DISTRIBUTION OF ABO AND RH (D) BLOOD GROUPS AND ASSOCIATED TRAITS: A STUDY OF THE COLLEGE OF NURSING AND MIDWIFERY, OBANGEDE, KOGI STATE

BY

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DEPARTMENT OF HUMAN ANATOMY,
FACULTY OF MEDICINE,
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OCTOBER, 2016

DECLARATION

I, Abdulganiyu Adeiza Aliyu declare that the work in the dissertation titled ‘‘**Distribution of ABO and Rh(D) Blood Groups and associated traits: A study of the College of Nursing and Midwifery, Obangede, Kogi State**” was carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The information used for my literature review was fully acknowledged in the text and references. This dissertation has not been presented in any Scientific gathering, neither has it been presented for another degree or diploma at any University.

Abdulganiyu Adeiza Aliyu	____________________ **Signature**	____________________ **Date**

CERTIFICATION

The project thesis titled **Distribution of ABO and Rh(D) Blood Groups and associated traits: A study of the College of Nursing and Midwifery, Obangede, Kogi State by Abdulganiyu Adeiza ALIYU** meets the regulations governing the award of degree of Master of Science in Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

I dedicate this work to almighty Allah who gave rise to my creation, to the nurture of me, to the nourishment of me and to the goodness towards me. He has made this possible, for the race is not to the swift, nor the battle to the strong and my beloved parents, Alh and Mrs M. A. Aliyu.

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ABSTRACT

In the last five decades, genetic structure of populations are being studied in via ABO blood groups system, mtDNA and Y chromosome makers to understand history of human migration and relatedness of ethnic groups settled in close proximity. In the present study, the distribution of ABO and Rh blood groups and associated traits among various ethnic groups in Kogi State was analysed to determine the genetic composition of various ethnic groups. The study was carried out using students of Kogi State College of Nursing and Midwifery, Obangede. Participants for the study included 1,863 students of the college (1,427 females and 436 males) aged 16-40 years. The study is both retrospective and prospective. Records of admitted students into the college spanning 11 years (2002–2013) consecutively were retrospectively and prospectively collected from the various record units of the College. ABO, Rh blood group and Hb-electrophoresis tests were carried out on a white porcelain tile using blood grouping antisera and Hb-electrophoresis machine. From the 1,863 records reviewed for the ABO blood group, the phenotypic frequencies were highest for ABO*O (965, 51.80%) followed by ABO*B (404, 21.70%). The frequencies for ABO*A was observed as (379, 20.30%), whereas the frequency of ABO*AB group was observed to be lowest (115, 9.00%). It was observed that ABO*O > ABO*B > ABO*A > ABO*AB for each sex. The same pattern was observed for all the ethnic groups with a slight deviation in Bassa and Nupe where group A>B. The distribution of Rh antigen shows that Rh D positive was the highest with 1,755 (94.20%) while Rh D Negative was the lowest with 108 (5.8%). The genotypic frequency for haemoglobin was discovered to be highest for AA 1,095(58.50%) followed by AS 768 (41.00%) SS was observed to be comparatively very low 9(0.50%). The present study reveals association of ABO with malaria, hepatitis and level of academic performance of the subjects. There is also a weak association with birth order, BMI, height and weight.

The result from this study is in line with the observed trend in other studies in Nigeria and also suggest that the ABO blood group disease association may affect more of metabolic than infectious diseases.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background of Study

Blood is defined as “the red viscous fluid that circulates round the body supplying O_2 and nutritive substances absorbed from the gastrointestinal tract to the tissues, returns CO_2 to the lungs and other products of metabolism to the kidneys, functions in the regulation of body temperature, and distributes hormones and other agents that regulate cell function” (Saladin, 2003; Barrett *et al*., 2010).

Blood has always had a special mystique. From time immemorial, people have seen blood flow in the body and with it, the life of individual depends. People thus presumed that blood carried a mysterious “vital force,” and Roman gladiators drank it to fortify themselves for battle. From ancient Egypt to nineteenth-century America, physicians drained “bad blood” from their patients to treat everything from gout to headaches, from menstrual cramps to mental illness (Saladin, 2003).

“Blood group” can be defined as inherited allogeneic variation detected on the surface of blood cells (Daniels, 2002). The term applies to a defined system of red blood cell antigens (blood group substances) controlled by a genetic locus having a variable number of alleles e.g. A, B, and O in the ABO system (Murray *et al.,* 2003; Daniels, 2002). The blood group is determined by the genetic make-up of the alleles of a system (Bakare *et al.,* 2006; Bhuvnesh *et al.,* 2011).

There are at least other 29 known blood groups with a total of more than 240 antigens, including the MN, Duffy, Kell, Kidd, and Lewis groups (Storry and Olsson, 2004). These rarely cause transfusion reactions. The Kell, Kidd, and Duffy groups occasionally cause

Haemolytic Disease of the Newborn (Iyiola *et al*., 2011).The most famous blood groups are those of ABO and Rhesus (Rh) series (Khan *et al.,* 2009). The ABO blood group are the first red cell antigens while the Rhesus blood group are the most immunogenic red cell antigens discovered (Chima *et al.,* 2012). Both are routinely typed for in any blood bank or blood transfusion service (Bakare *et al.,* 2006; Enosolease and Bazuaye, 2008).

Blood-group antigens may be carbohydrate structures on red cell surface glycoproteins or glycolipids (Storry and Olsson, 2004; Akinnuga, 2011), or they may be determined primarily by the amino acid sequence of polypeptides or glycoproteins (Suzuki, 2005). At least 23 red cell surface proteins express blood-group polymorphism (Daniels, 2002; Yamamoto *et al.*, 2012).

The ABO blood group system of carbohydrate antigen expression on the surface of human red blood cells (RBCs) was first described by Karl Landsteiner in 1900 and represented an important step towards development of safer blood transfusions (Owen**,** 2000; Loscertales et. al., 2007; Iyiola *et al.,* 2011; Chandra and Gupta**,** 2012). Alfred von Decastello and Adriano Sturli discovered the 4th type, AB, in 1902 (Eweidah *et al.,* 2011) while Landsteiner and Weiner in 1940 discovered the Rhesus (Rh) blood group (Iyiola *et al.,* 2011).

Based on RBC agglutination patterns, individuals could be divided into 4 major groups A, B, AB, and O (Saladin, 2003; Suzuki, 2005; Yamamoto *et al.,* 2012). ABO and Rhesus (Rh) blood group antigens are hereditary characters and are useful in population genetic studies, researching population migration patterns, as well as resolving certain medico-legal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice (Enosolease and Bazuaye, 2008; Reddy and Sudha, 2009; Yamamoto *et al.,* 2012). ABO and Rh genes and phenotypes vary widely across ethnic

groups, races and geographical boundaries despite the fact that the antigens involved are stable throughout life (Bhuvnesh *et al;* 2011; Iyiola *et al.*, 2011; Chandra and Gupta, 2012).

ABO gene is located on the long arm of the ninth human chromosome (9q34.1) while the Rh D and RHce genes encoding the Rh proteins (d and cc/ee, respectively) are located on chromosome 1p34-p36 (Rai *et al.,* 2009; Iyiola *et al.*, 2011). The ABO blood group gene is known to code for a glycosyltransferase, which acts at the last step of sequential extension of oligosaccharide chains attached to glycoproteins or glycolipids (Suzuki, 2005).

The Rh blood group is named for the rhesus monkey, in which the Rh antigens were discovered in 1940. It is the most complex of the human blood-group systems with 52 well-defined antigens, the most immunogenic of which is D (RHD) (Daniels, 2002; Saladin, 2003). The Rh blood groups rank with ABO groups in clinical importance because of their relation to haemolytic disease of the newborn (HDN) and their importance in blood transfusion (Adeyemo and Soboyejo, 2006; Bakare *et al.,* 2006). The Rh is genetically complex but it is simply described in terms of a single pair of alleles, D and d This group is determined by three genes called C, D, and E, each of which has two alleles: C, c, D, d, E, e. The Rh blood type is tested by using an anti-D reagent (Daniels, 2002; Saladin, 2003).

Rh frequencies vary among ethnic groups just as ABO frequencies. Between 82% and 88% of Caucasians, about 95% of black Africans, and almost 100% of people from the Far East are D-positive (Iyawe *et al.,* 1999; Daniels, 2002). The first discovery that the frequencies of the blood groups differed from one population to another was made in the early 20th century. Subsequent results from practically all countries of the world have

corroborated this, and have also shown that frequency figures are valid only for the specific population from which they are derived (Mourant *et al.,* 1976). In contrast to the ABO group, anti-D antibodies are not normally present in the blood. They form only in Rh- individuals who are exposed to Rh+ blood (Saladin, 2003).The antigens of the Rh system are encoded by two genes, RHD and RHCE. They are highly homologous and have very similar genomic organization, each containing 10 coding exons arranged in opposite orientation on chromosome 1 (Daniels, 2002).

1.2 Statement of Problem

In the last five decades, numerous studies have been carried out on the genetic composition of various population groups around the world including some parts of Nigeria. However, genetic studies among the ethnic groups of Kogi State are non-existent. Hence, the need for the present study to determine the frequencies of ABO and Rhesus blood groups among the ethnic groups of Kogi State. The present study attempts to provide initial data of genetic composition of ethnic groups in Kogi State, using ABO blood group system.

1.3 Justification/Significance of Study

The main purpose of the study is to establish the distribution of ABO and Rh blood groups among the various ethnic groups in Kogi State with a view to providing useful data for the government and health care providers in tackling health-related problems. The present study will help to correlate the ethno-historical, mythological and other related records in association with genetic data that can be useful in demonstrating the origin of a population and the genetic relationship between populations. The knowledge of the frequencies of ABO and Rh blood groups at local and regional levels are helpful in the effective management of blood banks and in blood transfusion services. Data from

this study will be of immense use to the geneticists, biologists, blood transfusion services, policy makers and clinicians. To create awareness for the Primary Health Care (PHC) Centres on the frequency distribution pattern of ABO and Rhesus (RHD) blood groups and associated traits in people of Kogi State. The findings will add to the existing literature on gene frequencies of ABO and Rh blood groups in the Nigerian populations.

1.4 Aims and Objectives of the Study

1.4.1 The aim of the study

This study aims to investigate the genetic structure and variation of population in Kogi State, Nigeria, with respect to ABO blood group and Rhesus factor.

1.4.2 Objectives of the study

The objectives of the study are to:

i. describe the genetic structure of Kogi State indigenes with respect to ABO and Rh blood groups.
ii. describe the pattern of distribution of ABO and Rh blood groups among the ethnic groups of Kogi State.
iii. investigate any association between blood groups and some disease and physiological traits.
iv. provide baseline data on ABO and Rh blood groups for scientists, health professionals, health-care providers and policy makers in Kogi State

1.5 Research Hypothesis

i. There is variation in the distribution of ABO and RH (D) blood groups among different ethnic groups in Kogi State.

ii. There is association between blood group and some physical and physiological traits.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Blood Group Antigens

The cells that make up the body's tissues and organs are covered with surface markers, or antigens (Dean, 2005). Antigens are foreign substance which, when injected parenterally into an animal lacking that substance, cause the production of antibodies to those antigens (Faller *et al.,* 2004; Rastogi, 2008). For well over a century, blood group antigens have been recognized as differences between the red blood cells (RBCs) of one person and another. Antigens have been defined by human antibodies, immune and 'naturally occurring', as well as those deliberately stimulated in animals (Storry and Olsson, 2004). RBC of individual have either A or/ and B surface antigens often called agglutinogens (blood group A, B or AB) or neither both and are referred to as blood group O (Martini, 2004).

Human blood is not the same, people belong to different blood groups, depending upon the surface markers found on the red blood cell (Dean, 2005). The two main blood groups are called ABO (with blood types A, B, AB, and O) and Rh (with Rh D-positive or Rh D-negative blood types). Both are useful in blood transfusion and organ transplantation (Khan *et al.,* 2009, Eweidah *et al.*, 2011). The system was first described with three antigens, six genotypes and four phenotypes, but has now been shown to be highly polymorphic (Chester and Olsson, 2001). In addition to the well known ABO classified groupings, and Rh factor, there are over 260 "minor" antigens that can complicate blood transfusions have been identified. About one person in 1,000 inherits a rare Blood type. These antigens may appear in varying combinations. The presence or absence of these

specific "minor" antigens single out that particular blood type as being "rare" (Beckman, 2008).

ABO blood groups are defined by carbohydrate moieties on the extracellular surface of the red blood cell membranes (Turcot *et al.,* 2003; Jeremiah, 2006). Along with their expression on red blood cells, ABO antigens are also highly expressed by epithelial and endothelial cells, and in secretor type individuals they are also expressed on mucins secreted by exocrine glands (Yamamoto *et al.,* 2014). Red blood cell antigens have various functions, including membrane structural integrity, transportation of molecules through membranes, and adhesion (Anstee, 2010, Xie *et al.,* 2010; Than *et al*., 2011).

The antigens expressed on the red blood cell determine an individual's blood group. The associated anti-A and anti-B antibodies are usually IgM antibodies, which are usually produced in the first years of life between 2-8 months after birth (Garatty, 2005) by sensitization to environmental substances such as food, bacteria, and viruses (Dean, 2005; Damulak *et al.,* 2011). Hence, individuals of blood group O contain antibodies to A and B antigens (anti AB), group A contains anti B and group B has anti A in their serum (Yamamoto *et al.*, 2012).

Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane. For example, the antigens of the ABO blood group are sugars (Turcot *et al.,* 2003). They are produced by a series of reactions in which enzymes catalyze the transfer of sugar units. A person's DNA determines the type of enzymes they have, and, therefore, the type of sugar antigens that end up on their red blood cells. In contrast, the antigens of the Rh blood group are proteins. A person's DNA holds the information for producing the protein antigens (Dean, 2005; Criswell, 2008).

The red blood cell membrane contains three types of protein that carry blood group antigens: single-pass proteins, multi-pass proteins, and glycosylphosphatidylinositol (GPI)-linked proteins as shown in fig 2.1(Dean, 2005).

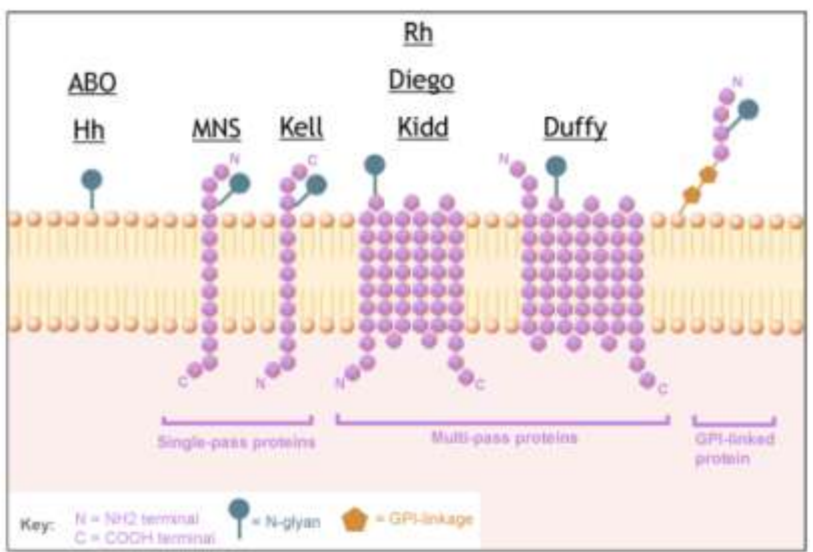

Fig 2.1. Types of red cell membrane protein (Dean, 2005)

2.2 Classification of Blood Cell Antigens

Red cell surface antigens are validated and classified by the International Society of Blood Transfusion (ISBT), which currently recognizes about 328 antigens, 284 belonging to one of 30 blood-group systems. Each system consists of between one and 52 antigens encoded either by a single gene or by two or three closely linked homologous genes (Daniels, 2002).

Traditionally, newly discovered red blood cell antigens were named alphabetically (e.g. ABO, MNS, P) or were named for the first person who produced antibody against them (e.g. Duffy, Diego). In 1980, The International Society of Blood Transfusion (ISBT) Working Party on Terminology for Red Cell Surface antigens was formed to create a standard for blood group terminology. Under this terminology, each blood group antigen has a number, and it belongs to a blood group system, a collection, or a series (Daniels *et al.,* 2004).

There are about 29 blood group systems, including the ABO, Rh, and Kell blood groups which contain antigens that can provoke the most severe transfusion reactions. Each blood group antigen is assigned a six-digit number by the ISBT. The first three digits represent the blood group (e.g., ABO is 001, Rh is 004), and the last three identify the antigen in the order it was discovered. For example, for ABO, the A antigen was the first to be discovered and has the number 001.001 whereas the B antigen was next and is designated 001.002 (Daniels, 2002).

2.2.1 ABO blood group

The ABO system was discovered by Karl Landsteiner in 1901 and consists of three main alleles: two co dominant A and B and one silent and recessive allele called O (Owen,

2000; Loscertales *et al.,* 2007). The A and B alleles code for glycosyltransferases that add an N-acetyl galactosamine or a galactose, respectively, to various substrates generically referred to as H substance (Turcot *et al.,* 2003; Anstee, 2010; Yamamoto *et al.,* 2012). These products result in A or B blood group specific antigens. In blood group O individuals, the RBCs express H antigen, but fail to express either A or B antigenic determinants. Thus, H antigen is present on all RBCs, except in the rare Bombay phenotype (Contreras and Lubenko, 1999). However, quantitative H antigen expression varies significantly between different groups (O > A2 > A2B > B > A1 > A1B) .The combinations of the three main alleles result in six major genotypes in the ABO blood group, O, A2, A2B, B, A1, A1B (Contreras and Lubenko, 1999; Than*.,* 2011) and four major phenotypes, namely A, B, AB, and O (Ghasemi *et al.*, 2012; Yamamoto *et al.,* 2012) which are characterized by the presence (or absence) of A and B antigens on the surface of red cells and the presence in the serum of natural antibodies against the antigen absent at the surface of red blood cells (Calafell*,* 2008; Criswell, 2008).

In clinical blood transfusion practise, human erythrocytes are typically grouped into six main ABO phenotypes, O, A1, A2, B, A1B and A2B, using serological methods. The difference between the A1 and A2 subgroups is partly quantitative and only a quarter of the A or AB phenotypes possess the A2 antigen (Clausen and Hakomori, 1989).

Some authors have suggested that the ABO blood group antigens should be termed ABH histo-blood group antigens to emphasize that they are primarily tissue antigens.ABH antigens appear earlier in evolution in ectodermal or endodermal tissue than in mesenchymal hematopoietic tissue and cells, including RBCs.

Although RBCs are the main carriers of ABH antigens, these antigens are actually also expressed on white blood cells (WBCs), platelets, vascular endothelium and in a soluble form in the plasma. Moreover, ABH antigens have also been identified on most epithelial tissues and their secretions (of ABH secretors), but not in connective tissues (Ravn and Dabelsteen, 2000). ABO alleles A and B thus codes for glycosyltransferases which transfer GalNac and galactose respectively (Kitano *et al.,* 2009), while O is a null allele incapable of coding for a functional glycosyltransferases (Daniels, 1997).

ABO blood groups are defined by carbohydrate moieties on the extracellular surface of the red blood cell membranes (Storry and Olsson, 2004). Along with their expression on red blood cells, ABO antigens are also highly expressed on the surface of epithelial cells (Xie*,* 2010; Than *et al.,* 2011). On RBCs, they are mostly glycosphingolipids. The antibodies against red cell antigens are called agglutinins and individuals are divided into four major blood groups A, B, AB and O according to the presence of these antigens and agglutinins (Jeremiah, 2006). A and B antigens can only be formed in the tissues of patients with an active *FUT2* by the action of alpha-glycosyltransferases capable of transferring *N*-acetyl D-galactosamine or D-galactose to carbon 3 of the same glycans . The ability of the two enzymes to distinguish between the A and B donors is largely determined by a single amino acid residue (Patenaude *et al.*, 2002). The secretions and tissues of a person with an active *FUT2* (a secretor) can express A, B, H, and Le^b antigens in those secretions according to the glycosyltransferase genes inherited (Yamamoto *et al*., 2012).

In humans, ABH antigens are widely expressed on a variety of cell surface molecules in many tissues and also in secretes, depending on the ABO genotypes of the individuals. These include glycoproteins and glycolipids on mucous cells, nerve cells, red cells,

epidermis, and vascular endothelium (Suzuki, 2005; Than *et al*., 2011; Yamamoto *et al.,* 2011). ABO blood types are also present in some other animals, for example apes such as chimpanzees, bonobos, and gorillas (Dean, 2005; Yamamoto *et al.,* 2011).

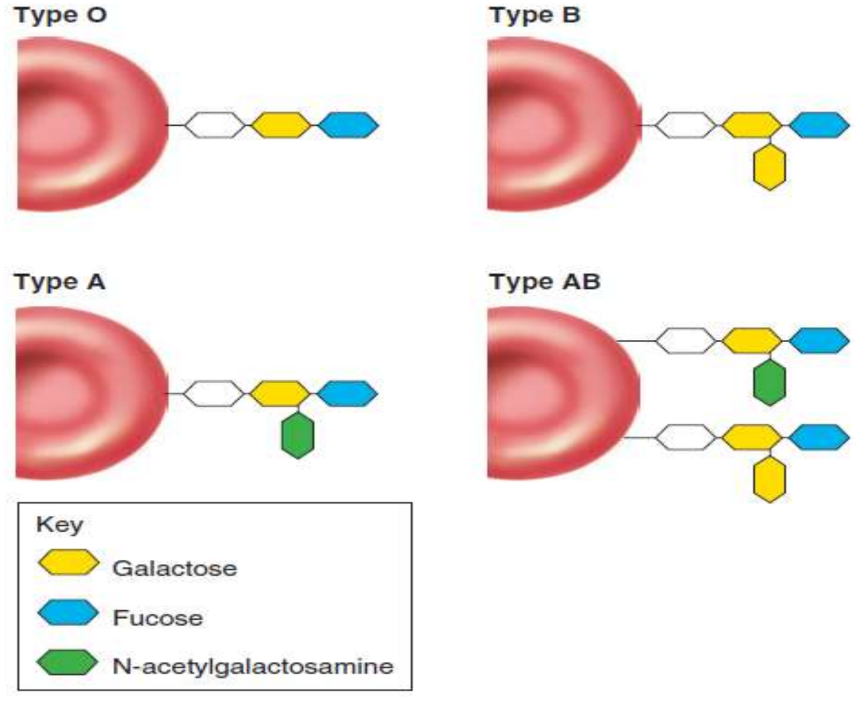

Fig 2.2: Chemical structure of ABO Blood group (Saladin, 2003)

2.2.2 Characteristic feature and lifestyle of various blood groups

There have been reports of associations of blood groups with disease and certain traits. Some of these are early statistical associations, some are more recent associations based on scientific findings while some of these are rather strange (Garraty, 2005).

It has also been reported that hangover was worse in individuals of group A and that group B's defecate the most while group O's have the best teeth. Bohmer found an increased incidence of group B amongst criminals (Garraty, 2005). Other unusual associations appear to this day in respectable scientific journals. In 1973, the highly respected scientific journal Nature published a paper by Gibson *et al*. 1973, on a relationship between ABO groups and intelligence. Group A2 were found to have the highest IQ; A2 and O were found to have a higher IQ than group A1(Garratty, 2005). In 1984, Nature published a paper entitled “Blood group and socioeconomic class”, which purported to show that, in the British population, group A is significantly more common among members of the higher socioeconomic groups (Garratty, 2005). This generated a wealth of correspondence in response; these letters, with responses from the authors, make entertaining reading (Beardmore and Karimi-Booshehri, 1983).

Blood Group A: the person of this blood type is a collaborator person who is intelligent, and has a sense of delicate, and maudlin despite his/her hiding for concern. According to that, the leadership positions do not suit him/her not because he/she is not good in doing this but by instinct. Therefore he/she needs for relaxation and focus exercises such as yoga. It is advisable to eat vegetables like beans, fruits like pineapples, and seafood for his/her digestive and immune system is sensitive and consequently will be susceptible to some diseases like diabetes, anaemia, and heart diseases. And the most famous figures are

among this blood group such as Adolph Hitler and some of the United States presidents like Richard Nixon, Jimmy Carter, and Johnson (Atoom, 2014).

