GENOTYPIC AND ALLELIC FREQUENCIES OF THE ABO AND Rh-D BLOOD GROUPS AMONG PRIMARY AND SECONDARY SCHOOL STUDENTS OF HIRNA, WEST HARARGHE ZONE OF OROMIA

MSc Thesis

Girma Legesse

October 2016

Haramaya University, Haramaya

Genotypic and Allelic Frequencies of the ABO and Rh-D Blood Groups among Primary and Secondary School Students of Hirna, West Hararghe Zone of Oromia

A Thesis Submitted to the Department of Biology,
Postgraduate Directorate
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOLOGY

Girma Legesse

October 2016
Haramaya University, Haramaya

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this Thesis entitled 'Genotypic and Allelic Frequencies of the ABO and Rh-D Blood Groups among Primary and Secondary School Students of Hirna, West Hararghe Zone of Oromia' prepared under my guidance by Girma Legesse. I recommend that it be submitted as fulfilling the thesis requirement.

Tamru Oljira (PhD)	_____	_____
Major Advisor	Signature	Date
Yohannes Petros (PhD)	_____	_____
Co- Advisor	Signature	Date

As a member of the Board of Examiners of the *MSc* Thesis Open Defense Examination, I certify that I have read and evaluated the Thesis prepared by Girma Legesse and examined the candidate. I recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Biology.

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent upon the submission of its final copy to the Council of Graduate Directorate (CGD) through the candidate's department or postgraduate directorate committee (PGDC).

DEDICATION

This thesis is dedicated to: my mother Alemitu Abebe, my wife Alemnesh Yifru, my sister Emebet Legesse and my two daughters.

STATEMENT OF THE AUTHER

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis, and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

This Thesis is submitted in partial fulfillment of the requirement for a *Master of Science degree in Biology* at the Haramaya University. The Thesis is deposited in the Haramaya University Library and is made available to borrowers under the rules of the Library. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotation from this Thesis may be made without special permission provided that accurate and complete acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this Thesis in whole or in part may be granted by the Head of the Directorate or Department when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances however, permission must be obtained from the author of the Thesis.

Name: Girma Legesse____ Signature: _____

Date: _____

Directorate/Department: Postgraduate/Biology

BIOGRAPHICAL SKETCH

The author was born in December 1972 in Oromia Regional State, West Hararghe Zone, Chiro Wereda, Assebe Teferi (Chiro) town. He attended his primary education (grades 1-5) in Assebe Teferi (Chiro) № 3 Primary School, (grades 6-8) in Assebe Teferi № 1 Junior Secondary School and his Secondary School (grades 9-12) in Chercher Comprehensive Secondary School. After completing grade 12 formal education, he attend the one year professional studies for primary school teachers conducted at Nazereth (Adama) Teacher Training Institute in the academic year of 1988/89 and employed as primary school teacher. Next, he attended project 1700-distance education prepared by MOE for upgrading primary school teachers to diploma under governance of Adama Teachers College and awarded the diploma in October 10, 2004. Then immediately he joined AAU in summer in order to study his B.Edu in Biology and certified in September 15, 2009. Then having four years of *B.Ed degree* experiences in teaching Biology, in Hirna Secondary School, he joined the Postgraduate Directorate at Haramaya University in 2012 to study for his *MSc degree* in Biology.

ACKNOWLEDGEMENTS

The author expresses his sincere thanks to his advisors, Dr Tamiru Oljira and Dr Yohannes Petros, who put him in the right track of the research. The success and timely accomplishment of this study would have been difficult without generous, tireless assistance from the early title selection until the final write up of the thesis through providing their constructive and even teaching comments. Indeed their guidance made this thesis research possible. Furthermore, he expresses his sincere thanks to Hirna Health Center for their cooperative works, Tullo Wereda Health Bureau, Education Bureau, and Hirna Primary and Secondary School for giving permission to carry out laboratory work for this study.

The author expresses his sincere thanks to the Haramaya University for their facilitate finance for these study. Finally, he is grateful and will never forget Wondwosen Legesse, Zeyede Belayneh and all the participants involved formally or informally for they consciousness and spontaneous participation in this study.

ACRONYMS AND ABBREVIATIONS

HDN:	Hemolytic Disease of the New Born
HWE:	Hardy-Weinberg Equilibrium
IG:	Immunoglobulin
RBC:	Red Blood Cell
Rh:	Rhesus

TABLE OF CONTENTS

continued…

LIST OF TABLES

Table **page**

LIST OF FIGURES

Genotypic and Allelic Frequencies of the ABO and Rh-D Blood Groups among Primary and Secondary School Students of Hirna, West Hararghe Zone of Oromia

ABSTRACT

This study was aimed at identifying the distribution patterns of ABO and Rh-D blood group system alleles among Hirna primary and secondary schools in 2015/16 academic year. Six hundred volunteer students were involved to determine the ABO and Rh-D blood group phenotypes, and to estimate allelic, and genotypic frequencies of the blood groups. The blood sample was collected by qualified laboratory technicians using the standard clinical procedures with sterilized needle from each student. The blood group of every individual was determined on the basis of agglutination reaction with anti-sera and recorded as blood group A^+, B^+, AB^+, O^+ or A^-, B^-, AB^- and O^-. The study revealed that blood group O is at the highest frequency (47.83%) followed by blood group A (26.50%), B (20.00%) and the least percentage frequency was blood group AB (5.67%). The allelic frequency distribution of ABO revealed that i allele is highly frequent (0.687) followed by I^A (0.176), and I^B (0.137).The Rh-D distribution also varies among the four ABO blood groups. For Rh-D positive, O^+ was the highest frequent (44.33%) compared with A^+ (24.17%), B^+ (18.67%), AB^+(4.83%) and for Rh-D negative, O^- was the highest frequent (3.5%) compared with A^- (2.33%), B^- (1.33%), and AB^- (0.83%) respectively. The total percentage of Rh-D positive was 92% and that of Rh-D negative was found to be 8% as well as 'D' allele was highly frequent (0.7172) than 'd' allele (0.2828). The genotypic frequency for ABO blood types shows that ii is the most frequent (0.4714) followed I^AI^O (0.2415), I^BI^O (0.1888), I^AI^B (0.0484), I^AI^A (0.0309), and I^BI^B (0.019) respectively, for Rh-D I^DI^D is most frequent (0.5144) followed I^DI^d (0.406) and I^dI^d (0.0800). Generally, O blood phenotype is the highest frequent and AB blood phenotype is the least frequent in the study population.

Key words: ABO, Allele, HDN, Hirna, Rhesus factor,

1. INTRODUCTION

The blood plays more roles than one might expect, it is involved in respiration, nutrition, waste elimination, thermoregulation, immune defense, water and acid-base balance, and internal communication. Most adults have 4 to 6 L of blood. Erythrocytes are also known as red blood cells (RBCs), leukocytes are also known as white blood cells (WBCs) and Platelets. Erythrocytes have two principal functions. (1) to pick up oxygen from the lungs and deliver it to tissues elsewhere and (2) to pick up carbon dioxide from other tissues and unload it in the lungs. An erythrocyte is a disc-shaped cell with a thick rim and a thin sunken center where the nucleus used to be. It is about 7.5µm in diameter and 2.0 µm thick at the rim (Saladin, 2003). The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules (Daniels, 2002).

The ABO and Rh-D 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. Blood group or blood type is based on the presence or absence of inherited antigenic substance on the surface of red blood cells (RBC) that can be determined by specific antibodies (Firkin *et al*., 1989). More than 600 surface antigens have been finding on red blood cells (Waters, 1995). Several of these antigens that stem from one allele or very closely linked genes collectively form a blood group system (Waite, 2009). Development of these antigens are genetically controlled and they appear early in fetal life and remain unchanged until death (Firkin *et al.*, 1989).

The ABO and Rh-D blood groups are among the most important blood groups (Seeley *et al*., 1998). In the ABO blood group system, individuals are divided into four major blood groups; A, B, AB and O. According to the presence of the antigens; type A blood has type A antigen, type B blood has type B antigen, type AB blood has both types of antigens, and type

O blood has neither A nor B antigens. In addition, plasma from type A blood contains type B antibodies, which act against type B antigens, whereas plasma from type B blood contains type A antibodies, which act against type A antigens. Type AB has neither type of antibody and type O blood has both A and B antibodies (Seeley *et al.,* 1998). People are Rh positive if they have a certain Rh-D antigen on the surface of their erythrocytes, and people are Rh negative if they do not have this Rh-D antigen (Daniels, 2007).

