FREQUENCY OF ABO AND Rh BLOOD GROUP ALLELES AMONG OROMO, AMHARA AND WOLAYITA ETHNIC GROUP STUDENTS IN ROBE SECONDARY AND PREPARATORY AND ZEBELA PRIMARY SCHOOL, BALE ZONE, ETHIOPIA

M.Sc. Thesis

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October, 2013
Haramaya University

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A Thesis Submitted to Department of Biology,

School of Graduate Studies, Haramaya University

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN GENETICS

By

Nigusu Girma

October, 2013

Haramaya University

SCHOOL OF GRADUATE STUDIES
HARAMAYA UNIVERSITY

As *Thesis* Research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Nigusu Girma, entitled **Frequency Of ABO and Rh Blood Group Alleles Among Oromo, Amhara and Wolayita Ethnic Group Students in Robe Secondary and Preparatory and Zebela Primary Schools in Bale Zone, Ethiopia.** We recommend that it can be submitted as fulfillment of the *Thesis* requirement.

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DEDICATION

To all my family, whose advice and support have given me the strength to take on the challenges that life presents.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author was born to his father, Girma Negewo, and his mother, Mebirat Mammo, on October 17, 1980 in Oromia Region, Bale Zone, Ginnir Woreda, Jafera Huluko Kebele, Ethiopia. He attended his elementary education in Jafera Hulluko Primary School, his junior education at Dello Sebiro Junior School and his secondary education at Ginnir Senior Secondary school in Ginnir town. Then, he joined Addis Ababa University, Science Faculty, and graduated with B.Sc. degree in Biology in 2001.

After graduation, he taught Biology and Chemistry in Dinsho Secondary School. He also taught professional science at 2020 Open Crivate college. Then after, he served as Head of Sinana Woreda Education Office. He also worked in Bale Zone Administration Office as Process owner of monitoring and evaluation of government job. From 2011 onwards he has been working as process owner of Environmental protection in Bale Zone Rural Land Administration and Environmental Protection Office. He joined the School of Graduate Studies of Haramaya University in 2011 to pursue M.Sc. study in Genetics.

ACKNOWLEDGEMENTS

I am grateful to my advisor, Dr. Yohannes Petros, for his encouragement, guidance, and professional expertise. Successful and timely accomplishment of this study would have been very difficult without his generous devotion from the early design of the research proposal, data collection and to the final write-up of the thesis. Special appreciation and thanks also to my co-advisor, Dr. Habtamu Zeleke, as he added valuable and constructive comments which enabled me to complete the research work and thesis write-up. Also, I would like to express my heartfelt thanks and sincere appreciation to Dr. Mebiasilasie Andargie, for his material support, guidance, experience sharing and directing me as far as the research topic is concerned.

Among all, I would like to thank all the school communities of Zebela Primary and Robe Secondary and Preparatory School for their special support and willingness to participate in the research activity. I also like to extend my great thanks to Bale Zone Administration and Rural Land Administration and Environmental Protection Offices and to all my colleagues and friends, for their patience, moral support, advice and contributions during my study. And also great thanks go to Ato Sisay Hurisa, Ato Tilahun Fikadu, W/ro Almaz Abate, for their special contribution during my study. I don't have smart words to thank Dr. Mashila Dejene, Associate SGS Dean of Haramaya University, and Dr. Sisay Menkir, Biology Departement Head, for their special support in realizing my goal. Simply let me say here is your result.

Last, but not least, I would like to express my heart-felt thanks, gratitude and appreciation to my beloved wife Rahel Tesfaye, my child Yohannes Nigusu, Mahilet Nigusu and my parents, and all my sisters and brothers for their generous assistance, moral support and helpful encouragement during my graduate study with all their kindness and affection.

LIST OF ACRONYMS AND ABBREVIATIONS

CSA	Central Statistical Authority
DIC	Disseminated Intravascular Coagulation
EDTA	Ethyldiamine tetra acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
ETB	Ethiopian Birr
FFP	Fresh Frozen Plasma
FMC	Flinders Medical Centre
HDN	Hemolytic Disease of the Newborn
HEWs	Health Extension Workers
HWE	Hardy-Weinberg Equilibrium
HWP	Hardy-Weinberg Principle
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
NRBCs	Nucleated red blood cells
PCR	Polymerase chain reaction
pH	Power of Hydrogen
RBCs	Red Blood Cells
Rh	Rhesus
WBCs	White Blood Cells
WHO	World Health Organization

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FREQUENCY OF ABO AND Rh BLOOD GROUP ALLELES AMONG OROMO, AMHARA AND WOLAYITA ETHNIC GROUP STUDENTS IN ROBE SECONDARY AND PREPARATORY AND ZEBELA PRIMARY SCHOOL, BALE ZONE, ETHIOPIA

ABSTRACT

The Frequencies Blood types of genotypes and alleles of the ABO and Rh blood group system varies worldwide and are not found in equal numbers even among ethnic groups. Therefore, this study is aimed at getting information on the frequencies of alleles, phenotypes and genotypes of ABO and Rh D blood group systems among the study of Oromo, Amhara and Wolayita ethnic groups at Robe Secondary and Preparatory and Zebela Primary school, Robe town, Bale Zone Oromia region. A total sample of 200 students from each ethnic group were purposively were tested for ABO and Rh blood types. Blood samples were taken from finger pricks and open slide method of testing was followed. A drop of each of the antisera, anti-A, anti-B and anti-D was added and mixed with each blood sample and rocked gently for about 60 seconds to observe agglutination. All materials were single use, and disposable. Differences in allelic, phenotypic and genotypic frequencies of the (ABO) and Rh D blood groups among the students of the three ethnic groups were observed. Blood group O in which 42%, 43%, and 44.5% for Oromo, Amhara and Wolayita ethnic groups respectively has the highest allelic and phenotypic frequencies while blood group AB has the lowest allelic and phenotypic frequencies in all the three ethnic groups. With respect to rhesus factor Rh D +ve (93.5%, 94.5% and 94.5%) for Oromo, Amhara and Wolayita ethnic groups respectively is the most dominant over Rh –ve blood. The distribution and proportion of individuals belonging to each ethnic group did not differ significantly from those expected under the Hardy Weinberg law (P>0.05).

Key Words: ABO blood groups, Allele, Ethnic group, Frequency, Genotype, Phenotype, Rh blood groups,

1. INTRODUCTION

Blood is the most important body fluid. This is responsible for circulation of important nutrients, enzymes and hormones all across the body, besides, the most crucial substance, oxygen. The human red blood cell (RBC) contains different types polysaccharide antigens called agglutinogen. The A and B antigens are important complex oligosaccharide antigens on their external surface. Antibodies are produced in the blood plasma against these A and B antigens and continued to be produced throughout a person's life. According to the presence of antigens and agglutinins, individuals are divided into four major blood groups A, B, AB and O (Novak, 1995). The ABO blood groups and Rhesus (RhD) blood group antigens are the most frequently studied genetic markers populations' worldwide (Mourant *et al.*, 1976).

Since blood carries several antigens within it, which form the basis of its reactivity, it is not possible to mix the blood of all humans without initiating an immune reaction. Only the blood samples, which share the same antigenic identity, do not initiate an immune response, and hence are termed as compatible. The utility of these antigens is not only for blood transfusion or organ transplantation, but have also been utilized in genetic research, anthropology and tracing of ancestral relation to human beings (Eastlund , 1998).

Blood group testing plays a key role in medical treatment prior to blood transfusion and child birth. The blood group of a person does not change with in one's own life time and so it is considered as a unique genetic marker for research. The blood group is determined by the genetic make-up of the alleles of a system (Gupta, 1999).

Furthermore, the discovery of ABO and Rh blood groups has contributed immensely to blood banking services and transfusion medicine in order to avoid morbidity and mortality in both adults and children. The ABO and Rh blood group alleles vary worldwide and are not found in equal frequencies even among the same ethnic groups. For example, among African-Americans, the distribution of ABO blood group is type O is 46%; A, 27%; type B, 20%; and type AB, 7%. Among Caucasians in the United States, the distribution of type O

is 47%; type A, 41%; type B, 9%; and type AB, 3%. Also, among Western Europeans, type O is 46%; type A, 42%; type B, 9% and type AB, 3% (Adeyemo and Soboyejo, 2006).

The human blood groups have been studied extensively for their involvement in incompatibility selection. Various studies on ABO incompatibility have produced evidence of high frequency of prenatal death among incompatible mating (Srikumari *et al.,* 1987).

The ABO and Rhesus 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. The need for blood group prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Green *et al.,* 1995). Estimates of gene frequencies provide very valuable information on the genetic similarity of different populations and to some extent on their ancestral genetic linkage, despite the cultural and religious differences of the two populations (Meade *et al.,* 1994).

Blood grouping has improved with the advent of monoclonal antibodies and the automation of testes. Although different advanced techniques, such as micro plate method, PCR based typing, mini sequencing analysis, fluorescent immune micro plate technique, sandwich ELISA method, etc… are available for ABO genotyping, the Manual(Serological) method has its own significance not only in blood typing but also measuring its genotypic frequency by Hardy- Weinberg law (Srikumary *et al.,* 1987).

ABO and Rh blood group systems in humans are two important genetic markers that are routinely analyzed prior to blood transfusion and medical treatment. The ABO blood group system is governed by a single gene (the ABO gene) with three alleles (I^A, I^B and I^O), of which I^A, and I^B alleles are co-dominant but both of them are dominant over the recessive alleles I^O in intra allelic interaction (Povety *et al.,* 1976).

Since no similar study was reported in the literature regarding the frequencies of ABO and Rh D blood goup phenotypes, genotypes and alleles in the population and different ethnic groups of Bale zone, Robe town. This study aims to investigate the frequency distribution of the blood group alleles of the ABO and Rh D among students of Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory and Zebela Primary School, and compare the same with Ethiopian populations to stablish certain specific features in the genetic structures.

As it is described above, different countries of the world have had well organized documents on the frequency of alleles, phenotypes and genotypes of ABO and Rh D blood group among their different ethnic groups and made the information available for the purpose of blood transfusion and other blood related activities, and hence reduced problems with respect to blood transfusion and HDN. In most parts of Ethiopia, including the present research site – Bale zone, Robe town, there was no prior study of this type and literature that provides information and creates awareness to reduce complication in relation to blood transfusion and HDN. So, this study is significant in coming up with document that shows the phenotypic, genotypic and allelic frequencies of ABO and Rh D blood groups of the three major ethnic groups - Oromo, Amhara and Wolayita that serves as a base line information in creating awareness to reduce complication occurred during blood transfusion activities and HDN. It also used in adding knowledge to the already existing body of knowledge and serves as a reference material for another research of the same type or researches of different version of this topic carried in the zone or other places of our country.

This research was done with the following objectives

Genera objectives

The general objective of the research was to generate data on the ABO and Rh blood group systems among Robe Secondary and Preparatory and Zebela Primary School Students belonging to Oromo, Amhara and Wolayita ethnic groups.

The Specific Objectives

The Specific objectives of the research were to determine:-

- ✓ ABO blood type phenotypic frequency in the students of the three ethnic groups.
- ✓ Rh blood type frequency in the students of the three ethnic groups.
- ✓ Allelic frequency in the students of the three ethnic groups.
- ✓ Genotypic frequency in the students of the three ethnic groups.
- ✓ Testing whether the frequencies are in Hardy-Weinberg equilibrium or not.

2. LITERATURE REVIEW

2.1. History of ABO Blood Grouping

In the 1901, an Austria Scientist Karl Landsteiner established the existence of the first known blood group system. Landsteiner named the first two blood groups antigens A and B, using the first two letters of the alphabet while RBCs not reacting with anti- A and anti- B were called type C. Classification of the blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies present in the serum of individuals directed against these antigens. Where no agglutination had occurred, either the antigen or the antibody was missing from the mixture. In 1902, Von Decastello and Sturli described RBCs reacting with both anti-A and anti-B, but did not give these type a name, but continued calling RBCs that did not react with anti-A and ant-B type C (Garratty *et al.*, 2000).

In 1911, Von Dungern and Hirszfeld were the first to use the term O to describe RBCs not reacting with anti-A and anti-B and the term AB for RBCs reacting with both anti-A and anti-B. The ABO blood groups are genetically determined antigens present on the surface of the red cells and most other body cells (Mollison, 1994).