Blood Group B**:** persons who belong to this blood type are characterized by flexibility and creativity. And they live in a balance way, love to do exercises, eat in a balance way, and carry in their inside the essence of the modern man that strengthens over difficulties in life. They also combine the intellectual activity with a delicate sense and have harmony in addition to inner peace. As a result, that makes them less tended to challenge and confront. Any imbalance leads them to difference in the functions of the organs of the body and thus gets susceptible for diseases and rare viruses. So, it is advisable to eat all kinds of food but in a balanced way. The holders of this blood group form the vast majority of the peoples of China, Japan, and Southeast Asia (Atoom, 2014).

Blood Group (AB): holders of this blood group are called *spirituals* because they gently receive all kinds of life and without any negative perception of the consequences which will happen due to this. And they are the most charming and exciting people which may sometimes fall them in an emotional problems. It is advisable to avoid eating red meats and beans. And it is advisable to eat fish, vegetables, and milk products. It also advisable to do mild sport with long walk on feet mild as well as cycling and swimming. Among the most famous figures in this category are John F. Kennedy and Marilyn Monroe (Atoom, 2014).

Blood Group (O): the holders of this blood type are having in their lives physical and personal strength, stamina, self-reliance, courage, pursuit of success, leadership features, power, and optimism. And they are advised to do all kinds of sports especially the combat ones. In addition, it suits them all kinds of food as meat, fish, vegetables, and fruit and they are advised to focus on proteins. Among the most prominent figures of this category

are U.S. President Ronald Reagan, the seventh Soviet Union President Mikhail Gore Bachev, Britain's Queen Elizabeth II, and Prince Charles (Atoom, 2014).

Table 2.1 Characteristic feature and lifestyle of various blood groups (Atoom, 2014).

Blood grp	Characteristics	Advisable diet	Disease susceptibility	Famous figure in gp
A	Intelligent, Not suitable for Leadership positions by instinct. Needs much relaxation and focus exercises such as yoga.	Beans, fruits like pineapples, and seafood for his/her digestive and immune system is sensitive	diabetes, anaemia, and heart diseases.	Adolph Hitler and some US presidents like Richard Nixon, Jimmy Carter, and Johnson.
B	Flexibile, creative, live and eat in a balance way, love exercises, strengthens over difficulties in life. High intellectual activity, have harmony in addition to inner peace. Less tendency to challenge and confront	All kinds of food but in a balanced way	Susceptible for diseases and rare viruses.	Forms vast majority of the peoples of China, Japan, and Southeast Asia.
AB	Called spirituals. Gently receive all kinds of life and without any negative perception of the consequences. most charming and exciting	fish, vegetables, and milk products.	emotional problems	John F. Kennedy and Marilyn Monroe.
O	Have physical and personal strength, stamina, self-reliance, courage, pursuit of success, leadership features, power, and optimism. And they are	meat, fish, vegetables, and fruit and they are advised to focus on proteins.	Infectious diseases	Ronald Reagan of US, Soviet Union President Mikhail Gore Bachev and Queen Elizabeth II

2.2.3 Rh blood group

The Rhesus factor also known as Rh factor is an antigen that exists on the surface of red blood cells in most people. People who have the rhesus factor are considered to have a positive(+) blood type such as A+ and B+ while those who don't are considered as negative(-) blood type, such as O- or AB- (Dubroff and Joseph, 2003; Adeyemo and Soboyejo, 2006). It is clinically the most important protein based blood group system with 49 antigens so far described; it is the largest of all the 29 blood group system (Flegel, 2007). In contrast to the situation encountered in antigen A and B, the plasma of Rh negative individual does not necessarily contain ant-Rh antibodies (Martini, 2004).

Several other red cell blood groups hence have been discovered but the rhesus (RHD) blood groups are of utmost importance clinically and anthropologically. Cells which have Rhesus antigen on their surface are described as Rhesus positive while those without it are known as Rhesus negative (Faller *et al.*, 2004; Eweidah *et al.,* 2011). The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells (Saladin, 2003).

The rhesus (Rh D) group system is important in blood transfusion because the Rh D immune response in Rh D negative women is the primary etiology for hemolytic disease of the newborn (Apoil and Blancher, 2000 and Okeke *et al.,* 2012). It is important because the Rh antibody unlike the IgM antibodies found against the ABO antigens, IgG antibodies do cross the placenta. This can lead to a very harmful condition during pregnancy (even several years after immunization) called severe haemolytic disease of the newborn (HDN) (Gurevitz, 2010; Iyiola *et al.,* 2012).

Unlike the ABO antibodies, Rh antibodies do not occur naturally, but are formed only when blood from Rh-positive donors is transfused into Rh-negative recipients. In such a case the recipients become sensitized to the Rh antigen, that is, they form antibodies against Rh-positive erythrocytes. Subsequently, during a second transfusion, large numbers of antibodies are formed rapidly, and these promptly agglutinate the erythrocytes of the Rh-positive donor (Bakare *et al.,* 2006).

Similarly, Rh antibodies may be formed automatically during pregnancy, for example, when the mother is Rh-negative and the father and child are Rh-positive. During labor, Rh-positive erythrocytes from the child can pass through leaks in the placenta into the maternal bloodstream, eliciting the formation of so-called anti-D antibodies (Rh antibodies) in the mother (Saladin 2003; Faller *et al.*, 2004).

2.2.4 Other blood groups

In addition to the ABO and Rh groups, there are more than 29 other known blood groups with a total of more than 240 antigens, including the MN, Duffy, Kell, Kidd, and Lewis groups (Martini *et al.,* 2004; Storry and Olsson, 2004). These rarely cause transfusion reactions, but they are useful for such legal purposes as paternity and criminal cases and for research in anthropology and population genetics (Faller *et al*., 2004). The Kell, Kidd, and Duffy groups occasionally cause HDN (Saladin, 2003).

The Bombay blood group lacks H gene and therefore cannot make H antigen (H substance). Since the H substance is the precursor for the A and B antigens, these antigens also are not made. The cells type as O and the serum has anti-A, anti-B, and anti-H since the individual lacks all of these antigens. Anti-H agglutinates O cells. In the extreme rare Bombay phenotype (Oh), the H genotype is silent (hh) and no H transferase is produced; therefore, no H substance is made, so A and B genes if present cannot be

expressed. Such individuals have anti-A, anti-B, and anti-H in their plasma and can, therefore, only be safely transfused with Oh blood (Chima *et al*., 2012). The only cells Bombay individuals do not agglutinate are from other Bombay blood people since they lack the H antigen (Chima *et al*., 2012).

2.3 Historical Discovery of ABO and Rh Blood Groups

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types :A, B, and O in 1900 (Owen, 2000; Dean 2005; Adeyemo and Soboyejo, 2006; Loscertales., 2007; Ghasemi *et al*., 2010; Iyiola *et al*., 2012). He was awarded the Nobel Prize in Physiology or Medicine in 1930 for his work. Due to inadequate communication at the time it was subsequently found that Czech serologist Jan Janský had independently pioneered the classification of human blood into four groups, but Landsteiner's independent discovery had been accepted by the scientific world while Janský remained in relative obscurity.

Alfred von Decastello and Adriano Sturli discovered the 4th type, AB, in 1902 (Blancher and Socha, 1997; Eweidah *et al*., 2011). Ludwik Hirszfeld and E. von Dungern discovered the heritability of ABO blood groups in 1910–11, with Felix Bernstein demonstrating the correct blood group inheritance pattern of multiple alleles at one locus in 1924 (Dean, 2005).

Watkins and Morgan, in England, discovered that the ABO epitopes were conferred by sugars, to be specific, N-acetylgalactosamine for the A-type and galactose for the B-type. Later, Yamamoto's group showed the precise glycosyl transferase set that confers the A, B and O epitopes (Dean, 2005). In 1907, Dr. Reuben Ottenberg carried out the first ever safe blood transfusion by matching blood groups. Blood transfusion became quite

common in the following years. It helped save thousands of lives in the First World War (American Red Cross, 2005).

Charles Drew was the first black person to pursue the advanced degree of Doctor of Science in Medicine, for which he studied transfusion and blood-banking procedures at Columbia University in 1933. He organized numerous blood banks during World War II and saved countless lives by convincing physicians to use plasma rather than whole blood for battlefield and other emergency transfusions (Saladin, 2003). Discovery of the Rh blood group system by Landsteiner and Alexander Weiner in 1939 (Okuda and Kajii, 2002).

2.4 Evolution of ABO Genes

In any polymorphism, one of the alleles must have or had a selective advantage in order to achieve a frequency of greater than 1% in a large population, but we do not know what that advantage is or was. It is now apparent that glycoproteins and glycolipids carrying blood group activity, either on red cells or in other tissues, are exploited by pathogenic micro-organisms as receptors for attachment to the cells and subsequent entry and it is probable that pathogens have played the major part in the evolution of blood group polymorphisms (Daniels, 1997). Evolutionary pressure from various pathogens is generally thought to be responsible for the generation of genetic variants, the host effects of which determine whether or not they will survive over time. This has been discussed with a main focus on microbial pathogens, e.g. relating to the differences in carbohydrates expressed on cell surfaces (Storry and Olsson, 2004)

A/B antigens are not restricted to humans but are widely present in nature (Yamamoto *et al.*, 2001).The kind of ABO types varies depending on species. For example, only A and O groups are known to exist in chimpanzees, whereas only the B group is found in

gorillas (Socha *et al.,* 1995). This is in contrast to A, B, AB, and O groups that are found in humans (Damulak *et al*., 2011). The limited repertoire of ABO groups is also observed in mammals other than primates. For instance, only A and O are found in pigs. It has been that pig O gene lacks a major portion of the gene. It was also shown that mouse ABO gene encodes an enzyme with dual specificity that is capable of synthesizing both A and B antigens in vitro, although B antigen is rarely produced in vivo. Because of DNA sequencing efforts, genes orthologous to ABO have been identified in various species of organisms (Blancher and Socha, 1997; Damulak *et al.,* 2011).

The erythrocyte expression of ABO antigens is not constant in mammalian species. Among primates, erythrocyte expression is limited to humans and apes (chimpanzee, gorilla, orangutan, and gibbon). In other mammals, some pigs express A on erythrocytes due to passive absorption of A glycolipids in the red blood cell membrane (Turcot *et al*., 2003). Evolutionary analysis of nucleotide sequences of this gene for great apes and Old World monkeys as well as those for human suggested that this gene is under some kind of positive selection. Further sequence analyses of this gene for great apes and Old World monkeys confirmed the unique feature of this gene (Kitano *et al*., 2009). In the current version of the Ensembl database, a total of 58 ABO orthologues in 29 species, including African clawed frog and zebra fish, are deposited (Damulak *et al*., 2011).

A/B antigens can be expressed in microbes as well. For example, gram-negative Escherichia coli O86 exhibit strong blood group B and weak blood group A activity by the O-polysaccharide antigen. The B transferase gene from *E. coli* and the A transferase gene from *Helicobacter mustelae* were initially cloned and sequenced. Homologous sequences to these genes have been identified in several dozen bacterial species and a cyanophage (Yamamoto *et al*., 2012).

There is a general increase in the frequency of the type O allele and in many populations a drop in the type B allele. But as expected, the frequencies for each allele are close to what they could have been at the start of human history or with Noah's family. The shift in frequency (the increase in type O and decrease in type B) can be caused by migration of people with manifestations in groups that had a higher or lower frequency for one of the alleles at the time of migration. It could also result from random genetic drift, or from a mutation that renders glycosyltransferase inactive which would result in blood type O from type A and is likely one cause for the increase in the frequency of the O allele (Criswell, 2009). The main mutation giving rise to the null O allele is likely to have appeared at least three times in human evolution, giving rise to allele lineages O02, O01, and O09 (Calafell *et al.*, 2008). A combination of selection against infectious diseases, such as plague and smallpox, and genetic drift and founder effects in small populations (resulting from migration patterns of early humans) may ultimately explain the allele frequencies observed today (Anstee, 2010).

Group O is presumed to have arisen in Africa before the migration of early humans. Severe malaria results in the death of millions each year before they reach child-bearing age, and therefore selects survival genes (Anstee, 2010). Experimental support for the hypothesis is provided by Fry *et al*. (2008) and by Rowe *et al.* (2007). Rowe *et al.* (2007) report reduced rosetting of *Plasmodium falciparum* isolates from group O Malian children compared with non-O blood groups (Anstee, 2010). Parasitized red cells form rosettes with uninfected red cells and adhere to vascular endothelium, causing vasocclusion and severe disease (Calafell *et al*., 2008 and Anstee, 2010). However, the genealogy changes along the gene and variations of both numbers of branches and of their time depth were observed, which could result from a combined action of recombination and selection (Calafell *et al.,* 2008).

Molecular evolutionary time scales place modern humans at roughly 200,000 years ago as reported by Cann in 1987, a timeframe too short to increase the O allele frequency to 60 percent of all people alive today within a population of 10,000. Certainly a biblical timeframe would be far too short for such fixation (Yamammoto *et al*., 2011). The deletion responsible for converting an A allele to an O allele is not present in chimpanzees, and sequence comparisons between humans and chimps indicate this allele is unique to the human lineage, further complicating an evolutionary scenario for the origin of blood type O (Kitano *et al*., 2009). This scenario would fit better if the O allele was rare in the population today and appeared in a specific people group. However, the O allele is by far the most common allele globally, indicating that if it did originate via a mutational event, it had to occur when the human population was extremely small and before humans divided into ethnic groups and spread across the globe (Criswell, 2009).

There are more than 180 variations (polymorphisms) for the ABO gene listed on the National Center for Biotechnology Information website, and each one of these polymorphisms can be assigned to one of the three ABO alleles. Most of these polymorphisms do not change glycosyltransferase activity or blood type, but can identify ethnic groups that formed after humans migrated across the globe. Mutation and chromosome crossing-over events are the most plausible cause of these variants (Criswell, 2009). For example the mutation giving rise to sickle cell disease (SCD; HbS) may have arisen at 3 different sites in Africa (Atlantic West Africa, Central West Africa, and Bantu-speaking Central and Southern Africa) with expansion of the mutation occurring 2000 to 2500 years ago (Anstee, 2010).

2.5 Distribution of ABO Blood Group

Various populations intermingle throughout the world with relative freedom, resulting in uniform distribution of different blood groups (Yadav *et al.*, 2011). However, a significant regional heterogeneity is also found in ABO and RhD blood group allele frequencies (Bhasin *et al.,* 1994). The distribution of the four ABO blood types, A, B, AB, and O, varies in populations throughout the world despite the fact that the antigens involved are stable throughout life (Martini *et al.,* 2004; Reddy and Sudha, 2009, Periyavan *et al*. 2010). It is determined by the frequency of the three alleles of the ABO gene in different populations. Blood type O is the most common worldwide, followed by group A. Group B is less common, and group AB is the least common (Calafell *et al*., 2008). However Blood group B is the most common group in Pakistan as evident from various studies, More than 60% of the population in Pakistan has blood group B (Lutfullah *et al.,* 2010).

Studies based on the distribution pattern of ABO and Rh system are very useful for studying complicated evolutionary history and human migration pattern. These studies are of immense assistance in finding out which disease is more prevalent in a particular blood group (Yadav *et al*., 2011). The hypothesis that ABO groups may be of importance is supported by the observation that their geographical distribution varies markedly, suggesting that positive selective factors may have influenced gene spread (Loscertales *et al*., 2007). People with blood type O are said to be "universal donors" because their blood is compatible with all ABO blood types. It is also the most common blood type in populations around the world, including the USA and Western Europe. Among indigenous populations of Central and South America, the frequency of O blood type is extremely high, approaching 100%. It is also high among Australian aborigines.

Type A is common in Central and Eastern Europe. In countries such as Austria, Denmark, Norway, and Switzerland, about 45-50% of the population have this blood type, whereas about 40% of Poles and Ukrainians do so. The highest frequencies are found in small, unrelated populations. For example, about 80% of the Blackfoot Indians of Montana have blood type A. Blood type B is relatively common in Chinese and Indians, being present in up to 25% of the population. It is less common in European countries and Americans of European origin, being found in about 10% of these populations. Blood type AB individuals are known as "universal receivers" because they can receive blood from any ABO type. It is also the rarest of the blood groups. It is most common in Japan, regions of China, and in Koreans, being present in about 10% of these populations (Dean, 2005).

2.6 Chemistry of ABO Blood Group System

2.6.1 Biochemical structure and synthesis of A and B substance

ABO antigens are characterized structurally by carbohydrate chains that are joined covalently to a hydroxyl of a Ser/Thr residue (O-linked) or to the amide nitrogen of an Asn residue (N-linked), or to sphingosine, one class of membrane lipids (Suzuki, 2005).

The antigenic determinants of ABO are synthesized in Golgi by sequential action of glycosyltransferases, which add single nucleotide sugar donor substrates to acceptor substrates (Suzuki, 2005, Patenaude *et al*., 2002). On the erythrocyte surface, the A and B antigens are trisaccharides, GalNAc α1→3 (Fucα1→2) Gal- and Gal α1→3 (Fucα1→2) Gal- for A and B antigen respectively, that are attached to different glycolipids and glycoproteins (Turcot *et al.,* 2003, Yamamoto *et al.,* 2014). An enzyme glucotransferase is necessary for the production of A and B antigens (Patenaude, 2002). On the other hand, blood group ‘O’ carries a disaccharide H antigen (Fucα1-2Galb1) due to the absence of the enzyme glucotransferase (Panda *et al*., 2011). The common precursor H antigen is

synthesized by fucosyltransferase 1 (H locus) in RBCs and by fucosyltransferase 2 (Se locus) in the secretory epithelium of gastrointestinal and respiratory tracts of ''secretor'' individuals (Than *et al*., 2011). H substance itself is formed by the action of a fucosyltransferase**,** which catalyzes the addition of the terminal fucose in Fucα1→2 linkage onto the terminal Gal residue of its precursor: The H locus codes for this fucosyltransferase.

The expression of ABH antigens in tissues and body fluids other than blood cells is regulated by the secretor gene (*FUT2*) (Mäkivuokko *et al*., 2012), which encodes an alpha 1,2-fucosyltransferase capable of transferring L-fucose to carbon 2 of galactose (beta, 1-3) *N*-acetyl D glucosamine-containing glycans. In the absence of an active FUT2 gene (nonsecretor), the structure created is the Lea antigen. The product of the Le gene is an alpha 1,3/4 fucosyltransferase (*FUT3*), which transfers L-fucose to carbon 4 of the penultimate *N*-acetyl-D glucosamine residue of the same glycans. The structure created in tissues by the combined action of *FUT2* and *FUT3* is the Leb antigen (Anstee, 2010). Secretor- and Lewis-genes control the secretion of ABO blood group antigens to all bodily liquid secretions, such as tears, milk, saliva and gastrointestinal mucus, and to secreting organs, such as pancreas and liver (Henry, 1996). The *h* allele of the H locus codes for an inactive fucosyltransferase; therefore, individuals of the *hh* genotype cannot generate H substance, the precursor of the A and B antigens. Thus, individuals of the *hh* genotype will have red blood cells of type O, even though they may possess the enzymes necessary to make the A or B substances (Murray *et al*., 2003).

At the last step of the synthesis, the allelic products of the ABO gene, A- or B-transferases, produce the A antigen by catalyzing alpha-1,3 glycosidic bond between the C3 hydroxyl of acceptor oligosaccharide chain (the H antigen) and the C1 hydroxyl of the

reducing donor N-acetylgalactosamine or the B antigen by catalyzing the same bond between the H antigen and galactose. The O allele, however, produces no active transferase, resulting in the H antigen left as it is (Suzuki, 2005). Previous studies underscored the importance of the amino acid at position 268 with a glycine determining A activity versus an alanine determining B activity (Patenaude *et al.*, 2002; Yamamoto and McNeill, 1995).

The glycosyltransferase specific enzyme for antigen A and antigen B synthesis (namely, A101 andB101) differs by just four amino acid residues: A101 carries 176 Arg, 235 Gly, 266 Leu, 268 Gly, while B101 carries 176 Gly, 235 Ser, 266 Met, 268 Ala) out of 354, and there are several DNA sequence differences in the alleles that code for the A- and O-specific enzyme (Calafell *et al.*, 2008 and Criswell 2008) . Previous studies underscored the importance of the amino acid at position 268 with a glycine determining A activity versus an alanine determining B activity (Patenaude *et al.*, 2002; Yamamoto and McNeill, 1995).

The four differences between the A and B glycosyltransferase are enough to allow the enzyme to specify the characteristic terminal sugar that distinguishes antigens A and B. A single DNA deletion in the A-specific allele results in a truncated version of the glycosyltransferase gene product, eliminating enzymatic activity and effectively resulting in blood type O (Criswell, 2009). Two major types of the H antigen are available for the ABO transferase, one (Type 1 H) in secretions, plasma, and endodermally derived tissues and the other (Type 2 H) in ectodermally or mesodermally derived tissues including red blood cells (Suzuki, 2005).

In view of the structural findings, it is not surprising that A substance can be synthesized in vitro from O substance in a reaction catalyzed by a GalNAc transferase, employing

UDP-GalNAc as the sugar donor. Similarly, blood group B can be synthesized from O substance by the action of a Gal transferase, employing UDP-Gal (Patenaude *et al*., 2002). It is crucial to appreciate that the product of the *A* gene is the GalNAc transferase that adds the terminal GalNAc to the O substance. Similarly, the product of the *B* gene is the Gal transferase adding the Gal residue to the O substance (Patenaude, 2002). Individuals of type AB possess both enzymes and thus have two oligosaccharide chains, one terminated by a GalNAc and the other by a Gal. Individuals of type O apparently synthesize an inactive protein, detectable by immunologic means; thus, H substance is their ABO blood group substance (Murray *et al*., 2003).

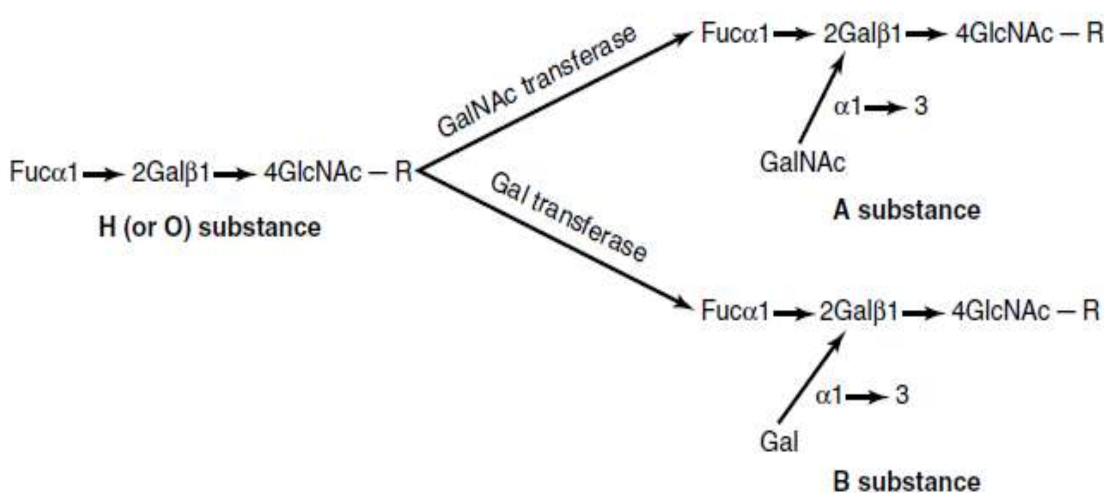

Fig 2.3 Diagrammatic representation of the structures of the H, A and B blood group substances.

R represents a long complex oligosaccharide chain, joined either to ceramide where the substances are glycosphingolipids, or to the polypeptide backbone of a protein via a serine or threonine residue where the substances are glycoproteins (Murray *et a*l., 2003).