The frequencies of ABO and Rh-D blood groups vary from one population to another and from time to time in the same region. The knowledge of distribution of ABO and Rh-D blood groups at local and regional levels is helpful in the effective management of blood banks and safe blood transfusion services (Patel *et al*., 2012). 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 (Platt *et al.,* 1985). Thus, this shows that the need for estimates of blood group and gene's frequency studies provides very valuable information on the genetic similarity of different populations and to some extent on their ancestral genetic relation, despite the cultural and religious differences of the populations (Khurshid *et al*., 1992).

In Nigeria, Sudan, Bangladesh, Saudi Arabia, India, and Pakistan and in other countries in the world, there are a number of literatures that report the frequency of ABO and Rh blood group alleles. However, a few studies have been carried out in Ethiopia on the distribution of ABO and Rh-D blood groups. There are no studies which have been conducted relating distribution of ABO and Rh blood groups in West Hararghe zone Tullo Wereda, Hirna town. In this study, an attempt is made to study the distribution of ABO and Rh-D blood group systems among the students.

Therefore, the General Objective of the Study was-To identify the distribution patterns of ABO and Rh-D blood group alleles among students of Hirna primary and secondary schools.

The Specific Objectives of the Study were;

1) To determine the distribution of the ABO and Rh-D blood groups phenotypes among the students.
2) To estimate the allelic frequencies of the ABO and Rh-D blood groups among the students.
3) To estimate the frequencies of the ABO and Rh-D blood groups genotypes among the students.
4) The check if the population is at Hardy Weinberg to ABO and Rh-D blood group systems.

2. LITERATURE REVIEW

2.1 History of ABO and Rh-D Blood Groups

Karl Landsteiner first described the ABO blood group in 1900, and it served the beginning of blood banking and transfusion medicine (Ali *et al.,* 2005). Even after 116 years, the single most important test performed in blood banking services is determination of ABO blood groups to avoid morbidity and mortality (Honig and Bore, 1980). Furthermore, the presence of Rhesus system was recognized in 1939 and it was confirmed within few years (Landsteiner and Weiner, 1940).

The ABO blood group system derives its importance from the fact that A and B are strongly antigenic and anti A and anti B naturally occurring antibodies present in the serum of persons lacking the corresponding antigen and these antibodies are capable of producing intravascular haemolysis in case of incompatible transfusion (Harmening and Firestone, 2005).

The Rh-D antigens are also highly immunogenic and at present 49 *Rh* antigens have been identified (Dean, 2005). Rh-D antigen is the most significant and Rh-D negative individuals produce anti-D if they encounter the Rh-D antigen through transfusion or pregnancy and causes haemolytic transfusion reaction, or haemolytic disease of fetus and newborn. For this reason, the *Rh-D* status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be (Bethesda, 2005).

2.2. Inheritance of Blood Groups

The ABO blood group and the Rhesus factor or Rh-D blood group are two of the most notable blood groups in humans due to their importance and association with blood transfusion (Khattak *et al*., 2008). A single gene (the ABO gene) controls the ABO blood type with three alleles. *i*, I^A, I^B. Blood group of an individual is determined by two alleles of a gene, which are inherited from both parents. The I^A allele gives type A, I^B gives type B, and *i* gives type O. As

both, I^A and, I^B are dominant over *i*, individuals with, I^AI^A or I^Ai gives type A blood, Individuals with, I^BI^B or I^Bi have type B blood, only *ii* people have type O blood. Individuals with I^AI^B have both antigens, because A and B express a special dominance relationship called co-dominance, which means the type A and B parents can have an AB child. A type A and a type B couple can also have a type O child if they are both heterozygous (I^Ai, I^Bi). The cis-AB phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A or B red blood cells, which can help solve the problem of an apparently genetically impossible blood group (Yazer, 2006).

The Rh-D antigen was name after the rhesus monkey, *Macaca mulatta* (Zimmerman) where it was initially detect. The inheritance of these antigens is complex, and there are two theoretical models attempt to explain the pattern of inheritance. The Wiener system postulates a single gene locus with a series of at least ten multiple alleles. The Fisher system assumes the existence of at least three closely linked loci designated as C, D, and E. Both are currently in use and are still being studying. However, only the presence of the D antigen in the Fisher system serves as the basis for classification of the Rh blood group; this way, the mode of inheritance is simply single gene inheritance with accompanying dominance (Dennis *et al*., 1998).

2.3. ABO Blood Group System

Although the ABO blood group name consists of three letters, ABO blood typing designates the presence or absence of just two antigens, A and B. Both are glycoproteins. The ABO locus is located on chromosome 9 specifically in the segment 9q34.1-q34.2 (Narahara et al. 1986) contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. The ABO locus has three main allelic forms: I^A, I^B *i*. The I^A allele encodes a *glycosyltransferase* that bonds α-*N-acetylgalactosamine* to the D-galactose end of the H antigen, producing the A antigen. The I^B allele encodes a

glycosyltransferase that bonds α-D-galactose to the D-galactose end of the H antigen, creating the B antigen (Bhasin *et al*., 1992) (Appendix vii).

People whose erythrocytes have A antigens on their erythrocyte membrane surfaces are designated blood type A, and those whose erythrocytes have B antigens are blood type B. People can also have both A and B antigens on their erythrocytes, in which case they are blood type AB. people with neither A nor B antigens are designated blood type O. Normally the body must be exposing to a foreign antigen before an antibody can produced. This is not the case for the ABO blood group. Individuals with type A blood without any prior exposure to incompatible blood have preformed antibodies to the B antigen circulating in their blood plasma. These antibodies, referred to as anti-B antibodies, will cause agglutination and haemolysis if they ever encounter erythrocytes with B antigens. Similarly, an individual with type B blood has pre-formed anti-A antibodies. Individuals with type AB blood, which has both antigens, do not have preformed antibodies to either of these. People with type O blood lack antigen A and antigen B on their erythrocytes, but both anti-A and anti-B antibodies circulate in their blood plasma (American RC 'US', 2013).

Table 1: Summary of human blood groups phenotype and genotype with its surface antigen and serum antibodies.

Phenotype	**Genotype**	**Antigen (RBC)**	**Antibodies (Serum)**
A	I^AI^A	A	Anti-B
	I^AI^O	A	Anti-B
B	I^BI^B	B	Anti-A
	I^BI^O	B	Anti-A
AB	I^AI^B	A and B	None
O	I^OI^O	None	Anti-A and B

2.4. Rh-D Blood Group System

The term Rh refers not only to a specific red blood cell antigen but also to a complex blood group system that currently composed of 58 different antigenic specificities (Chou and Westhoff, 2011). The Rh locus is located on the long arm of chromosome 1 (on 1p36-p34). It contains the Rh-D genes. Rh-D contains 10 exons and spans a ~75-kb DNA sequence. The Rh-D gene is flanked by two 9-kb, highly homologous sequences called "Rhesus boxes" (Wagner et al., 2005). An individual either has, or does not have, the "Rhesus factor" on the surface of their red blood cells. This term strictly refers only to the most immunogenic D antigen of the Rh blood group system. The status is usually indicated by Rh positive (Rh+ does have the D antigen) or Rh negative (Rh- does not have the D antigen) suffix to the ABO blood type. However, other antigens of this blood group system are also clinically relevant. In contrast to the ABO blood group, immunization against Rh can generally only occur through blood transfusion or placental exposure during pregnancy in women (Daniels, 2007).

2.5 Distribution of ABO and Rh-D Blood Groups

In Ethiopian Sodo, Silte and Meskan ethnic groups, the blood group “O” was predominant with 36.73%, 40.14%, and 46.26% respectively. In all the Rh positive subjects whereas blood group “A” was predominant (5.4%) in the Rh negative subjects only in the Sodo ethnic group among the three ethnic groups. The percentage of Rh-D positive and negative subjects was 91.16%, 93.19%, 91.84% and 8.84%, 6.81%, 8.16% in Sodo, Silte and Meskan ethnic groups, respectively. The frequency of ABO blood groups in both Rh-D positive and negative subjects among the three ethnic groups of the Silte Zone, Ethiopia was O > A > B > AB, except in the Sodo ethnic group where the blood group A was the commonest among Rh-D negative subjects (Kassahun *et al*., 2014).