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. Watkins and Morgan, in England, discovered that the ABO epitopes were conferred by sugars, to be specific, N-acetylgalactosamine for the A-type and galactosyl for the B-type. After much published literature claiming that, the ABH substances were all attached to glycosphingolipids (Mollison, 1994).

2.2. Historical Overview of Immunohematology

Immunohematology is one of the specialized branches of medical science. It deals with the concepts and clinical techniques related to modern transfusion therapy. Efforts to save human lives by transfusing blood have been recorded for several centuries. The era of blood transfusion, however, really began when William Harvey described the circulation of blood in 1616 (Bryant, 1994).

In 1665, an English physiologist, Richard Lower, successfully performed the first animal-to-animal blood transfusion that kept ex-sanguinated dogs alive by transfusion of blood from other dogs. In 1667, Jean Bapiste Denys, transfused blood from the carotid artery of a lamb into the vein of a young man, this at first seemed successful. However, after the third transfusion of lamb's blood the man suffered a reaction and died. Denys also performed subsequent transfusions using animal blood, but most of them were unsuccessful (Bryant *et al*., 1994). Later, it was found that it is impossible to successfully transfuse the blood of one species of animal into another species. Due to the many disastrous consequences resulting from blood transfusion, transfusions were prohibited from 1667 to 1818- when James Blundell of England successfully transfused human blood to women suffering from hemorrhage at childbirth (Bryant, 1994).

Such species-specific transfusions seemed to work about half the time but mostly the result was death. Blood transfusions continued to produce unpredictable results, until Karl Landsteiner discovered the ABO blood groups in 1901, which introduced the immunological era of blood transfusion. It became clear that the incompatibility of many transfusions was caused by the presence of certain factors on red cells now known as antigens. Two main postulates were also drawn by this scientific approaches:- 1.Each species of animal or human has certain factor on the red cell that is unique to that species, and 2. Even each species has some common and some uncommon factor to each other. This landmark event initiated the era of scientific-based transfusion therapy and was the foundation of Immunohematology as a science (Bryant, 1994).

2.3. General Properties of Human Blood

Red blood cells, also known as erythrocytes, average about 7.5 µm in diameter with a thickness of about 2.4 µm at the edges and 1.0 µm in the center (Tsinopoulos *et al.*, 2002). Red blood cells make up about 40 – 50% of the composition of blood. A normal human will have about 5 million red blood cells per cubic millimeter of blood. The red blood cell is composed of about 64% water, 28% hemoglobin, 7% lipids or fatty materials, and the rest consists of sugars, salts, enzymes, and other proteins. The main purpose of red blood cells is to carry oxygen throughout the body from the lungs and return carbon dioxide to the lungs. They are able to do this because they contain a protein known as hemoglobin which is able to carry out the oxygen-carbon dioxide exchange blood (5 x 10 /L). They are made in the bone marrow and, when they are mature, enter the bloodstream where they have a lifespan of approximately 120 days. After this, they break down and are removed by cells of the reticuloendothelial system. These cells are highly specialized and are scattered throughout the body. They are found mainly in the bone marrow, liver, spleen and lymph glands. (Prakash and Arara, 1998).

2.4. Basic Biochemistry of ABO Blood Group

2.4.1. The Role of H-Gene in the Expression of ABO Genes

Inheritance of A and B genes usually results in the expression of A and B antigens on erythrocytes, but H, A and B antigens are not the direct products of the H, A, and B genes, respectively. Each gene codes for the production of a specific transferase enzyme as shown in Table 1, which catalyses the transfer of a monosaccharide molecule from a donor substance to the precursor substance, and enable the conversion of the basic precursor substance to the particular blood group substance (Laine and Rush, 1988).

Table 1 ABH Genes and Their Enzymatic Products

Gene	Enzyme
H	L- fucosyl transferase
A	3- N-acetyl- D- galactosaminyl transferase
B	3-D- galactosyl transferase
O	None

Source: - (Boorman *et al*., 1988.)

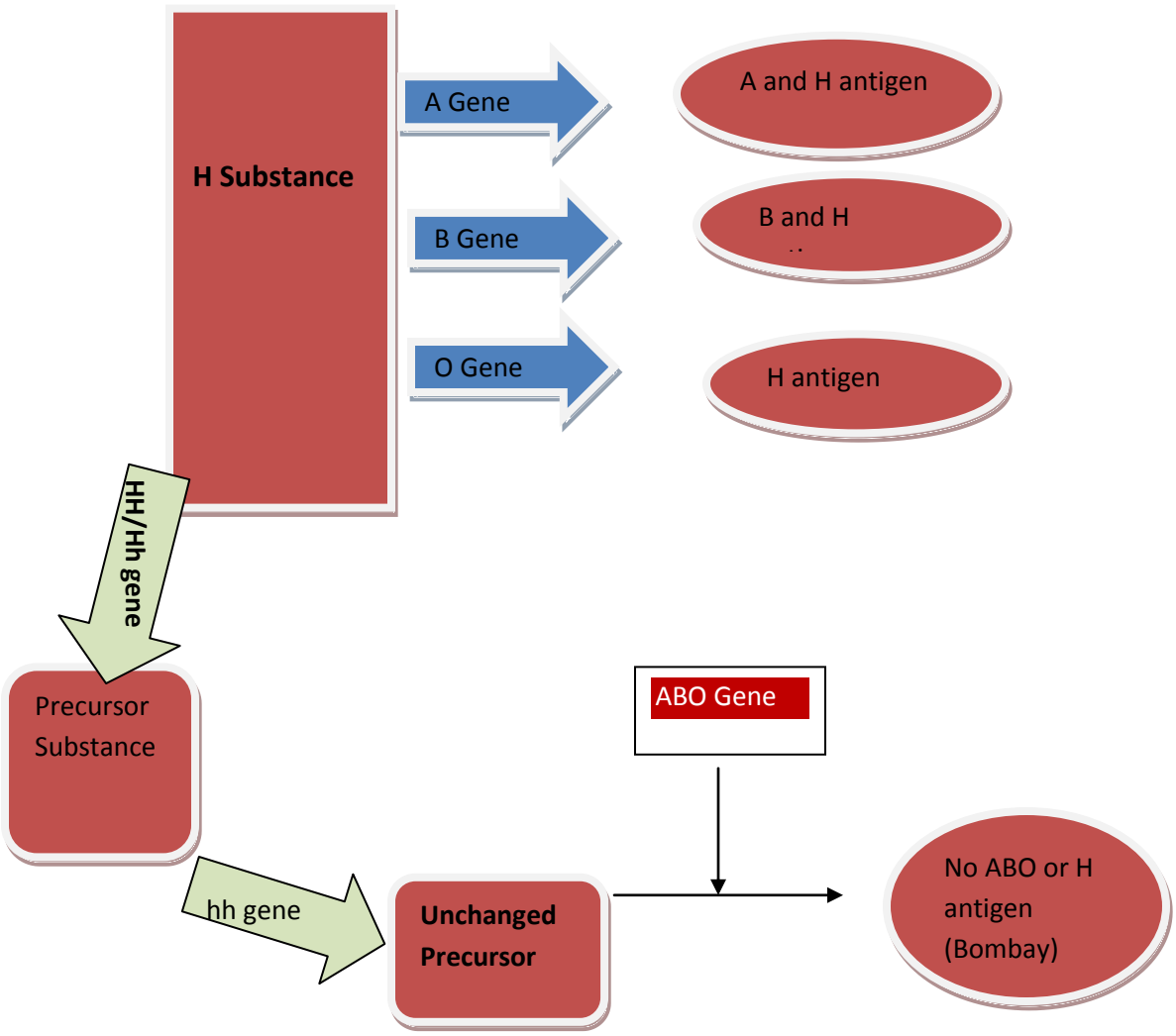

Figure 1 A, B and H antigen formation pathway

Source: - (Boorman *et al*., 1988.)

ABO Blood Phenotypes, antigens and antibodies

Blood type	**Antigen presents**	**Antibodies**
A	A + H	B
B	B + H	A
AB	A + B + H	None
O	H	A + B
Oh	None	A + B + H

Source: - (Boorman *et al*., 1988.)

As shown in Table 1`and Fig 1, the H gene (HH/Hh) encodes for an enzyme, which converts the precursor on red blood cells into H substance (H antigen). The H locus is located on chromosome 19 at 19q13.3. A and B genes encode specific transferase enzymes which convert H substance into A and B red cell antigens. Some H substance remains unconverted (the H substance is partly converted). O gene encodes for an inactive enzyme, which results in no conversion of the substance in-group O red cells. This indicates group O individual contains the greatest concentration of H antigen. Persons who do not inherit H gene (very rare hh genotype) are unable to produce H substance and therefore even when A and B genes are inherited, A and B antigens cannot be formed. This rare group is referred to as Oh (Bombay group). 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 are typed 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. The only cells Bombay individuals do not agglutinate are from other Bombay blood people since they lack the H antigen (Boorman *et al.*, 1988).

The first person that was discovered to have the Bombay phenotype seemed to have an interesting blood type that reacted to other blood types in a way never seen before. The serum contained antibodies that reacted with all RBCs' normal ABO phenotypes. The RBCs' appeared to lack all of the ABO blood group antigens plus an additional antigen that was previously unknown (Dean, 2005).

Individuals with the rare Bombay phenotype (hh) do not express H antigen the antigen which is present in blood group O. As a result, they cannot make A and B antigen on their red blood cells, whatever alleles they may have of the A and B blood-group genes, because A antigen and B antigen are made from H antigen. For this reason people who have Bombay phenotype can donate RBCs to any member of the ABO blood group system (unless some other blood factor gene, such as Rhesus, is incompatible), but they cannot receive blood from any member of the ABO blood group system (which always contains one or more of A and B and H antigens), but only from other people who have Bombay phenotype (Dean, 2005).

It is very important, in order to avoid any complications during a blood transfusion, to detect Bombay phenotype individuals because the usual tests for ABO blood group system would show them as group O. Since Anti-H immunoglobulins can activate the complement cascade, it will lead to the lysis of RBCs while they are still in the circulation, provoking an acute hemolytic transfusion reaction. This, of course, cannot be prevented unless the lab technologist that is involved has the means and the thought to test for Bombay group (Dean, 2005).

2.4.2. Antigens and Antibodies

An antigen can be defined as any substance which, when introduced into an individual who himself lacks the substance, stimulates the production of an antibody, and which, when mixed with the antibody, reacts with it in some observable way. Foreign substances, such as erythrocytes, can be immunogenic or antigenic if their membrane contains a number of areas recognized as foreign. These are called antigenic determinants or epitopes. The immunogenicity of a substance is influenced by a number of characteristics: such as foreignness, molecular weight, structural stability, structural complexity and route of administration (Garratty *et al.*, 2000).

Antibodies are serum proteins produced in response to stimulation by a foreign antigen that is capable of reacting specifically with that antigen in an observable way. Five major immunoglobulin (Ig) classes exist; which are called IgG, IgA, IgM, IgD and IgE, with heavy chains gamma (γ), alpha (α), mu (μ), delta (δ), and epsilon (Є) respectively. Each is unique and possesses its own characteristic. Blood group antibodies are almost exclusively IgG, IgM and IgA. **IgG** is the predominant immunoglobulin in normal serum, accounting for about 85% of the total immunoglobulin. IgG is the only immunoglobulin to be transferred from mother to fetus through the placenta, a fact that explains its role in the etiology of HDN**.** It is predominantly produced during the secondary immune response. **IgM** accounts for about 10% of the immunoglobulin pool, with a concentration of about 1.0 g/l in normal serum. It is the predominant antibody produced in a primary immune response. Because of its large size, IgM cannot pass through the placental barrier to the fetus. Both IgG and IgM are characterized by complement binding. **IgA** is the predominant immunoglobulin in secretions such as, tears, saliva, colostrums, breast milk, and intestinal secretions and it does not fix complement and is not transported across the human placenta (Daniels, 2002).

2.5. The Genetics of ABO Blood group systems

A blood group system may be defined as a genetically discrete group of antigens controlled by a single gene or by a cluster of two or more closely linked homologous genes with virtually no recombination occurring between them (Yazdanbakhsh *et al.,* 2001).

The classification of blood groups into type A, B, AB and O in ABO system, Rh- positive and Rh-negative in Rh system is based on the presence or absence of inherited antigenic substances on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoproteins, glycolipids depending on the blood group system (Hasna *et al.,* 2010).