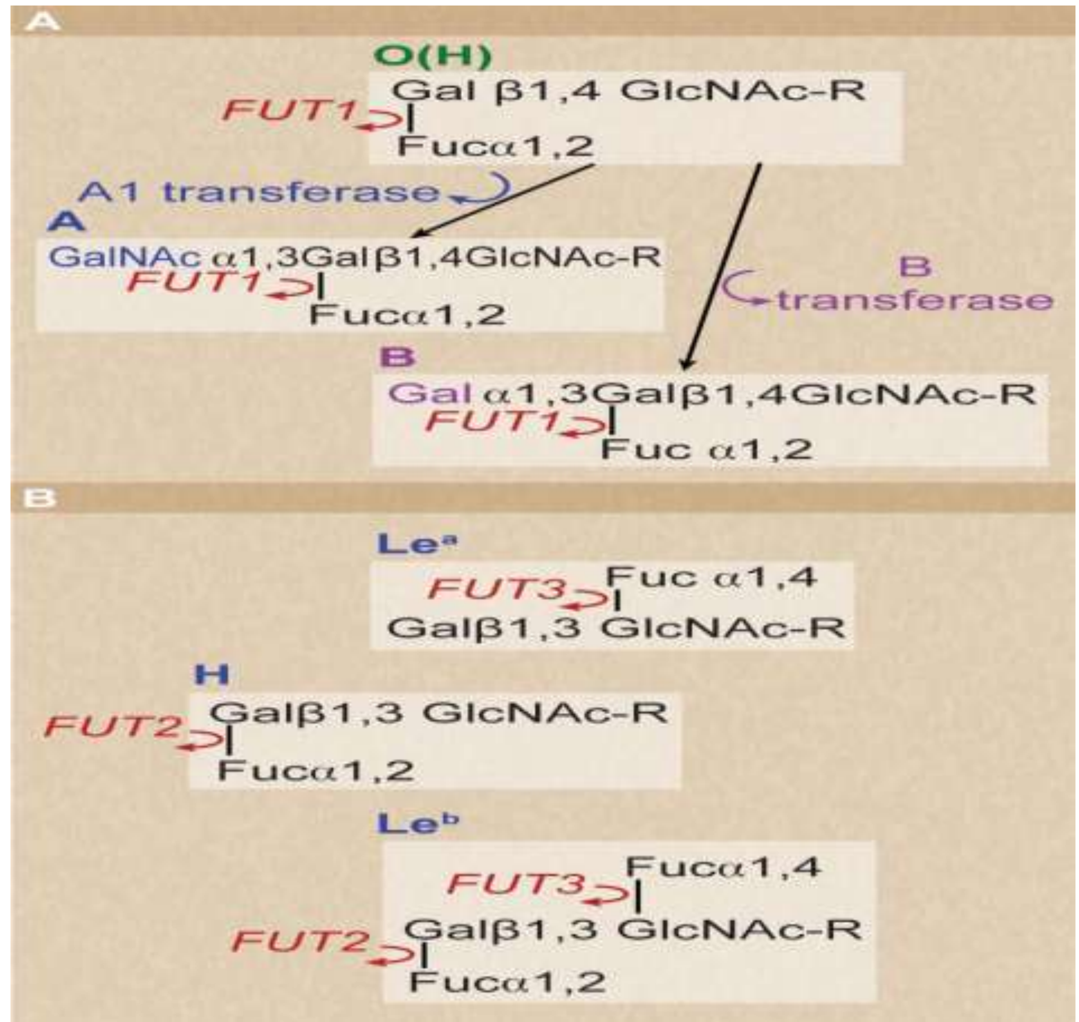

Fig 2.4: Structure of ABO, H, and Lewis antigens (Anstee, 2010).

(A) Structure of ABO and H antigens on human red cells. H antigen formed by the action of FUT1 on oligosaccharide precursor chains in which the terminal D-galactose residue is linked to carbon 4 of the penultimate N-acetyl D-glucosamine residue (type II chain).
(B) Structure of Le blood group antigens in bodily secretions. Secretor gene (FUT2) regulates the production of H antigen, which can be converted to A or B antigen if the corresponding active ABO glycosyltransferase is present. The ABH, Leb-active structures are formed on oligosaccharide precursor chains in which the terminal D-galactose residue is linked to carbon 3 of the penultimate N-acetyl D-glucosamine residue (type I chain) If FUT 2 is deficient the Lea active structure predominates.

2.6.2 Molecular genetic basis of ABO and RH blood groups

The molecular basis for O phenotype was clearly explained by 261delG, which introduces a premature stop codon after nt 352 in exon 6 otherwise identical to that of A1C by shifting the reading frame. Another O allele with the same deletion and 8 additional base substitutions along exons 3–7 was also shown in one of the cDNA sequences (FY-68-6) and later found to occur at high frequencies in various populations (Suzuki, 2005).

Currently, over 70 alleles have been described at the molecular level, and ABO seems to be one of the most polymorphic genes in humans. The main A and B alleles (namely, A101 and B101) differ at four amino acid residues: A101 carries 176 Arg, 235 Gly, 266 Leu, 268 Gly, while B101 carries 176 Gly, 235 Ser, 266 Met, 268 Ala (Daniels, 1997; Yamamoto *et al*., 2014).

Four amino acid substitutions were identified between A and B transferases (R176G, G235S, L266M, and G268A). Among the 4 amino acid substitutions at codons 176, 235, 266, and 268 between the human A and B transferases, the third and fourth substitutions were shown to be crucial for different donor nucleotide-sugar substrate specificity whereas the second is influential and the first is not so important (Yamamoto *et al.,* 2014). Two kinds of O alleles were identified that are different from one another by several nucleotide substitutions, but both types contained a single nucleotide deletion (261delG) that were relatively close to the N-terminus of the coding sequence A single nucleotide deletion (261delG) was found in O alleles.. It was concluded that O alleles are nonfunctional because of a frameshift of codons (Yamamoto *et al.,* 2012).

In vitro expression studies, cisAB alleles (i.e., alleles coding for an enzyme that can transfer both N-acetyl galactosamine and galactose), and B sequences in other primates have shown that the determining amino acid residues at codon 266 and 268 (Yamamoto *et*

al., 2001, Patenaude *et al*., 2002; Yamamoto *et al.,* 2014). The two functional A and B allele classes were revealed to contain numerous sequence variants. For example, the A201 allele responsible for a serologically detectable A2 phenotype with a 20- to 50-fold reduction in A activity, displays when compared to the A101 allele an insertion at genomic position 1,061, which results in a frameshift adding 21 additional amino acid residues to the protein (Daniels, 1997). Non-sense mutations, such as nucleotide deletions or insertions, often abolish or decrease blood group expression by causing a shift in the open reading frame of the sequence such that the amino acids encoded after the mutation are completely different (Storry and Olsson, 2004).

The silent allele O is also greatly heterogeneous when studied at the gene sequence level. The most frequent human O alleles are O01 and O02, which have been found at high frequencies in all populations studied so far. They differ in exons 6 and 7 by nine nucleotide substitutions (Olsson and Chester, 1996; Yamamoto *et al*., 2001), and by an additional 14 positions in intron 6 (Roubinet *et al*. 2004) but share a point deletion of a G at position 261 in exon 6. This deletion, referred to as 261 as per the numbering in Yamamoto *et al*., (2001), induces a frame shift and creates a premature stop codon (nucleotides 352–354), resulting in a truncated (117 amino acids) protein deprived of any glycosyltransferase activity (Yamamoto et al. 2001). Numerous variants of O01 and O02 alleles have been described. They differ from O01 or O02 by a few point mutations, or result from inter-allelic exchanges between them or with A or B alleles (Chester and Olsson, 2001; Roubinet *et al*. 2004; Yamamoto *et al*., 2001). Rarer alleles such as O03 carry diVerent inactivating mutations. Previous studies of the exon 6 to exon 7 region had showed three main lineages: A101/O01, B101, and O02 (Roubinet *et al*., 2004).

Most null blood group phenotypes are the result of molecular changes in the gene that encodes the carrier molecule. However, there are important interactions between proteins at the cell surface and with the cytoskeleton and therefore mutations that change the expression of an interactive protein can affect the proteins around it. Mutations in RHAG that stop the expression of the Rh-associated glycoprotein (RhAG) also prevent the expression of the RhD and RhCE proteins, the so-called regulator type of Rhnull phenotype (Storry and Olsson, 2004).

ABO gene is located on the long arm of the ninth human chromosome (9q34.1) (Suzuki, 2005, Murray *et al.,* 2003; Kitano *et al.,* 2009; Rai *et al.,* 2009; Carpeggiani, 2010; Ghasemi *et al.,* 2010) while the Rh(D) gene encoding the Rh protein is located on chromosome 1p34–p36 (Iyiola *et al.,* 2012).

There are three alleles, two of which are codominant (A and B) and the third (O) recessive; these ultimately determine the four phenotypic products: the A, B, AB, and O substances (Murray *et al.,* 2003; Ségurela *et al*., 2012). The human A and B alleles of the ABO blood group gene code for glycosyltransferases, which transfer N-acetylgalactosamine and galactose, respectively, to a common precursor (Yamamoto *et al*., 2001, Patenaude *et al*., 2002). Two critical sites for the distinction between A and B activities of the glycosyltransferase have been identified in exon 7 (Yamamoto and Hakomori, 1990), and these nucleotide differences were concordant with serological studies (Kitano *et al*., 2009). A difference of four nucleotides is apparently responsible for the distinct specificities of the A and B glycosyltransferases. On the other hand, the *O* allele has a single base-pair mutation, causing a frameshift mutation resulting in a protein lacking transferase activity (Murray *et al*., 2003).

The RH locus on chromosome 1p34-p36 is composed of the homologous RHD and RHCE genes (96% identity), which are tandemly organized in opposite orientation {RHCE (50- >30)-(30 < -50) RHD} and interspersed by a third gene, SMP1, whose function is presently unknown (Van Kim *et al*., 2006).

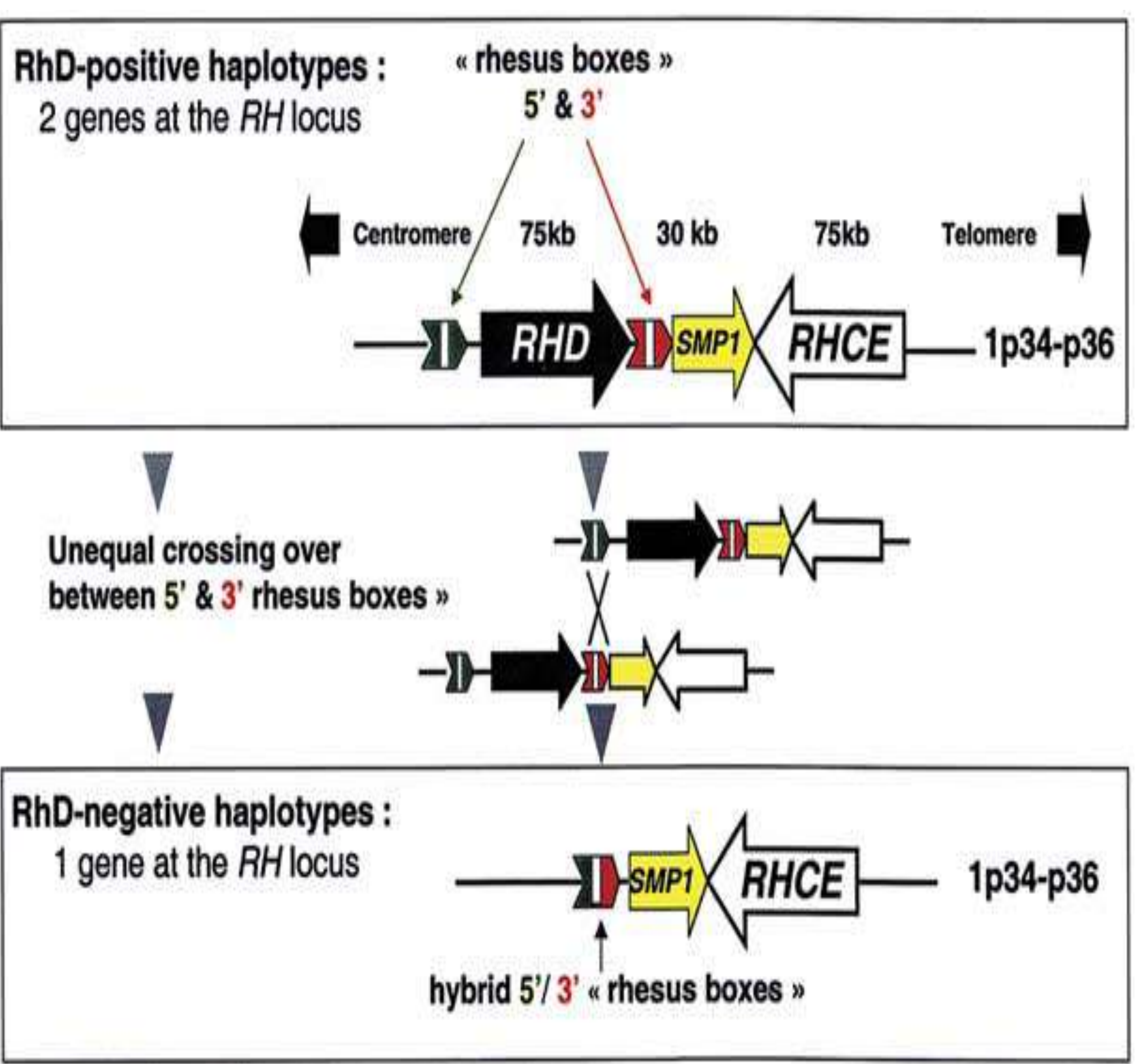

Fig 2.5: RhD-positive/RhD-negative polymorphism (Van Kim *et al*., 2006).

Furthermore, the RHD gene is flanked by two repeated sequences (9,000 bp) exhibiting 98.6% homology called ‘‘rhesus boxes’’ (Wagner and Flegel, 2000). The deletion of the RHD gene in the RhDnegative haplotypes is most probably the result of an unequal crossing over in the rhesus boxes, as deduced from the presence of a hybrid rhesus box (Van Kim *et al.,* 2006).

RhD and RhCE proteins differ by 34 to 37 amino acid substitutions, depending on which RHCE allele is considered (RHce, cE, Ce, CE), but their respective role in the D specificities remain unknown . In few cases, the RhD-negative phenotype in Caucasians can result from gene rearrangements or point mutations leading to stop codons(Wagner and Flegel, 2000) The RhD negative phenotypes in Africans is in most cases due to the presence of a pseudogene, RHDw, exhibiting different mutations, including a 37 bp duplication in intron 3 and exon 4 which disrupt the open reading frame (Van Kim *et al.*, 2006). RhD variants. RhD and RhCE proteins differ by 34 to 37 amino acid substitutions depending on which RHCE allele is considered (RHce, cE, Ce, CE), but their respective role in the D specificities remain unknown (Van Kim *et al*., 2006).

Most blood group polymorphisms are due to missense mutations resulting in amino acid substitutions, but other types of mutation are involved including gene deletion, single base deletion, and exchange of genetic material between homologous genes. All the polymorphisms of the Kell system, for example, result from missense mutations. Comparison of the position of these mutations within the *KEL* gene with clusters of antigens determined by an immunochemical technique, suggests that the Kell-system antigens are not linear and are probably discontinuous (Daniels, 1997).

The biological significance of the proteins and glycoproteins carrying many of the blood group antigens is known, or at least can be speculated upon. Identification of the nonsense

mutations responsible for some null-phenotypes has shown that these macromolecules are not usually essential for a healthy life. Little is known, however, about the biological significance of the blood group polymorphisms. In the MNS system, two amino acid substitutions within glycophorin A are responsible for MN polymorphism and one substitution within glycophorin B for the Ss polymorphism (Kitano *et al.,* 2009).

In the Rh system, four missense mutations within the *RHCE* gene are associated with the Cc polymorphism, and different single missense mutations within *RHCE* are responsible for the Ee polymorphism and for C w and VS, which are also polymorphic (Daniels, 1997). Missense mutations responsible for the carbohydrate polymorphisms (ABO and Lewis) do not directly encode changes in red cell surface antigens, but affect the activity and specificity of glycosyltransferases involved in the biosynthesis of carbohydrate chains on glycoproteins and glycolipids (Daniels, 1997).

Other types of mutation are also responsible for blood group polymorphisms. The only red cell surface antigen polymorphism that results from gene deletion is the D polymorphism of the Rh system, in which the D-negative phenotype usually derives from a complete absence of *RHD,* the gene encoding the D polypeptide. Deletion of a single nucleotide from a gene disrupts the tTiplet code and the resultant shift in the reading frame usually means that no functional protein is produced and often introduces a nonsense codon, responsible for premature termination of mRNA translation (Daniels, 1997). This is what happens in the common form of O allele at the *ABO* locus, so no active glycosyltransferase and, consequently, no A or B antigen, is produced. In the A 2 allele of the *ABO* gene, a single base deletion at the 3' end of an A 1 gene, in the codon before the normal translation stop codon, results in the abolition of that stop codon. This extends the length of the encoded A-transferase by 21 amino acids and affects its

specificity, but does not destroy its activity. In the complex systems involving two or more structural genes (MNS, Rh, Chido/Rodgers), some of the polymorphisms may involve exchange of genetic material between homologous genes (Daniels, 1997).

Mutations in blood group genes may occur which do not adversely affect the function of the membrane glycoprotein or glycolipid, but may make it less suitable as a receptor for a parasite. This might have nothing to do with red cells; the target for the parasite may be other cells which carry the protein. The mutant form would then have a selective advantage and may establish itself as a polymorphism. Subsequently, the selective advantage could disappear, either because the parasite evolves to be able to utilise the alternative form of the membrane structure, or because the parasite is no longer present to infect the host (Daniels, 1997).

2.6.3 Structural and functional diversity of blood group antigen

ABO blood types are determined by a cell surface marker that identifies the cell as belonging to "self" or to that individual. These cell surface markers are characterized by a protein or lipid that has an extension of a particular arrangement of sugars. The arrangement of sugars that determines each of the A, B, and O blood types is identical, except that types A and B have an additional sugar: N-acetylgalactosamine for A, and galactose for B (Loscertales *et al.,* 2007; Criswell, 2008).

These antigens are oligosaccharide antigens whose immunodominant structures are defined as GalNAc $\alpha1\rightarrow3$ (Fuc$\alpha1\rightarrow2$) Gal- and Gal $\alpha1\rightarrow3$ (Fuc$\alpha1\rightarrow2$) Gal- for A and B antigen, respectively. Functional alleles at the ABO locus encode enzymes that catalyze the final step of synthesis (Murray *et al*., 2003 and Loscertales *et al*., 2007). A alleles encode for A transferase, which transfers the GalNAc residues from the UDPGalNAc nucleotide-sugar to the galactose residue of the acceptor H substrates defined by

Fucα1→2 Gal-. B alleles encode for B transferase that transfers the galactose residue from UDP-galactose to the same H substrates. O alleles are non-functional, null alleles (Yamamoto *et al.*, 2012).

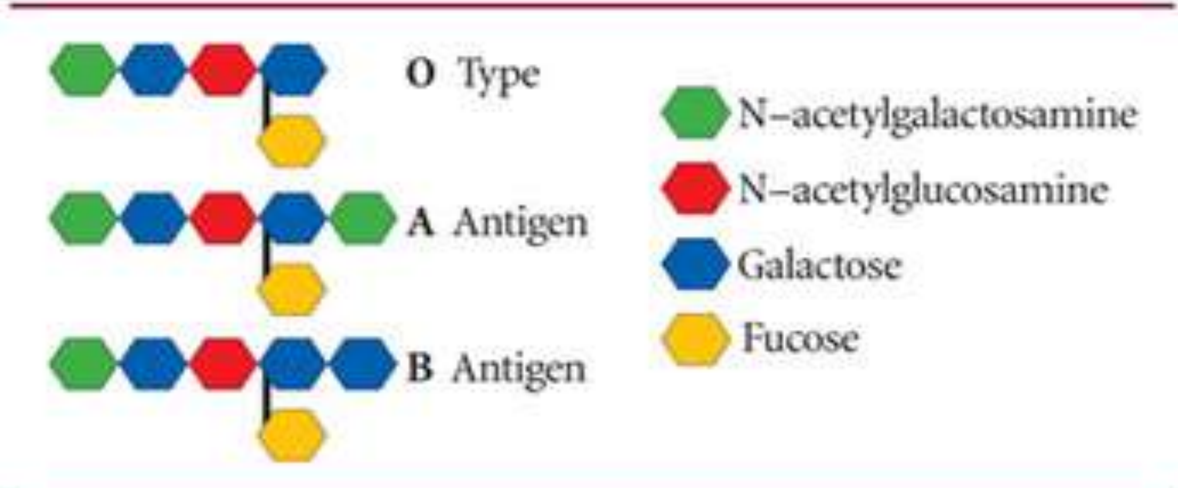

Fig 2.6: ABO antigen specificity. The ABO antigens differ by just one sugar at the antigen terminus. Only the carbohydrate portion of the antigen is illustrated (Criswell, 2008) .

Based on a comparison of information stored in database and observations on structural homology, prediction of secondary structure, and the well characterized biological function of certain molecules, blood group antigens have been tentatively classified into five functional categories:

– Transporters and channels;

– Receptors for exogenous ligands, viruses, bacteria and parasites;

– Adhesion molecules;

– Enzymes;

– Structural proteins

It should be noted, however, that some blood group antigens may exhibit several functions and fit into several categories. For instance, protein Band 3 is an anion exchanger, but also plays a role in membrane structure (attachment site for skeletal proteins) and in the cytoadherence of *P. falciparum*-infected erythrocytes to the vascular endothelium.

Table 2.2 Functional classification of Human Blood Groups

Molecular class	*Gene symbol*	*Symbol ISBT*	*Chromosome*	*Protein or lipid*[b]	*Size (kDa)*[a]	*Copies per RBC*	*Biological function*
Transporter or channel	DI	010	17q21	Band 3 (CD233)	90	10^6	Anion exchanger [AE1]
	CO	015	7p14	AQP-1/CHIP28	28**	$2x10^5$	Water channel
	JK	009	18q11-q12	Kidd	50	$15x10^3$	Urea Transporter [hUT-B1]
	RH	004	1p34-p36	Rh (CD240)	30-32	$2x10^5$	RhAG(CD241)* - Amonium Transporter
	XK	019	Xp21.1	Kx	37		Transporter?
Receptor	FY	008	1q22-q23	DARC (CD234)	35-45	$15x10^3$	Receptor *P. vivax* / chemokines / (HIV-1 ?)
	KN	022	1q32	CR1 (CD35)	170-280	10^3	Receptor *P. falciparum* / C3b, C4b
	MNS	002	4q28-q31	GPA/B (CD235A/CD235B)	36/20	$10^6/3x10^5$	Receptor *P. falciparum (EBA-175)*/ bacteria / viruses
	CROM	021	1q32	DAF (CD55)	70	$6-15x10^3$	Receptor *E. coli* / Enterovirus
	P	003	22q11-ter	Globoside		$10x10^6$	Receptor Parvovirus B19
Adhesion	IN	023	11p13	CD44	80	$5-10x10^3$	Ligands- Hyaluronate, Collagens I and VI, fibronectin, Serglycin, ETA-1
	LW	016	19p13	ICAM-4 (CD242)	42	$3-5x10^3$	Ligands- integrins α4β2 (and α4β1, αvβ3 ?)
	LU	005	19q12-q13	Lu/B-CAM (CD239)	78-85	$1.5-4x10^3$	Ligand- Laminin (chain α5)
	XG	012	Xp22-p32	XG1(Xg[a])/XG2(CD99)	22-29	150/960	? ligand ?
	OK	024	19p13.2	EMMPRIN (CD147)/Ok[a]	54-65		Leukocyte adhésion molecule M6 (ligand - ?)
	JMH	026	15q23-q24	JMH/SEMA7A (CDw108)	75-80		Semaphorin 7A (Cell attachment through RGD sequence ?)
Enzyme	ABO	001	9q34-q34.2	GlycosylTransferase	40-42		3-α-D-GalNAc/Gal-transferases (A/B)
	H	018	19q13	GlycosylTransferase			2-α-L-fucosyltransferase (H- FUT1 / SE- FUT2)
	LE	007	19p13	GlycosylTransferase			3/4-α-L-fucosyltransferase (FUT3)
	YT	011	17q22.1-22.3	Cartwright	160	$3x10^3$	Acetylcholinesterase
	KEL	006	7q32-q36	Kell (CD238)	93	$3-6x10^3$	Zn-Metalloproteinase
	DO	014	12p13.1-13.2	Dombrock	54-57		ADP-ribosyltransferase ?
Structure*	GE	020	2q14-q21	GPC/D (CD236C/CD36D)	32/23	$2x10^5$	Mechanical/elastic properties of red cell membrane and receptor *P. falciparum (BAEBL)*
Others	CH/RG	017	6p21.3	C4A/CAB fragments			*Complement fracions adsorbed on RBCs*
	SC	013	1p32-p34	Scianna	*60*		*unknown*
	RAPH	025	11p15	MER2	*40#*	*70-500#*	*unknown*

Source: (Catron and Collins, 2001)

2.7 ABO and Body Mass Index, Height and Weight

In a study by Jafari *et al.* (2012), height, weight and BMI were not associated with ABO blood groups. In several studies, there was no association between anthropometric measures and ABO blood groups. However a few studies have shown an association between anthropometric measures and ABO blood groups. In one study conducted among 898 young men, blood group B (B, AB) subjects were taller than non-B (A, O) subjects. In another study on Brazilian infants, weights of females with blood group A were significantly more than other blood types. This difference was not found among male infants group B (B, AB) subjects were taller than non-B (A, O) subjects (Ainee *et al.,* 2014). In another study on Brazilian infants, weights of females with blood group A were significantly more than those with other blood types. This difference was not found among male infants (Jafari *et al.,* 2012).

