)	28 (18.7)	38 (25.3)	22 (14.7	15 (10.0)	150
Water melon	1 (0.7)	34 (22.7)	20 (13.3)	23 (15.3)	28 (18.7)	13 (8.7)	9 (6.0)	22 (14.7)	150
Pawpaw	-	29 (19.3)	22 (14.7)	20 (13.3)	27 (18.0)	18 (12.0)	11 (7.3)	23 (15.3)	150
Pineapple	1 (0.7)	25 (16.7)	20 (13.3)	23 (15.3)	11 (7.3)	25 (16.7)	13 (8.7)	32 (21.3)	150
Coconut	-	18 (12.0)	16 (10.7)	13 (8.7)	16 (10.7)	23(15.3)	15(10.0)	49(32.7)	150
Avocado (Pear)	37 (24.7)	40 (26.7)	24 (16.0)	17 (11.3)	10 (6.7)	15(10.0)	5(3.3)	2(1.3)	150

Appendix VIId: Food frequency consumption of vegetables

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Vegetables									
Okro	4 (2.7)	6 (4.0)	6 (4.0)	6 (4.0)	23 (15.3)	30 (20.0)	39 (26.0)	36 (24.0)	150
Aleefu	92 (61.3)	18 (12.0)	11 (7.3)	5 (3.3)	5 (3.3)	8 (5.3)	4 (2.7)	7 (4.7)	150
Garden eggs	4 (2.7)	19 (12.7)	9 (6.0)	5 (3.3)	17 (11.3)	50 (33.3)	31 (20.7)	15 (10.0)	150
Turkey berries (bedru)	45 (30.0)	27 (18.0)	16 (10.7)	14 (9.3)	13 (8.7)	13 (8.7)	13 (8.7)	9 (6.0)	150
Tomato	1 (0.7)	-	-	2 (1.3)	1 (0.7)	3 (2.0)	21 (14.0)	122 (81.3)	150
Kontomire	5 (3.3)	26 (17.3)	23 (15.3)	28 (18.7)	15 (10.0)	27 (18.0)	17 (11.3)	9 (6.0)	150
Bokoboko	59 (39.3)	30 (20.0)	15 (10.0)	10 (6.7)	14 (9.3)	9 (6.0)	9 (6.0)	4 (2.7)	150
Bitter leaf	86 (57.3)	22 (14.7)	16 (10.7)	10 (6.7)	5 (3.3)	7 (4.7)	3 (2.0)	1 (0.7)	150
Ayoyo	99 (66.0)	20 (13.3)	12 (8.0)	6 (4.0)	7 (4.7)	2 (1.3)	2 (1.3)	2 (1.3)	150
Cassava leaves	76 (50.7)	17 (11.3)	12 (8.0)	6 (4.0)	12 (8.0)	16 (10.7)	6 (4.0)	5 (3.3)	150
Cabbage	5 (3.3)	34 (22.7)	25 (16.7)	17 (11.3)	19 (12.7)	26 (17.3)	20 (13.3)	4 (2.7)	150
Lettuce	5 (3.3)	35 (23.3)	22 (14.7)	19 (12.7)	21 (14.0)	26 (17.3)	18 (12.0)	4 (2.7)	150
Carrot	6 (4)	34 (22.7)	21 (14.0)	20 (13.3)	16 (10.7)	24 (16.0)	25 (16.7)	4 (2.7)	150
Green pepper	7 (4.7)	26 (17.3)	22 (14.7)	13 (8.7)	22 (14.7)	36 (24.0)	18 (12.0)	6 (4.0)	150
Dandelion	95 (63.3)	25 (16.7)	6 (4.0)	5 (3.3)	8 (5.3)	3 (2.0)	7 (4.7)	1 (0.7)	150