A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type in one of the many possible combinations of blood-group

antigens. Across the thirty blood groups, over 600 different blood group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups (Seltsam *et al.,* 2003).

Almost always, an individual has the same blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. Another more common cause in blood type change is a bone marrow transplant. Bone-marrow transplants are performed for many leukemia's and lymphomas, among other diseases. If a person receives bone marrow from someone who is a different ABO type (e.g. a type A patient receives a type O bone marrow), the patient's blood type will eventually convert to the donors type (Masushita *et al.,* 1983).

2.5.1. Alleles of the ABO and Rh D blood Locus

The ABO locus is located on chromosome 9 at 9q34.1-q34.2. It contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most O alleles and results in a loss of enzymatic activity. The A and B alleles differ from each other by seven nucleotide substitutions, four of which translate into different amino acids in the gene product (R176G, G235S, L266M, and G268A). The residues at positions 266 and 268 determine the A or B specificity of the glycosyltransferase blood group A and B encode. The O allele differs from the A allele by deletion of guanine at position 261. The deletion causes a frame shift and results in translation of an almost entirely different protein that lacks enzymatic activity (Yamamoto *et al.,* 1990).

2.5.2. Genotypes of the ABO Blood group system

In addition to the current practices of serologic testing of blood types, the progress in molecular diagnostics allows the increasing use of blood group genotyping. In contrast to serologic tests reporting a direct blood type phenotype, genotyping allows the prediction of

a phenotype based on the knowledge of the molecular bases of the currently known antigens. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for patients with needs for much transfusion to prevent alloimmunization (Cummings, 2008).

Ludwik Hirszfeld and E. von Dungern discovered the heritability of ABO blood groups in 1910–11. In 1924 Bernstein postulated the existence of three allelic genes. According to the theory of Bernstein the characters A, B and O are inherited by means of three allelic genes, also called I^A, I^B and I^O. He also proposed that an individual inherited two genes, one from each parent, and that these genes determine which ABO antigen would be present on a person's erythrocytes. The O gene is considered to be silent (amorphic) since it does not appear to control the development of an antigen on the red cell. Every individual has two autosomal chromosomes each carrying either A, B or O, one from each parent, thus the possible ABO genotypes are I^AI^A, I^AI^O, I^BI^B, I^BI^O, I^AI^B and I^OI^O. ABO typing divides the population into the four groups, group A, B, O and, AB (Benjamini *et al.*, 2000).

Table 2 The ABO mating with possible genotype and phenotype of children.

Mating		Children	
Phenotypes	**Genotypes**	**Genotypes**	**Phenotypes**
A x A	1. I^{AA} x I^{AA} 2. I^{AA} x I^{AO} 3. I^{AO} x I^{AO}	1. I^{AA} 2. I^{AA} and I^{AO} 3. I^{AA}, I^{AO} and I^{OO}	A and O
A x B	1. I^{AA} x I^{BB} 2. I^{AA} x I^{BO} 3. I^{AO} x I^{BB} 4. I^{AO} x I^{BO}	1. I^{AB} 2. I^{AB} and I^{AO} 3. I^{AB} and I^{BO} 4. I^{AB}, I^{BO}, I^{AO} and I^{OO}	A, B, AB and O
A x AB	1. I^{AA} x I^{AB} 2. I^{AO} x I^{AB}	1. I^{AA} and I^{AB} 2. I^{AB}, I^{BO} and I^{AA}	A, B and AB A, B and AB
A x O	1. I^{AA} x I^{OO} 2. I^{AO} x I^{OO}	1. I^{AO} 2. I^{AO} and I^{OO}	A and O
B x B	1. I^{BB} x I^{BB} 2. I^{BB} x I^{BO} 3. I^{BO} x I^{BO}	1. I^{BB} 2. I^{BB} and I^{BO} 3. I^{BB}, I^{BO} and I^{OO}	B and O
B x AB	1. I^{BB} x I^{AB} 2. I^{BO} x I^{AB}	1. I^{AB} and I^{BB} 2. I^{AB}, I^{BB}, I^{AO} and I^{BO}	A and AB
B x O	1.I^{BB} x I^{OO} 2. I^{BO} x I^{OO}	1. I^{BO} 2. I^{BO} and I^{OO}	B and O
AB x AB	1. I^{AB} x I^{AB}	1. I^{AA}, I^{BB}, I^{AB}	A, B and AB
AB x O	1. I^{AB} x I^{OO}	1. I^{AO}, I^{BO}	A and O
O x O	1. I^{OO} x I^{OO}	1. I^{OO}	O

Source :- (Bryant Neville, 1994).

2.5.3. Association between Blood Groups and Diseases

Compared to non-O blood group (A, AB, and B) individuals, O group individuals have 14% reduced risk of squamous cell carcinoma and 4% reduced risk of basal cell carcinoma It is also associated with a reduced risk of pancreatic cancer .The B antigen links with increased risk of ovarian cancer. Gastric cancer has reported to be more common in blood group A and least in group O (Xie *et al.*, 2010).

Some blood types are associated with inheritance of other diseases: for example, the Kell antigen is sometimes associated with McLeod syndrome. Certain blood types may affect susceptibility to infections, an example being the resistance to specific malaria species seen in individuals lacking the Duffy antigen. The Duffy antigen, presumably as a result on natural selection, is less common in ethnic groups from areas with high incidences of malaria (Chown *et al.,* 1975).

The mechanism by which blood group O confers some protection against severe malaria compared to blood groups A, B and AB is not fully understood. However, lower rosette formation by patient RBCs of group O as shown by some studies have established that parasitized erythrocytes form rosettes more readily with RBCs of either A, B or AB blood groups than with those belonging to blood group O. Also, it is well established that this parasite triggered RBC rosette formation is associated with the severity of clinical disease (Zinaye Tekeste and Beyene Petros, 2010)

2.5.4. Frequency variations among different world population

The ABO system is the most important blood-group system in human-blood transfusion. The associated anti-A and ant-B antibodies are usually immunoglobulin M, abbreviated IgM, antibodies. ABO IgM antibodies are produced in the first years of life by sensitization to environmental substances such as food, bacteria and viruses (Khurshid *et al.,* 2008).

The ABO blood group distribution varies among the different racial and ethnic groups all over the world. For example, blood group B has its highest frequency in northern India and neighboring Central Asia, and its incidence diminishes both towards the West and the East, falling to single digit percentages in Swiss, it is believed to have been entirely absent from Native American and Australian Aboriginal populations prior to the arrival of Europeans in those areas. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian

Aborigine populations and the black foot Indians of Montana (ISBT, 2006). Table 3 shows the distribution of the ABO blood types along racial and ethnic lines.

Table 3 Racial and ethnic distribution of ABO (without Rh) blood types

PEOPLE GROUP	O (%)	A (%)	B (%)	AB (%)
Aborigines	61	39	0	0
Abyssinians/Ethiopia	43	27	25	5
Ainu (Japan)	17	32	32	18
Albanians	38	43	13	6
Grand Andamanese	9	60	23	9
Arabs	34	31	29	6
Armenians	31	50	13	6
Asian (in USA - General)	40	28	27	5
Austrians	36	44	13	6
Bantus	46	30	19	5
Basques	51	44	4	1
Belgians	47	42	8	3
Blackfoot (N. Am. Indian)	17	82	0	1
Bororo (Brazil)	100	0	0	0
Brazilians	47	41	9	3
Bulgarians	32	44	15	8
Burmese	36	24	33	7
Buryats (Siberia)	33	21	38	8
Bushmen	56	34	9	2
Chinese-Canton	46	23	25	6
Chinese-Peking	29	27	32	13
Chuvash	30	29	33	7
Czechs	30	44	18	9
Danes	41	44	11	4
Dutch	45	43	9	3
Egyptians	33	36	24	8
English	47	42	9	3
Eskimos (Alaska)	38	44	13	5
Eskimos (Greenland)	54	36	23	8
Estonians	34	36	23	8
Fijians	44	34	17	6
Finns	34	41	18	7

French	43	47	7	3
Georgians	46	37	12	4
Germans	41	43	11	5
Greeks	40	42	14	5
Gypsies (Hungary)	29	27	35	10
Hawaiians	37	61	2	1
Hindus (Bombay)	32	29	28	11
Hungarians	36	43	16	5
Icelanders	56	32	10	3
Indians (India - General)	37	22	33	7
Indians (USA - General)	79	16	4	1
Irish	52	35	10	3
Italians (Milan)	46	41	11	3
Japanese	30	38	22	10
Jews (Germany)	42	41	12	5
Jews (Poland)	33	41	18	8
Kalmuks	26	23	41	11
Kikuyu (Kenya)	60	19	20	1
Koreans	28	32	31	10
Lapps	29	63	4	4
Latvians	32	37	24	7
Lithuanians	40	34	20	6
Malaysians	62	18	20	0
Maori	46	54	1	0
Mayas	98	1	1	1
Moros	64	16	20	0
Navajo (N. Am. Indian)	73	27	0	0
Nicobarese (Nicobars)	74	9	15	1
Norwegians	39	50	8	4
Papuas (New Guinea)	41	27	23	9
Persians	38	33	22	7
Peru (Indians)	100	0	0	0
Filipinos	45	22	27	6
Poles	33	39	20	9
Portuguese	35	53	8	4
Romanians	34	41	19	6
Russians	33	36	23	8
Sardinians	50	26	19	5

Scots	51	34	12	3
Serbians	38	42	16	5
Shompen (Nicobars)	100	0	0	0
Slovaks	42	37	16	5
South Africans	45	40	11	4
Spanish	38	47	10	5
Sudanese	62	16	21	0
Swedes	38	47	10	5
Swiss	40	50	7	3
Tartars	28	30	29	13
Thais	37	22	33	8
Turks	43	34	18	6
Ukrainians	37	40	18	6
USA (US blacks)	49	27	20	4
USA (US whites)	45	40	11	4
Vietnamese	42	22	30	5
Mean	43.91	34.80	16.55	5.14
Standard deviation	16.87	13.80	9.97	3.41

Source: - (ISBT, 2006).

Table 4 Frequencies of ABO blood groups studied in different areas of Nigeria and Pakistan

Population	A	B	AB	O	References
Mandi Bahauddin	0.1583	0.2832	0.0448	0.5522	Anees *et al.*, 2007
Swat,Pakistan	0.2792	0.3240	0.1058	0.2910	Khattak *et al.*, 2008
Gujrat .Pakistan	0.1740	0.2229	0.0435	0.5596	Anees and Mirza, 2005
Ogbomoso, Nigeria	0.2290	0.2130	0.0590	0.5000	Bakare *et al*., 2006
Gujrat .Pakistan	0.2372	0.2009	0.0297	0.5322	Enosolease and Bazuaye, 2008
Ibadan, Nigeria	0.2160	0.2140	0.0280	0.5420	Omotade *et al*., 1999
Portharcourt (Nigeria)	0.2290	0.1710	0.0484	0.5516	Jeremiah, 2006
Lagos, Nigeria	0.2530	0.1670	0.0270	0.5530	Adeyemo and Soboyejo, 2006
Adamawa (Nigeria)	0.1650	0.2130	0.1170	0.5060	Abdulazeez *et al.*, 2008
Nigeria	0.2443	0.2388	0.0275	0.4894	Falusi *et al*., 2006
Northern Nigeria	0.2305	0.2995	0.0440	0.4660	Kulkarni *et al*., 1985

Source: - (World Journal, 2011).

2.6. Rh Blood Group System and Its Inheritance

The Rh system is the second most significant blood group system in human blood transfusion with currently 50 antigens identified. There are five main rhesus antigens; D, C, c, E and e which are only expressed on red cells and which are encoded by two adjacent gene loci, the RHD gene which encodes the RhD protein with the D antigen and the RHCE gene which encodes the RhCE protein with the C, E, c and e antigens. They are not found in body fluids like saliva, amniotic fluid and not detected on leucocytes or platelets. The'd' gene is not expressed and there is no'd' antigen, it only implies the absence of 'D'. Individuals who lack any of these antigens may be stimulated to produce the corresponding antibodies (anti-D, anti- C, anti-c, anti-E, and anti-e) by transfusion or pregnancy. Antigen D, having antigen site between 110,000 and 202,000 per erythrocyte, is the most important of the rhesus antigens medically, because it is highly antigenic than the other Rhesus antigens and most likely to provoke an immune system response of the five main antigens (Flegel *et al.,* 1997).