Kelso *et al.,* (1992) found evidence for an association between blood groups and body weight and recently, Kelso *et al.* (1992) in an unpublished data reviewed new evidence for an association between blood group phenotypes and body weight. They found variable results among studies of ABO Blood groups in males. However in females those of AB phenotypes were usually the heaviest, and A women are usually heavier than O or B women.

2.8 ABO and Intelligence

In a study by Atoom (2014) on Blood Groups and their Relation with Intelligence among Sample of Jordanian Universities students, students whose blood group is (AB) got the highest cumulative average and the highest grade in the test, while students whose blood group is (B) got the lowest cumulative average and the lowest grade. When calculating the correlation coefficient between GPA and the test's results it is found that it is equal

(0.98) and it is a highly value and interpreted as logical because IQ correlates positively and highly. These findings are consistent with what was published on the *Japanese-Zodiac* site. The holders of (AB) blood type are the highest ones in the percentage of their intelligence. And that scientists and geniuses in this blood group are more than any other holders of other blood groups (Atoom, 2014).

Dr. Safaa Abdel-Kader, a consultant psychiatrist, says that it has been scientifically proven that each person has characteristics different from other people, and that is due to blood type. He points that each type of the four blood groups has different characteristics (Atoom, 2014).

2.9 ABO and Diseases

Research on ABO group system has been of immense interest, due to its medical importance in different diseases (Chandra and Gupta, 2012). The ABO blood type, an easily accessible factor in patient's genetic makeup, has been associated with many diseases (Akhtar *et al.,* 2010). Already in 1960's and 1970's, correlations between human ABO blood group phenotype and susceptibility to develop several diseases were broadly postulated based on data from large epidemiological studies carried out around the world (Mäkivuokko *et al.,* 2012).

ABO blood groups have shown to have some association with various non-infectious (Umit *et al*., 2008) and infectious diseases (Jefferys *et al*., 2005). Host genetic and environmental factors may be important in the genesis of diseases. ABO blood groups are one set of agglutinogens (antigens), which are genetically determined carbohydrate molecules carried on the surface membranes of the red blood cells (Naeini *et al.,* 2010). With the discovery of ABO blood group system in 1901 by Landsteiner, it was studied as etiological factor of many diseases for example peptic ulcer and carcinoma of stomach

(Lutfullah *et al*., 2010). ABO antigens might have been evolutionarily advantageous in conferring resistance against pathogens. The susceptibility to various diseases, such as infections, cancer, cardiovascular diseases and hematologic disorders, have been associated with ABO blood groups (Than *et al.,* 2011).

In most people A and B antigens are secreted by the cells and are present in the blood circulation. It seems that nonsecretors are susceptible to a variety of infections (Naeini *et al.,* 2010). The possible pathogenesis for this susceptibility is that as many organisms that may bind to polysaccharide on cells and soluble blood group antigens may block this binding (Odeigah, 1990; Jefferys *et al*., 2005). Daniels (1997) reported that the ABO polymorphism can prevent that the species carrying it be endangered by a pathogen using a given carbohydrate as receptor. On the other hand, the ABO polymorphism leads to a polymorphic production of anti-A and anti-B natural antibodies, which potentially protect individuals from the various and numerous infectious agents expressing A and B motifs (Calafell *et al.,* 2008).

2.9.1 ABO and malaria

The association between ABO and malaria was first suggested by Athreya and Coriell, (1967) when they reported that group B confers a selective advantage to malarial infection. By 1978, a marked excess of group A patients, as compared with groups O and B, was recognized from combined data analysis. However, the types of malaria were rarely mentioned in the literature (Yamamoto *et al*., 2012).

There are some differences in Lewis antigen level according to ABO phenotype since A, B and AB cells carry less Le antigen than O cells, because their respective transferases use the same precursors (Loscertales *et al.,* 2007). A recent hypothesis has been presented

that *falciparum* malaria may account for the three-fold higher prevalence of the Le (a_ b_) phenotype among people of African ancestry (Cserti and Dzik, 2007).

Although the contribution of ABO blood group system to protection against malaria has not drawn much attention, it has been reported that blood group O may confer resistance to severe *falciparum* malaria through the mechanism of reduced rosetting (Iyiola *et al.,* 2012). Sequestration and rosetting are linked to the pathogenesis of severe malaria. The former is the process whereby *P falciparum*–infected RBCs roll on and adhere to the microvascular endothelium and then disappear from the circulation. The latter signifies the formation of aggregates by infected RBCs with uninfected RBCs and/or platelets (Yamamoto *et al.,* 2012). The microcirculatory obstruction by cytoadhesion may result in reduced oxygen and substrate supply (Yamamoto *et al.,* 2012). The rosetting receptor in RBCs is the complement receptor type 1 (CR1). CR1 appears to carry the Knops blood group antigens with the S1 (a) phenotype forming fewer rosettes (Rowe *et al*., 1997).

The primary requirement for entry of *plasmodium falciparum* merozites into human red cells is the recognition of a carbohydrate structure present in glycophorin A or B which include sialic acid and galactose, but is not necessarily clustered at the N terminal end of the molecule (Catron *et al.,* 1983). Sialic acid that is common to some pathways that use glycophorins as ligands has also been related to ABO phenotypes. Sialic acid is one of the essential molecules for parasite attachment and it has even been suggested that the human–chimpanzee differences in malaria susceptibility are related to the genetic changes in sialic acid molecules present in glycophorins (Martin *et al*., 2005).

In regions highly endemic for *Plasmodium falciparum* malaria, it is well recognised that a range of RBC polymorphisms associated with resistance to severe disease have undergone positive selection (Min-Oo and Gros, 2005). Many macromolecules carrying

blood group activity are receptors for pathogenic micro-organisms and these pathogens may well have played an important part in the evolution of blood group polymorphism (Turcot *et al*., 2003). The Duffy-glycoprotein is a chemokine receptor, but also a receptor for the malaria parasite *Plasmodium vivax.* A mutation within an erythroid-specific transcription factor binding site within the FY gene, common in people of African origin but rare in other ethnic groups, results in the absence of the glycoprotein from red cells and, therefore, resistance to *P. vivax* infection, but presence of the glycoprotein in other tissues, reducing any potential selective disadvantage (Daniels, 1997).

2.9.2 ABO and infectious disease

Actually, ABO polymorphism has been associated with certain infectious diseases (Boren *et al.,* 1993; Cserti and Dzik; 2007; Anstee, 2010). The presence/absence of A/B antigens and concordant absence/presence of anti-A/B antibodies provide strong defensive lines against infection. Having ABO gene should be beneficial because many vertebrate species maintain this gene. However, having both functional A and B genes ubiquitously within species might not be so advantageous because they may eventually lose anti-A/B antibodies. Rather, frequent gene conversion of A/B specificity producing amino acid substitutions or recombination with non-functional partial genes may have conferred an adaptation against microbial attacks (Yamamoto *et al.,* 2014).

Because infectious agents often use cell-surface glycoconjugates as receptors for attachment, glycosylation polymorphisms such as ABO may affect host-pathogen interactions and result in differential susceptibility among individuals with different glycosylation profiles. It should be remembered that certain microbial parasites share blood group antigens with their hosts (molecular mimicry). Early etiological studies

identified associations between ABO and infectious diseases such as cholera (Yamamoto *et al.,* 2012).

Mourant *et al.,* (1976) discussed suggestions that the major differences seen in ABO blood groups in different parts of the world may be due to epidemics that have occurred in the past. It was suggested that some major differences were due to the presence of an "A-like" antigen on the smallpox virus, and an "H-like" antigen on the plague bacillus (and cholera). This would make individuals who have anti-A (group B and O) more resistant to smallpox, and individuals who can make anti-H (A1 and B) more resistant to the plague and cholera (Garratty, 2005). Anstee (2010) reported that once a person is infected with cholera (*Vibrio cholerae* strains O1 El Tor and O139) the phenotype group O confers a greater likelihood of severe infections than non-O blood group phenotypes. Patients of group O were more susceptible in an outbreak of gastrointestinal infections caused by Escherichia coli O157 in Scotland in 1996. A total of 87.5% of patients who died were group O. Susceptibility to norovirus infection is also closely linked to the expression of ABH and Le antigens in the gastrointestinal tract. Nonsecretors appear more susceptible to infections by *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* and urinary tract infection caused by E coli.

The association between ABO and peptic ulcer was one of the first to be identified. It was shown that group O individuals had higher susceptibility to peptic ulcers (Yamamoto *et al.,* 2012). Gastritis and ulceration of stomach/duodenum were later correlated to infection with the bacterium H pylori. It was also found that patients could be cured from peptic ulcer by eradicating the bacteria with antibiotics and acid secretion inhibitors. It was reported that the fucosylated antigens H type 1 and Lewis b (Leb) mediate H pylori attachment to human gastric mucosa and that soluble glycoproteins presenting Leb or

antibodies to the Leb antigen could inhibit the bacterial binding. Furthermore, the conversion of Leb to ALeb by the addition of a terminal GalNAc diminished the bacterial binding. This may explain the reduced infectivity of groups A/B/AB individuals as compared with group O individuals (Yamamoto *et al.,* 2012).

There are other examples of infectious diseases in which the severity of infection can be directly linked to ABO phenotype. The authors of numerous studies have shown that once a person is infected with cholera (*Vibrio cholerae* strains O1 El Tor and O139) the phenotype group O confers a greater likelihood of severe infections than non-O blood group phenotypes (Anstee, 2010).

2.9.3 ABO and cardiovascular disease

During the last few decades several reports have suggested that ABO blood groups, in particular non-O blood groups, are associated with the risk of ischemic heart disease and of developing severe manifestations of atherosclerosis (Amirzadegan *et al.,* 2005). Genetic factors contribute to the development of coronary artery disease CAD) and influence individual response to risk factor modification. The most significant cardiovascular risk factors—arterial hypertension, diabetes mellitus, hypercholesterolemia, and family history for ischemic heart disease are, at least in part, genetically transmitted. Of these, family history very accurately predicts coronary events (Carpeggiani, 2010).

ABO blood groups are genetically transmitted as well, through chromosomes 9 at locus 9q34. The ATP-binding cassette 2 (ABCA2) genes, which plays a role in cholesterol homeostasis, is located at locus 9q34. In addition, a genetic variation significantly affecting the risk for developing CAD has been recently discovered on the 9p21

chromosomal region. Much of the familial aggregation of CAD might be linked to heritable risk factors, and inheritance of ABO groups could play an important role in this context (Carpeggiani *et al.,* 2010; Yamamoto *et al*., 2012).

ABO blood-group antigens are linked to the protein backbone of coagulation factor VIII and von Willebrand factor and critically affect coagulation. Indeed, patients with blood-group O are prone to excess bleeding because of the approximately 25% lower plasma concentrations of these coagulation factors, which is the consequence of the increased clearance of these glycoproteins, a phenomenon that is related to the H antigen linked to their backbone (Anstee, 2010). Conversely, the elevated plasma concentrations of coagulation factor VIII and von Willebrand factor in non-O blood-group individuals has been implicated in the increased risk for thromboembolic disease and ischemic heart disease (Than *et al.,* 2011).

Interestingly, ABO blood group is a key determinant of coagulation factor VIII and von Willebrand factor plasma concentrations. Low plasma concentrations of these glycoproteins in blood-group O individuals may lead to excess bleeding, while elevated plasma concentrations of these factors in non-O blood-group individuals have been implicated in increasing the risk of thromboembolic and ischemic heart diseases (Anstee, 2010). Preeclampsia, a syndrome unique to human pregnancy and one of the leading causes of maternal and fetal morbidity and mortality, is also associated with maternal blood group. Patients with blood group AB have an increased risk of severe-, early-onset, or intrauterine growth restriction (IUGR) associated forms of preeclampsia.

Placental Protein 13 is considered to be an early marker for preeclampsia. It is a galectin (galectin-13) that binds beta-galactosides, such as N-acetyl-galactosamine, galactose,fucose, located at terminal positions on ABO blood-group antigens. While

elevated plasma concentrations of these factors in non-O blood-group individuals have been implicated in increasing the risk of thromboembolic and ischemic heart diseases. PP13 is primarily produced by the placenta in anthropoid primates and is predominantly localized to the syncytiotrophoblast apical membrane, from where it can be secreted and/ or shed into the maternal circulation (Than *et al.,* 2011). Similarly, ABO was linked to myocardial infarction in the presence of coronary atherosclerosis (Yamamoto *et al.,* 2012).

2.9.4 ABO and cancer

Although the relation between ABO blood group and cancer was the subject of intensive research in the mid 1900's, there has been renewed interest after the recent publication of reports establishing an association between ABO blood group and pancreatic cancer (Wolpin *et al.,* 2009, Iodice *et al.,* 2010; Wolpin *et al.,* 2010). Simultaneously, a genome-wide association study (GWAS) identified pancreatic cancer susceptibility loci in the ABO gene (Amundadottir *et al.,* 2009; Iodice *et al.,* 2010).

The first association between the ABO blood group and cancer risk was reported in 1953 in English patients with stomach cancer, where blood group A was associated with increased risk of stomach cancer and blood group O conferred a protective advantage. Early independent studies showed association of rectal, cervical, leukemia, pancreatic, breast, ovarian, gastric cancers among individuals with blood groups A, AB, or B more likely to have elevated risk of pancreatic cancer than individual belonging to blood group O (Iyiola *et al.,* 2012; Sharma *et al*., 2010) found more prevalence of lung and oral cancer in males with blood group B, while prevalence of cervical cancer was also more in females with blood group B.

Since the first report showing an association between blood group A and gastric cancer, numerous other reports have documented a relation between susceptibility to cancer and blood group. There has been an increasing incidence of association of ABO blood group with several types of genetic diseases including cancers (Iyiola *et al.,* 2012). Some researchers have proved the relation between the ABO blood groups and pancreatic cancer and similarly for duodenal ulcer, peptic ulcer (Krishnakanth *et al.,* 2012).

In oral cancer, loss of the A and B antigens is associated with increased cell migration, in non-small cell lung cancer, the A blood group is associated with improved survival, and in ovarian cancer the B antigen is associated with increased risk (Yamamoto *et al.,* 2012). In addition to the association with blood group antigens, variability of SNPs within the ABO genomic region has been associated with circulating levels of tumour necrosis factor alpha (TNFα), soluble intracellular adhesion molecule-1 (sICAM-1) and alkaline phosphatase. Because these tumor-promoting factors may also provide an environment favoring breast tumor development, variation of rs505922 may contribute to increased risk of breast cancer development (Rummel *et al*., 2012).

Previous studies suggest a possible association between ABO blood group and the risk of some epithelial malignancies, including pancreatic and gastric cancer. Several plausible mechanisms, including inflammation, immune-surveillance for malignant cells, intercellular adhesion, and membrane signalling have been proposed to explain the observed association between ABO blood groups and cancer risk (Xie *et al*., 2010). For instance early independent studies showed association of rectal, cervical, leukemia, pancreatic, breast, ovarian, gastric cancers among individuals with blood groups A, AB, or B more likely to have elevated risk of pancreatic cancer than individual belonging to

blood group O (Iyiola *et al.,* 2012). Similarly, ABO was linked to myocardial infarction in the presence of coronary atherosclerosis (Yamamoto *et al*., 2012).

2.10 Inheritance of ABO and RH (D) Blood Groups

Blood group antigens are stable characteristics controlled by genes inherited in a simple Mendelian manner (Loscertales *et al*., 2007; Yamamoto *et al.,* 2012). The ABO blood group locus is located on chromosome 9, with co-dominant A and B alleles that express glycosyltransferases that add a *N*-acetyl galactosamine or a galactose, respectively, to various substrates generically referred to as H substance (Calafell *et al.,* 2008). These products result in A or B blood group specific antigens, as well as silent alleles with no observable expression (Loscertales *et al.,* 2007).

People inherit two genes for blood type; or, more accurately, two alleles, one from each parent. A set of three possible alleles at an autosomal locus are responsible for four blood types. The gene symbols i or I^0, I^A and I^B, are often used to denote these alleles, where I stands for isoimmunogglutinin. The superscript indicates the specifics antigen (Khan *et al.,* 2004). These alleles are represented as I^A for type A, I^B for type B, and I^O or i for type O (Criswell, 2008). These three alleles combine to yield six genotypes and four phenotypes (Khan *et al.,* 2004). Both glycosyltransferase alleles for antigens A and B are expressed when inherited together, producing both antigens and resulting in blood type AB. When the allele for blood type A or B is inherited with type O, the individual will be either type A or B. This is not necessarily because the type O allele is silenced or recessive, but is instead a result of the activity of the A or B glycosyltransferase, while the glycosyltransferase for the O allele is inactive. A type O individual has both alleles for the inactive glycosyltransferase (Criswell, 2008).

Is it possible for the two people of the Creation account (Adam and Eve) or the eight people on Noah's Ark to give rise to all of the ABO blood types present in humans today? If Adam and Eve were heterozygous for blood types A and B, respectively (one allele for type O and one allele for either type A or B), they could have produced children that had any of the ABO blood types, as illustrated in Fig. 2.6.

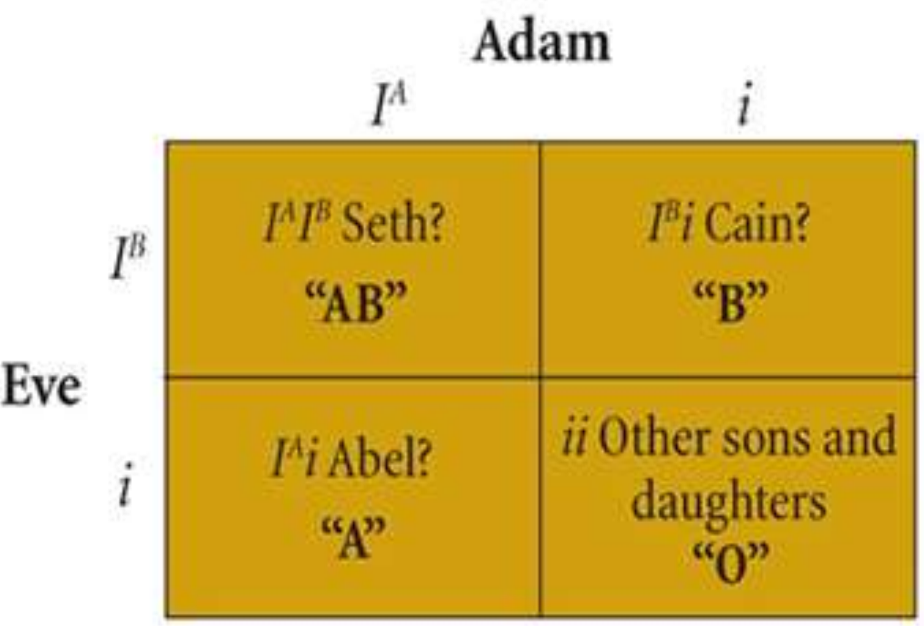

Fig. 2.7: The possible inheritance of four blood types from Adam and Eve.

Alleles for blood type I^A = A, I^B = B, i = O (Khan *et al*., 2004).

2.11 ABO and Incompatibility

2.11.1 Transfusion reaction

For purposes of blood transfusion, it is particularly important to know the basics of the ABO and Rh systems (Murray *et al*., 2003). The ABO typing is the most important test performed in transfusion practice today and the single most common cause of transfusion related fatalities is due to patient being transfused with ABO incompatible blood (Rai *et al.,* 2009). Transfusion of ABO-incompatible blood may result in RBC hemagglutination, kidney failure, and occasional death of the recipient. Transplantation of ABO-incompatible cells/tissues/organs may, without immunosuppression, result in acute rejection (Yamamoto *et al.,* 2012).

ABO incompatibility leads to complement activation against the donor RBCs, leading to intravascular hemolysis. This is extremely dangerous and can lead to mortality. Unfortunately, the most common cause of this situation is clerical error, such as misidentification of a patient (Gurevitz, 2010). Blood types are determined by antigenic glycoproteins and glycolipids on the RBC surface. Incompatibility of one person's blood with another result from the action of plasma antibodies against these RBC antigens (Saladin, 2003; Rai *et al.,* 2009). The membranes of the red blood cells of most individuals contain one blood group substance of type A, type B, type AB, or type O. Individuals of type A have anti-B antibodies in their plasma and will thus agglutinate type B or type AB blood. Individuals of type B have anti-A antibodies and will agglutinate type A or type AB blood. Type AB blood has neither anti-A nor anti-B antibodies and has been designated the universal recipient. Type O blood has neither A nor B substances and has been designated the universal donor (Murray *et al.,* 2003; Saladin, 2003).

Most transfusions are not associated with adverse reactions. However, reactions can occur with any blood component. The reaction may occur at the time of the transfusion, such as:

- Abrupt high fever (called a "febrile reaction") or
- The destruction of the transfused red cells (called a "hemolytic reaction").
- Transfusion-related acute lung injury (TRALI) is the term for new-onset of acute lung injury (ALI) that occurs within six hours after the transfusion of a plasma-containing blood product. The cause of TRALI is currently not fully understood. TRALI is treatable with supportive care, but can be fatal if recognition of TRALI is delayed.
- Other deleterious effects, such as the transmission of viruses, are not apparent until weeks or months later, after the incubation period and the onset of the viral disease.

The symptoms of most of the reactions that occur either during or soon after transfusion are similar. These include

- The development of a fever, Chills and Nausea,
- Pain at the site of the transfusion (an arm vein) or in the back
- Shortness of breath
- A drop in blood pressure, passing dark or red urine or a rash (American Red Cross, 2005).

Rare blood types can cause blood supply problems for unprepared Blood banks and hospitals. For example, the rare blood type, Duffy-negative blood, occurs much more frequently in people of African ancestry. The relatively rarity of this rare blood type in the rest of the North-American population can result in a shortage of that rare blood type for

patients of African ethnicity, in need of a blood transfusion. Keep in mind, if you have a rare blood type, there may be some risk in traveling to parts of the world where your rare blood type may be in short supply (Beckman, 2008).

2.11.2 Haemolytic disease of the newborn (HDN)

The major clinical disease associated with the Rh blood group system is hemolytic disease of the fetus and newborn (Anstee, 2010).