In Nigeria, the blood group identified were 21.4% blood group B, 21.6% blood group A, 54.2% blood group O and 2.8% blood group AB of the total blood samples. Overall gene frequencies for the O, A and B genes were 0.7398, 0.1305, and 0.1298 respectively. For the

Rh-D gene, 95.2% were Rh-positive while 4.8% were Rh-negative (Muwangi *et al*., 1999). Similarly the phenotypic distribution of ABO blood groups in Kuwaiti population was 26.7% with A, 24.1% with B, 4.6% with AB, and 44.6% with O and the calculated gene frequencies were 0.6678 for O, 0.1768 for A, and 0.1554 for B (Al-Bustan *et al*., 2002). Blood group B has highest frequency in Northern India and neighboring Central India and neighboring Central Asia, and its incidence diminutions both toward the west and toward the east, falling to single digit percentage in Spain. It is believed to have been entirely absent from Native American and Australian Aboriginal population prior to the arrival of European in those area (Encyclopedia Britannica, 2002). 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 population and Blackfoot Indians of Montana (Dean, 2005) (Table 2).

Table 2: Comparison of frequency and percentage of ABO and Rhesus blood group in different countries of the world

Population	Reference	A	B	AB	O	Rh+	Rh$^-$
USA	Frances (2002)	41.0	9.0	4.0	46.0	85.0	15.0
Britain	Behra and Joshi (2013)	42.0	8.0	3.0	47.0	83.0	17.0
Australia	ARC (2013)	38	10	03	49	NA	NA
Saudi Arabia	Bashwari et al. (2001)	24.0	17.0	4.0	52.0	93.0	7.0
Pakistan	Rahman and Lodhi (2004)	22.40	32.40	8.40	30.50	93.0	7.0
Kuwaiti	Al-Bustan et al. (2002)	26.7	24.1	4.8	44.6	NA	NA
Punjab	Sidhu (2003)	21.91	37.56	9.30	31.21	97.30	2.70
Khatmandu, Nepal	Pramanik et al. (2000)	26.2	22	4.4	47.5	80.3	19.7
Nepal	Pramanik et al. (2000)	34.0	29.0	4.0	32.50	96.70	3.30
Eastern Ahmedabad	Wadhwa et al. (1998)	23.3	35.5	8.8	32.5	94.2	5.8
Western Ahmedabad	Patel et al. (2012)	21.94	39.40	7.86	30.79	95.05	4.95
Surat(14)	Mehta and Swadas (2012)	24.10	34.89	8.69	32.32	94.18	5.82
New Guinea	Loua et al. (2007)	22.50	23.70	4.70	48.90	95.90	4.10
Nigeria	Mwangni (1999)	21.60	21.40	2.80	54.20	95.20	4.80
Nairobi, Kenya	Lyco et al. (1992)	20.0	32.2	6.1	41.7	92.8	7.2
In South Ethiopia							
Sodo	Kasahun et al. (2014)	31.97	25.85	5.44	36.74	91.156	8.843
Silte	Kasahun et al. (2014)	28.57	23.13	5.44	42.86	93.197	6.8
Meskan	Kasahun et al. (2014)	23.81	21.09	5.44	49.66	91.84	8.16

NB: “NA” Stands for not available.

2.6. Clinical Significance of the ABO and Rh-D Blood Group System

2.6.1. Blood Transfusion

Transfusion medicine is a specialized branch of hmatology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and other blood products. Across the world, a medical doctor must prescribe blood products in a similar way as medicine. Much of the routine work of a blood bank involve testing blood from both donors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfuse between a donor and recipient, a severe acute hemolytic reaction with RBC 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 haemolysis of the transfused blood. Patients should ideally receive their own blood or type specific blood products to minimize the chance of a transfusion reaction. Risks can be further reducing by cross matching blood. However, this may skipped when blood is required for an emergency (Bruce, 2002).

2.6.2. Hemolytic Disease of the New Born (HDN)

The Rh-D antigens are highly immunogenic, and most of the Rh antibodies should be consider as potential causes of hemolytic transfusion reactions and HDN. Whereas most blood types are, determine by red blood cell antigens that differ by one or two amino acids of its enzymes, the Rh blood group contains the D antigens that differs from the C/c and E/e antigens by 35 amino acids its enzymes. This large difference in amino acids is the reason why the Rh-D antigens are potent at stimulating an immune response (Westhoff, 2004).

The most notable medical importance of this blood group system is the occurrence of Rh-D incompatibility between mother and fetus, which is a major factor in the development of erythroblastosis fetalis or haemolytic disease of the newborn (Dennis, *et al.*, 1998). Rh-D

incompatibility can pose a major problem in some pregnancies when the mother is Rh-D negative and the foetus is Rh-D positive (Avent, 1999). If foetal blood leaks through the placenta and mixes with the mother's blood, the mother becomes sensitize to the Rh-D antigen. The mother produces Rh antibodies that cross the placenta and cause agglutination and haemolysis of foetal erythrocytes. This disorder is called Hemolytic disease of the newborn (HDN), or erythroblastosis foetalis, and it may be fatal to the foetus (Dennis *et al*., 1998).

On the other hand ABO blood group incompatibilities between the mother and the child does not usually cause hemolytic disease of the new born (HDN) because antibodies to the *ABO* blood groups are usually of the IgM type, which do not cross the placenta.(Yazer,2006).

2.6.3. Universal Donors and Universal Recipients

With regard to transfusion of 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 reaction of the recipient's anti-A and anti-B antibodies to transfused red blood cells. Blood donors with particularly strong anti-A, anti-B or any atypical blood group antibody are excluded from blood donation. The possible reaction of anti-A and anti-B antibodies present in the transfused blood to the recipients RBCs need not be consider, because a relatively small volume of plasma containing antibodies is transfused (Fauci, 1998).

Blood group AB individuals have both A and B antigens on the surface of their RBCs, and their blood plasma do not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood can receive blood from any group but cannot donate blood to either A, O or B group. They are known as universal recipients. Blood group A individuals have the A antigen on the surface of their RBCs, and blood serum containing IgM antibodies against the B antigen. Therefore, a group A individual can receive blood only from individuals of group A or O, and can donate blood to individuals with type A or AB. Blood group B individuals have the B antigen on the surface of their RBCs, and blood serum containing IgM

antibodies against the A antigen. On the contrary, blood group B can receive blood only from individuals of groups B or O, and can donate blood to individuals with type B or AB. Blood group O individuals do not have either A or B antigens on the surface of their RBCs but their blood serum contains IgM anti-A and anti-B antibodies against the A and B blood group antigens. On the other hand, a group O individual can receive blood only from a group O individual, but can donate blood to individual of any ABO blood group (Appendix iv). If a patient in a hospital situation is in need of a blood transfusion in an emergency, and if the time taken to process the recipient's blood would cause a detrimental delay, O negative blood can be issue, they are universal donors (Hillier, 2008).

2.6.4. Blood Group Genotyping

In addition to the current practice 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 basis 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 particularly for patients that needed many transfusions to prevent alloimmunization (Anstee, 2009).

2.6.5. Blood Products

A blood product is any component of the blood, which is collect from a donor for use in a blood transfusion. Whole blood is un commonly used in transfusion medicine at present; blood products may also called blood- based product to differ from blood substitute, which generally refer to artificially produced products. Whole blood may be classifies as a blood product or as a separate entity. Also, although many blood products have the effect of volume expansion, the group is usually distinguished from volume expanders, which generally refer to artificially produced substance and within the scope of blood substitutes (Henrik Bendixen., *et al*., 2012).

2.7. The Hardy-Weinberg Genetic Equilibrium

The Hardy-Weinberg model describes a mathematical relationship that allows the prediction of the frequency of offspring genotypes based on parental allele frequencies. It also predicts that allele frequencies will not change from one generation to the next, indicative of non-evolution (Klug and Cummings, 2002; Mayo, 2008). For a population, to be in Hardy-Weinberg equilibrium, the following assumptions are required to hold: random mating, no mutation, no migration, no stochastic effects or genetic drift due to small population size, and equal fertility for all genotype groups so that no selection is occurring (Minnelli, *et al*., 2008). Violation of any of these assumptions can result in evolutionary change in terms of allelic frequency distribution (Mayo, 2008). These conditions, however, seldom occur simultaneously, resulting to most populations not exhibiting Hardy-Weinberg equilibrium and are therefore evolving.