It is common for D-negative individuals not to have any anti-D or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following sensitizing events: possibly a fetomaternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with D-positive RBCs. Rh disease develops in these cases (Moise, 2008).

Rh-negative blood types are much less in proportion of Asian populations than they are in white as shown in Table 3, the presence or absence of the Rh antigens is signified by the + or – sign, so that for example the A- groups does not have any of the Rh antigens (Cummings, 2008).

A person is grouped as Rhesus (Rh) positive or negative based on the presence or absence of antigen D. Rh positive (+) is a person who inherits gene D and the red cell express antigen D. Whereas Rh negative (-) is a person who does not inherit gene D and the red cells do not express antigen D. For transfusion purpose, Rh positive blood can be given to Rh positive individuals and Rh negative blood can be given to both Rh+ and Rh- individuals. Rh+ blood should never be given to Rh- individuals especially to women of child bearing age. Rh antibodies are generally developed from two to six months after the initial immunization by red cells. Their production is consistent with the classical immune response in that the earliest antibody to appear is IgM, followed by IgG, some IgA have also been identified. The predominant Rh antibodies however, are immunoglobulin class IgG (Moise, 2008).

The D antigen is inherited as one gene (RHD) (on the short arm of the first chromosome, 1p36.13-p34.3 with various alleles. Though very much simplified, one can think of alleles that are positive or negative for the D antigen. The gene codes for the Rh D protein on the red cell membrane. D-individuals who lack a functional RHD gene do not produce the D antigen (Weiner *et al.*, Retrieved 2010).

Table 5 ABO and Rh blood type distribution by country (population averages)

Country	Population	O+	A+	B+	AB+	O-	A-	B-	AB-
Australia	21,262,641	40.0%	31.0%	8.0%	2.0%	9.0%	7.0%	2.0%	1.0%
Austria	8,210,281	30.0%	33.0%	12.0%	6.0%	7.0%	8.0%	3.0%	1.0%
Belgium	10,414,336	38.0%	34.0%	8.5%	4.1%	7.0%	6.0%	1.5%	0.8%
Brazil	198,739,269	36.0%	34.0%	8.0%	2.5%	9.0%	8.0%	2.0%	0.5%
Canada	33,487,208	39.0%	36.0%	7.6%	2.5%	7.0%	6.0%	1.4%	0.5%
China	1,339,724,852	47.7%	27.8%	18.9%	5.0%	0.3%	0.2%	0.1%	0.03%
Czech Republic	10,532,770	27.0%	36.0%	15.0%	7.0%	5.0%	6.0%	3.0%	1.0%
Denmark	5,500,510	35.0%	37.0%	8.0%	4.0%	6.0%	7.0%	2.0%	1.0%
Estonia	1,299,371	49.0%	36.9%	9.2%	4.9%	8.0%	5.9%	1.2%	0.9%
Finland	5,250,275	27.0%	38.0%	15.0%	7.0%	4.0%	6.0%	2.0%	1.0%
France	62,150,775	36.0%	37.0%	9.0%	3.0%	6.0%	7.0%	1.0%	1.0%
Germany	82,329,758	35.0%	37.0%	9.0%	4.0%	6.0%	6.0%	2.0%	1.0%
Hong Kong SAR	7,055,071	40.0%	26.0%	27.0%	7.0%	0.3%	0.2%	0.1%	0.1%
Iceland	306,694	47.6%	26.4%	9.3%	1.6%	8.4%	4.6%	1.7%	0.4%
Ireland	4,203,200	47.0%	26.0%	9.0%	2.0%	8.0%	5.0%	2.0%	1.0%
Israel	7,233,701	32.0%	34.0%	17.0%	7.0%	3.0%	4.0%	2.0%	1.0%
Korea	73,000,000	36.6%	32.8%	21.0%	9.0%	0.4%	0.2%	0.09%	0.03%
Netherlands	16,715,999	39.5%	35.0%	6.7%	2.5%	7.5%	7.0%	1.3%	0.5%
New Zealand	4,213,418	38.0%	32.0%	9.0%	3.0%	9.0%	6.0%	2.0%	1.0%
Norway	4,660,539	34.0%	42.5%	6.8%	3.4%	6.0%	7.5%	1.2%	0.6%
Poland	38,482,919	31.0%	32.0%	15.0%	7.0%	6.0%	6.0%	2.0%	1.0%
Portugal	10,707,924	36.2%	39.8%	6.6%	2.9%	6.0%	6.6%	1.1%	0.5%
Saudi Arabia	28,686,633	48.0%	24.0%	17.0%	4.0%	4.0%	2.0%	1.0%	0.3%
South Africa	49,320,000	39.0%	32.0%	12.0%	3.0%	7.0%	5.0%	2.0%	1.0%
Spain	40,525,002	36.0%	34.0%	8.0%	2.5%	9.0%	8.0%	2.0%	0.5%
Sweden	9,059,651	32.0%	37.0%	10.0%	5.0%	6.0%	7.0%	2.0%	1.0%
Turkey	76,805,524	29.8%	37.8%	14.2%	7.2%	3.9%	4.7%	1.6%	0.8%
United Kingdom	61,113,205	37.0%	35.0%	8.0%	3.0%	7.0%	7.0%	2.0%	1.0%
United States	307,212,123	37.4%	35.7%	8.5%	3.4%	6.6%	6.3%	1.5%	0.6%
Country	**Population**	**O+**	**A+**	**B+**	**AB+**	**O-**	**A-**	**B-**	**AB-**
Weighted mean	2,261,025,244	36.4%	28.3%	20.6%	5.1%	4.3%	3.5%	1.4%	0.5%

Source: - (ISBT, 2006).

Table 6 Frequencies of Rh blood groups studied in different areas of Nigeria

Population	Rh+	Rh-	References
Lagos (Nigeria)	0.9400	0.0600	Adeyemo and Soboyejo, 2006
Ogbomoso (Nigeria)	0.9670	0.0330	Bakare *et al*., 2006
Benin (Nigeria)	0.9388	0.0603	Enosolease and Bazuaye, 2008
Adamawa (Nigeria)	0.9740	0.0260	Abdulazeez *et al*., 2008
Portharcourt (Nigeria)	0.9677	0.0323	Jeremiah, 2006
Ibadan (Nigeria)	0.9500	0.0480	Omotade *et al*., 1999
Nigeria	0.9430	0.0570	Falusi *et al*., 2006

Source: - (World Journal, 2011).

2.7. Clinical Significance of Blood Group Typing

2.7.1. Blood Transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide transfusion services for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor, licensed physician or surgeon in a similar way as medicines. Blood and blood products are used for a number of purposes, but the three main reasons for blood transfusion are: to correct anemia, to replace blood lost by bleeding, either during surgery or because of an accident to replace other constituents of blood such as coagulation factors (Daniels, 2006).

Much of the routine work of a blood bank involves testing blood from both donors and recipients to insure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between a donor and recipient, a severe acute hemolytic reaction with hemolysis (RBCs destruction), renal failure and shock is likely to occur, and death is a possibility. Antibodies can be highly active and can

attack RBCs and bind components of the complement system to cause massive hemolysis of the transfused blood (Nickel *et al*,. 1999).

Patients should ideally receive their own blood or type-specific blood products to minimize the chance of a transfusion reaction. Risks can be further reduced by cross-matching blood, but this may be skipped when blood is required for an emergency. Cross-matching involves mixing a sample of the recipient's serum with a sample of the donor's red blood cells and checking if the mixture agglutinates, or form clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination with a microscope. If agglutination occurs, that particular donor's blood cannot be transfused to that particular recipient. In a blood bank it is vital that all blood specimens are correctly identified, so labeling has been standardized using a barcode system known as ISBT128 (Bruce, 2002).

The complete process of blood coagulation is extremely complex and it is outside the scope of this study to explain the intrinsic (surface contact) and extrinsic (tissue injury) pathways of coagulation in detail. In simple terms, however; damage or injury to a blood vessel will trigger the coagulation pathway or cascade, resulting in the change of soluble fibrinogen to fibrin, which forms a stable clot and prevents further bleeding (Mc Clelland, *2001*).

Rare blood types can cause supply problems for blood banks and hospitals. For example Duffy-negative blood occurs much more frequently in people of African origin (Nickel et al,. 1999). And the rarity of this blood type in the rest of the population can result in a shortage of Duffy-negative blood for patients of African race. Similarly for Rh D negative people, there is a risk associated with travelling to parts of the world where supplies of Rh D negative blood are rare, particularly East Asian, where blood services may endeavor to encourage Westerners to donate blood (Bruce, 2002).

As far as transfusion compatibility is concerned, it is not strictly as simple as matching A, B, and O groups. In other words, no individual will ever receive a blood transfusion based on the ABO system alone. The Rhesus factor must also be considered. Together, the Rhesus

factor and ABO blood grouping are the two most important compatibility factors to consider. (Greenwalt, 1997).

2.7.2. Universal Donors and Universal Recipients

With regard to transfusions of whole blood or packed red blood cells, individuals with type O Rh D negative blood are often called universal donors, and those with type AB Rh D positive blood are called universal recipients; however, these terms are only generally true with respect to possible reactions of the recipient's anti-A and anti-B antibodies to transfused red blood cells, and also possible sensitization to Rh D antigens. One exception is individuals with hh antigen (also known as the Bombay blood group) who can only receive blood safely from other hh donors, because they form antibodies against the H substance (Fauci *et al.*, 1998).

Blood donors with particularly strong anti-A, anti-B or any blood group antibody are excluded from blood donation. The possible reactions of anti-A and anti-B antibodies present in the transfused blood to the recipients RBCs need not be considered, because a relatively small volume of plasma containing antibodies is transfused (Daniel, 2002).

By way of example, considering the transfusion of O Rh D negative blood (Universal donor blood) into a recipient of blood group A Rh D positive, an immune reaction between the recipient's anti-B antibodies and the transfused RBCs is not anticipated. However; relatively small amount of plasma in the transfused blood contains anti-A antibodies, which could react with the A antigens on the surface of the recipients RBCs, but a significant reaction is unlikely because of the dilution factors. Rh D sensitization is not anticipated. Additionally, red blood cell surface antigens or other than A, B and Rh D, might cause adverse reactions and sensitization, if they can bind to the corresponding antibodies to generate an immune response. Transfusion are further complicated because platelets and white blood cells (WBCs) have their own systems of surface antigens, and sensitization to platelets or WBC antigens can occur as a result of transfusions (Avent, 2009).

With regard to transfusions of plasma, this situation is reversed. Type O plasma, containing both anti-A and anti-B antibodies, can only be given to O recipients. The antibodies will attack the antigens on any other blood type. Conversely, AB plasma can be given to patients of any ABO blood group due to not containing any anti-A or anti-B antibodies (Anees, 2009).

2.7.3. Hemolytic Disease of the Newborn (HDN)

Hemolysis is the break down or rupture of the red cell membrane by specific antibody (hemolysin) through the activation of complement with the release of hemoglobin, and the librated hemoglobin can easily be observed staining the supernatant fluid (Louise, 1995).

Hemolytic disease of the newborn, originally known as erythroblastosis fetalis, results from blood group incompatibility in which maternal antibodies destruct fetal red cells. An infant having inherited an antigen from the father, which is absent in the mother, causes her to form the corresponding antibodies. These antibodies pass through the placenta by active transport mechanism, coat the fetal erythrocytes and cause damage to them. It is only IgG immunoglobulin that is capable of passing the placental barrier and which is found in cord blood in a concentration equivalent to that found in maternal blood. IgM agglutinin though produced in response to fetal red cells in uterus plays no part in the cause of HDN, and are either present in much lower concentration in the new born than the mother or entirely absent (Louise, 1995).

Fetal hematopoietic tissue (liver, spleen and bone marrow) respond to hemolysis by increased production of RBC, predominantly NRBCs. Increased destruction of red cells leads the fetus to develop anemia and jaundice from the hemoglobin breakdown product, bilirubin. If this bilirubin reaches excessive levels in the newborn or infants circulation it causes mental retardation or death. HDN is mainly caused by Rh blood group incompatibility (Louise, 1995).