Appendix VIIe: The food frequency animal and animal products

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Animal and animal Products									
Egg	-	3 (2.0)	7 (4.7)	16 (10.7)	26 (17.3)	20 (13.3)	32 (21.3)	46 (30.7)	150
Milk	1 (0.7)	9 (6.0)	19 (12.7)	16 (10.7)	19 (12.7)	28 (18.7)	18 (12.0)	40 (26.7)	150
Cheese/Waagashie	45 (30.0)	28 (18.7)	16 (10.7)	4 (2.7)	9 (6.0)	13 (8.7)	20 (13.3)	15 (10.0)	150
Yogurt	9 (6.0)	28 (18.7)	34 (22.7)	26 (17.3)	28 (18.7)	15 (10.0)	3 (2.0)	7 (4.7)	150
Burkina	73 (48.7)	19 (12.7)	22 (14.7)	3 (2.0)	12 (8.0)	2 (1.3)	7 (4.7)	12 (8.0)	150
Mutton	87 (58.0)	26 (17.3)	8 (5.3)	5 (3.3)	6 (4.0)	9 (6.0)	3 (2.0)	6 (4.0)	
Chicken	-	9 (6.0)	8 (5.3)	5 (3.3)	10 (6.7)	24 (16.0)	26 (17.3)	68 (45.3)	150
Beef (Cow meat)	1 (0.7)	14 (9.3)	14 (9.3)	15 (10.0)	16 (10.7)	25 (16.7)	19 (12.7)	46 (30.7)	150
Fish	3 (2.0)	3 (2.0)	4 (2.7)	2 (1.3)	7 (4.7)	20 (13.3)	20 (13.3)	91 (60.7)	150
Pork	106 (70.7)	16 (10.7)	6 (4.0)	8 (5.3)	6 (4.0)	3 (2.0)	1 (0.7)	4 (2.7)	150
Chevon (Goat meat)	22 (14.7)	72 (48.0)	16 (10.7)	13 (8.7)	15 (10.0)	5 (3.3)	6 (4.0)	1 (0.7)	150
Turkey	68 (45.3)	45 (30.0)	11 (7.3)	9 (6.0)	9 (6.0)	4 (2.7)	3 (2.0)	1 (0.7)	150
Guinea fowl	101 (67.3)	27 (18.0)	8 (5.3)	5 (3.3)	5 (3.3)	2 (1.3)	2 (1.3)	-	150
Game	102 (68.0)	23 (15.3)	10 (6.7)	6 (4.0)	5 (3.3)	1 (0.7)	3 (2.0)	-	150
Crab	36 (24.0)	27 (18.0)	29 (19.3)	24 (16.0)	14 (9.3)	13 (8.7)	5 (3.3)	2 (1.3)	150
Lobster	36 (24.0)	29 (19.3)	30 (20.0)	22 (14.7)	10 (6.7)	17 (11.3)	2 (1.3)	4 (2.7)	150
Oyster	44 (29.3)	28 (18.7)	24 (16.0)	20 (13.3)	14 (9.3)	14 (9.30	3 (2.0)	3 (2.0)	150
Snail	62 (41.3)	27 (18.0)	20 (13.3)	14 (9.3)	14 (9.3)	8 (5.3)	3 (2.0)	2 (1.3)	150
Shrimp	41 (27.3)	30 (20.0)	23 (15.3)	19 (12.7)	17 (11.3)	13 (8.7)	5 (3.3)	2 (1.3)	150

Appendix VIIf: Food frequency consumption of beverages

	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Beverages									
Coffee	28 (18.7)	30 (20.0)	18 (12.0)	11 (7.3)	8 (5.3)	24 (16.0)	13 (8.7)	18 (12.0)	150
Tea	22 (14.7)	38 (25.3)	20 (13.3)	7 (4.7)	12 (8.0)	14 (9.3)	13 (8.7)	24 (16.0)	150
Milo	2 (1.3)	40 (26.7)	27 (18.0)	19 (12.7)	22 (14.7)	19 (12.7)	16 (10.7)	5 (3.3)	150
Fresh juice	9 (6.0)	32 (21.3)	14 (9.3)	18 (12.0)	11 (7.3)	17 (11.3)	12 (8.0)	37 (24.7)	150
Guinness	140 (93.3)	9 (6.0)	-	1 (0.7)	-	-	-	-	150
Wine	141 (94.0)	6 (4.0)	1 (0.7)	1 (0.7)	-	1 (0.7)	-	-	150
Beer	134 (89.3)	8 (5.3)	5 (3.3)	-	1 (0.7)	2 (1.3)	-	-	150
Hard liquor	146 (97.3)	1 (0.7)	2 (1.3)	-	-	1 (0.7)	-	-	150

Appendix VIIg: Food frequency consumption of processed food items, deep fried foods and pastries

Food Group	Never	Seldom	Once a month	Twice a month	1-2x/ week	3-4x/ week	5- 6x/ week	Daily	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N
Processed food items									
Pastries	7 (4.7)	26 (17.3)	22 (14.7)	13 (8.7)	26 (17.3)	26 (17.3)	16(10.7)	14 (9.3)	150
Dried fruits	139 (92.7)	9 (6.0)	-	1 (0.7)	-	1 (0.7)	-	-	150
Deep fried foods and Pastries									
Koose	9 (6.0)	4 (2.7)	5 (3.3)	5(3.3)	9(6.0)	27 (18.0)	20 (13.3)	71 (47.3)	150
Maasa	54 (36.0)	25 (16.7)	159 (10.0)	9(6.0)	6(4.0)	6 (4.0)	5 (3.3)	30 (20.0)	150