2.7.1 Extension of the Hardy- Weinberg Law to Loci with more than Two Alleles

When a single locus with two alleles, the Hardy Weinberg law tells us that 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 extend to any number of alleles that are sampled two at a time into a diploid zygote (Daniel *et al.*, 2007). The frequencies of the genotype at equilibrium were compute by the square of the allelic frequencies. $(p + q + r)^2 = p^2 (I^A I^A) + 2pr (I^A i) + q^2 (I^B I^B) + 2qr (I^B i) + 2pq (I^A I^B) + r^2 (ii)$, p, q and r were allelic frequency; $I^A I^A$, $I^A i$, $I^B I^B$, $I^B i$, $I^A I^B$, ii were genotype of ABO blood groups respectively (Griffith, *et al*., 2008).

Table 3: Punnet square showing Hardy-Weinberg frequencies for three autosomal alleles of ABO blood group.

		Male gamete		
Female gamete		I^A(p)	I^B(q)	I^O(r)
I^A(p)	$I^A I^A$	$I^A I^B$	$I^A I^O$
	p^2	pq`	pr
I^B(q)	$I^A I^B$	$I^B I^B$	$I^B I^O$
	pq	q^2	qr
I^O(r)	$I^A I^O$	$I^B I^O$	$I^O I^O$
	pr	qr	r^2

NB: p,q,r = frequency of allele I^A, I^B and I^O, respectively

5.8. Ethical Considerations

Research Ethical Review Committee of the Tullo Wereda Health Bureau approved the study. Participants were voluntarily participating after they become informs about the objective of the study. There was no risk associated with the process of blood collection.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was carried out at Hirna primary and secondary schools. Hirna is located in West Hararghe Zone, Oromia Region. It is about 43 Km from Chiro, West Hararghe zonal town and 369 Km from Finfinne (Addis Ababa) the capital city of Oromia and the Federal Democratic Republic of Ethiopia. Hirna town lies between 9^0 13' N and 41^0 06' E latitude and 9^0 21' N and 41^0 10' E longitude. The altitude of the town is 1723m-2318m and has 16^0C-18^0C average temperature with 250mm-900mm annual rainfall. Its population was 11,650 according to 2007 census (CSA, 2007) and the calculated growth based on the country growth rate indicates as, it might have reached 14,937 in 2016. The population consists of different ethnic groups but among these the Oromo is the largest and the most dominant one. Hirna town has three primary schools and one secondary school. For this study, Ethiopia Tikdem Primary School, which had 510 students (grade 5-8), and Hirna Secondary School which had 1558 students (9-10) during the 2015/2016 academic year were selected.

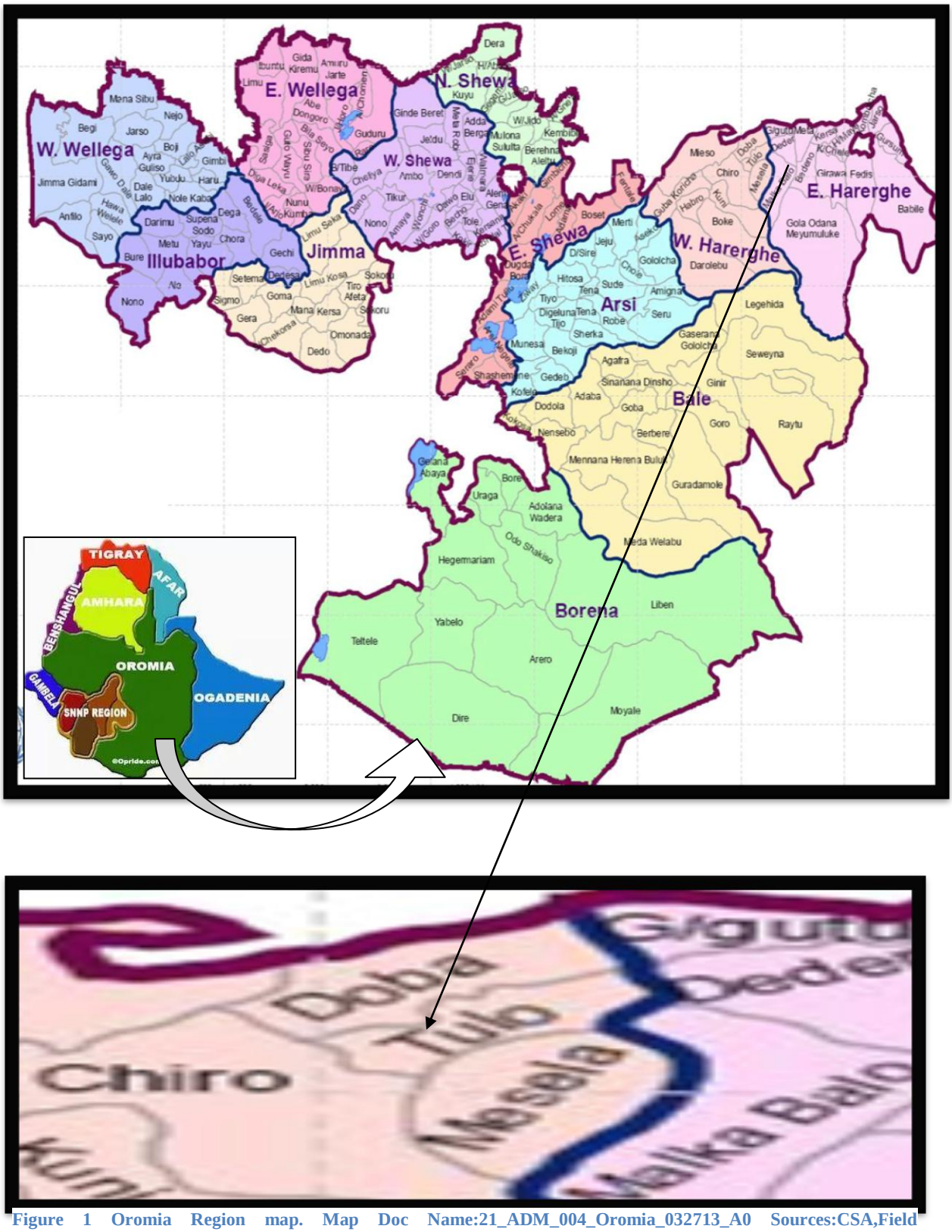


Figure 1 Oromia Region map. Map Doc Name:21_ADM_004_Oromia_032713_A0 Sources:CSA,Field SurveFeedback:ocha-eth@un.org http;//ochaonline.un.org/ethiopia

3.2. Study Sample and Sampling

For this study, 600 voluntary students were purposively selected from the two schools. From Ethiopia Tikdem School (Hirna) 95 students (72 male and 23 female) were selected. The largest portion (n=505) was taken from Hirna Secondary School; 324 of them were males and the remaining 181 were female students. All the subjects included in the study were 15 - 25 age groups. The research was conducted from January 2016 - August 2016.

3.3 Blood Sample Collection and Typing

The blood samples were collect from each student who was willing to participate in the research process. Traditional slide method (antigen - antibody compatibility test) was used to determine blood group of the participants which was also used in other published studies (Rahman, 1975) and the advantage of this method includes easy screening of blood groups in large number of samples within short period of time (Waters, 1995). Before taking the blood samples, students were made aware and signed the agreement form voluntarily (Appendix 1). Qualified laboratory technician took the blood samples. The blood samples were taken from each volunteer student by left hand finger pricks with sterile lancet and placed on three clean slides and a drop of one of the anti-sera, that is anti-A, anti-B, and anti-D, were added to individual's blood samples and mixed using single clean stick rod for each. The result was record correspondingly with respect to the donor code according to the blood sample agglutinated by anti-sera, as blood group A^+, B^+, AB^+, O^+ or A^-, B^-, AB^- and O^-. This means if the blood is agglutinated with both anti-A and anti-D but not agglutinate with anti-B it is recorded as A^+ and if only agglutinated with anti-A but not agglutinate with anti-B and anti-D it is recorded as A^-. If it is agglutinated with both anti-B and anti-D but not agglutinate with anti-A it is recorded as B^+ and if only agglutinated with anti-B but not agglutinate with anti-A and anti-D it is recorded as B^-. If agglutinated with all it is recorded as AB^+ and if it is not agglutinate with anti-D but not with the others it is recorded as AB^-. If as blood not agglutinate with anti-A and anti-B but agglutinated with anti-D it is recorded as O^+ and recorded as O^- if not agglutinate with all anti sera.

Table 4: Summary of determine Blood type using Anti-sera on the blood sample

No.	Anti-A	Anti-B	Anti-D	Blood type
1	✓	X	✓	A^+
2	✓	X	X	A^-
3	X	✓	✓	B^+
4	X	✓	X	B^-
5	✓	✓	✓	AB^+
6	✓	✓	X	AB^-
7	X	X	✓	O^+
8	✓	✓	X	O^-

NB: ✓- Agglutinates, X- not agglutinates.