A person's Rh type is generally most relevant with respect to pregnancies. During pregnancy, an Rh+ fetus developing in the womb of an Rh- woman runs the risk of pp developing Rhesus disease, also called Rh disease or hemolytic disease of the newborn. Only Rh- women risk having children with this disease; an Rh+ woman can carry an Rh- child without developing this condition (Dubroff and Joseph, 2003).The first pregnancy is likely to be uneventful because the placenta normally prevents maternal and fetal blood from mixing. However, at the time of birth, or if a miscarriage occurs, placental tearing exposes the mother to Rh- fetal blood. She then begins to produce anti-D antibodies. If she becomes pregnant again with an Rh- fetus, her anti-D antibodies may pass through the placenta and agglutinate the fetal erythrocytes. Agglutinated RBCs hemolyze, and the baby is born with a severe anaemia called hemolytic disease of the newborn (HDN), or erythroblastosis fetalis (Saladin, 2003). The maternal production of anti-Rh antibodies can be prevented by administering such antibodies (available under the name Rhogram) to the mother in the last three months of pregnancy and during and after delivery (Martini *et al.,* 2004). Rhogam is given only as a prophylaxis and is useless once sensitization has occurred (Okeke *et al.,* 2012). Not all HDN is due to Rh incompatibility, however about 2% of cases result from incompatibility of ABO and other blood types. About 1 out of 10 cases of ABO incompatibility between mother and fetus results in HDN (Anstee, 2010).

The severity of anti-D HDN is highly variable: the most severely affected fetuses die *in utero* from about the 17th week of gestation onwards; in less severe cases, hydrops fetalis may occur. In those severely affected infants born alive, jaundice may develop rapidly and lead to kernicterus. About 70% of infants who develop kernicterus die within a few days; of those who survive, many have permanent cerebral damage (Daniels, 2002).

There are preventative measures to protect against Rhesus disease and its effects. Women should be tested early in their first pregnancies to determine if they are Rh- and if they are sensitized. Sensitization might occur not only through normal pregnancies, but any time a woman and her child's or fetus' blood mix, including miscarriages, ectopic pregnancies and blood transfusions (Dubroff and Joseph, 2003).

2.12 Solution to ABO Antigen Problem

Advances have also been made in attempts to tackle and overcome limited blood supply. These include improved surgical procedures that minimize bleeding, the use of erythropoietin and novel erythropoiesis-stimulating protein, preparations of hemoglobin-based oxygen carriers and perfluorocarbon- based oxygen carriers, and the generation of RBCs in vitro from hematopoietic stem cells of diverse origins, from embryonic stem cells, and from induced pluripotent stem cells (Yamamoto *et al.,* 2012).

Scientist from the University of British Columbia have created an enzyme that potentially solve the antigen problem. It works by snipping off the problem antigen and effectively turning A and B blood into O. The team created the enzyme by a process known as “directed evolution” the enzyme was able to remove the vast majority of antigen from type A and B blood; they were not able to remove all of them. As the immune system is incredibly sensitive to blood groups so much so that even small amount of residual

antigen can trigger an immune response, the scientist must first be certain that all antigens are absent (David, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

The study was carried out in Kogi State College of Nursing and Midwifery, Obangede located in Okehi LGA (in the central senatorial District) of the State. The college was established in 1980 by the then Kwara State Government and is the only Government owned training institution for Nurses in the State, and hence admission into the college is on quota basis.

Approval of the authority was obtained for this study from the College management for access to health and other records of the students. Records of admitted students into the College of different ages (16 and 40years old) and sexes spanning 11 years (2002–2013) consecutively was retrospectively and prospectively collected from the various record units of the College (Registry, Exams Office and Sickbay).

3.2 Study Population

This study was designed to investigate the gene frequencies for the ABO and Rh (D) blood groups in a population consisting of different tribes, sexes and ages in Kogi State, Nigeria, (using those that attended Kogi State College of Nursing and Midwifery, Obangede) over a period spanning 11 years (2002–2013).

Kogi state is one of the 36 states in Nigeria, is located in the middle belt region, north-central Nigeria. The State was founded in 1991 from parts of Kwara State and Benue State ($6^0$37′N $5^0$29′E and $8^0$41′N $7^0$33′E) with a total land area of 29,833km^2 (11,519 sq miles).The State as presently constituted is made up of 21 Local Government Areas,

There are three main ethnic groups and languages in Kogi: Igala, Ebira, and Okun with other minorities like Bassa, a small fraction of Nupe, Koton, Oworo and Ogori Magongo community with a total population of 3,314,043. The climatic condition of the state is Savannah and the inhabitants are largely farmers.

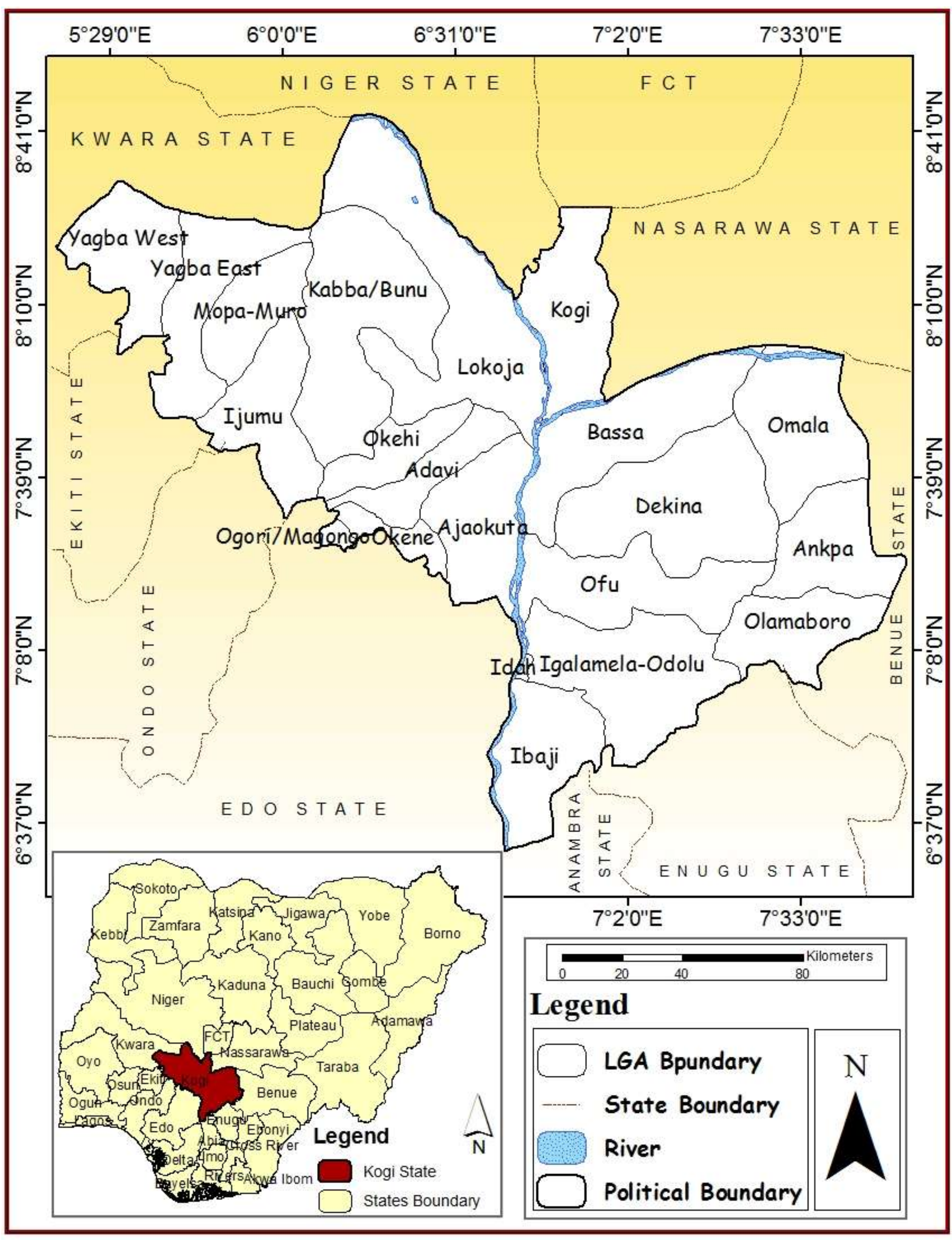

Fig 3.1: Map of Kogi State showing its 21 L.G.A. Adapted from administrative map of Nigeria

3.3 Method and Instrument of Data Collection

For the retrospective part of the study, data were collected from the records and registers in the record unit of the College, while for the prospective part of the study, ABO and Rh blood group tests were carried out for 2011/2012 and 2012/2013 sets (Set 38 & 39) according to the method by Bhasin *et al.* (1996). on a white porcelain tile and or micro slide using blood grouping sera (Lome Laboratories Ltd., UK, BIOTEC Laboratories Ltd., UK.). In brief, about 20 ul of whole blood was taken on a clean slide and 20 ul of anti A, anti B and anti D blood group sera applied, mixed by means of an applicator stick and results were noted. The haemoglobin type was determined using Haemoglobin – electrophoresis. Blood samples were collected by venipuncture into ethylenediamine tetracetic acid (EDTA) salt and used for haemoglobin genotype determination. A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris – EDTA – Borate buffer at pH 8.9. The electrophoresis was then allowed to run for 15 – 20 mins at an electro motive force (emf) of 160 V to find different type of haemoglobin. The results were read immediately. Stadiometer was used to measure height and weight following standard protocol and BMI was calculated using the formular BMI=weight (kg) / height (m^3).

3.4 Sampling Technique/Method

Sample for this study were collected from student's records in Kogi State College of Nursing and Midwifery, Obangede while anthropometric variables (height and weight) were measured using stadiometer.

3.5 Sampling Size Determination

The sample size for this study was obtained using the formula as described by Naing *et al*, (2006)

$$n = \frac{z^2 p q}{d^2}$$

Where:

n = sample size,

Z = Z statistic for a level of confidence, usually set at 1.96 (≈2.0)

P = expected prevalence or proportion (proportion in the population having the particular trait (when no estimate, 5% is used , P = 0.5), and

q=1.0 - p

d = precision (in proportion of one; if 5%, d = 0.05).

Therefore,

$$n = \frac{(1.96)^2(0.5)(0.5)}{(.05)^2} = 384$$

But for the purpose of this study 1,863 subjects (males n=436 and females n=1,427) participated in the study.

3.6 Inclusion and Exclusion Criteria

3.6.1 Inclusion criteria

Subjects considered for this study

i Must be indigene of Kogi State

ii Must have attended the College within the period under study

3.6.2 Exclusion criteria

i. Non Kogi State indigene were excluded

ii Non students were also excluded

3.7 Ethical Consideration

Approval to conduct the study was granted by the Health Research Ethics Committee of the Ahmadu Bello University Teaching Hospital, Shika Zaria. An introduction letter was obtained from the Department of Human Anatomy, Ahmadu Bello University, Zaria which was presented to the Provost of the College for permission to carry out the research.

Plate I: Collection of blood sample by finger pricking for blood typing

Plate II: Preparing blood sample for genotype testing with the assistance of lab technologist

Plate III: Measurement of height and weight using Stadiometer

3.8 Limitations of the Study

The problems encountered during the studies is that some students prefer to treat themselves of diseases like malaria and typhoid within their hostel (as nurses in training) or in the General hospital without visiting the sickbay thereby very scanty information on disease conditions.

3.9 Statistical Analyses

Data were presented as numbers and percentages. Allele frequencies was calculated under the assumption of Hardy–Weinberg equilibrium and expressed as percentages. Chi-square test was used to compare blood group and ethnic background and blood group disease association. Sigmastat 2.0 for Windows (Systat Inc., San Rafael, CA) was used for the data analysis. $P<0.05$ was deemed statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Analyses of Study Population

The scope of this study was restricted to the students of Kogi State College of Nursing and Midwifery, Obangede in Okehi Local Government Area who are indigene of the State. Participants (males n= 436 and females n=1427) aged 16-40 years collected constituted the analytic subjects. It is interesting to note here that the low sex ratio in male students is due to having pretty high number of female sex studying Nursing sciences as compared to the male gender. Data were recorded after obtaining necessary permission from Health Research Ethic Committee of Ahmadu Bello University, Zaria and from relevant authority of the school after reviewing the study protocols (guidelines). The anthropometrics including height and weight of each subject were measured using standard height and weight (mechanical) scale respectively, following a standard technique by trained investigators on resumption in the College.

4.2 Descriptive Statistics of Study Population

The mean and standard deviation (SD) of mean values of age and anthropometrics of the participants are shown in Table 4.1. Age and anthropometric pattern of female and male subjects were not uniform ($p < 0.05$) except BMI that appears to be uniform for both sexes ($p > 0.05$). The mean height of female subjects was found to be smaller than that of their male counterparts. Male subjects were also heavier and older than their female counterparts.

Table 4.1: Mean and standard deviation of age, height, weight and body mass index (BMI) for study participants

	All (n = 1863)		Female (n = 1427)		Male (n = 436)		T-test	
Variables	Mean ± SD	Min – Max	Mean ± SD	Min – Max	Mean ± SD	Min – Max	t	p
Age (yrs.)	28.50 ± 6.92	16.00 – 40.00	28.22 ± 5.09	16.00 – 40.00	28.91 ± 4.83	17.00 – 40.00	-2.5	0.013
Height (cm)	160.15 ± 6.15	64.00 – 181.00	159.99 ± 5.44	145.00 – 180.00	160.90 ± 6.58	150.00 – 181.00	-2.91	0.004
Weight (kg)	61.99 ± 19.34	48.50 – 81.50	61.14 ± 5.68	48.50 – 81.50	62.18 ± 5.91	49.50 – 81.50	-3.31	0.001
BMI (kg m^{-2})	24.24 ± 7.94	16.63 – 33.56	23.91 ± 2.14	16.63 – 30.44	24.05 ± 2.20	16.90 – 33.56	-1.21	0.225

4.3 Genotypic Frequency Distribution

The genotype of students, reflecting observed frequencies is given in Table 4.2. Overall genotypic frequency for the complete students was in the following order i.e. AA>AS>SS. The genotypic frequency were highest for AA (58.50%) followed by AS (41.00%) (Fig. 4.1). The frequency of SS was observed to be comparatively very low (0.50%). This pattern of genetic frequencies was not ethnic dependent ($\chi^2 = 11.53$, $p = 0.644$). Interestingly, the genetic frequencies were also not sex dependent as there was no significant association that was observed between genotypes and sex, 58.40% and 58.70% of females and males are AA respectively, 41.30% and 40.10% of females and males are AS, while 0.30% and 1.10% of females and males ($\chi^2 = 14.79$, $p = 0.393$ and $\chi^2 = 16.57$, $p = 0.280$) are respectively SS.

Table 4.2: Genotype frequency distribution (%) for sample population classified according to ethnicity and sex

	All			Female			Male		
	AA	AS	SS	AA	AS	SS	AA	AS	SS
Ethnicity	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Bassa	38 (61.30)	24 (38.70)	-	32 (68.10)	15 (31.90)	-	6 (40.00)	9 (60.00)	-
Ebira	291 (60.80)	186 (38.80)	2 (0.40)	204 (60.50)	133 (39.50)	-	87 (61.30)	53 (37.30)	2 (1.40)
Igala	291 (57.70)	212 (42.10)	1 (0.20)	213 (56.30)	165 (43.70)	-	78 (61.90)	47 (37.30)	1 (0.80)
Koto	40 (50.60)	38 (48.10)	1 (1.30)	30 (58.80)	21 (41.20)	-	10 (35.70)	17 (60.70)	1 (3.60)
Nupe	26 (52.00)	23 (46.00)	1 (2.00)	17 (48.60)	18 (51.40)	-	9 (60.00)	5 (33.30)	1 (6.70)
Ogori	56 (57.70)	41 (42.30)	-	44 (58.70)	31 (41.30)	-	12 (54.50)	10 (45.50)	-
Okun	318 (59.60)	212 (39.70)	4 (0.70)	271 (59.20)	183 (40.00)	4 (0.80)	47 (61.80)	29 (38.20)	-
Oworo	35 (52.20)	32 (47.80)	-	25 (50.00)	25 (50.00)	-	10 (58.80)	7 (41.20)	-
Total	1095 (58.50)	768 (41.00)	9 (0.50)	836 (58.40)	591 (41.30)	4 (0.30)	259 (58.70)	177 (40.10)	5 (1.10)
	$\chi^2 = 11.53$, $p = 0.644$			$\chi^2 = 14.79$, $p = 0.393$			$\chi^2 = 16.57$, $p = 0.280$		

Values are n (%). Genotypes: AA, normal adult haemoglobin; AS, sickle cell trait; SS, sickle cell disease.

4.4 ABO Blood Groups Frequency Distribution

The results of ABO blood groups of participants reflecting observed frequencies is given in Table 4.3. Overall phenotypic frequency for all participants was in the order ABO*O > ABO*B > ABO*A > ABO*AB. The phenotypic frequencies were highest for ABO*O (965, 51.80%) followed by ABO*B (404, 21.70%). The frequencies for ABO*A was observed as (379, 20.30%), whereas the frequency of ABO*AB group was observed to be comparatively very low (115, 9.00%). Similar pattern was observed for ABO blood group phenotype distribution frequencies for each sex (Table 4.4). It was observed that ABO*O > ABO*B > ABO*A > ABO*AB for each sex. This shows that there is poor association between allelic frequencies of ABO blood group and sex of this group of individuals with $\chi^2 = 29.41$, $p = 0.105$ and $\chi^2 = 22.37$, $p = 0.378$ for female and male subjects respectively.

Table 4.3: Comparison of ABO blood group types across various ethnicities of the subjects

	Blood groups				T-value	
Ethnicity	A n (%)	AB n (%)	B n (%)	O n (%)	χ^2	p
Bassa	13 (21.00)	3 (4.80)	11 (17.70)	35 (56.50)	30.15	0.089
Ebira	85 (17.80)	28 (5.90)	90 (18.90)	274 (57.40)		
Igala	112 (22.30)	26 (5.20)	114 (22.70)	251 (49.90)		
Koto	15 (19.20)	5 (6.40)	16 (20.50)	42 (53.80)		
Nupe	13 (26.50)	6 (12.00)	8 (16.30)	22 (44.90)		
Ogori	23 (23.70)	10 (10.30)	31 (32.00)	33 (34.00)		
Okun	104 (19.60)	31 (5.80)	118 (22.30)	277 (52.30)		
Oworo	14 (20.90)	6 (9.00)	16 (23.90)	31 (46.30)		
Total	379 (20.30)	115 (6.20)	404 (21.70)	965 (51.80)		

Table 4.4: Comparison of ABO blood group types characterized by ethnicity and sex

	Females				Males			
Ethnicity	A n (%)	AB n (%)	B n (%)	O n (%)	A n (%)	AB n (%)	B n (%)	O n (%)
Bassa	12 (25.50)	2 (4.30)	6 (12.80)	27 (57.40)	1 (6.70)	1 (6.70)	5 (33.30)	8 (53.30)
Ebira	62 (18.40)	17 (5.00)	57 (16.90)	201 (59.60)	23 (16.40)	11 (7.90)	33 (23.60)	73 (52.10)
Igala	82 (21.70)	21 (5.60)	84 (22.20)	191 (50.50)	30 (24.00)	5 (4.00)	30 (24.00)	30 (48.00)
Koto	10 (19.60)	3 (5.90)	11 (21.60)	27 (52.90	5 (18.50)	2 (7.40)	5 (18.50)	15 (55.60)
Nupe	11 (31.40)	4 (11.40)	5 (14.30)	15 (42.90)	2 (14.30)	2 (14.30)	3 (21.40)	7 (50.00)
Ogori	16 (21.30)	6 (8.00)	26 (34.70)	27 (36.00)	7 (31.80)	4 (18.20)	5 (22.70)	6 (27.30)
Okun	92 (20.30)	26 (5.70)	102 (22.50)	234 (51.50)	12 (15.80)	5 (6.60)	16 (21.10)	43 (56.60)
Oworo	8 (16.00)	5 (10.00)	10 (20.00)	27 (54.00)	6 (35.30)	1 (5.90)	6 (35.30)	4 (23.50)
Total	293 (20.50)	84 (5.90)	301 (21.10)	749 (52.50)	86 (19.70)	31 (7.10)	103 (23.60)	216 (49.50)
	$\chi^2 = 29.41$, $p = 0.105$				$\chi^2 = 22.37$, $p = 0.378$			

4.5 Rhesus and ABO Blood Groups Frequency Distribution

Out of 1863 subjects, 1755 (94.20 %) have Rh-D positive while the remaining 108 (5.8 %) are Rh-D negative (Table 4.5). for the distribution of subjects according to blood group, Rhesus factors and sex (Tables 4.6 and 4.7), the most common blood group and Rhesus factor observed for both female and male subjects was ‘O’ positive 701 (93.59 %) followed by ‘B’ positive 285 (94.68 %) for female subjects. AB positive, O negative, and AB negative are rarer being present in 82 (97.62 %), 48 (6.41 %) and 2 (2.38 %) respectively (Table 4.6). Males and Females have similar incidence of B positive 285 (94.68 %) and 94 (91.26 %) and O positive 701 (93.59 %) and 205 (94.91 %) respectively. On the contrary, ‘A’ positive blood group is found more in males while O negative is found more in females. However, AB negative and B negative are found more in males than in females (Tables 4.6 and 4.7).

Table 4.5: Rh-D antigen distribution among study participants

	Rh-D antigens			
Ethnicity	Rh +ve n (%)	Rh –ve n (%)	χ^2	P
Bassa	60 (96.80)	2 (3.20)	6.78	0.452
Ebira	444 (93.10)	33 (6.90)		
Igala	473 (94.00)	30 (6.00)		
Koton	70 (89.70)	8 (10.30)		
Nupe	47 (95.90)	2 (4.10)		
Ogori	91 (93.80)	6 (6.20)		
Okun	506 (95.50)	24 (4.50)		
Oworo	64 (95.50)	3 (4.50)		
Total	1755 (94.20)	108 (5.80)		

Table 4.6: Distribution of ABO and Rhesus blood groups for female subjects according to ethnic groups

	A		AB		B		O		Total	
Ethnicity	Rh +ve n (%)	Rh –ve n (%)	Rh +ve n (%)	Rh –ve n (%)	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)
Bassa	12 (100.00)	-	1 (50.00)	1 (50.00)	5 (83.30)	1 (16.70)	27 (100.00)	-	45 (95.74)	2 (4.26)
Ebira	55 (88.70)	7 (11.30)	16 (94.10)	1 (5.90)	55 (96.50)	2 (3.50)	191 (95.00)	10 (5.00)	317 (94.07)	20 (5.93)
Igala	77 (93.90)	5 (6.10)	21 (100.00)	-	80 (95.50)	4 (4.80)	176 (92.10)	15 (7.90)	354 (93.65)	24 (6.35)
Koton	7 (70.00)	3 (30.00)	3 (100.00)	-	10 (90.90)	1 (9.10)	24 (88.90)	3 (11.10)	44 (86.27)	7 (13.73)
Nupe	11 (100.00)	-	4 (100.00)	-	5 (100.00)	-	14 (93.30)	1 (6.70)	34 (97.14)	1 (2.86)
Ogori	15 (93.80)	1 (6.20)	6 (100.00)	-	25 (96.20)	1 (3.80)	25 (92.60)	2 (7.40)	71 (94.67)	4 (5.33)
Okun	91 (98.90)	1 (1.10)	26 (100.00)	-	95 (93.10)	7 (6.90)	220 (94.00)	14 (6.00)	432 (95.15)	22 (4.85)
Oworo	8 (100.00)	-	5 (100.00)	-	10 (100.00)	-	24 (88.90)	3 (11.10)	47 (94.00)	3 (6.00)
Total	276 (94.20)	17 (5.80)	82 (97.62)	2 (2.38)	285 (94.68)	16 (5.32)	701 (93.59)	48 (6.41)	1344 (94.18)	83 (5.82)
	χ^2 = 19.80, P= 0.013		χ^2 = 21.99, P= 0.003		χ^2 = 3.71, 0.813		χ^2 = 5.31, P = 0.622		χ^2 = 7.61, P = 0.368	

Table 4.7: Summary statistics of ethnicity based distribution of ABO and Rhesus blood groups for male subjects

	A		AB		B		O		Total	
Ethnicity	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)	Rh +ve n (%)	Rh -ve n (%)
Bassa	1 (100.00)	-	1 (100.00)	-	5 (100.00)	-	8 (100.00)	-	15 (100.00)	-
Ebira	22 (95.70)	1 (4.30)	11 (100.00)	-	29 (87.90)	4 (12.10)	65 (89.00)	8 (11.00)	127 (90.71)	13 (9.29)
Igala	28 (93.30)	2 (6.70)	5 (100.00)	-	29 (96.70)	1 (3.30)	57 (95.00)	3 (5.00)	119 (95.20)	6 (4.80)
Koton	5 (100.00)	-	2 (100.00)	-	4 (80.00)	1 (20.00)	15 (100.00)	-	26 (96.30)	1 (3.70)
Nupe	2 (100.00)	-	2 (100.00)	-	2 (66.70)	1 (33.30)	7 (100.00)	-	13 (92.86)	1 (7.14)
Ogori	7 (100.00)	-	2 (50.00)	2 (50.00)	5 (100.00)	-	6 (100.00)	-	20 (90.91)	2 (9.09)
Okun	12 (100.00)	-	5 (100.00)	-	14 (87.50)	2 (12.50)	43 (100.00)	-	74 (97.37)	2 (2.63)
Oworo	6 (100.00)	-	1 (100.00)	-	6 (100.00)	-	4 (100.00)	-	17 (100.00)	-
Total	83 (96.51)	3 (3.49)	29 (93.55)	2 (6.45)	94 (91.26)	9 (8.74)	205 (94.91)	11 (5.09)	411 (94.27)	25 (5.73)
	$\chi^2 = 2.14$, P = 0.95		$\chi^2 = 14.43$, P = 0.044		$\chi^2 = 6.46$, P = 0.487		$\chi^2 = 9.65$, P = 0.209		$\chi^2 = 7.49$, P = 0.380	

4.6 Genotype and ABO Blood Groups Frequency Distribution

Table 4.8 shows the distribution of the genotypes among ABO blood group for all the subjects and for subjects based on sex. More number of subjects were found in blood group O with genotype AA for all (fig. 4.8), female (fig. 4.9) and male (fig. 4.10) subjects 554 (50.60 %), 437 (52.30 %) and 117 (59.40 %) respectively. In both sexes there are more individuals in AA genotype than in AS genotype. The pattern of distribution of the genotype with blood group for all the subjects is as follows AA*O > AA*A >AA*B >AA*AB. This is similar to the pattern observed among the females. The male students however, have their pattern as follows AA*O> AA*B> AA*A> AA*AB. The relative lower sample size of the males may be responsible for the trend observed. These pattern of distribution is followed by the distribution of the AS genotype with the blood group. There is weak association between blood groups and genotype as observed in the table for all, female and male subjects respectively $\chi^2 = 5.94$, 0.114, $\chi^2 = 5.38$, $p = 0.146$ and $\chi^2 = 6.26$, $p = 0.099$.