2.7.3.1. Hemolytic Disease of the Newborn due to Rh D Blood Group Incompatibility

Hemolytic disease of the newborn due to anti-Rh D occurs when mother and infant are always incompatible with respect to the Rh factor: The mother Rh D negative, and the infant Rh (D) positive (inherited the D factor from the father). ABO incompatibility between the mother and fetus reduces the chance of maternal immunization to the D antigen. This is probably because the fetal cells, which are incompatible with the maternal ABO antibodies, are destroyed by existing ABO antibodies before they have a chance to act as an antigenic stimulus. The first Rh-D incompatible infant is usually unaffected because the number of fetal cells that cross the placenta during pregnancy (after 24 weeks gestation) is small and insufficient to cause IgG anti D production, unless a prior transfusion of D positive blood has been given. During transplacental hemorrhage, the amount of fetal blood that enters the maternal circulation increases and in six months time after delivery only 10% of these Rh negative women could produce detectable antibodies. The actual production of anti-D antibodies depends on the dosage and antigenecity of the D antigen, and the mother's ability to respond to these foreign antigens (Wagner *et al.,* 2002).

During a second pregnancy with Rh positive fetus, small number of fetal cells cross the placenta stimulating the antibody to high concentration, mainly IgG anti- D that passes into the fetal circulation is destroying fetal red cells. The severity of the disease increases with each Rh positive pregnancy (Wagner *et al.,* 2002).

2.7.3.2. Hemolytic Disease of the Newborn due to ABO Blood Group Incompatibility

Hemolytic disease of the newborn due to ABO blood grouping usually occurs when the mother is invariably group O (posses IgG anti-A and B), the infant group A or B and when the mother and infant are Rh compatible. The fetal red cells cross the placenta into the maternal circulation stimulating the existing anti-A and B to high titters; the "immune" anti-A and B stimulated is largely IgG. ABO HDN occurs in the first pregnancy because anti-A and anti-B are always present and therefore readily stimulated. Although ABO

incompatibilities between mother and baby occur frequently and represent a common form of HDN, the clinical course of ABO HDN is relatively mild, probably because of the antigenic development of the fetal red cells and may also be due to the presence of A and B substances in the fetal tissues and fluids that will neutralize the anti-A and anti-B antibodies before they can attack the fetal red cells (Benjamini *et al.*, 2000).

2.7.4. Prevention of Hemolytic Disease of the Newborn

One of the major advances of the twentieth century medicine was to prevent this disease by stopping the formation of anti-D antibodies by D-negative mothers with an injectable medication called Rh D immunoglobulin. Antibodies associated with some blood groups can cause severe HDN, others can only cause mild HDN and others are not known to cause HDN (Cummings, 2008).

Fetal red cells in the maternal circulation might be destroyed by administration of suitable quantity of IgG anti- D to prevent Rh immunization of the mother, given to Rh-negative women within 72 hours of delivery. This dramatically decreases the incidence of anti-D HDN. Combined prenatal-postnatal treatment is more effective than postnatal treatment alone in suppressing Rh immunization. All pregnant Rh-ve women should receive Rh immunoglobulin even if the Rh status of the fetus is unknown because fetal D antigen is present in fetal erythrocytes as early as 38 days of conception. Treatment of Infants Suffering from HDN For infants who develop hyperbilirubinemia and/or anemia due to HDN, exchange transfusion is usually carried out (Cummings, 2008).

2.7.5. Blood Products

To provide maximum benefit from each blood donation and to extend shelf-life, blood banks fractionate some whole blood into several products. The most common of these products are packed RBCs, plasma, platelets, cryoprecipitate, and fresh frozen plasma (FFP). FFP is quick-frozen to retain the labile clotting factors V and VIII, which are usually

administered to patients who have a potentially fatal clotting problem caused by a condition such as advanced liver diseases, over dose of anti coagulant, or disseminated intravascular coagulation (DIC). Units of packed red cells are made by removing as much of the plasma as possible from whole blood units. Clotting factors synthesized by modern recombinant methods are now in routine clinical use for hemophilia, as the risks of infection transmission that occur with pooled blood products are avoided (Benjamini *et al.,* 1996).

2.8. Population Genetics and the Hardy–Weinberg principle

Population genetics considers how the frequencies of alternative states of genes in populations are maintained or changed from generation to generation. The Hardy-Weinberg law states that in a large, random-mating population with no mutation, no migration, and no selection affecting the gene, there is a simple mathematical relationship between the allele frequencies and genotype frequencies. Significant deviations from Hardy-Weinberg expectations in a sample of subjects would lead to the rejection of the hypothesis of genetic equilibrium in the population. Blood type a trait determined by three alleles at a single locus. The alleles are commonly denoted A, B, O. These alleles combine to give the following phenotypic blood types: AA and AO (type A), BB and BO (type B), AB (type AB), and OO (type O). Denote the frequencies of alleles A, B, O as p, q, r respectively. Under the assumptions of Hardy-Weinberg, we would expect the genotypic frequencies: AA with frequency p2, AB with frequency 2pq, AO with frequency 2pr, BB with frequency q2, BO with frequency 2qr, and OO with frequency r2. Notice that, again, these frequencies can be calculated by squaring the appropriate multinomial. This time it is $(p + q + r)^2$ = p2+2pq +2pr + q2+2qr + r2 (Kalmes and Huret., 2001).

3. RESEARCH METHODOLOGY

3.1. Description of the Study Area

The study was conducted in Oromia Regional State, Bale zone, Robe town (Robe Secondary and Preparatory School and Zebela Primary School which have got over 2752 students belonging to different ethnic groups. The town is located at an altitude of 2492 meter above sea level and it is located 430Km southeast of Addis Ababa and the town is situated at $7^0$5'0"N and $40^0$0'0"E. Ethiopian National House and Population Census reported that Robe town has a total population of 57,385 out of which 29,148 are male and 28,237 are female (Projected by the annual growth rate of 2.93%). Out of this population 88.9% is Oromo, 7.6% is Amhara while the rest 3.2% are from other ethnic group (CSA, 2007).

3.2. Sampling Procedure and Sample Size

Robe Secondary and Preparatory School and Zebela Primary School have 2752 students of which Robe Secondary and Preparatory school comprises of 1237 students while the later has 1515 students enrolled in the academic year 2012/2013 G.C. The latter school was included in the research activity to increase the chance of getting students' of Amhara and Wolayita ethnic groups due to the fact that this school is the only primary school that uses Amharic language as medium of instraction and hence the majority of Amhara and Wolayita ethnic group children attend their primary education here. The study was conducted on 600 purposively sampled students comprising approximately 22% of the students' population in the two schools. The sample students were selected purposively so as to include equal number of students from the three ethnic groups: - Oromo, Amhara and Wolayita in the two schools. Thus, the sampled students were consisting of 200 individuals from each ethnic group. The students expressed their willingness to participate in the study by filling all their profile and signing on the consent agreement format. The profile filled by the participating students accepted after the two school directors signed and stamped for its correctness. The information about ethnic groups was reapproved by directly asking the students during his

or her actual participation. Students from parents of two different ethnic groups were not included in the research activity due to the difficulty in determining the ethnic group of such individuals.

3.3. Blood Sample Collection and Typing Method

The Blood sample was collected from each individual after they agreed to participate in the research process and signed on the consent form that assures their willingness. The ABO and Rh blood group test was performed by using standardized and packed lancet, to obtain blood from finger pricks for each sample students. Blood samples were taken from finger pricks, and open slide method of testing for ABO and Rh D blood groups was followed (Bhasin and Chahal, 1995). The blood was placed on a clean slide in three places and a drop of one of the Anti sera that is antibody coated, Anti-A, Anti-B and Anti-D was added to each of an individual's blood samples and mixed using a glass rod. Standardized anti sera, Anti-A, Anti-B and Anti-D, lancet, slides were obtained commercially from Robdan Medical Drugs and Chemicals Distributor in Robe-Bale. Blood groups were determined on the basis of agglutination, and recorded as blood group A^+, B^+, AB^+, and O^+ and A^-, B^-, AB^-, and O^-. The blood samples were collected and tested by qualified laboratory technicians using the standard clinical procedure (Bhasin and Chahal, 1995)

All instruments and materials were single use and disposable. Tested samples and specimens used were collected in garbage baskets and then after, at the end of daily activity, it was burned with the application of little fuel at a site far from the reach of human beings, animals and flammable materials. Finally, what left was dipped into toilet.

3.4. Method of Data Analysis

The genetic structure of a population was determined by the total number of all alleles (the gene pool) in the case of sexually interbreeding individuals; the structure was also characterized by the distribution of alleles into genotypes. The genetic structure could be

described in terms of allelic and genotypic frequencies (Russe, 2005). For this study, the frequency of the blood group genotypes was used to calculate the frequency of the ABO blood group alleles by using the extension of Hardy-Weinberg principle (HWP), and chi-square (χ^2) test was used during comparison of observed frequencies of ABO and Rh blood group with expected frequencies (Griffiths *et al.*, 2008). Extension of the Hardy Weinberg law to loci with more than two alleles was used to analyze the genotypic and allelic frequencies based on Hardy–Weinberg equations. Chi-square goodness-of-fit statistic was calculated to compare observed and expected frequencies and to investigate heterogeneity.

3.4.1. Formula for the Calculation of Phenotypic frequency

The percentage frequency of each blood group was calculated using the formula

$$\% \text{ Frequency} = \frac{\text{Number observed}}{\text{Total sample}} \times 100$$

3.4.2. Formula for the Calculation of Genotypic frequency

When two alleles are present at a locus, the Hardy Weinberg law tells us at equilibrium the frequencies of the genotype is $p^2 + 2pq + q^2$, which is the square of allelic frequencies $(p + q)^2$. This is the simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are sampled two at a time into a diploid zygote (Dnaniel and Clark, 2007). For this study three alleles are computed (I^A, I^B and I^O), with frequencies equal to p, q and r respectively. The frequencies of the genotype at equilibrium will be computed by the square of thee allelic frequencies.

$(p + q + r)^2 = p^2 (I^AI^A) + 2pq (I^AI^B) + q^2 (I^BI^B) + 2pr (I^AI^O) + 2qr (I^BI^O) + r^2 (I^OI^O)$ (Griffiths *et al.*, 2008). It is also expressed using punnet square as follows

Table 7 Punnet square showing Hardy Weinberg frequencies for ABO alleles

Female gametes		**Male gamete**			
		Allele	**I^A**	**I^B**	**I^O**
Allele	**Frequency**	**Frequency**	**p**	**q**	**r**
I^A	p		I^AI^A p2	I^AI^B pq	I^AI^O pr
I^B	q		I^AI^B pq	I^BI^B q2	I^BI^O qr
I^O	r		I^AI^O pr	I^OI^B rq	I^OI^O **r^2**

It is summarized as:-

Phenotype (Blood group)	**Genotype**	**Phenotypic frequency**	**Genotypic frequency**	**Expected frequency**
A	$I^AI^A + I^AI^O$	nA	$nI^AI^A + n\ I^AI^O$	$p2 + 2pr$
B	$I^BI^B + I^BI^O$	nB	$nI^BI^B + nI^BI^O$	$q2 + 2pr$
AB	I^{AB}	nAB	nI^{AB}	$2pq$
O	I^OI^O	nO	nI^OI^O	$r2$
Total		n	n	1

P^2 is the frequency of genotype I^AI^A

q^2 is the frequency of genotype I^BI^B

2pq is frequency of genotype I^AI^B

2pr is frequency of genotype I^AI^O

2qr is the frequency of genotype I^BI

r^2 is the frequency of genotype I^OI^O

Source: - (Hartl and Clark, 1989).

3.4.3. Formula for the Calculation of Allelic frequency

ABO allele frequencies were estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates were calculated as: $p = 1 - \sqrt{B+O}$, $q = 1 - \sqrt{A+O}$, $r = \sqrt{O}$ (p, q, r denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O. A correction factor (d) will be calculated according to $d = 1 – p – q – r$. The final allele frequencies were then calculated as follows: $p1 = p (1 + d/2)$; $q1 = q (1 + d/2)$; $r1 = (r + d/2) (1 + d/2)$ [where p1, q1, and r1 denote corrected allele frequencies. Rh (D) allele frequencies were calculated according to the Hardy-Weinberg equation (Al-Arrayed, *et al.,* 2007). Observed and expected genotype frequencies in Hardy-Weinberg were calculated on the basis of gene's frequency and Chi-square tests was done to test the independence and the goodness of fit for genotype frequencies (Chakraborty, 2010).