3.4 Method of Data Analysis

For the present study, the frequencies of the blood group phenotypes were calculate as percentage. The frequency of Three alleles (I^A, I^B, i) are computed from phenotypic data with frequencies equal to p(A), q(B) and r(O) by using Hardy Weinberg law as:

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

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

$r=\sqrt{O}$

$D=1-d$

$d=\sqrt{dd}$

The obtained value for p, q and r was correct by using correction factor d.

$d = 1- (p + q + r)/2.$

$p^- = p(1 + d)$

$q' = q(1+ d)$

$r' = 1 – (p' + q')$

p', q', r', D, d were represent allele frequency and A, B, O, and d were phenotype frequencies (Al-Rubeai, 1975).

The frequency of the ABO and Rh-D blood group genotypes were calculated from estimated allelic frequency using the extension of Hardy Weinberg principle as employed by (Griffith *et al*., 2008).

The frequency of ABO blood group genotypes: $p'^2 + 2p'r' + q'^2 + 2q'r' + 2p'q' + r'^2 = 1$, were

$p'^2 = I^A I^A$ for Homozygous A

$2p'r' = I^A I^o$ for Heterozygous A

$q'^2 = I^B I^B$ for Homozygous B

$2q'r' = I^B I^O$ for Heterozygous B

$2p'q' = I^A I^B$ for Heterozygous AB

$r'^2 = I^O I^O$ for Homozygous O

The frequency of Rh-D blood group phenotypes: $D^2 + 2Dd + d^2 = 1$, were

D^2 = DD for Homozygous +Ve

2Dd for Heterozygous +Ve

d^2 =dd for Homozygous -Ve

In addition, a chi square test was use to test for the goodness of fit of Observed and Expected phenotype categories. Furthermore, the obtained results were displayed using tables.

$$\chi\mathbf{2} = \frac{\Sigma\,(\mathbf{Observed} - \mathbf{Expected})\mathbf{2}}{\mathbf{Expected}} =$$

Number of Expected phenotype was,

$p'^2 * n + 2p'r' * n$ ------- for A

$q'^2 * n + 2q'r' * n$ ------- for B

$2p'q' * n$ ------- for AB

$r'^2 * n$ ------- for O

NB: “n” Represent total sample population.

4. RESULTS AND DISCUSSION

The ABO blood group and the Rhesus factor or Rh-D blood group are two of the most known groups in humans due to their importance and association with blood transfusion (Khattak *et al.*, 2008). This observational study was carried out to determine the phenotype, allele, and genotype frequency distribution patterns of ABO and Rh-D blood groups among a total of 600 (396 male, 204 female) students of Hirna Primary and Secondary Schools. The classification of blood groups into type A, B, AB and O in ABO system; Rh-positive and Rh-negative in Rh-D system is based on the presence or absence of inherited antigenic substances on the surface of the red blood cells. The presence of a blood group antigen or phenotype is determine by testing RBCs from a study population with a specific antibody and calculating the percentage of positive and negative reactions. Its allelic frequency was estimate from calculated phenotypes and the genotypes were estimate from allelic frequency by using the low of Hardy-Weinberg equilibrium as described in section 3.4 of this thesis.

4.1. The Distribution of Blood Group Phenotypes

4.1.1. The Distribution of the ABO and Rh-D Blood Group Phenotypes

The percentage distribution of the ABO blood groups phenotypes for the sample population in this study is shown in Table 5. The results reveal that among male blood group percentage distribution, blood group O was predominant (48.74%), followed by group A (24.75%), group B (20.45%), and group AB (6.06%). Among female also O was predominant (46.08), followed by group A (29.90%), group B (6.50%), and group AB (4.90%). In general, blood group O was predominant (47.83%), followed by group A (26.50%), group B (20.00%), and group AB (5.67%).

The distribution of ABO blood group varies regionally, ethnically and from one population to another (Patel *et al*., 2012**)**. This finding is in agreement with other previous studies of different parts of the world such as in Southern Ethiopia Sodo, Silte and Meskan ethnic groups

(Kassahun *et al.*, 2015); in Nigeria (Mwangni, 1999); in Kuwaiti (Al-Bustan *et al*., 2002); in Lagos, Nigeria (Adeyemo, 2006). in Ogbomoso, Oyo State, Nigeria (Bakare *et al*., 2006); in the United States (Frances, 2002); among African American, among Western Europeans (Pramanik and Pramanik, 2000); South East and Western part of Bangladesh (Majumder and Roy, 1982); in Northern district of Dinajpur (Pathan *et al*., 2008) (Table 2) in which, blood group O has been found to be the most common blood group followed by blood groups A, B, and AB in the order of O > A > B > AB.

Thus, the segregation of the genes responsible for the ABO blood group system has always taken a particular pattern for its distribution. Even though, the distribution pattern of the ABO blood group of this study were in order of O > A > B > AB, however, female blood group A (29.90%) was greater than male blood group A (20.45%). With regard to blood group O, males were greater than that of the females (i.e. male 48.74% and female 46.08% respectively). Also from other previous studies, such as, in Nepal blood group A is the most common and followed by blood groups O, B, and AB (Pramanik and Pramanik, 2000). As well as in Eastern Ahmedabad (Wadhwa *et al*., 1998); in Western Ahmedabad (Patel *et al*., 2012) and Surat (Mehta and Swadas, 2012) blood group B is the most common followed blood group O, A, and B (Table 2). This difference might be the result of genetic and environmental condition. In addition, it can be seen from this study that blood group AB has the least percentage, which is most of the time very rare and similarly to the results obtained from other previous studies.

The percentage distribution of Rh-D blood group phenotype for the sample in this study is shown in Table 5. The results revealed that Rh-D^+ were more frequent in both male (92.68%) and female (90.69%) than Rh-D^- of male (7.32%) and female (9.31%). The total Rh-D positive were more frequent than Rh-D negative with a frequency of 92.0% and 8.0% respectively. Of the total 48 Rh negative samples, (9.31%) were females.

Table 5: The distribution of the ABO and Rh-D blood group phenotypes among student in the present study

Sex	**ABO blood group**				**Rh-D blood group**	
	A	B	AB	O	Rh-D^+	Rh-D^-
M(396)	98(24.75)	81(20.45)	24(6.06)	193(48.74)	367(92.68)	29(7.32)
F(204)	61(29.90)	39(19.12)	10(4.90)	94(46.08)	185(90.69)	19(9.31)
Total(600)	159(26.50)	120(20.00)	34(5.67)	287(47.83)	552(92.00)	48(8.00)

NB: The numbers in the body of this table represent the count of each of the categories and those in parenthesis represent the frequency in percentage of each cell by using row total for each category.

This study showed that the distribution of Rh-D +Ve (92.00%) was very high than Rh-D^- (8.00%) among the students. The percentage of male students (92.68%) Rh-D^+ was a little bit greater than female (90.69%). On the other hand, Rh-D^- in female was a little bit more frequent than male with (9.31%) and (7.32%) respectively. As it has been stated in the previous section of this study, it has been found that the prevalence of Rh-D positive remains very high compared to the Rh-D negative blood throughout the world. This study also followed the global trend with much higher Rh-D positive than Rh-D negative. The distribution pattern among ethnic groups in Southern part of Ethiopia Sodo, Silte and Meskan (Kassahun *et al.,* 2014); in Lagos, Nigeria (Adeyemo, 2006); in Nairobi, Kenya (Lyco *et al*., 1992) (Table 2) are almost comparable to this study. The distribution pattern in Nigeria (Mwangi, 1999); New Guinea (Loua *et al.*,); in Eastern Ahmedabad (Wadhwa *et al*., 1998); in Western Ahmedabad (Patel *et al*., 2012) and Surat (Mehta and Swadas, 2012) (Table 2) with small percentage Rh-D negative is also comparable to the present study. On the other hand, when compared to this study, higher frequency of Rh negative blood, Khatmandu, Nepal (Pramanik et al., 2000) 19.7%; in USA (Garratty *et al*., 2004) 15%; in Britain (Behra and Joshi, 2013) 17% and in Iran (Pour *et al*., 2001) 10.08% was observed.