Table 4.8: Distribution of genotypes in different blood groups

	All		Female		Male	
	AA	AS	AA	AS	AA	AS
Blood groups	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
A	240 (63.30)	139 (36.70)	186 (63.48)	107 (36.52)	54 (62.79)	32 (37.21)
AB	73 (63.50)	42 (36.50)	50 (59.52)	34 (40.48)	23 (74.19)	8 (25.81)
B	228 (56.40)	176 (43.60)	163 (54.15)	138 (45.85)	65 (63.11)	38 (36.89)
O	554 (57.40)	411 (42.60)	437 (58.34)	312 (41.66)	117 (54.17)	99 (45.83)
Total	1095 (58.80)	768 (41.20)	836 (58.60)	591 (41.40)	259 (59.40)	177 (40.60)
	$\chi^2 = 5.94$, 0.114		$\chi^2 = 5.38$, $p = 0.146$		$\chi^2 = 6.26$, $p = 0.099$	

4.7 Prevalence of Malaria, Typhoid and Hepatitis Based on Genotype

Table 4.9 summarizes the influence of genotypic alleles on malaria. The incidence of malarial fever is higher among subjects with AA genotype followed by AS subjects. Subjects with SS genotype record the lowest occurrence. The incidence of malaria fever among all, female and male subjects with AA genotype are respectively 58.50 %, 58.40 % and 58.70 % which is similar among these categories of subjects. The incidence of malarial fever is also similar among the AS genotype subject for the overall, female and male subjects (41.00 %, 41.30 % and 40.10 %) respectively. The proportion of subjects that reported frequent and occasional incidence of malaria are similar for the three genotypic alleles for both sexes. Male subjects reported having rarer incidence of malaria among the AA and SS subjects while more of the females with AS genotype. As shown in the table, there is no statistical significant association between genotype and severity of malarial fever $p > 0.05$.

Concerning typhoid fever (Table 4.10), subjects with AA genotype have highest incidence of the fever than those with AS and SS for each sex. 55.30 %, 58.70 % and 38.80 % of female subjects with AA genotype reported frequent, occasional and rare incidence of malaria while 62.60 %, 59.20 % and 54.90 % of the male subjects with AA genotype reported frequent, occasional and rare incidence of typhoid fever respectively. There is no statistical significant association between genotype and severity of typhoid fever for both sexes, $p > 0.05$.

As shown in Table 4.11, most subjects are negative to hepatitis antigens for both female and male subjects. At present, most subjects that are negative to the antigen have AA genotype followed by the AS genotype. However, there are more of the males who react positively with the antigen than those that react negatively. The size of male subjects may

be responsible for this variation. Subjects with sickle cell anaemia react negatively to the antigen. There is no statistical significant association between genotype and the antigen for hepatitis for all, female and male subjects respectively $\chi^2 = 0.18$, $p = 0.914$, $\chi^2 = 0.19$, $p = 0.912$ and $\chi^2 = 0.86$, $p = 0.652$.

Table 4.9: Prevalence of malaria fever according to genotype and sex

Prevalence	All			Female			Male		
	AA	AS	SS	AA	AS	SS	AA	AS	SS
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Frequently	483 (61.00)	307 (38.80)	2 (0.30)	376 (61.10)	238 (38.70)	1 (0.20)	107 (60.50)	69 (39.00)	1 (0.60)
Occasionally	282 (54.90)	230 (44.70)	2 (0.40)	215 (55.80)	169 (43.90)	1 (0.30)	67 (51.90)	61 (47.30)	1 (0.80)
Rarely	330 (58.30)	231 (40.80)	5 (0.90)	245 (56.80)	184 (42.70)	2 (0.50)	85 (63.00)	47 (34.80)	3 (2.20)
Total	1095 (58.50)	768 (41.00)	9 (0.50)	836 (58.40)	591 (41.30)	4 (0.30)	259 (58.70)	177 (40.10)	5 (1.10)
	$\chi^2 = 7.59$, $p = 0.108$			$\chi^2 = 4.07$, $p = 0.397$			$\chi^2 = 6.23$, $p = 0.183$		

Table 4.10: Prevalence of typhoid fever according to genotype and sex

	All			Female			Male		
Prevalence	AA n (%)	AS n (%)	SS n (%)	AA n (%)	AS n (%)	SS n (%)	AA n (%)	AS n (%)	SS n (%)
Frequently	328 (56.90)	246 (42.70)	2 (0.30)	246 (55.30)	199 (44.70)	-	82 (62.60)	47 (35.90)	2 (1.50)
Occasionally	386 (58.80)	265 (40.40)	5 (0.80)	293 (58.70)	203 (40.70)	3 (0.60)	93 (59.20)	62 (39.50)	2 (1.30)
Rarely	381 (59.50)	257 (40.20)	2 (0.30)	297 (61.00)	189 (38.80)	1 (0.20)	84 (54.90)	68 (44.40)	1 (0.70)
Total	1095 (58.50)	768 (41.00)	9 (0.50)	836 (58.40)	591 (41.30)	4 (0.30)	259 (58.70)	177 (40.10)	5 (1.10)
	$\chi^2 = 2.62, p = 0.624$			$\chi^2 = 6.53, p = 0.163$			$\chi^2 = 2.55, p = 0.635$		

Table 4.11: Prevalence of hepatitis according to genotype and sex

	All			Female			Male		
	AA	AS	SS	AA	AS	SS	AA	AS	SS
Prevalence	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Negative	1074 (58.50)	753 (41.00)	9 (0.50)	817 (58.40)	579 (41.40)	4 (0.30)	257 (58.90)	174 (39.90)	5 (1.10)
Positive	21 (58.30)	15 (41.70)	-	19 (61.30)	12 (38.70)	-	2 (40.00)	3 (60.00)	-
Total	1095 (58.50)	768 (41.00)	9 (0.50)	836 (58.40)	591 (41.30)	4 (0.30)	259 (58.70)	177 (40.10)	5 (1.10)
	$\chi^2 = 0.18$, $p = 0.914$			$\chi^2 = 0.19$, $p = 0.912$			$\chi^2 = 0.86$, $p = 0.652$		

4.8 Prevalence of Malaria, Typhoid and Hepatitis Based on ABO Blood Groups

In Table 4.12, the interaction between blood group and prevalence of malaria was considered. Chi square analysis showed that there was interaction between these variables for the overall, female and male subjects ($\chi^2 = 129.29$, $\chi^2 = 91.13$, $\chi^2 = 51.56$, $p < 0.001$) respectively. Subjects with blood group O reported frequent incidence of malaria while subjects with blood group AB reported less frequent incidence of malaria for female and male subjects. Though more females with blood group B (17.40 %) reported having more frequent incidence of malaria than those with blood group A (15.40 %) while, more of the male subjects with blood group A (14.10 %) reported more frequent incidence of malaria than those with blood group B (13.00 %).

Table 4.13 shows the prevalence of typhoid fever with ABO blood groups. There is however, no significant association between blood group and the incidence of typhoid fever ($p > 0.05$). Considering female subjects, the frequencies of the incidence of typhoid fever is as follows: ABO*O > ABO*B >ABO*A > ABO*AB for frequent, occasional and rare incidence of the fever. This is similar to that reported by male subjects that reported occasional and rare occurrence of typhoid fever while the pattern of occurrence for male subjects that reported frequent occurrence of the typhoid fever is: ABO*O > ABO*A > ABO*B >ABO*AB.

Data presented on Table 4.14 reveal that there is statistical significant association between blood group with the incidence of hepatitis among male subjects ($\chi^2 = 22.12$, $p = 0.001$). Female subjects did not show statistical significant interaction between their blood groups and the occurrence of hepatitis ($\chi^2 = 3.78$, $p = 0.287$). For both female and male subjects, most of the subjects reacted negatively to the antigen. The results also

showed that the pattern in which the subjects reacted negatively to the antigen is in the order ABO*O > ABO*B > ABO*A > ABO*AB for both male and female subjects.

Table 4.12: Prevalence of malaria fever according to ABO blood group and sex

		ABO Blood group					
	Prevalence	A n (%)	AB n (%)	B n (%)	O n (%)	χ^2	p
All	Frequently	120 (15.20)	30 (3.80)	130 (16.40)	512 (64.60)	129.29	0.001
	Occasionally	129 (25.10)	16 (3.10)	140 (27.20)	229 (44.60)		
	Rarely	132 (23.30)	69 (12.20)	134 (23.70)	231 (40.80)		
	Total	381 (20.40)	115 (6.10)	404 (21.60)	972 (51.90)		
Female	Frequently	95 (15.40)	24 (3.90)	107 (17.40)	389 (63.30)	91.13	0.001
	Occasionally	101 (26.20)	7 (1.80)	97 (25.20)	180 (46.80)		
	Rarely	97 (22.50)	53 (12.30)	97 (22.50)	184 (42.70)		
	Total	293 (20.50)	84 (5.90)	301 (21.00)	753 (52.60)		
Male	Frequently	25 (14.10)	6 (3.40)	23 (13.00)	123 (69.50)	51.56	0.001
	Occasionally	28 (14.10)	9 (7.00)	43 (33.30)	49 (38.00)		
	Rarely	35 (25.90)	16 (11.90)	37 (27.40)	47 (34.80)		
	Total	88 (20.00)	31 (7.00)	103 (23.40)	219 (49.70)		

Table 4.13: Prevalence of typhoid fever according to ABO blood group and sex

		ABO Blood group					
		A	AB	B	O	χ^2	P
	Prevalence	n (%)	n (%)	n (%)	n (%)		
All	Frequently	128 (22.20)	28 (4.90)	129 (22.40)	291 (50.50)	9.80	0.133
	Occasionally	133 (20.30)	50 (7.60)	148 (22.60)	325 (49.50)		
	Rarely	120 (18.80)	37 (5.80)	127 (19.80)	356 (5.60)		
	Total	381 (20.40)	115 (6.10)	404 (21.60)	972 (51.90)		
Female	Frequently	97 (21.80)	21 (4.70)	103 (23.10)	224 (50.30)	10.57	0.103
	Occasionally	107 (21.40)	36 (7.20)	108 (21.60)	248 (49.70)		
	Rarely	89 (18.30)	27 (5.50)	90 (18.50)	281 (57.70)		
	Total	293 (20.50)	84 (5.90)	301 (21.00)	753 (52.60)		
Male	Frequently	31 (23.70)	7 (5.30)	26 (19.80)	67 (51.10)	4.32	0.634
	Occasionally	26 (16.60)	14 (8.90)	40 (25.50)	77 (49.00)		
	Rarely	31 (20.30)	10 (6.50)	37 (24.20)	75 (49.00)		
	Total	88 (20.00)	31 (7.00)	103 (23.40)	219 (49.70)		

Table 4.14: Prevalence of hepatitis according to ABO blood group and sex

		ABO Blood group					
		A	AB	B	O	χ^2	p
	Prevalence	n (%)	n (%)	n (%)	n (%)		
All	Negative	374 (20.40)	112 (6.10)	399 (21.70)	951 (51.80)	1.60	0.660
	Positive	7 (19.40)	3 (8.30)	5 (13.90)	21 (58.30)		
	Total	381 (20.40)	115 (6.10)	404 (21.60)	972 (51.90)		
Female	Negative	286 (20.40)	84 (6.00)	297 (21.20)	733 (52.40)	3.78	0.287
	Positive	7 (22.60)	-	4 (12.90)	20 (64.50)		
	Total	293 (20.50)	84 (5.90)	301 (21.00)	753 (52.60)		
Male	Negative	88 (20.20)	28 (6.40)	102 (23.40)	218 (50.00)	22.12	0.001
	Positive	-	3 (60.00)	1 (20.00)	1 (20.00)		
	Total	88 (20.00)	31 (7.00)	103 (23.40)	219 (49.70)		

Table 4.15 shows association of academic performance to genotypic allele. More number of genotype AA subject have higher percentage performance in the general population . however chi square test shows no significant association between genotype and academic performance with $\chi2 = 4.01$, p = 0.405

The result in Table 4.16 reveals that there is significant association between blood group and academic performance $\chi2 = 28.24$, P=0.001 in the general population but when comparism is made in both male and female, however, the female subjects shows more statistical significance than their male counterparts $\chi2 = 21.47$, P=0.002 and $\chi2 = 0.60$, P= 0.197 respectively. Blood group O shows higher percentage performance with respect to those with fair and good performances while AB has least percentages O (54.40% and 47.50%) and AB (7.20% and 5.80%) respectively.

Table 4.15: Comparison of academic performance according to genotypic alleles

	All			Female			Male		
	AA	AS	SS	AA	AS	SS	AA	AS	SS
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Good	299 (57.70)	216 (41.70)	3 (0.60)	225 (59.70)	151 (40.10)	1 (0.30)	74 (52.50)	65 (46.10)	2 (1.40)
Fair	435 (61.00)	274 (38.40)	4 (0.60)	335 (60.60)	216 (39.10)	2 (0.40)	100 (62.50)	58 (36.20)	2 (1.20)
Poor	361 (56.30)	278 (43.40)	2 (0.30)	276 (55.10)	224 (44.70)	1 (0.20)	85 (60.70)	54 (38.60)	1 (0.70)
Total	1095 (58.50)	768 (41.00)	9 (0.50)	836 (58.40)	591 (41.30)	4 (0.30)	259 (58.70)	177 (40.10)	5 (1.10)
	$\chi2 = 4.01$, $p = 0.405$			$\chi^2 = 3.97$, $p = 0.410$			$\chi^2 = 3.69$, $p = 0.449$		

Table 4.16: Comparison of academic performance according to ABO blood group alleles

		ABO Blood group					
		A	AB	B	O	χ^2	p
	Performance	n (%)	n (%)	n (%)	n (%)		
All	Fair	115 (16.10)	51 (7.20)	159 (22.30)	388 (54.40)	28.24	0.001
	Good	144 (27.80)	30 (5.80)	98 (18.90)	246 (47.50)		
	Poor	122 (19.00)	34 (5.30)	147 (22.90)	338 (52.70)		
	Total	381 (20.40)	115 (6.10)	404 (21.60)	972 (51.90)		
Female	Fair	90 (16.30)	39 (7.10)	117 (21.20)	307 (55.50)	21.47	0.002
	Good	105 (27.90)	20 (5.30)	69 (18.30)	183 (48.50)		
	Poor	98 (19.60)	25 (5.00)	115 (23.00)	263 (52.50)		
	Total	293 (20.50)	84 (5.90)	301 (21.00)	753 (52.60)		
Male	Fair	25 (15.60)	12 (7.50)	42 (26.20)	81 (50.60)	8.60	0.197
	Good	39 (27.70)	10 (7.10)	29 (20.60)	63 (44.70)		
	Poor	24 (17.10)	9 (6.40)	32 (22.90)	75 (53.60)		
	Total	88 (20.00)	31 (7.00)	103 (23.40)	219 (49.70)		

Table 4.17 shows comparison between ABO blood groups with height. Blood group O shows average higher percentage tallness than others while blood group AB shows the least. However chi square test shows no significant association between blood groups with height $\chi^2 = 9.93$. p=0.128.

Table 4.18 shows comparison of ABO blood group with height, weight and BMI. The mean body weight, height and BMI of subjects in blood groups A, AB, B and O is not statistically significant.

Table 4.17: Comparison of ABO blood groups with height

	All				Females				Males			
Height	A n (%)	AB n (%)	B n (%)	O n (%)	A n (%)	AB n (%)	B n (%)	O n (%)	A n (%)	AB n (%)	B n (%)	O n (%)
Short	54 (22.10)	14 (5.70)	54 (22.10)	122 (50.00)	40 (21.60)	12 (6.50)	39 (21.10)	94 (50.80)	14 (23.70)	2 (3.40)	15 (25.40)	28 (47.50)
Average	305 (20.90)	86 (5.90)	309 (21.20)	760 (52.10)	242 (21.20)	62 (5.40)	237 (20.70)	603 (52.70)	63 (19.90)	24 (7.60)	72 (22.80)	157 (49.70)
Tall	20 (12.60)	15 (9.40)	41 (25.80)	83 (52.20)	11 (11.20)	10 (10.20)	25 (25.50)	52 (53.10)	9 (14.80)	5 (8.20)	16 (26.20)	31 (50.80)
Total	379 (20.30)	115 (6.20)	404 (21.70)	965 (51.80)	293 (20.50)	84 (5.90)	301 (21.10)	749 (52.50)	86 (19.70)	31 (7.10)	103 (23.60)	216 (49.50)
	$\chi^2 = 9.93$, $p = 0.128$				$\chi^2 = 9.20$, $p = 0.163$				$\chi^2 = 3.03$, $p = 0.805$			

Table 4.18: Comparison of ABO blood groups with weight, height and BMI

	Females				Males					
	A	AB	B	O	A	AB	B	O		
Variables	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	p
Weight (kg)	62.88 ± 3.34	62.12 ± 5.97	61.03 ± 5.50	61.90 ± 2.14	61.38 ± 5.90	64.90 ± 4.63	62.56 ± 7.08	61.93 ± 5.35	0.31	0.824
Height (cm)	159.70 ± 5.27	161.32 ± 6.05	159.82 ± 5.51	160.03 ± 5.39	160.07 ± 5.95	161.26 ± 6.97	161.46 ± 7.04	160.92 ± 6.55	0.21	0.097
BMI (kg/m^2)	.69 ± 13.39	23.89 ± 2.11	23.92 ± 2.08	24.18 ± 7.93	23.97 ± 2.09	25.05 ± 2.41	24.01 ± 2.39	23.96 ± 2.08	0.43	0.732

CHAPTER FIVE

5.0 DISCUSSION

5.1 Distribution of ABO Blood Groups

ABO and Rh genes and phenotypes are not equally distributed (Adeyemo and Soboyejo, 2006; Akinnuga, 2011) .There are wide variations in racial distribution of ABO antigens despite the fact that the antigens involved are stable throughout life (Enosolease and Bazuaye, 2008; Rai *et al.,* 2009; Chima *et al.,* 2012).

The study of distribution of blood groups is important as it plays a vital role in genetics, blood transfusion, organ transplantation, genetic research, human evolution (Harvey and David, 2005), forensic pathology and some groups have shown associations with diseases like duodenal ulcer (Ziegler *et al.,* 2004), diabetes mellitus (Akhta *et al.,* 2003), urinary tract infection (Qureshi and Bhatti, 2003) and Rh and ABO incompatibilities of newborn (Mourant *et al.*, 1978). Forensic application of blood group studies is of great value in detection of crime (Eweidah *et al.,* 2011).

In Caucasian, prevalence of 46%, 42%, 9%, and 3 % (Mourant *et al.,* 1976; Adeyemo and Soboyejo, 2006) were reported compared to 57%, 20.5%, 21.6%, and 1.4% for O, A, B, and AB in Blacks (Iyawe *et al.,* 1999). The resultant polymorphism remains important in population genetic studies, estimating the availability of compatible blood, evaluating the probability of haemolytic disease in the new born, resolving disputes in paternity/maternity and for forensic purposes (Enosolease and Bazuaye, 2008). The present study is, therefore, useful in providing information on the status of ABO and Rh blood group distribution in Kogi State of Nigeria using Students of Kogi State origin studying Nursing Sciences in College of Nursing and Midwifery, Obangede as subjects.

In the present study of Distribution of ABO and Rh-D blood groups among Students of Kogi State College of Nursing and Midwifery, Obangede, the order of ABO percentage frequencies are O>B>A>AB.

The group 'O' (51.80 %) is the most frequently encountered phenotype. This observation is in accordance with previous reports from other parts of Nigeria. In Southern Nigeria, Worlledge *et al.,* (1974) reported Binis and Yorubas to have group 'O' frequency of 57.7% and 51.1%, respectively, Bakare *et al.,* (2006) reported 50% in Lagos while in the Northern part, Ahmed and Obi (1998) reported it as 52%. The present study is equally not too different from earlier reports from other parts of Nigeria with blood group O prevalence of 56.3% in Port Harcourt (South-South) and 47.1% in Jos (North-Central Nigeria (Worlledge *et al.,* 1974*).* It is also similar to reports from the South-Western part among the Yorubas (Iyiola *et al.,* 2012), South-East among the Igbos and from Kaduna (North West) among the predominantly Hausa population (Ahmed and Obi, 1998) while in India blood group O is equally reported to be more predominant having frequency of 45% (Periyavan, *et al.* 2010; Krishnakanth, *et al.,* 2012).

With regard to the other phenotypes of ABO blood groups, the frequency of group 'A' as 379 (20.3%) in the present study is in proximity to the reported frequency of 24.9% for group 'A' among the Binis (Worlledge *et al.,* 1974). A slightly higher frequency for group A (27%) was reported in the North-East region of Nigeria (Ahmed and Obi, 1998). The little discrepancy between the findings of the present study and that reported by Worlledge *et al*. (1974) and Ahmed and Obi (1998) may be attributed to the ethnic difference among the population of Nigeria, due to the smaller sample size of the Binis as against a relatively larger sample size in the present study which cuts across the entire ethnic groups that constituted Kogi State. The scope of the present study has same

grounds with the report on the Yorubas, which was based on a large population with the frequency for groups ‘A’ and ‘B’ reported as 21.3% and 23.3%, respectively (Worlledge *et al., 1974*). More also the frequency of group ‘B’ is 404 (21.70%), while ‘AB’ group was the least encountered phenotype with a frequency of 115 (6.20%) among the samples studied.