3.5. Statistical method to Test the Goodness-of-fit

Observed and expected genotype frequencies in Hardy-Weinberg were calculated on the basis of gene's frequency and Chi-square tests was done to test the independence and the goodness of fit for genotype frequencies (Chakraborty, 2010). Chi-square test was used to compare observed allelic and genotypic frequency distributions of the blood group ABO and Rh anti-gens to that expected under the Hardy–Weinberg (Griffith *et al.,* 2008).

The Chi-square (χ^2) test statistic is then

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The number of degrees of freedom is simply the number of classes minus one; for intrinsic hypotheses it is usually determined as the number of classes minus one minus the number of independent parameters estimated from the sample. Hardy Weinberg equilibrium is a useful indicator of genotype frequencies within a population and whether they are based on a valid

definition of alleles and a randomly mating sample. HWE assumes a stable population of adequate size without selective pressures and is used in human genetic studies as a guide to data quality by comparing observed genotype frequencies to those expected within a population (Griffith *et al.,* 2008).

Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium and expressed as percentages. Chi-square test was used to compare observed allelic and genotypic frequency distributions of that expected under the Hardy–Weinberg (Chakraborty, 2010).

Ethical clearance was obtained from Bale zone Health Office after it was revised and got acceptance by the Health Management Committee of the Office.

4. RESULTS AND DISCUSSION

This chapter is mainly concerned with the analyses and interpretations of major findings to address the objectives of the research. The chapter is divided into three sections. All these were analyzed and pooled together to present results and discussion. Analyses of data generated were made using frequencies, percentages and Chi-square ($\chi 2$) test, Section 4.1 presents phenotypic frequencies of ABO and Rh blood group among Oromo, Amhara and Wolayita ethnic groups in the study area; Section 4.2 presents estimation of genotypic and allelic frequencies of ABO and Rh blood group among Oromo, Amhara and Wolayita ethnic groups in the study area; section 4.3 present statistical method to test the goodness-of-fit.

4.1. Phenotypic percentage of ABO and Rh Blood groups among students of the Oromo, Amhara and Wolayita Ethnic groups

This section shows the phenotypic percentage distribution of ABO and Rh blood group system among three ethnic groups namely Oromo, Amhara and Wolayita in which 200 students are purposively selected from each ethnic group and the overall population treated in the research.

Table 8 Numbers and Percentages of ABO and Rh Blood Group Systems phenotypes among the three ethnic groups (Oromo, Amhara and Wolayita)

Ethnic Group	ABO Blood Grouping System					Rh (Rhesus Blood Grouping System	
	O	A	B	AB	Total	Rh+	Rh-
Oromo	84 (42)	56 (28)	50 (25)	10 (5)	200	187 (93.5)	13 (6.5)
Amhara	86 (43)	58 (29)	46 (23)	10 (5)	200	189 (94.5)	11 (5.5)
Wolayita	89 (44.5)	54 (27)	48 (24)	9 (4.5)	200	189 (94.5)	11 (5.5)
Over all	259 (43.17)	168 (28)	144 (24)	32 (4.83)	600	565 (94.17)	35 (5.83)

Values in parentheses represent phenotype percentage occurrence

Table 8 presents the phenotypic percentage distribution of ABO and Rh blood group phenotypes of Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory School and Zebela Primary School. The phenotypes of 200 blood samples from each ethnic groups in this study were O (42%), A (28%), B (25%) and AB (5%) for Oromo ethnic group, O (43%), A (29%), B (23%) and AB (5%) for Amhara ethnic group and O (44.5%), A (27%), B (24%) and AB (4.5%) for Wolayita ethnic group.

With respect to Rhesus blood grouping system, 93.5% of the population sampled from Oromo ethnic group were Rh D +ve while 6.5% were Rh D-ve and 94.5% of both Amhara and Wolayita ethnic groups were Rh D +ve while 5.5% were Rh D -ve Table 8.

The overall ABO and Rh D blood phenotypic percentage distribution of 600 students is also presented in which O blood type is 43.17%, A type = 28%, B type = 24% and AB type = 4.83% from which 94.17% is Rh D +ve while 5.83% is Rh D –ve.

The phenotypic, genotypic and allelic frequencies of ABO and Rh blood group vary in different populations throughout the world. In this study, The researcher attempted to determine the phenotypic, genotypic and allelic frequencies of ABO and Rh blood group in three different ethnic groups Oromo, Amhara and Wolayita in Robe Secondary and Preparatory and Zebela Primary Schools, Bale zone, Ethiopia, and came-up with document that shows the existence of variations in the phenotypic, genotypic and allelic frequencies of ABO and Rh blood groups along ethnic lines thought, the difference is not statistically significant.

The data revealed that the ABO blood group phenotypic percentage distribution in Oromo ethnic group was found in the order O > A > B > AB (42%, 28%, 25% and 5%) respectively among 200 students purposively sampled. The order of ABO blood group phenotypic percentage distribution observed in Oromo ethnic group holds true for both Amhara and Wolayita ethnic groups in which O > A > B > AB and their frequencies were 43%, 29%, 23%, 5% and 44.5%, 27%, 24% and 4.5% respectively among 200 students purposively sampled for each ethnic groups. When compared with other reports from similar studies, the present data is consistent with previous findings from Ethiopia and other parts of the world like Brazilians: - O (47%) > A (41%) > B (9%) > AB (3%), Arabs: - O (34%) > A (31%) > B (29%) > AB (6%) (ISBT, 2006).

The present finding is consistent with many countries of ABO blood group distribution profile. In Britain, the percentage frequencies of the ABO blood group were 47%, 42%, 9%, and 3.0% for O, A, B, and AB blood groups respectively (ISBT, 2006). Findings reported from Asia (in USA-General) also showed that the percentage frequencies were 40%, 28%, 27%, and 5% for O, A, B, and AB blood groups respectively (ISBT, 2006) see Table 3. In the Northern part of Nigeria, (Kulkarni *et al.,* 1985) obtained frequencies of 46.6%, 29.95%, 23.05% and 4.4% for blood group O, A, B and AB respectively and frequencies of 55.3%, 25.3%, 16.7% and 2.7% in the order O > A > B > AB were also obtained among 150 students of Cell Biology and Genetics at the University of Lagos, Nigeria (Adeyemo and Soboyejo, 2006). Among the Caucasians in the United States of

America, the frequency of blood group O, A, and AB are 47%, 41%, 9% and 3 % respectively (Adeyemo and Soboyejo, 2006).

However; the present findings does not agree with the relative frequency of blood types determined to some population. For example, ABO blood group antigens from Ainu (Japan) where ABO blood group frequency occurred in the order A = B (32%) > AB (18%) > O(17%) (ISBT, 2006). It also seem not to agree with the results obtained from Swat district in Pakistan where the percentage frequencies were A=27.92%, B= 32.40 %, O = 29.10% and AB= 10.58% in which B > O > A > AB (Khattak *et al.,* 2008). It is also not consistent with ABO phenotypic frequency of Bororo (Brazil) in which 100% of the population are O blood groups (ISBT, 2006).

In the present study, when we see along ethnic line, 93.3% of the Oromo ethnic group (consisting 55.5% of DD individuals and 37.995% Dd individuals) were phenotypically Rh +ve while 6.5% were Rh -ve. In the case of Amhara and Wolayita ethnic groups 94.5% (consisting 58.5% of DD individuals and 35.96% Dd individuals) were phenotypically Rh +ve while only 5.5% were Rh –ve. These findings show that in all the three ethnic groups - Oromo, Amhara and Wolayita, the proportion of Rh -ve is far lower than for Rh +ve. The findings are consistent with reports 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 (see Table 5) (Kulkarni *et al.,* 1985, Ahmed and Obi, 1998; Omotade *et al.,* 1999; Ahmed *et al.,* 2004; Ahmed *et al.,* 2007; Jeremiah and Odumody, 2005, Bakare *et al.,* 2006, Akhigbe *et al.,* 2009, Adeyemo and Soboyejo, 2006).

The results, however; differ from the work reported by Yousaf and colleagues where the population sampled among Bahawalpur division of Pakistan population were all Rh D positive (Yousaf *et al.,* 1988). It also disagrees with that of Salmon *et al.* 1988 and Njoku *et al.,* 1996 who reported rhesus positive values of 100% for Eastern Highlands of Papua Guinea and Nigeria, respectively. In addition, it is dissimilar to that in Indians with a

preponderance of the Rh(d) of 89.7% over the Rh(D) gene of 10.3% (Thangaraj *et al.*, 1992).

Apart from ABO and Rh blood groups percentage distribution for the three ethnic groups, Table 8 also presents the percentage distribution of ABO and Rh blood groups for the overall students population treated in the research. So, it occurs in the order O (43.17%) > A (28%) > B (24%) > AB (4.83%). The findings are consistent with reports from previous studies including Ethiopia in which O (43%) > A (27%) > B (25%) > AB (5%) as indicated in Table 3 (ISBT, 2006).

4.2. Genotypic and Allelic Frequencies of ABO and Rh Blood groups among students of the Oromo, Amhara and Wolayita Ethnic groups.

This section presents the genotypic and allelic frequencies of ABO and Rh blood groups among three ethnic groups namely Oromo, Amhara and Wolayita in which 200 students are purposively selected from each ethnic group.

Table 9 Genotypic and Allelic frequencies of ABO and Rh blood groups for the three ethnic groups (Oromo, Amhara and Wolayita)

Ethnic Group	Allele	Allelic Freq.	Genotype	Genotypic Freq.
Oromo	I^O	0.6540	I^{OO}	0.4277
	I^A	0.1821	I^{AA}	0.0332
			I^{AO}	0.2382
	I^B	0.1639	I^{BB}	0.0269
			I^{BO}	0.2144
	-	-	I^{AB}	0.0597
			I^{DD}	0.5550
	I^D	0.745	I^{Dd}	0.3710
	I^d	0.255	I^{dd}	0.0650
Amhara	I^O	0.6600	I^{OO}	0.4356
	I^A	0.1881	I^{AA}	0.0354
			I^{AO}	0.2483
	I^B	0.1519	I^{BB}	0.0231
			I^{BO}	0.2005
	-	-	I^{AB}	0.0571
	I^D	0.765	I^{DD}	0.5852
			I^{Dd}	0.3596
	I^d	0.235	I^{dd}	0.0552
Wolayita	I^O	0.6722	I^{OO}	0.4519
	I^A	0.1729	I^{AA}	0.0299
			I^{AO}	0.2324
	I^B	0.1549	I^{BB}	0.0240
			I^{BO}	0.2082
	-	-	I^{AB}	0.0536

	I^D	0.765	I^{DD}	0.5852
			I^{Dd}	0.3596
	I^d	0.235	I^{dd}	0.0552
Overall	I^O	0.6621	I^{OO}	0.4384
	I^A	0.1810	I^{AA}	0.0328
			I^{AO}	0.2397
	I^B	0.1569	I^{BB}	0.0246
			I^{BO}	0.2078
	-	-	I^{AB}	0.0568
	I^D	0.7585	I^{DD}	0.5753
			I^{Dd}	0.3664
	I^d	0.2415	I^{dd}	0.0583

Table 9 presents the allelic and genotypic frequencies of ABO and Rh blood group of Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory and Zebela Primary School. The allelic frequencies of ABO blood group for Oromo ethnic group was I^O = 0.6540, I^A = 0.1821 and I^B was 0.1639. The allelic frequencies of ABO blood group for the Amhara ethnic group was also I^O = 0.6600, I^A = 0.1881 and I^B was 0.1519. Similarly, frequency of I^O = 0.6772, I^A = 0.1729 and I^B was 0.1549 for Wolayita ethnic groups. The allelic frequencies of ABO blood group for the overall students population is I^O = 0.6621, I^A = 0.1810 and I^B = 0.1569.

With respect to Rhesus blood group system, the allelic frequency of Oromo ethnic group was 0.745 for I^D and 0.255 for I^d. And the allelic frequency was found to be 0.765 for I^D and 0.235 for I^d for both Amhara and Wolayita ethnic groups.