4.1.2. CO-Distribution of ABO and Rh-D Blood Group Phenotypes

The percentage distribution of ABO Blood group with Rh-D phenotype for the study population is shown in Table 6. Among the total students, with Rh-D +Ve blood, O^+ is the highest (44.33%) followed by A^+, B^+, AB^+ with 24.17%, 18.67%, 4.83% respectively and with Rh negative, O^- is the highest (3.5%) followed by A^-, B^-, AB^-, with 2.33%, 1.33%, 0.83% respectively. Within the male percentage distribution of Rh-D^+, O^+ is highest (45.71%) followed by A^+, B^+, AB^+ with 22.47%, 19.19%, 5.30% respectively and for Rh-D^-, O^- is the highest (3.23%), followed by A^-, B^-, AB^-, with 2.27%, 1.26%, 0.76% respectively. Like male students, in the female students also O^+ is the highest (41.67%) followed by A^+, B^+, AB^+, with 27.45%, 17.65%, 3.92% respectively and for Rh-D^-, O^- is the highest (4.41%) followed by A^-, B^-, AB^- with 2.45%, 1.47%, 0.96% respectively. In all categories of the distribution patterns of blood group phenotypes for Rh-D^+ and Rh-D^-, it was observed that O > A > B > AB. However, the percentage of O^+, B^+ and AB^+ of male students are slightly greater than that their female counter parts. On the other hand, A^+ in female is found to be greater than the male students. In all Rh-D^- blood group phenotypes presented in Table 6, it was observed that female students are more frequent than male students are.

Table 6: Co-distribution of ABO and Rh-D blood group phenotypes among students in the present study

Sex	**A^+**	**A^-**	**B^+**	**B^-**	**AB^+**	**AB^-**	**O^+**	**O^-**
M	89	9	76	5	21	3	181	12
(n=396)	(22.47)	(2.27)	(19.19)	(1.26)	(5.30)	(0.76)	(45.71)	(3.03)
F	56	5	36	3	8	2	85	9
(n=204)	(27.45)	(2.45)	(17.65)	(1.47)	(3.92)	(0.98)	(41.67)	(4.41)
Total	145	14	112	8	29	5	266	21
(600)	(24.17)	(2.33)	(18.67)	(1.33)	(4.83	(0.83)	(44.33)	(3.5)

NB: The numbers in the body of this table represent the count of each of the categories and those in parenthesis represent the frequency in percentage of each cell by using row total for each category.

The Rh-D distribution varies among the four ABO blood groups. In this study blood type O is the most frequent blood types than A, B, AB blood types in order of O > A > B > AB in both Rh-D negative and Rh-D positive. This study is in agreement with the study done in Silte Zone, Ethiopia in Sodo, Silte and Meskan ethnic groups except that in the Sodo ethnic group the blood group A was the most common among the Rh-D negative subjects (Kassahun *et al*., 2014).

4.2. Allelic Frequencies of the ABO and Rh-D Blood Groups among the Students

Allelic frequencies of the ABO and Rh-D blood groups of the study population are shown in Table 7. The study revealed that, the allelic frequency for ABO blood is highest for *i* (0.6866) followed I^A (0.1759), I^B (0.1375) and for *Rh* is higher in *D* (0.7172) and followed *d* (0.2828).

The allelic frequency in this study population was calculated from observed phenotype frequency by using Hardy Weinberg's low (Al-Rubeai, 1975). The allele frequencies in this study shows a high frequency of the allele *i* over I^A and I^B for ABO, *D* allele over *d* for Rh-D respectively in the order of ($i > I^A > I^B$ and D > d). This allelic frequency distribution is in agreement with other previous study done in Silte Zone, Ethiopia Sodo, Silte and Meskan (Kassahun *et al*., 2014), and Nigeria (Omotade *et al*., 1999).

4.3. Genotypes Frequencies of the ABO and Rh-D Blood Groups among Students

Genotypes frequencies of the ABO and Rh-D blood group of the study population is that illustrated in Table 7 reveals that, for ABO blood types; *ii* genotypes is the highest (0.4714) followed by genotypes I^Ai (0.2415), I^Bi (0.1894), I^AI^B (0.0484), I^AI^A (0.0309), I^BI^B (0.0189), and for Rh-D; Rh-D positive homozygous *DD* is more frequent (0.5144), followed Rh-D positive heterozygous *Dd* with (0.4056). Rh-D negative homozygous *dd* was the least with a frequency of 0.08.

The genotypes frequency of individuals for the study population was calculate from estimated allele's frequency by using Hardy Weinberg law, which was stated by Griffith, *et al*., (2008). The genotype frequency occurred in the order of ($ii > I^Ai > I^Bi > I^AI^B > I^AI^A > I^BI^B$).

Table 7: Allelic and Genotypes frequencies of the ABO and Rh-D blood group among students of Hirna primary and secondary schools

Allele	Allelic frequency	Genotype	Genotype frequency
I^A	0.1759	I^AI^A	0.0309
		I^Ai	0.2415
I^B	0.1375	I^BI^B	0.0189
		I^Bi	0.1888
		I^AI^B	0.0484
i	0.6866	ii	0.4714
D	0.7172	DD	0.5144
		Dd	0.4056
d	0.2828	dd	0.0800

4.4. Chi Square Test for ABO and Rh-D Phenotypes

The chi square test for ABO phenotypes of the study population shown that the calculated χ^2 for ABO = 1.2035 (Table 8) and for Rh-D = 0 (Table 9).

Chi Square test for ABO phenotypes calculated to see whether the study population is at Hardy-Weinberg Equilibrium. Critical values for the chi-square are determined from a statistical table based on the significance level at which the test is being performed (Appendix ii). The calculated χ^2 for ABO is 1.2035 and the critical value at df = 3 is 7.82. The calculated chi-square value for ABO 1.2035 is to smaller than the critical value 7.89. The test of goodness of fit for ABO phenotypes at df = 3, P > 0.05 is insignificant. The calculated χ^2 for Rh-D = 0, no difference between the observed and expected value for Rh-D blood phenotypes. The distribution and proportion of individuals' ABO and Rh-D phenotypes did not differ from expected under Hardy - Weinberg equilibrium for Hirna students.

Table 8: Chi-square test for the goodness of fit of observed and Expected phenotype categories

Blood groups	**Observed number**	**Expected number**	**(Obs, -Expe.)**	$(\mathbf{Obs.-Expe.})^2$	$\frac{(\mathbf{Obs.-Expe.})^2}{\mathbf{Expected}}$
A	159	163.5	-4.5	20.25	0.1239
B	120	124.62	-4.62	21.3444	0.1713
AB	34	29.02	4.96	24.6016	0.8471
O	287	282.84	4.16	17.3056	0.0612
					$\Sigma =$ 1.2035

* P is significant at> 0.05

$$\chi\mathbf{2} = \Sigma\ \frac{(\mathbf{Observed}\ -\ \mathbf{Expected})^2}{\mathbf{Expected}} =\ 1.2035$$

Table 9. Expected and observed number of study population for Rh-D blood phenotypes

Blood groups	**Observed number**	**Expected number**	**(Obs. -Expe.)**	$(\mathbf{Obs.-Expe.})\mathbf{2}$	$\frac{(\mathbf{Obs.-Expe.})\mathbf{2}}{\mathbf{Expected}}$
Rh+Ve	552	552	0	0	0
Rh-Ve	48	48	0	0	0

$$\chi\mathbf{2} = \Sigma\ \frac{(\mathbf{Observed}\ -\ \mathbf{Expected})^2}{\mathbf{Expected}} = 0$$

5. SUMMARY, CONCLUSIONS AND RECOMANDATION

5.1. Summary

The main purpose of this study was to determine phenotype distribution, to estimate allele and genotype frequency of ABO and Rh-D blood group among students. The study was conducted in West Hararghe Zone Tullo Woreda's of two schools namely Hirna primary and secondary schools. Six hundred volunteer students were selected purposively from Hirna primary and secondary schools for the study, which took place from January 2016 to August 2016. The blood samples were taken by qualified laboratory technician from each volunteer student's left hand finger pricks by sterile lancet and placed on three clean slides and a drop of one of the anti-sera, that is anti-A, anti-B, and anti-D, were added to individual's blood samples and mixed using clean glass rod. The result was recorded according to the blood sample agglutinated by anti-sera, as blood group A^+, B^+, AB^+, O^+ or A^-, B^-, AB^- and O^-.

The frequencies of the blood group phenotypes were calculated as percentage. The frequency of three alleles (I^A, I^B, i) with frequencies equal to p(A), q(B) and r(O) for ABO blood types and two alleles (D, d) with frequencies equal to D (Rh +Ve) and d (Rh -Ve) for Rh-D blood types were computed from phenotypic data. The frequency of the ABO and Rh-D blood group genotypes were calculated from allelic frequency by using the extension of Hardy Weinberg principle. Beside, Chi square test was carried out to check whether the observed values for ABO and Rh-D blood groups phenotypes obey the Hardy Weinberg equilibrium principle or not. Finally, the obtained results were presented by using tables.