Blood group B (21.7%) was slightly more prevalent than blood group A (20.3%) in the present study. This closely support earlier report of 24.6% in Port Harcourt, 20.5% in Lagos and 20.7% in Kano where blood group B was also found to be the second most prevalent in the population (Ojo, *et al.,* 1987; Iyawe, *et al.,* 1999; Chima, *et al.,* 2012) and in other parts of the world among the Desuri Reddis of Chittoor District, Andhra Pradesh (Reddy and Sudha, 2009) and among the Rajputs and Warlis of Naroli Village Panchayat, india (Meitei and Kshatriya, 2009). While report from iran indicate that blood group O predominates (37.6%) in the general Iranian population, followed by group A (30.2%), group B (24.4%) and group AB (7.8%) (Amirzadegan *et al*., 2006)

A similarity in the distribution of ABO phenotypes reported in North American Blacks in 1915 with that of the Nigerian population may indicate influencing factors such as genetic drift and ancestral link with the people of Nigeria through the Trans-Atlantic trade because of the proximity to ocean between these countries (Enosolease and Bazuaye, 2008).

This is slightly different from the study by Bakare, *et al.* (2006) who reported phenotypic frequencies of 50% for O, 22.9% for A, 21.3% for B and 5.9% for AB among 7653 individuals sampled in Ogbomoso, South-West, Nigeria.

However, other findings in Nigeria (Kulkarni, *et al*., 1985; Onwukeme, 1990; Korubo-Owiye, 1994; Mathew and Godwin, 2008) and reports from Caucasian population show that blood group A is the next most common after O while blacks show B as next to O. The result from the present study is in tune with the latter. However more research is still required on ethnic percentage distribution of ABO blood group phenotype, especially in North Central part of the country.

On the predominance of blood group O over other blood groups in the population sampled, the present study is strongly of the opinion that O alleles may be predominant in Kogi State for the fact that it is dominated mostly by muslim whose belief encourages consanguineous marriages and also almost 95% of the entire citizen irrespective of religious background still imbibe traditional cultural practices where that type of marriage is still very much been practiced.

More also the study equally agreed with the suggestion of Bakare, *et al.* (2006), that predominance of O allele may also be as a result of the fact that many A's and B's may have been heterozygous carrying O allele silently thereby maintaining O allele in the heterozygous population.

From the findings of the present study, it is evident that the proportion and gene frequencies of individuals belonging to blood group O in the studied population was most predominant. This is consistent with previous reports from the studies conducted among Nigeria population and African countries. The implication of this finding is that blood type O is the most readily available blood group in the Nigerian population which is more advantageous for the population in the event of blood transfusion.

The higher proportion of blood group O in the studied population is also an advantage because Nigeria is a malaria endemic country and so therefore individuals belonging to

blood group O may be protected from severe malaria attack due to the mechanism of reduced rosetting (Iyiola *et al*., 2012).

The low frequency of blood group AB and O -ve reported in the present study and other studies among Nigerian population indicate the high risk individuals in that category of blood groups are likely to encountered should they required blood transfusion as scarcity of it may be experienced. Hence individuals of blood group AB and O -ve should be a willing donor to blood banks across the state and Nigeria at large so as to make available blood to others in that category when in need, however the higher frequency of blood group O will be of relieve in terms of the scarcity.

5.2 Distribution of Rh (D) Blood Group

With respect to Rhesus blood grouping system, the present shows that the frequency of Rh-D positive antigen was detected in 1755 (94.20%) subjects while Rh-D negative phenotype was found in108 (5.80%) subjects in the total sample size of 1863 studied. This is similar to previous studies done at Ibadan and Abraka in Nigeria (Onwukeme, 1995, Kotila *et al.,* 2005). This rate shows a low frequency of Rh D negative Rh D blood group system in this environment (Okeke *et al.,* 2012). The allelic frequencies were 0.78 and 0.22 for D and d alleles respectively. The finding is in agreement with Ekanem (2006) and Chima, *et al*., (2012) who reported Rhesus D positive to be the highest with 96.68% and 94.8% while Rhesus D Negative was the lowest with 3.30% and 5.2% respectively. Again this findings are consistent with report from previous similar studies among different sets of Nigerian population where the Rh(D) positive was found to be higher in the population sampled than the Rh (D) negative (Kulkarni *et al*., 1985; Ahmed and Obi, 1998; Omotade *et al.,* 1999; Bakare *et al.,* 2006; Adeyemo and Soboyejo, 2006).

The frequency of Rh-D phenotype among the samples studied with respect to the ABO blood groups. Rh-D negative was common to the blood group 'O' 48 (6.41%) of all blood groups as compared to blood group AB of only 2 (2.38%). The Rh group negativity for blood groups A and B were close 17 (5.80% and 16 (5.32%) respectively. This is in agreement with the similar study carried out in Benin of Niger Delta Area of Nigeria by Enosolease and Bazuaye (2008).

However, the present study reveals no association between Rh status and ABO blood group. The present study shows that the frequency of Rh-D negative phenotype is close to those reported among the Yorubas 5.46% (Worlledge *et al.,* 1974) and in Enugu South Eastern Nigeria 4.5% (Okeke, *et al.,* 2012) but differs greatly than that reported from North-Eastern Nigeria (1.44%). Moreover, this shows ethnic variability among the Nigerians. A reported frequency of around 5.1% of Rh-D negative phenotype from India (Raj and Reddy, 2010) is close to the one reported in the present series and in sharp contrast to the frequency of 15% Rh-D negative phenotype reported in the people of European origin (Mourant *et al*., 1976) While some studies documented Rh-positive as 95% in African-Americans, 100% in Africans and Rh negative as 5.5% in South India, 5% in Nairobi, 7.3% in Lahore, 4.8% in Nigeria (Mwangi, 1999; Omotade *et al*., 1999; Meitei and Kshatriya, 2009) .

In present study, there was no significant relationship between sex and ABO and Rh blood groups. However, chi square test showed that is no significant relationship between ABO blood groups and ethnicity. This is confirmed that ABO and Rh antigenic structure differs between regions and nations (Lialiaris, *et al.,* 2010).

5.3 Genotypic Distribution

Haemoglobin genotypes and blood groups are all inherited blood characters. The inherited disorders of haemoglobin are the most common gene disorders with 7% of the world's population being carriers (Jeremiah, 2006).

The prevalence of HbSS among the black population in the United States, was reported to be 9% and 30 – 40% generally for Africans (Richard, 1975; Lewis, 1970). In another report, the geographical distribution of sickle cell anaemia (SS) was given as follows: 3 – 9% for USA black Americans, 1 – 8% for USA whites, 3 – 7% for Europe (UK, Pakistanis - Blacks), 2 – 8% for other European countries (Mediterranean), 1 – 3% for Caribbeans, 1 – 3% for Middle East, 1 – 10% for Africa. The frequency of sickle cell trait (AS) was equally reported as follows: 8 – 16% for USA blacks, 8 – 10% USA (whites), 6 – 15% for Europe (UK, Pakistanis - Blacks), 1 – 15% for Europe (Mediterranean), 3 – 8% for Caribbeans, 7 – 8% for Middle East, 15 – 30.5% for Africa and 40.5% for West Africa and Nigeria (Sinou, 2003).

The frequency of HbAA was 1095 (58.50%) while HbAS was found to be 768 (41.0%). HbSS occur in only 9 (0.50%) subjects among the 1863 in this study thus presenting a wide variation from previous published reports. However, this study is in agreement with a study carried out in Kenya, East Africa, where the prevalence of HbSS was almost zero, a value of 74% and 97% for HbAA in lowland and highland areas and 26% and 3% for HbAS in lowland and highland areas respectively were obtained in that study (Moormann *et al.,* 2003). The zero frequencies observed in those studies and 0.5% in the present study possibly implies that the sickling gene pool is gradually reducing in our African population. It may not mean a complete absence because there are other published reports in Nigeria that carry 3.0% HbSS in the South-West region of Nigeria (Bakare *et al.,*

2004). Another paper also reported 4% HbSS in parts of South-South region of Nigeria (Nwafor *et al*., 2001).

The low prevalence of HbSS in the study population could be attributed to increased awareness of the disease, improved socio-economic conditions and other environmental and genetic factor which have an overall effect on the sickling gene pool. It is equally possible that the Hardy - Weinberg equilibrium must have been disturbed which has led to more people acquiring normal haemoglobin gene and sickle cell trait while the homozygous sickle cell gene is gradually tending to zero. It could also be due to the location where the study is carried out. It is possible that in an institution of higher learning especially those that teaches medical education with expected much stressful studying and practical activities (such as College of Nursing and Midwifery, Obangede) very few of individuals with HbSS would seek for and/or qualify for admission, their primary and secondary education having probably been disturbed by frequent illness. Also a high proportion of all HbS homozygotes in Africa die in infancy of sicke cell anaemia (SCA) (Cabannes *et al.,* 1987). The observed frequency 1095 (58.50%) of HbAA is within the normal range of 55 – 75% earlier reported for Black (Fleming and Lehman, 1982) . The frequency of HbAS 768 (41.00%) in this study is slightly above 20 – 30% quoted for Nigeria and corresponds to the value, 20 – 40% reported for Africa in general (Reid and Famodu 1988; Sinou, 2003; Fleming and Lehman, 1982).

5.4 The ABO Blood Groups and Disease

5.4.1 ABO blood groups, genotypes and malaria

Many authors reported that ABO blood group confers selective advantage to malaria through a mechanism of rossetting (Loscertales *et al.,* 2007; Fry *et al.,* 2008; Panda *et al.,* 2011). An association between 'O' blood group and lower rosetting capacity has been

demonstrated (Uneke, 2007). However, rosetting capacities of blood group 'A', 'B' or 'AB' have remained controversial (Panda *et al*., 2011). In 2007, Cserti and Dzik (2007) published a review article critically analyzing the literature that reported the association/non association between ABO and P falciparum malaria. Based on their analysis, they proposed a biological model emphasizing the role of ABO in cytoadhesion (Yamamoto *et al*., 2012). The microcirculatory obstruction by cytoadhesion may result in reduced oxygen and substrate supply (Loscertales *et al*., 2007; Panda *et al*., 2011; Yamamoto *et al.,* 2012).

In the present study Subjects with blood group O reported frequent incidence of malaria while subjects with blood group AB reported less frequent incidence of malaria for female and male subjects. Hence, the present finding seems to deviate from the hypothesis about a selective (survival) evolutionary advantage of *P. falciparum* infection on blood group O cells compared with other blood group types (A, B or AB) in areas where malaria is endemic (Tewodros *et al.,* 2011) . Also Carlson (1993) and Kun *et al.* (1998) who reported that differences in rosetting ability were also observed between RBCs of different ABO blood groups, with a diminished rosetting potential in blood group O RBCs. The ability of RBCs from healthy donors to form rosettes appeared to be greater in cells from group A or B than those of group O patients (Barragan *et al.,* 2000).

The overall higher percentage of blood group O 51.8 % in relation to other blood groups taking more than half of the study population might probably be responsible for the higher frequencies observed to have been recorded in association to various traits, the participant tends to skew towards O and hence the result obtained.

The mutation giving rise to sickle cell disease (SCD; HbS) may have arisen at 3 different sites in Africa (Atlantic West Africa, Central West Africa, and Bantu-speaking Central

and Southern Africa) with expansion of the mutation occurring 2000 to 2500 years ago (Nagel and Steinberg, 2001). In this case, patients who inherit an HbS gene from both parents have SCD, whereas those who are heterozygous inheriting the HbS gene from 1 parent and the normal HbA gene from the other parent have substantial protection against malaria. A similar protective effect for the heterozygote seems likely in South East Asia, where HbE is very common and red cells from patients of genotype HbAE are markedly less susceptible to malaria parasite invasion in vitro (Chotivanich *et al.,* 2002).

Jallow *et al.* (2009) reported the GWAS study on severe malaria. It identified hemoglobin-β (HBB) and 18 other loci exhibiting significant association with the threshold of $P < 10^{-4}$. The peak of the signal at HBB coincided precisely at the position of the S-hemoglobin (a variant form of hemoglobin found in people with sickle cell disease) causal variant. However, no SNPs at the ABO locus were associated by the GWAS study (Yamamoto *et al.,* 2012).

Looking at the association of genotypic alleles with malaria, in the present study, the incidence of malaria fever is higher among subjects with AA genotype followed by AS subjects. This is in agreement with the earlier report by Anstee (2010) that those who are heterozygous inheriting the HbS gene from 1 parent and the normal HbA (AS) gene from the other parent have substantial protection against malaria (Anstee, 2010). The most probable mechanism by which Hb AS persons are protected against *P. falciparum* is that oxygen consumption by the parasite in the red cell causes sickling, followed by phagocytosis, thus breaking the malaria cycle (Fleming and Lehman, 1982).

Motulsky (1964) reported that falciparum malaria confers a selective advantage on G6PD deficiency as well as sickle cell haemoglobin trait. The geographic distribution of G6PD deficiency led many workers to investigate and propagate the hypothesis that G6PD

deficiency is a polymorphism that confers resistance to infection with *falciparum* malaria (Motulsky, 1964) and this being known as G6PD/ malaria hypothesis (Sharma *et al*., 2010). On the other hand, absence of association between ABO system and malaria infection was also observed in other studies (Tewodros *et al.,* 2011).

Also, in contrast to our observation, Rowe *et al.,* (1997) documented absence of difference in the frequency of ABO blood groups between healthy controls and those with uncomplicated malaria, suggesting insignificant effect of the ABO blood groups on uncomplicated clinical malaria disease (Tewodros *et al.,* 2011).

5.4.2 ABO blood groups and hepatitis

Result from the present study showed that there is statistical significant association between blood group with the incidence of hepatitis among male subjects (χ^2 = 22.12, P = 0.001) while Female subjects did not show statistical significant interaction between their blood groups and the occurrence of hepatitis (χ^2 = 3.78, p = 0.287). Meanwhile the overall result obtained for both female and male subjects, most of the subjects reacted negatively to the antigen. The results also showed that the pattern in which the subjects reacted negatively to the antigen is in the order ABO*O > ABO*B > ABO*A > ABO*AB for both male and female subjects.

Although there are small studies in literature about association between ABO blood groups and chronic viral hepatitis, only few studies found relation between fibrosis severity in chronic viral hepatitis C (Armelle *et al.,* 2006). The overall result of the present study agreed with reports of the previous findings that reported blood group O to be more resistant to hepatitis than other blood groups (Mathew and Chan 1979, Erin *et al.,* 2008, Naeini *et al*., 2010). Acute viral hepatitis in some patients may progress to a state of chronic hepatitis with aggressive changes; a significant reduction of blood group

O patients has been reported and seems such patients are more resistant to the possibly dangerous sequelae of acute viral hepatitis (Erin *et al.,* 2008).

In one study by Mathew's, it also revealed that group O patients were more resistant to dangerous sequelae of acute viral hepatitis (Mathew and Chan 1979), In another study about ABO blood groups distribution in serum hepatitis (Hepatitis B) a disproportionate excess of blood group O was found in an outbreak of hepatitis B among patients and staff of a Hemodialysis Unit and more severe cases were also mostly of group O (Naeini *et al.,* 2010).

5.5 ABO Blood Groups and Body Mass Index (BMI)

In the present study, height, weight and BMI were not associated with ABO blood groups. This is in agreement with several studies which reported that, there was no association between anthropometric measures and ABO blood groups (Mascie-Taylor and Lasker, 1990; Lutfullah *et al.,* 2010, Jafari *et al.,* 2012). However a few studies have shown an association between anthropometric measures and ABO blood groups.

In one study conducted among 898 young men, blood group B (B, AB) subjects were taller than non-B (A, O) subjects (Borecki *et al.,* 1985). In another study on Brazilian infants, weights of females with blood group A were significantly more than other blood types. This difference was not found among male infants (Kelso 1992). In another study by Krishnakanth *et al.,* (2012) titled "Correlation between obesity and ABO Blood Group in School going Children in India." when data for boys and girls were separately analyzed they found overweight and obesity to be more prevalent in girls (19%) than boys (14%). The result of the present study therefore do not support the generalizability of a positive association of the presence of the B antigen with height suggested by Borecki *et al.* (1985) and Kark *et al.* (1986).

5.6 ABO Blood Groups with Age and Sex

The results of the present study failed to show any association of ABO blood group with age, sex**.** Mean age was found to be almost similar in different ABO blood group and sex in the present study. Similar results were also seen in other studies (Amirzadegan *et al.*, 2006; Abdollahi *et al*., 2009).

In table 4.4 of the present study, it was found out that Sex distribution have no significant association with blood group with P= 0.105 for males and P = 0.378 , this was also observed by Abdollahi *et al*. (2009) and by another study which was done in Pakistan (Khattak *et al*., 2008) in which they reported that there seems not to be any noticeable relationship between Sex and ABO blood group.

5.7 ABO Blood Group and Intelligence

Intelligence is an abstract word and hypothetical formation which does not refer to something tangible owned by the individual. And consequently it cannot be noticed directly, and therefore cannot be measured directly. But inference is made in its effects and results through mental tests. According to this, intelligence is the common denominator between mental processes and its connection with intelligence to varying degrees (Atoom, 2014).

The present study found that the blood group (O) received the highest average in the academic performance test, which taking into consideration those that are good and fairly good. And that the blood type (AB) was the lowest in the GPA in the midcourse and NPTS Exam usually used as ‘weeding’ examination in the college. This is in contrast with the work done by Atoom (2014) in six Jordanian Universities which shows that AB has the highest average CGPA while B has the lowest average CGPA.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Data from this study have provided information on the genetic variability and polymorphism of the blood group and rhesus antigens among the various ethnic groups that make up Kogi State, Nigeria. With a large sample used in this research, the result obtained shows that the ABO and Rh blood groups distribution pattern in Kogi State conforms with the general pattern across Nigeria and many parts of the world in which blood group O is most predominant while group AB is the lowest, however blood groups A and B shows close proximity. The result for RH blood group is also in conformity with the generally reported trend reported among African with RH positive being commonest. The frequencies of ABO and Rh blood groups however appeared to be stable and consistent with previous published data. The result indicates that there is association between ABO blood group/Genotype, malaria and intelligence. This information would be useful to the geneticists and to the clinicians especially in the planning of blood transfusion programmes since they play integral role of the genetic profile of the Nigerian population.

These reports clearly present the distribution and more importantly, the gene frequencies of the alleles controlling the ABO and Rh blood group system and haemoglobin variants for samples of the Nigerian population in Kogi State. Data obtained may serve as reference for other studies in this field. It may also be useful in the planning of blood transfusion programmes, since they are an integral part of the genetic profile of the Nigerian population.

The result from the present study shows there is a gradual decline in the prevalence of abnormal haemoglobin variants (HbSS) in our black population which could be traced to increase awareness among the people of the abnormality and hence guiding against the occurrence.

The present study is original in that, it is the first comprehensive study that documented the distribution of ABO and RHD blood groups among the different ethnic groups that make up Kogi State, Nigeria. This study could have significant implications for the major blood banks across Kogi State where certain blood groups are needed more than others in emergency conditions. Furthermore, the data generated in this study would be helpful to the researchers in the field of population genetic to explore the factors responsible for the observed distribution patterns of these genetic markers in this part of West Africa

In terms of association of ABO blood groups with certain traits, the present study reveals that there is association with diseases like malaria, hepatitis and academic performance of the subjects. There is also a weak association with BMI, height and weight.

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6.2 Recommendations

It is recommended that:

i. Further studies on this topic should be conducted due to its importance in the educational field and usefulness in the effective management of blood banks and in blood transfusion services, to geneticists, biologists, policy makers and clinicians.

ii. Studies should be carried out in the general population not limiting it to school environment in which some categories of people are naturally disadvantaged in gaining admission and coping with the college activities and hence not adequately represented.

iii. Studies should be carried out on all age groups to really ascertain if age has any association to the blood group

6.3 Contribution to Knowledge

The present study provides the initial data on the distribution of ABO and RH(D) blood groups in Kogi State, Nigeria and hence it will provide material and serve as reference point for further studies.

To the best of my knowledge,the present study is the first study to show association between blood groups and academic performance in Nigeria school with χ^2 = 28.24, p = 0.001.

The present study shows that there is association between ABO blood group and malaria infection with χ^2 = 129.29, p = 0.001. Blood group AB conferred more resistance and blood group O more susceptible to the disease.

The present study shows that there is association between ABO blood group and hepatitis B infection especially in males with $\chi^2 = 22.12$, $p = 0.001$ while in the general population, it is a weak association with $\chi^2 = 1.60$, $p = 0.660$

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APPENDIX I

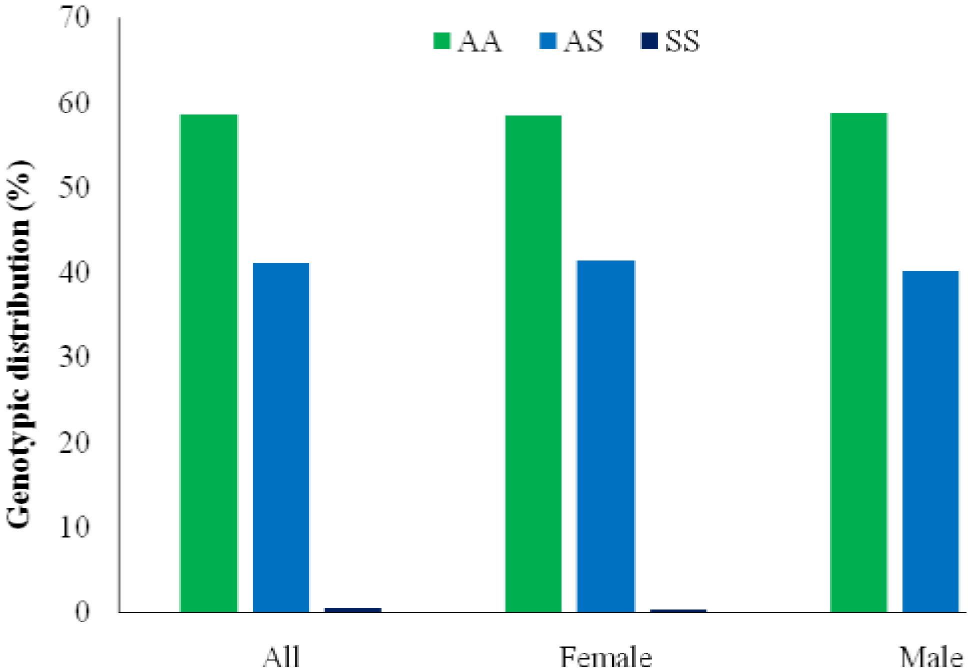

Fig. A1: Genotypic distribution characterized by sex and for all subjects. Normal subjects are higher in number than sickle cell trait carriers and patients across for both sexes and for all subjects. Chi square test does not show statistical significant association between genotype and sex for all, female and male subjects respectively (χ^2 = 11.53, p = 0.644, χ^2 = 14.79, p = 0.393 and 16.57, p = 0.280).