Table 9 also presents the frequencies of the various genotypes in the ABO and Rh systems. So that I^OI^O = 0.4277, I^AI^A = 0.0332, I^AI^O = 0.2382, I^AI^B = 0.0597, I^BI^B = 0.0269 and I^BI^O =

0.2144 in Oromo ethnic group. It follows the same patterns of distribution in both Amhara and Wolayita ethnic groups. The frequency of the genotypes for Rh blood group in Oromo ethnic group were 0.5550 for I^DI^D, 0.380 for I^DI^d and 0.065 for I^dI^d and 0.5852 for I^DI^D, 0.3596 for I^DI^d and 0.0552 for I^dI^d both for Amhara and Wolayita ethnic groups.

As it is shown in Table 9, in all the three ethnic groups the allelic frequencies of ABO blood group was occurred in the order $I^O > I^A > I^B$. It shows similar patterns of allelic frequencies with those documented from earlier studies among various segments of the world population including Ethiopia in which I^O (0.66) > I^A (0.1759) > I^B (0.1638) (computed from phenotypic frequencies) indicated in Table 3. For instance similar study by Bakare *et al,* 2006 in Ogbomoso,South-west Nigeria, Omotade *et al,* 1999 among a healthy infant population in Ibadan, Nigeria, Yan *et al,* 2005 on Chinese populations and Hussain *et al,* 2001 among Balochistan in Pakistan all found the allelic frequencies to occur in $I^O > I^A > I^B$ order.

With respect to Rhesus factor, allele D is far higher in frequency than allele d in all the three ethnic groups and in the overall student population sampled.

On the predominance of blood allele O over other blood alleles in the population sampled, the researcher agreed with the suggestion of Bakare *et al.,* 2006, that predominance of O allele may be as a result of heterozygous advantage that many A and B blood group have been heterozygous carrying O allele silently thereby maintaining O allele in the heterozygous population. For example, this finding shows that in Amhara ethnic group, the frequency of I^AI^A genotype was 0.0356 while I^AI^O genotype was 0.2483. Thus, among those who are blood group A, 13.17 % were homozygous I^AI^A, while about 86.83% were heterozygous I^AI^O. Similar deduction can be made for O allele to be carried silently in I^BI^O heterozygous form in blood group B in all the three ethnic groups. However; it is the researcher's candid opinion that molecular characterization of O allele could assist in elucidating the possible causes of blood group O predominance in various populations.

4.3. Observed versus expected frequency of ABO and Rh Blood groups

This section presents the observed versus expected number of ABO and Rh blood group among the three ethnic groups namely Oromo, Amhara and Wolayita in which 200 students are purposively selected from each ethnic group. It also deals with the observed versus expected genotypic frequencies of ABO and Rh blood group among the three ethnic groups and the overall students population treated in the research.

Table 10 Observed and Expected Number and Frequencies of ABO and Rh blood group for the three ethnic groups

Ethnic Group	ABO Blood system					Rh Blood System				
	Blood group	**Obs. No**	**Obs. Freq.**	**Exp. Freq**	**Exp. No**	**Blood Group**	**Obs. No**	**Obs. Freq.**	**Exp. Freq.**	**Exp. No**
Oromo	O A B AB	84 56 50 10	0.42 0.28 0.25 0.05	0.4356 0.2713 0.2413 0.0579	85.54 54.28 48.26 11.94	Rh(D) Rh(d)	187 13	0.935 0.065	0.9350 0.0650	186.995 13.005
$\chi^2 = 0.4601, p < 0.95$										
Amhara	O A B AB	86 58 46 10	0.43 0.29 0.23 0.05	0.4356 0.2837 0.2236 0.0571	87.12 56.74 44.72 11.43	Rh(D) Rh(d)	189 11	0.945 0.055	0.9448 0.0552	188.96 11.04
$\chi^2 = 0.2556, p > 0.95$										
Wolayita	O A B AB	89 54 48 9	0.445 0.27 0.24 0.045	0.4519 0.2623 0.2322 0.0536	90.38 52.46 46.45 10.72	Rh(D) Rh(d)	189 11	0.945 0.055	0.9448 0.0552	188.96 11.04
$\chi^2 = 0.3965, p \leq 0.95$										
Overall	O A B AB	259 168 144 29	0.4317 0.28 0.24 0.0483	0.4384 0.2725 0.2324 0.0568	263.04 163.50 139.44 34.08	Rh(D) Rh(d)	565 35	0.941 0.058	0.9417 0.0583	565.02 34.98
$\chi^2 = 1.082, p \leq 0.8$										

Table 10 presents the observed proportions of ABO and Rh individuals in the studied population when compared with expected proportions. It also shows the chi-square (χ^2) and probability (p) value for all the three ethnic groups separately and for the overall student population sampled in the study.

As it is shown in Table 10, the application of extended Hardy-Weinberg principle for three or more alleles yields little variation in the observed and expected genotypic frequencies and numbers which serves as a base in determining the chi-square (χ^2) values that further used in determining the goodness-of- fitness. Hence, the chi-square value for Oromo ethnic group is 0.4601 with $p \leq 0.95$, for Amhara ethnic group $\chi^2 = 0.2556$ with $p > 0.95$, for Wolayita ethnic group $\chi^2 = 0.3965$ with $p \leq 0.95$ and for the overall student population sampled, the chi-square value is 1.082 with $p \leq 0.8$ values. With determined degree of freedom 3 and 0.05 significance level.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

There has been no known data on the distribution pattern and frequency of ABO and Rh blood group phenotypes, genotypes and alleles in the population and different ethnic groups of Bale zone. This study aims at providing information on the distribution pattern of the phenotypes, genotypes, and the allelic frequencies among students of Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory and Zebela Primary School with a view of contributing to existing knowledge on the subject matter.

In this study, the percentage frequency distribution of blood group O is the highest with percentage frequency of 42%, 43% and 44.5% in Oromo, Amhara and Wolayita ethnic groups respectively, followed by blood group A (28%, 29% and 27%) and blood group B (24%, 23% and 24%), and the least percentage frequency is that of blood group AB (5%, 5% and 4.5%) in the three ethnic groups. Moreover, this study further confirmed that Rh (D) positive has the highest percentage frequency while Rh-negative has the lowest percentage frequency as observed among the three ethnic groups. There is also a similar trend in overall student population sampled in which blood group O (43.17%) > A (28%) > B (24%) > AB (4.83%). Frequency of Rh(D) 94.17% is by far greater than that of Rh(d), which scores 5.83%. With respect to allelic frequencies, allele O records the highest frequencies (0.6540, 0.66 and 0.6722) in Oromo, Amhara and Wolayita ethnic groups, respectively. This is followed by allele A (0.1821, 0.1881 and 0.1729) while allele B records the least frequencies (0.1639, 0.1519 and 0.1549). In the case of Rhesus factor allele D has a frequency distribution far higher than d allele in all the three ethnic groups under this study.

5.2. Conclusion

From this findings, it is evident that the proportion and allele frequencies of individuals belonging to blood group O in the studied population are most predominant. The implication of this finding is that blood type O is the most readily available blood group in the study area 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 an advantage because some parts of Bale zone is a malaria epidemic zone and so therefore individuals belonging to blood group O may be protected from severe malaria attack due to the mechanism of reduced rosetting.

The phenotypic percentage distribution of ABO and Rh blood group presented in Table 9, the allelic and genotypic frequencies presented in Table 10 shows little variations from one ethnic group to the other and the country's ABO and Rh blood group profile. On the researcher's opinion, this might happened as a result of inter-marriage situation by their grand and great grandparents as many ethnic groups live together, share culture and freely married with one-another in the study area.

To sum up, this study provide information on the Phenotypic, genotypic and allelic frequencies of ABO and Rh blood group in Oromo, Amhara and Wolayita ethnic groups of Robe Secondary and Preparatory and Zebela Primary school of Bale zone – Ethiopia. We hope that it will serve as a reference for other studies and future utilities in health planning.

5.3. Recommendations

The following recommendations are drawn from the study:-

- The present study is the first study that documents the phenotypic, genotypic and allelic frequencies of ABO and Rh D blood groups among the Oromo, Amhara and Wolayita ethnic groups in Bale, Robe town. So Data obtained from this research will be used in the planning of blood transfusion programmes and reducing HDN since it is an integral part of the genetic profile of Ethiopian population.

- There are techniques like PCR, ELISA that allows a more detailed determination of blood group typing and therefore a better match for transfusion. So, the researcher recommend to all concerned governmental and non-governmental organizations to facilitate the use of such blood group typing mechanisms in hospitals where blood transfusion services are highly prevalent.

- It is very important, in order to avoid any complications during a blood transfusion, to detect Bombay phenotype individuals because the usual tests for ABO blood group system would show them as group O. Since Anti-H immunoglobulins can activate the complement cascade, it will lead to the lysis of RBCs while they are still in the circulation, provoking an acute hemolytic transfusion reaction. This, of course, cannot be prevented unless the lab technologist that is involved has the means and the thought to test for Bombay group. In the researcher's opinion, it needs an urgent call for comprehensive effort to technology and technique innovators and implementers of this field to fill the gap in identifying and determining the proportion of Bombay (Oh/hh) blood group from O blood phenotype to reduce transfusion.

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7. APPENDICES

7.1. Appendix Tables

Appendix Table 1: Data collection and Registration format

No	Sex	Ethnic Group			Blood group result/Phenotypic result							
		Parents		Students	With Out Rh Factor				With Rh Factor			
		Father	Mother		O-	A-	B-	AB-	O+	A+	B+	AB+

Oromo O+/- = **A+/- =** **B+/- =** **AB+/- =** **Amhara O+/- =** **A+/- =** **B+/- =** **AB+/=**

Wolayita O+/- = **A+/- =** **B+/- =** **AB+/- =**

Researcher's Name &Sign_____ **School Director's Name& Sign_____**

Appendix Table 2: **Students Agreement Consent Form**

No	Full Name	Sex	Ethnic group	Signature	Remarks

Remark O = Oromo, A = Amhara, W= Wolayita

Appendix Table 3: A p-value of 0.05 or less is usually regarded as statistically significant, i.e. the observed deviation from the null hypothesis is significant.

Degrees of freedom (df)	**χ^2 value**										
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59
P value (Probability)	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
	Insignificant								**Significant**		

Source: - NIST (2006).

Study Group	ABO Blood Grouping System					Rh (Rhesus Blood Grouping System	
	O	A	B	AB	Total	Rh+	Rh-
Oromo	84 (42)	56 (28)	50 (25)	10 (5)	200	187 (93.5)	13 (6.5)
Amhara	86 (43)	58 (29)	46 (23)	10 (5)	200	189 (94.5)	11 (5.5)
Wolayita	89 (44.5)	54 (27)	48 (24)	9 (4.5)	200	189 (94.5)	11 (5.5)
Over all	259 (43.17)	168 (28)	144 (24)	32 (4.83)	600	565 (94.17)	35 (5.83)

Formulas Used to calculate the Genotypic and Allelic frequencies of ABO Blood Group

Calculation of allelic, genotypic frequencies and expected number of each blood group

For oromo ethnic group

Calculating the allelic frequencies of A, B and O blood group

$r = \sqrt{O}$

$r = \sqrt{0.42} = 0.6481$

$p = 1 - \sqrt{B+O}$

$p = 1 - \sqrt{0.25 + 0.42} = 0.1815$

$q = 1 - \sqrt{A+O}$

$q = 1 - \sqrt{0.28 + 0.42} = 0.1633$

A correction factor (d) will be calculated according to $d = 1 – p – q – r$.

So, $d = 1 – 0.1815 – 0.1633 – 0.6481$, $d = 0.0071$

The final allele frequencies were then calculated as follows: $p1 = p (1 + d/2)$; $q1 = q (1 + d/2)$; $r1 = (r + d/2) (1 + d/2)$ where p1, q1, and r1

So p1 (corrected frequencies of blood group A) will be:-

$p1 = p(1+d/2)$

$p1 = 0.1815(1 + 0.0071/2) = 0.1821$

$q1 = q(1 + d/2)$

$q1 = 0.1633(1 + 0.0071/2) = 0.1639$

$r1 = (r + d/2)(1 + d/2)$

$r1 = (0.6481 + 0.0071/2)(1 + 0.0071/2) = 0.6540$

So the allelic frequencies of $I^A = 0.1821$, $I^B = 0.1639$, $I^O = 0.6540$ for Oromo ethnic group.