5.2. Conclusions

The results of this study showed higher frequency of blood group O followed by blood group A, B and the least frequent was AB. On the other hand allele i is highly frequent than allele I^A and allele I^B in order of ($i > I^A > I^B$). Rh^+ blood group was also highly frequent among the

students' than Rh^-. The ABO and Rh-D factor data from this study follows the same pattern found in most populations of the world surveyed.

Study on the distribution of ABO and Rh-D blood groups within the population are important in terms of blood transfusion for effective management of blood bank inventory; in population genetics to infer population migration patterns in prehistoric time; and in forensics for Medico-legal issues, for paternity tests, and to avoid haemolytic diseases of the newborn baby (HDN).

This study of blood grouping not only generates a simple database for collection of blood and blood products which will be important for establishment of blood bank but also creates a great social awareness about self-blood grouping, safe blood transfusion and how to avoid Hemolytic disease of the new born (HDN) among the population of a country.

The study has a significant implication regarding the management of blood bank and transfusion services in this area. Knowledge of blood group distribution is also important for clinical studies, for reliable geographical information and for forensic studies in the population. Besides, this study will help a lot in reducing the maternal mortality rate, as access to safe and sufficient supply of blood will help significantly in reducing the preventable deaths.

5.3 Recommendations

- The data generated would be helpful as a base, for researchers who are interested to conduct studies on the association between blood groups and Varity of diseases in Hirna population.
- It also gives hints for blood banks to have the more needed blood types for the area.
- This study limited on the schools. Therefore, it is advisable for further study out of the schools to get precise data regarding the distribution on these blood groups.

7. REFERENCE

American Red Cross (US) 2013. Blood types [Internet]. c2013. Available from :http://www.redcrossblood.org/learn-about-blood/blood-types

Adeyemo, 2006. Frequency Distribution of ABO, Rh Blood Groups and Blood Genotypes among the Cell Biology and Genetics students of University of Lagos, Nigeria. *African Journal of Biotechnology*, 5(22) 2062-2065.

Al-Bustan S, El-Zawahri M, Al-Azmi D, Al-Bashir AA 2002. Allele frequencies and molecular genotyping of the ABO blood group system in a Kuwaiti population. *Int J Hematol*, 75:147-53.

Alimba, Adekoya, Oboh, 2010. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood groups, and haemoglobin variants among a Nigerian population *Egypt J Med Hum Genet*, 153–158

AL-Rubeai, 1975. ”Taste sensitivity to phenylthiocarbamide and blood groups in the Iraqi population (sample from Baghdad) *Bull. Coll. Sci*., 16 (2): 205 -215.

Ali N, Anwar M, Bhalti FA, Nadeem M, Nadeem A, Ali M, 2005. Frequency of ABO and Rh blood groups in major ethnic groups and casts of Pakistan. *Pakistan J. Med.Sci*., 21:26–29.

Anstee DJ, 2009. "Red Cell Genotyping and the Future of Pretransfusion Testing". Blood 144 (2): 248-56.

Australian Red Cross Society, 2013. All about blood. Available at/URL: www.donateblood.com.au/all-aboutblood/ blood-types: Accessed on: 21st July.

Avent ND, 1999. The rhesus blood group system: insights from recent advances in molecular biology. *Transfusion Med*. Rev., 13:245–266.

Bakare AA, Azeez MA, Agbolade JO 2006. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, south-west Nig. *Afr. J. Biotechnology*, 5(3):224 –229.

Bashwari LA, Al-Mulhim AA, Ahmad MS, Ahmed MA 2001. Frequency of ABO blood groups in Eastern region of Saudi Arabia. *Saudi Med J. 22*. 1008-12.

Behra R, Joshi YR. 2013. Distribution of ABO blood group and RH(D) factor in Western Rajasthan. *National J Medical Res*., 3:73-5.

Bethesda (MD): National Center for Biotechnology Information (US). Chapter 7, *The Rh Blood Group*. Available from http://www. Ncbi.nim.gov/books/NBK2269/

Bethesda D.L., 2005. Blood Groups and Red Cell Antigens. In: The Rh blood group. USA: National Center for Biotechnology Information. P.1-6. (Retrieved on 23 April 2013 from http://www.ncbi.nlm.nih.gov/books/NBK2269/pdf/ch07Rh. pdf)

Bhalti FA. Amin 1996. Spectrum of ABO and D blood groups of donors at Rawalpindi/Islamabad. *Pakistan J.Pathol*, 7(2):26 – 28.

Bhasin MK, Walter H, Danker-Hopfe H, 1992. The Distribution of Genetically Morphological and Behavioural Traits among the Peoples of Indian Region.

Bruce, M.G., 2002. BCF–Members – Chairman's Annual Report. The Blood Care Foundation. http://www.bloodcare.org.uk/html/resources_chairman_2001.htm.

Central Statistics Autority, 2007. Addis Ababa, Ethiopia.

Chou ST., Westhoff CM. 2011. The Rh System. In Roback JD, Grossman BJ, Harris T, editors. Technical manual standards. New York: AABB press, P.39 1-2.

Daniels G. 2002. Human Blood Groups, 2nd ed. Blackwell Science,

Daniels G L, Anstee D J, 2005. Blood group terminology: ISBT working party on terminology for red cell surface antigens. *Vox Sang*, 69:265-279.

Daniels H.L., and A.G.C lark. 2007. *Principle of Genetics* 4th ed. Sinauer Associates.

Dean L. 2005. Blood Groups and Red Cell Antigen (internet).

Dennis Y.M, Hylem NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF (1998). Prenatal diagnosis of fetal Rh-D status by molecular analysis of material plasma. New Engl. J. Med., 337:1734–1738.

Encyclopaedia Britannica, 2002. The New Encyclopaedia Britannica. Encyclopaedia Britannica, Inc. ISBN 0-85229-787-4.

Frances TF 2002. Blood groups (ABO groups). In: *Common Laboratory and rd Diagnostic Tests*. 3 Edition, Philadelphia: Lippincott, 19-25.

Firkin F, Chesterman C, Penington D, Rush B 1989. Blood groups; blood transfusion;acquired immune deficiency syndrome. In: Haque et al. Int J Pharm PharmSci, Vol 5, Issue 3, 814-816 816b de *Gruchy's Clinical Hematology in Medical Practice*. 5th ed, NewDelhi, Oxford University Press, 475-96.

Fauci, Anthony S., Eugene Braunwald, Kurt J. Isselbache ,Jean D.Wilson, JosephB. Martin,Dennis L.Hauser, Dan L. Longo 1998. *Harison's Principle of Internal Medicine.* New York McGraw-Hill.PP719

Garratty, G, Glynn SA, McEntire R 2004. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United states. *Transfusion*, 44(5):703-06.

Griffiths A.F, S.R, Wesser, R.C, Lewontin., and Carrol., 2008. *Introduction to Genetic Analysis*. 9th edition.W.H. Freeman and company. New York. P838.

Hartl D.L and Clark, A.G 1989. *Principle of Population Genetics*, 2nd ed. Sinauer. (Hardly-Weinberg law, EM algorism).

Harmening M.D, Firestone D 2005. The ABO blood group system. In: Harmening MN, editor. *Modern Blood Banking and Transfusion Practices*. 5th ed. USA: FA Davis Company, Philadelphia, USA, 108-32.

Henrik Bendixen, Fredericck Manning, and Linette Sparacino, 2012. Forum on Blood safety and Blood Availability, Institute of Medicine Texas University of A. and M. Christies Corpus MC. Nar.

Hillier C.D Shaz B.H, winkler A.M, Reid M. 2008. Department of pathology and Laboratory Medicine, Emory University,Atlanta, GA 30321,htt:ibgrl.blood.co.ulc/isbt%20.pages.

Honig C.L, Bore J.R 1980. Transfusion associated fatalities: a review of Bureau of Biologic reports 1976 –1978.*Transfusion*, 20:653–661.

Kassahun T. 2015. Frequency distribution of ABO and Rh (D) blood group alleles in silte zone, Ethiopia. *Egyptian Journal of Medical Human Genetics*, 16:571-76.