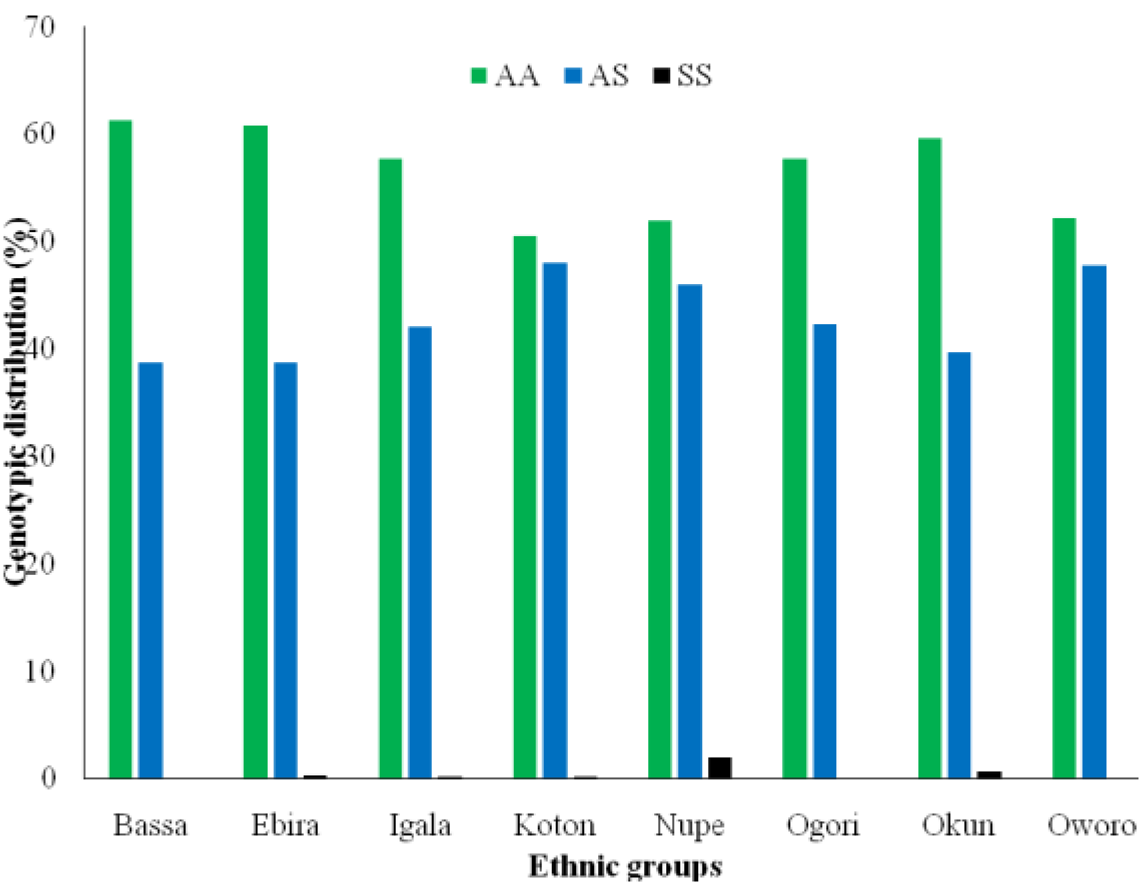

Fig.A2: Genotypic distribution characterized by ethnicity. Normal subjects are higher in number than sickle cell carriers and patients across for both sexes and for all subjects. Chi square test does not show statistical significant association between genotype and ethnicity ($\chi^2 = 11.53$, $p = 0.644$).

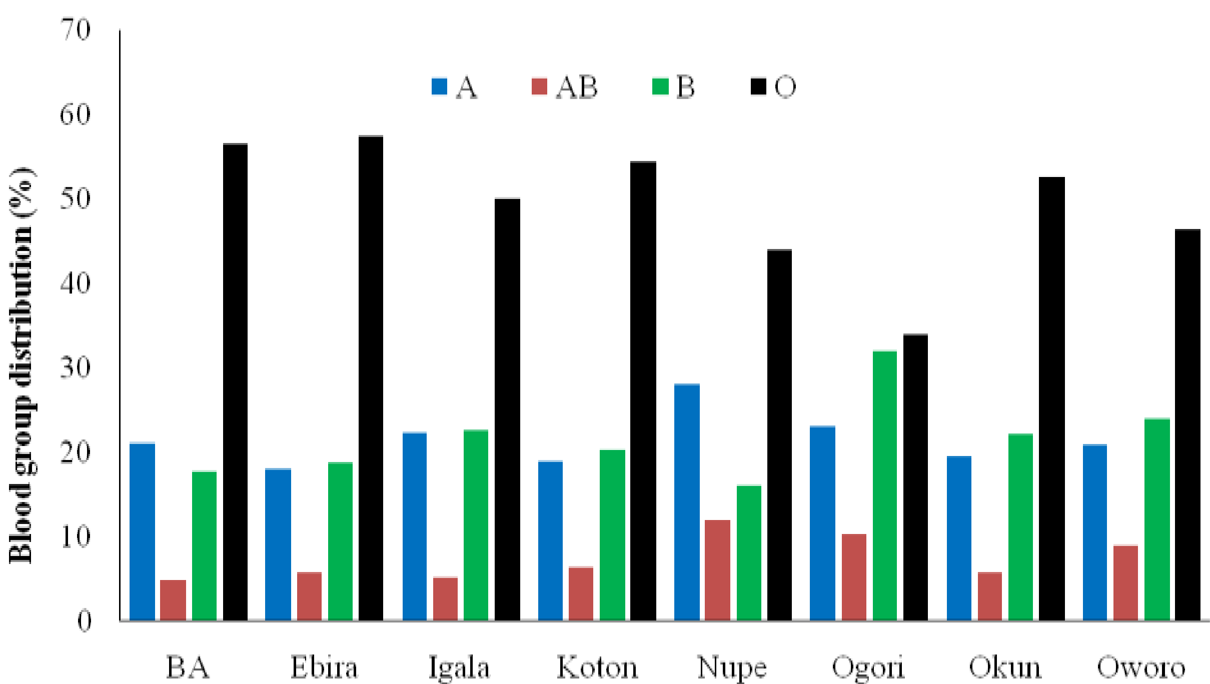

Fig A3: Comparison of ABO blood group across the ethnic groups. Overall frequency followed the order ABO*O>ABO*B>ABO*A>ABO*AB. However, chi square test did not indicate statistical significant association between blood group and ethnicity (χ^2 = 30.82, P = 0.08).

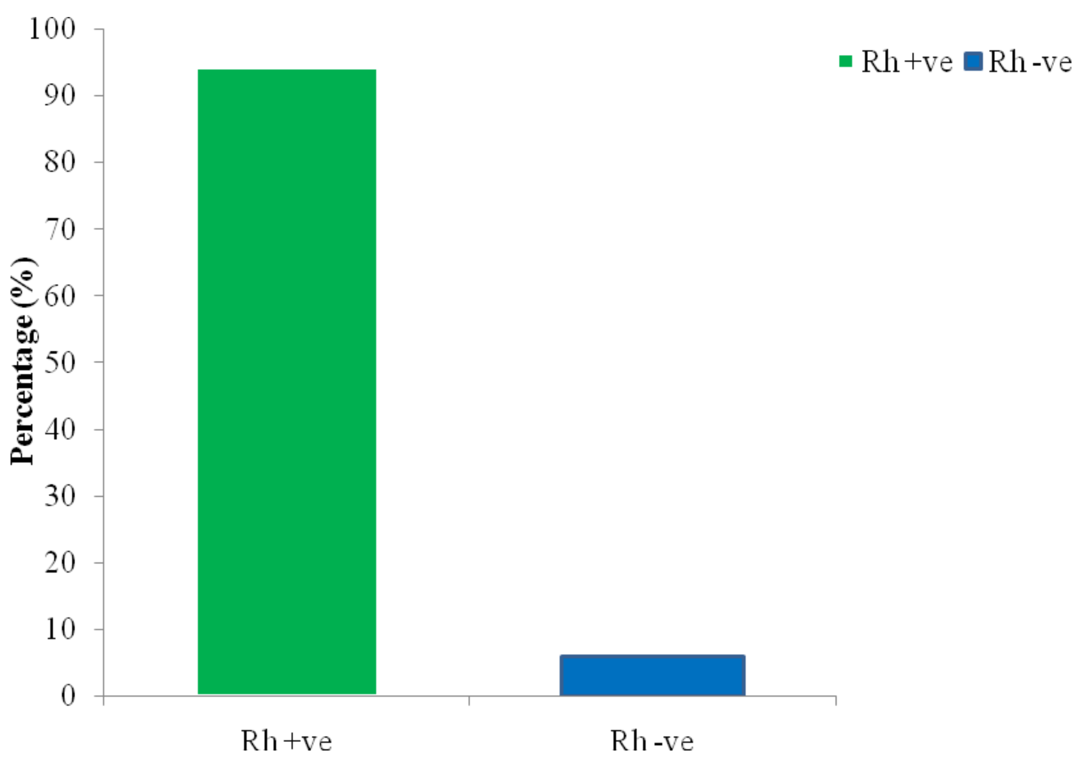

Fig. A4: Distribution of various Rh phenotypes among the samples studied (n=1863).

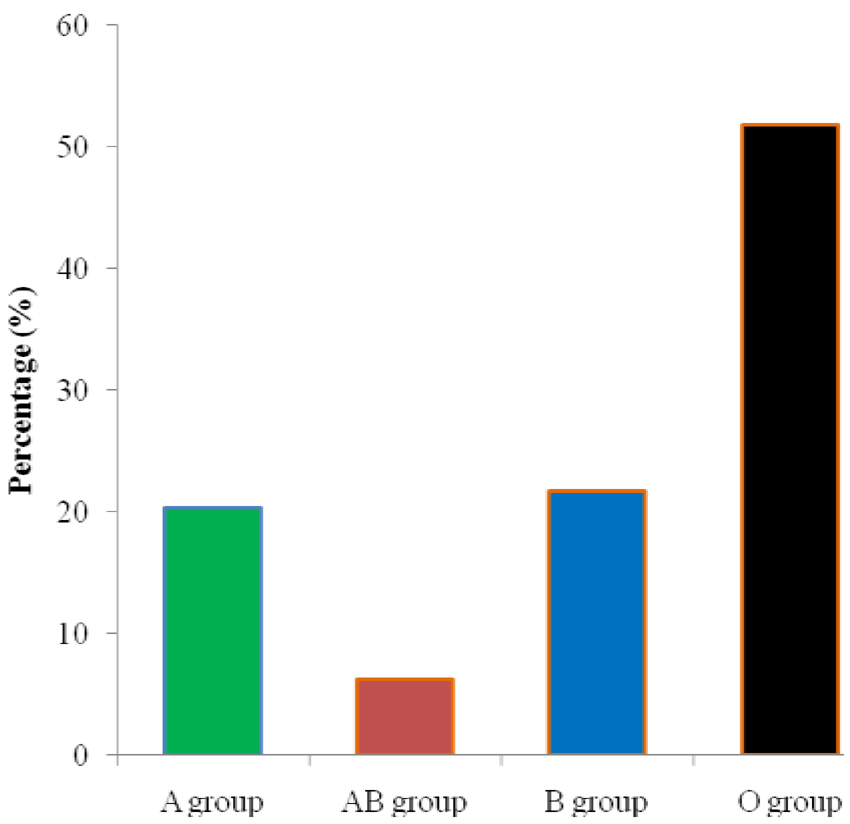

Fig. A5: Distribution of various ABO blood groups among the samples studied (n=1863). Overall frequency followed the order ABO*O>ABO*B>ABO*A>ABO*AB.

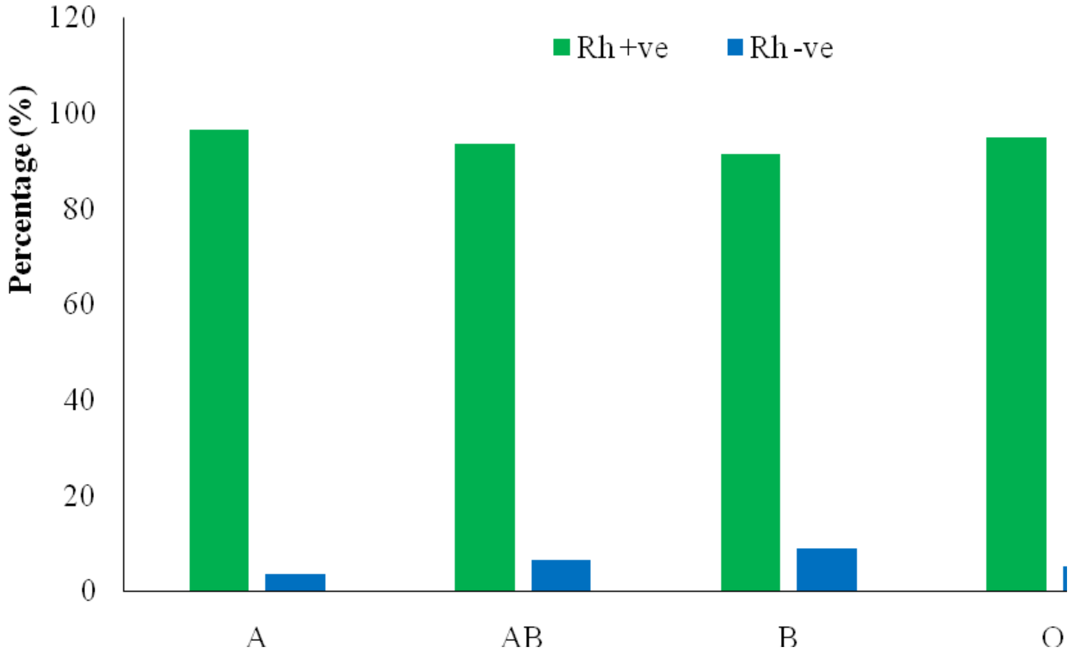

Fig. A6: Distribution of ABO blood groups with Rh factors for female subjects. Chi square test showed statistical significant association between blood groups A and AB with Rh factors (χ^2 = 19.80, P = 0.013 and χ^2 = 21.99, P = 0.003) respectively. Blood groups B and O did not show significant association with Rh factors (χ^2 = 3.71, 0.813 and χ^2 = 5.31, P = 0.622) respectively.

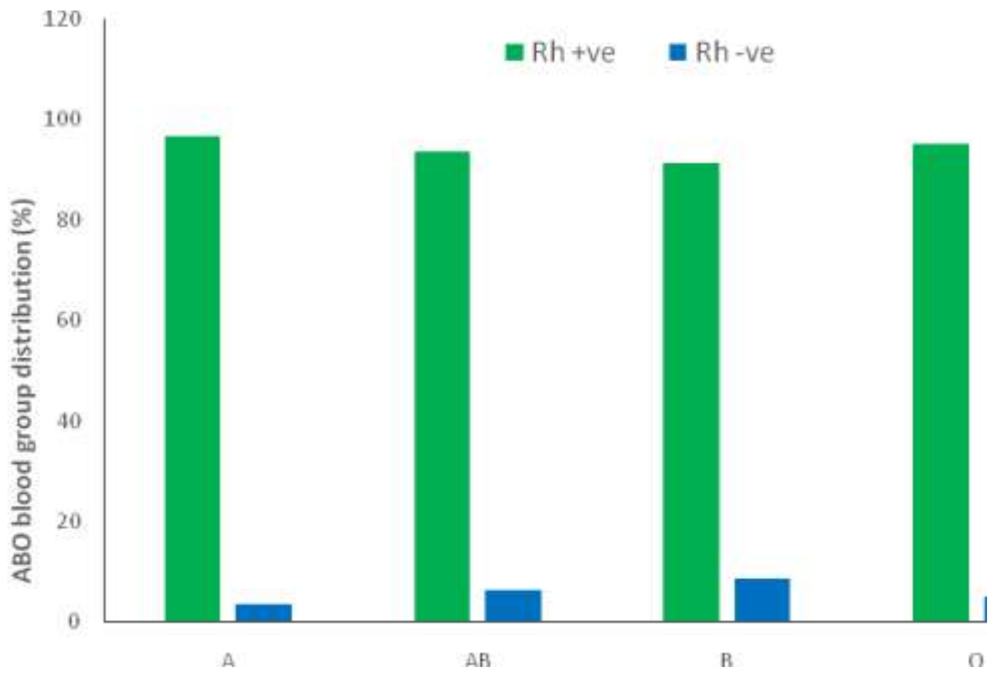

Fig. A7: Comparison of distribution of ABO blood groups with Rh factors for male subjects. Chi square test showed statistical significant association between blood groups AB with Rh factors (χ^2 = 14.43, P = 0.044). Blood groups A. B and O did not show significant association with Rh factors

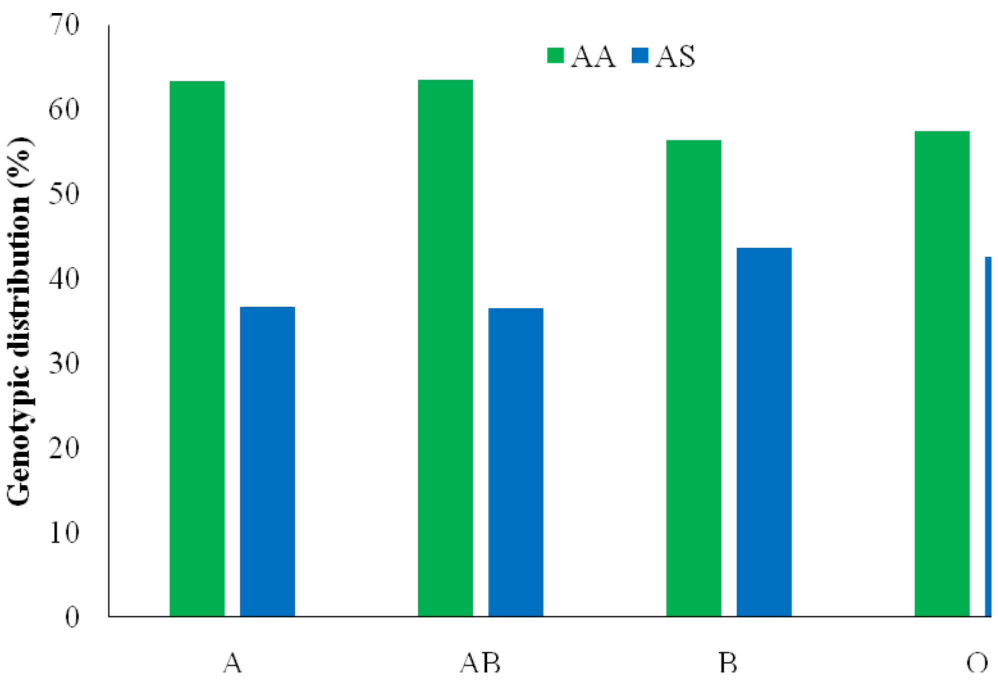

Fig. A8: Comparison of genotypes in different blood groups for all subjects. Chi square test did not indicate significant association between genotype and blood groups (χ^2 = 5.94, p = 0.114).

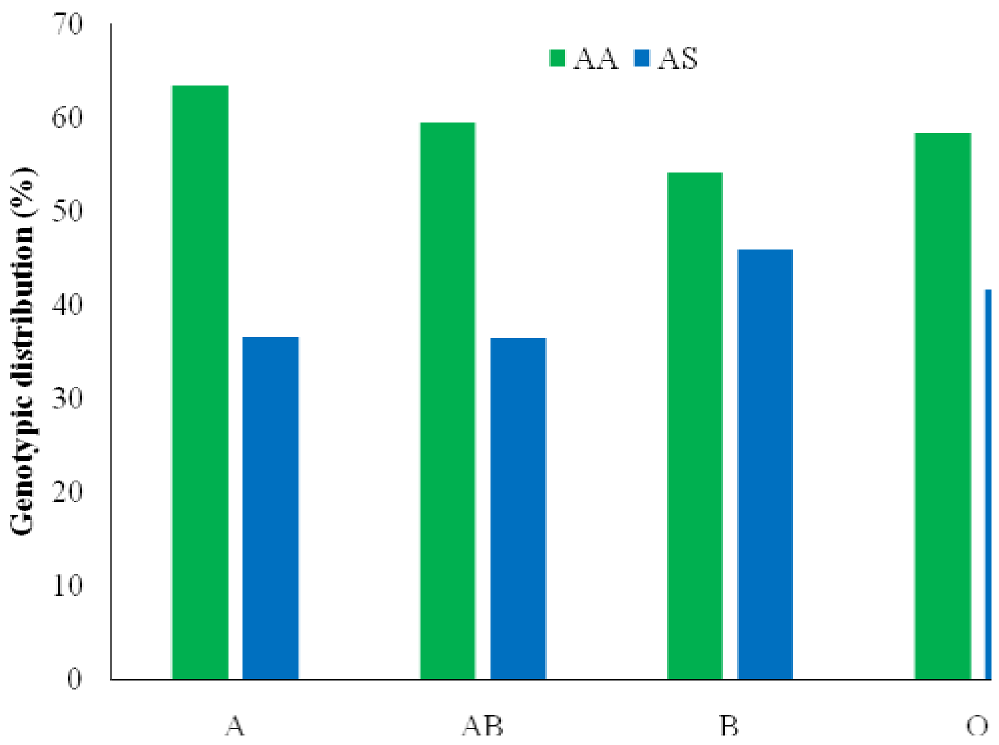

Fig. A9: Comparison of genotypes in different blood groups for Female subjects. Chi square test did not indicate significant association between genotype and blood groups $\chi^2 = 5.38$, $p = 0.146$.

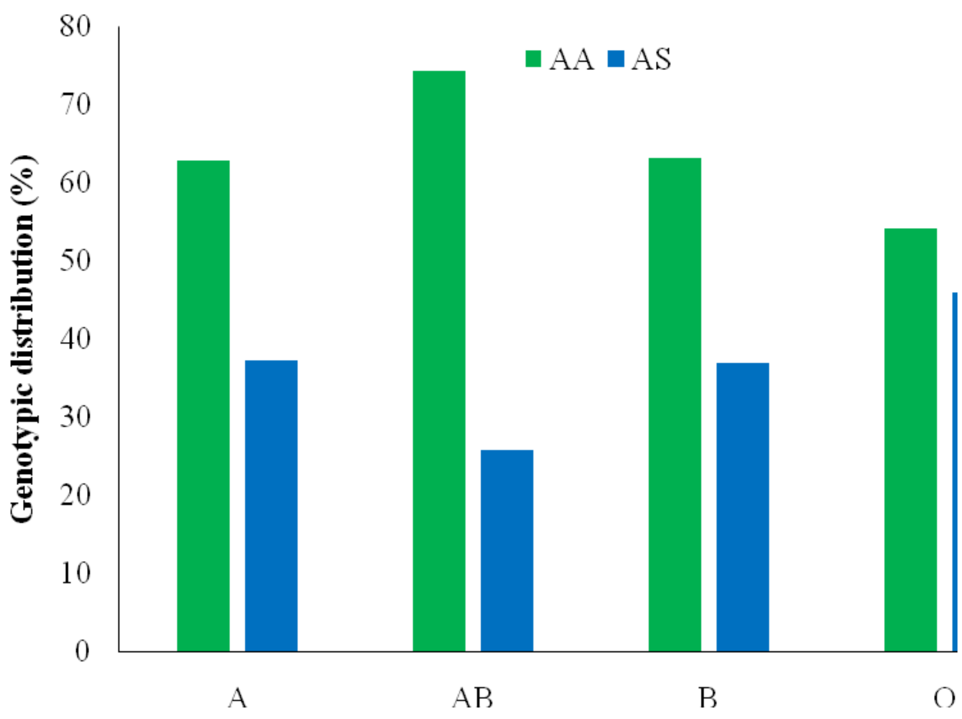

Fig. A10: Comparison of genotypes in different blood groups for Male subjects. Chi square test did not indicate significant association Between genotype and blood groups $\chi^2 = 6.26$, $p = 0.099$.

APPENDIX II

RECORD SHEET FOR RESEARCH PROJECT

DATA OF PARTICIPANT

- **Sex: ………………….** **Blood group…..……….**
- **L.G.A.: ………………** **Rh factor..……………..**
- **Tribe: ………………..** **Genotype………………**
- **Date of birth: ………..** **Height…………..………**
- **Age: ………………….** **Weight……….………..**
- **Season of birth………** **Birth order……………..**
- **Academic performance…………………...**
- **Blood disease association: Malaria…………**

  Typhoid................

  Hepatitis B.............

APPENDIX III

HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuthshika@yahoo.com website: www.abuth.org

Chairman of Board: Chief. Shuaib Oyedokun Afolabi Fnil

Chief Medical Director: Prof. Lawal Khalid, MBBS, FMCS, FWACS, FRCS(ED) mni

Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, MBBS, FWACP, FICS

Director of Administration: Barr. Ishak Bello, LL.B, BL., LL.M, PGDM, AHAN, FCAI

Our Ref: ABUTH/HREC/TRG/36

Date: 26th February, 2015

Your Ref: ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

RE: *"ABO and Rh (D) Blood Groups Distribution and Associated Traits among Students of Kogi State College of Nursing and Midwifery Obangede ."*

ABUTH Ethics Committee assigned number: - ABUTH/HREC/M40/2015

Name of the principal Investigator: - Abdulganiyu Adeiza Aliyu

Address of the Principal Investigator: - Department of Human Anatomy

Faculty of Medicine

A.B.U. – Zaria.

Date of receipt of valid application: - 13/02/15

Date of meeting when final determination

On ethical approval was made: - 26th February, 2015

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and ***given full approval by the ABUTH Ethics Committee.***

Please note: this approval dates from **26th February, *2015* to26th February, *2016*.** No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

Prof. Aisha.I.Mamman