Calculating the Genotypic frequencies and Expected number

- Genotype $I^{AA} = p^2 = (0.1821)^2 = 0.0332$, Exp. No. = 0.0332 x 200 = 6.64
- Genotype $I^{AO} = 2pr = 2(0.1821x0.6540) = 0.2382$, Exp. No.= 0.2382x200=47.64

So the expected number of blood group A is 54.28

- Genotype $I^{BB} = q^2 = (0.1639)^2 = 0.0269$, Exp. No.= 0.0269 x200 = 5.38
- Genotype $I^{BO} = 2qr = 2(0.1639x0.6540) = 0.2144$, Exp. No.= 0.2144x200= 42.88

So the expected number of blood group A is 48.26

- Genotype $I^{AB} = 2pq = 2(0.1821x\ 0.1639) = 0.0597$, Exp. No = 0.0597x200=11.94

The expected number of blood group AB is 11.94

- Genotype $I^{OO} = r^2 = (0.6540)^2 = 0.4277$, Exp. No = 0.4277 x 200 = 85.54

The expected number of blood group O is 85.54

The Chi-square (χ^2) test statistic is then

$$\chi^2_{ABO\,(Oromo)} = \sum \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2_{ABO\,(Oromo)} = \frac{(84-85.54)^2}{85.54} + \frac{(56-54.28)^2}{54.28} + \frac{(50-48.26)^2}{48.26} + \frac{(10-11.94)^2}{11.94} = 0.4601$$

The degree of freedom is 4-1 = 3

So the p value is $p < 0.95$

For Amhara ethnic group

Calculating the allelic frequencies of A, B and O blood group

$r = \sqrt{O}$

$r = \sqrt{0.43} = 0.6557$

$p = 1 - \sqrt{B+O}$

$p = 1 - \sqrt{0.23 + 0.43} = 0.1876$

$q = 1 - \sqrt{A+O}$

$q = 1 - \sqrt{0.29 + 0.43} = 0.1515$

A correction factor (d) will be calculated according to $d = 1 – p – q – r$.

So, $d = 1 – 0.1876 – 0.1515 – 0.6557$, $d = 0.0052$

The final allelic frequencies were then calculated as follows: $p1 = p (1 + d/2)$;

$q1 = q (1 + d/2)$; $r1 = (r + d/2) (1 + d/2)$ where p1, q1, and r1

So p1 (corrected frequencies of blood group A) will be:-

$p1 = p (1+d/2)$

$p1 = 0.1876 (1 + 0.0052 /2) = 0.1881$

$q1 = q (1 + d/2)$

$q1 = 0.1515 (1 + 0.0052 /2) = 0.1519$

$r1 = (r + d/2) (1 + d/2)$

$r1 = (0.6557+ 0.0052 /2) (1 + 0.0052/2) = 0.6600$

So the allelic frequencies of $I^A = 0.1881$, $I^B = 0.1519$, $I^O = 0.6600$ for Amhara ethnic group.

Calculating the Genotypic frequencies and Expected number

- Genotype $I^{AA} = p^2 = (0.1881)^2 = 0.0354$, Exp. No. = 0.0354 x 200 = 7.0763
- Genotype $I^{AO} = 2pr = 2(0.1881x\ 0.6600)=0.2483$, Exp. No.= 0.2483x200=49.66

  So the expected number of blood group A is 56.7363
- Genotype $I^{BB} = q^2 = (0.1519)^2 = 0.0231$, Exp. No.= 0.0231 x200 = 4.62
- Genotype $I^{BO} = 2qr = 2(0.1519x0.6600)=0.2005$, Exp. No.= 0.2005x200= 40.1016

  So the expected number of blood group A is 44.7216
- Genotype $I^{AB} = 2pq = 2(0.1881x\ 0.1519) = 0.0571$, Exp. No = 0.0571x200=11.42

The expected number of blood group AB is 11.42

- Genotype $I^{OO} = r^2 = (0.6600)^2 = 0.4356$, Exp. No = 0.4356 x 200 = 87.12

  The expected number of blood group O is 87.12.

The Chi-square (χ^2) test statistic is then

$$\chi^2_{ABO}\,(\text{Amhara}) = \sum \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2_{ABO\ (Amhara)} = \frac{(86-87.12)^2}{87.12} + \frac{(58-56.7363)^2}{56.7363} + \frac{(46-44.7216)^2}{44.7216} + \frac{(10-11.42)^2}{11.42} = 0.2556$$

The degree of freedom is 4-1 = 3

So the p value is p > 0.95

For Wolayita ethnic group

Calculating the allelic frequencies of A, B and O blood group

$r = \sqrt{O}$

$r = \sqrt{0.445} = 0.6671$

$p = 1 - \sqrt{B+O}$

$p = 1 - \sqrt{0.24 + 0.445} = 0.1724$

$q = 1 - \sqrt{A+O}$

$q = 1 - \sqrt{0.27 + 0.445} = 0.1544$

A correction factor (d) will be calculated according to $d = 1 – p – q – r$.

So, $d = 1 – 0.1724 – 0.1544 – 0.6671$, $d = 0.0061$

The final allelic frequencies were then calculated as follows: $p1 = p\,(1 + d/2)$;

$q1 = q\,(1 + d/2)$; $r1 = (r + d/2)\,(1 + d/2)$ where p1, q1, and r1

So p1 (corrected frequencies of blood group A) will be:-

$p1 = p\,(1+d/2)$

p1 = 0.1724 (1 + 0.0061 /2) = 0.1729

q1 = q (1 + d/2)

q1 = 0.1544 (1 + 0.0061/2) = 0.1549

r1 = (r + d/2) (1 + d/2)

r1 = (0.6671+ 0.0061/2) (1 + 0.0061/2) = 0.6722

So the allelic frequencies of I^A= 0.1729, I^B = 0.1549, I^O = 0.6722 for Wolayita ethnic group.

Calculating the Genotypic frequencies and Expected number

- Genotype $I^{AA} = p^2 = (0.1729)^2 = 0.0299$, Exp. No. = 0.0299 x 200 = 7.98
- Genotype I^{AO} = 2pr = 2(0.1729 x 0.6722)=0.2324, Exp. No.= 0.2324x200=46.48

  So the expected number of blood group A is 52.46
- Genotype $I^{BB} = q^2 = (0.1549)^2 = 0.0240$, Exp. No.= 0.0240 x200 = 4.8
- Genotype I^{BO} = 2qr = 2(0.1549 x0.6722)=0.2082, Exp. No.= 0.2082x200= 41.64

  So the expected number of blood group A is 46.44
- Genotype I^{AB} = 2pq = 2(0.1729x 0.1549) = 0.0536, Exp. No = 0.0536x200=10.2

  The expected number of blood group AB is 10.72
- Genotype $I^{OO} = r^2 = (0.6722)^2 = 0.4519$, Exp. No = 0.4356 x 200 = 90.38

  The expected number of blood group O is 90.38.

The Chi-square (χ^2) test statistic is then

$$\chi^2_{ABO}\,(\text{Wolayita}) = \sum \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2_{ABO\ (Wolayita)} = \frac{(89-90.38)^2}{90.38} + \frac{(54-52.46)^2}{52.46} + \frac{(48-46.44)^2}{46.44} + \frac{(9-10.72)^2}{10.72} = 0.3965$$

The degree of freedom is 4-1 = 3

So the p value is $p \leq 0.95$

Overall results for ABO and Rh(D) blood groups

Calculating the allelic frequencies of A, B, O, D and d blood group

$r = \sqrt{O}$

$r = \sqrt{0.4317} = 0.6570$

$p = 1 - \sqrt{B+O}$

$p = 1 - \sqrt{0.24 + 0.4317} = 0.1804$

$q = 1 - \sqrt{A+O}$

$q = 1 - \sqrt{0.28 + 0.4317} = 0.1564$

A correction factor (d) will be calculated according to $d = 1 – p – q – r$.

So, $d = 1 – 0.1804 – 0.1564 – 0.6570$, $d = 0.0062$

The final allelic frequencies were then calculated as follows: $p1 = p (1 + d/2)$;

$q1 = q (1 + d/2)$; $r1 = (r + d/2) (1 + d/2)$ where p1, q1, and r1

So p1 (corrected frequencies of blood group A) will be:-

$p1 = p (1+d/2)$

$p1 = 0.1804 (1 + 0.0062 /2) = 0.1810$

$q1 = q (1 + d/2)$

$q1 = 0.1564 (1 + 0.0062 /2) = 0.1569$

$r1 = (r + d/2) (1 + d/2)$

$r1 = (0.6570+ 0.0062 /2) (1 + 0.0062/2) = 0.6621$ and for Rh(D) blood group

$d = \sqrt{q}$

$d = \sqrt{0.0588} = 0.2415$

$D = 1-q$

$D = 1-0.2415 = 0.7585$

So the overall allelic frequencies of $I^A = 0.1810$, $I^B = 0.1569$, $I^O = 0.6621$, $D = 0.7585$ and $d=0.2415$.

Calculating the Genotypic frequencies and Expected number

- Genotype $I^{AA} = p^2 = (0.1810)^2 = 0.0328$, Exp. No. = 0.0328 x 600 = 19.68

- Genotype I^{AO} = 2pr = 2(0.1810 x 0.6621)=0.2397, Exp. No.= 0.2397600=143.82
  So the expected number of blood group A is 163.68
- Genotype $I^{BB} = q^2 = (0.1569)^2 = 0.0246$, Exp. No.= 0.0246 x600 = 14.76
- Genotype I^{BO} = 2qr = 2(0.1569 x0.6621)=0.2078, Exp. No.= 0.2078x600= 124.68
  So the expected number of blood group A is 139.44
- Genotype I^{AB} = 2pq = 2(0.1810 x 0.1569) = 0.0568, Exp. No = 0.0568x600=34.08
  The expected number of blood group AB is 34.08
- Genotype $I^{OO} = r^2 = (0.6621)^2 = 0.4384$, Exp. No = 0.4384 x 600 = 263.04
  The expected number of blood group O is 263.04. And the genotypic frequencies and expected number of Rh(D) blood group will be
- Genotype DD = $p^2 = (0.7585)^2 = 0.5753$, Exp. No = 0.5753 x 600 = 345.18
- Genotype of Dd = 2pq = 2(0.2415x0.7585)= 0.3664, Exp. No = 0.3664x600=219.84
  So the expected number of Rh(D) +ve blood group is 563.02
- Genotype dd = $q^2 = (0.2415)^2 = 0.0583$, Exp. No = 0.0583x600= 34.98
  The Chi-square (χ^2) test statistic is then

  χ^2_{ABO} (Overall) $= \sum \frac{(O_i - E_i)^2}{E_i}$

  $$\chi^2_{ABO\ (overall)} = \frac{(259-263.04)^2}{263.04} + \frac{(168-163.68)^2}{163.68} + \frac{(144-139.44)^2}{139.44} + \frac{(29-34.08)^2}{34.08} = 1.082$$

  The degree of freedom is 4-1 = 3
  So the p value is $p \leq 0.8$

Figure 2 Blood sample testing in Zebela Primary School

Figure 3 Awareness Creation on Research topic by preparing Coffee- tea Ceremony.

8. ETHICAL CLEARANCE LETTER

Ref.No BaMU-43/6048/11

Date 08/05/05

To Robe town Health Office

Robe

To Nigusu Girma Negewo

Issue:- Giving Ethical Clearance and Support Letter for a Research

Application Title:- Frequency of ABO and Rh Blood Group Alleles among Oromo, Amhara and Wolayita Students in Robe Secondary and Preparatory and Zebela Primary School, Bale Zone, Ethiopia.

Department:- Biology

Programme:- Genetics

University:- Haramaya University

Research ethics exist to ensure that the principles of justice, respect and avoiding doing harm are upheld, by using agreed standards. These basic principles are universal, though there are of course many subtleties and diversities, and the contingent aspects of how principles are understood, interpreted and practiced can vary from place to place. However, all variations tend to revolve around the question of how to balance the interests of the individual with those of the community/society/family and the goals of research studies.

So, here is an application attached with research proposal inquiring research ethical clearance letter. Hence, it is revised by Bale Zone Health Office Management Committee and has got acceptance.

Here by, we feel happy when we recommend Mr. Nigusu Girma to get the necessary assistance from any concerned body, and our office require to get one copy of the final research result to use it in future health planning.

Sincerely