Khattak I.D., T.M. Khan, P. Khan, S.M. Ali Shah, Khattak, and Ali. 2008. Frequency of ABO and Rhesus blood groups in District Swat. *Pakistan. Journal of Ayub Medical College Abbotabad*, 20(4): 127-129.

Khan M.S., F. Subhan, F. Tahir, B.M. Kazi, A.S. Dil& S. Sultan, 2004. Prevalence of blood groups and Rh factor in Bannu region NWFP (Pakistan). *Pakistan Journal of Medical Research*, 43(1):8–10.

Khurshid M, Naz M, Hassan S.F, 1992. Blood Frequency of ABO and Rh (D) blood groups in district Swabi, NWFP, Pakistan J Sci. Technol University peshawar, 16: 5–6

Klug W.S. and M.R. Cummings, 2002. *Essentials of Genetics.* 4th ed. Prentice Hall. New Jersey, USA, pp. 508

Landsteiner K, Weiner A.S 1940. An agglutinable factor in human blood recognized by immune sera for rhesus blood. *Proc.Soc. Exp. Biol. Med*., 43:223 – 224.

Loua A, Lamah MR, Haba NY, Camara M 2007. Frequency of blood groups ABO and Rhesus D in the Guinea population. *Transfuse Clin. Biol*, 14(5):435-9.

Lyco J, Gaertner H, Kaviti JN, Kariithi MW, Akoto B 1992. Blood-group systems ABO and RH in the Kenyan population. *Folia Med Cracov*, 33:85-92.

Laura Dean, M.D 2005. Blood group and red cell antigens: National Center for Biotechnology information, United States Government. ISBN 1-932811-05-2.

Majumder P.P, Roy J 1982. Distribution of ABO Blood groups on the Indian subcontinent: A cluster-analytic approach. *Current Anthropology*, 23(5):539-66.

Markovic´ M.S 1994. The ABO system of blood groups and the Rh factor in 360 pairs of twins and in 11 triplets from Belgrade Adam Mickiewicz University, Faculty of Biology,Pozna*Variability and Evolution*, 4: 97–102

Mayo O, 2008. A century of Hardy-Weinberg Equilibrium Twin Research and Human Genetics, 11(3): 249-256.

Mawuagi J (1999). Blood group distribution in an urban population of patient targeted blood donors. *East Afr. Med*, J., 76(11):615 – 618.

Mehta Nidhi, Swadas Bhawna, 2012. Prevalence of ABO Blood groups at Mahavir Heart Institute Surat. *Asian J. Trans.Sci*, 6:1:74.

Minelli, C., Thompson, J.R., Abrams K.R., Thakkinstian, A., Attia J. 2007. How should we use information about HWE in the meta-analyses of genetic association studies? *International Journal of Epidemiology*, 37: 136-146.

Molison PL.1979. *Blood Transfusion in clinical medicine*. 6th ed. Oxford UK. Blackwell Scientific Publication.

Narahara K, Y. Takahashi, K. Kikkawa, Y. Wakita, S. Kimura & H. Kimoto, 1986. Assignment of ABO locus to 9q31.3-qter by study of a family in which an intra chromosomal shift-involving chromosome 9 is segregating. *Journal of Human Genetics*, 31(3): 289-296.

Nandy CK 1986. Frequencies of the ABO blood groups in Jessore (Bangladesh). *Journal of IPGMR*, 1:40-42.

National Health Service. Blood and transplant. Blood group basics. National Blood Service. (Retrieved on 23 April 2013 from: http://www.blood.co.uk/about-blood/blood-group basics/)

Nydegger UE, Riedler GF, Flegel WA 2007. Histoblood groups other than HLA in organ transplantation. *Transplant Proc.*, 39(1):64-68.

Omotade OO, Adeyemo AA, Kayode CM, Falade SL, Ikpeme S, 1999. Gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, Nig. *West Afr. J. Med*. 18:294–297.

Patel P, Patel S, Shah J and Oza HV 2012. Frequency and distribution of blood groups in blood donors in Western Ahmedabad–A hospital based study. *Nat Journal Medical Res*, 2(2): 205-206.

Pathan AH, Apu AS, Jamaluddin ATM, Asaduzzaman M, Rahman ZM, Rahman A et al 2008. Prevalence of ABO blood groups and Rh factor in Bangladesh. *Bangladesh J. Life Science*, 20(2):131-35.

Platt, Milberg, Kiehl R, 1985. Schmitt-Ruth ABO blood group system, age, sex, risk factors and cardiac infarction. *Arch Gerontol Geriatr*, 4: 241–249

Pour, Fatollah AA, Oody A, Honarkaran N. 2001. Geographical distribution of ABO and Rh (D) blood groups among Indian blood donors in the year 1361 (1982) as compared with that of the year 1380. *Sci J Blood Transfus Organ*, 1(1):11-17.

Pramanik T, Pramanik S., 2000. Distribution of ABO and Rh blood groups in Nepalese Medical students: A report. *Eastern Mediterranean Health J*, 6(1):156 – 15

Rahman M. 1975.Incidence of important blood groups in Bangladesh. Bangladesh *Med. Res. Council Bull*, 1(1):60-63.

Rahman M and Lodhi Y., 2004. Frequency of ABO and Rhesus blood groups in blood donors in Punjab. *Pak J Med Sci*., 20:315-8.

Saladin K (2003). Anatomy and Physiology. *The Unity of Form and Function*: 3rd Edition. The McGraw-Hill Companies, USA, Pp.679-698.

Seeley RR, Stephens TD, Tate P. 1998. *Anatomy and Physiology*: 4th edition. The McGraw Hill Companies, Inc. USA, p.1098.

Sidhu S. 2003. Distribution of the ABO Blood Groups and Rh (D) Factor among the Scheduled Caste Population of Punjab. *Anthropologist*, 5(3):203-04.

Storry JR 2003. Human blood groups: inheritance and importance in transfusion medicine J. *Infuse Nurse*, 26(6):367-72.

Talaro, Kathleen P. 2005. *Foundations in Microbiology*: 5th Ed. New York. McGraw-Hill, pp. 510–1.

Wadhwa MK, Patel SM, Kothari DC, Pandey M, Patel DD 1998. Distribution of ABO and Rhesus-D groups in Gujarat, India: a hospital based study. *Indian J Ped Oncol*, 19(4):137-41.

Wagner F F, Moulds J M, Flegel W A 2005. Genetic mechanisms of Rhesus box variation. *Blood*, 45(3):338–44.

Waters AH. 1995. Red cell blood group antigens and antibodies: In: Dacie VJ and Lewis SM, Editors. *Practical Haematology*: 8th ed. London, Churchill Livingstone, p. 445-64.

Westhoff CM. 2004. The Rh blood group system in review: a new face for the next decade. *Transfusion*, 44:1663–73.

Waite GN.2009. Blood Components. In: Rhoades RA, Bell DR, editors. *Medical Physiology, Principles for Clinical Medicine*: 3rd ed. USA. Lippincott Williams and Wilkins, Philadelphia.p., 169 - 86.

Yazer M. Olsson M., Palcic M. 2006."Thecis-ABO group phenotype fundamental lesson in glycol biology". *Transfuse Med. Rev*., 20(3): 207-17.

8. APPENDIXIES

i. Agreement Form

Volunteer students sign in front of their name on this agreement form for their willingness to donate blood only for the research purpose.

№	Full name	Sex	Age	Sign

ii. Probability Values for Chi-Square Analysis

	Probabilities								
Df	0.95	0.90	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	.004	.016	.15	.46	1.07	1.64	2.71	3.84	6.64
2	.10	.21	.71	1.39	2.41	3.22	4.61	5.99	9.21
3	.35	.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35
4	.71	1.06	2.20	3.36	4.88	5.99	7.78	9.49	13.28
5	1.15	1.61	3.00	4.35	6.06	7.29	9.24	11.07	15.09
6	1.64	2.20	3.83	5.35	7.23	8.56	10.65	12.59	16.81
7	2.17	2.83	4.67	6.35	8.38	9.80	12.02	14.07	18.48
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.21
	Acceptable								Unacceptable

Note. From Stistical Tables for Biological and Medical Research (6th ed.), Table IV, by R.Fisher and F.Yates, Edinburgh: Longman Essex, 1963.

iii. Hirna Health Bureau and the Institutional Research Ethical Review Committee (IRERC)

Address: Tel._____ P.O.Box. _____Fax. _____ Hirna Health Bureau Email_____

Institutional Research Ethical Review Response Form

Name of the institution: Hirna Health Bureau

Name of PI: Girma Legesse

Phone **0912418768** Email_____P.O Box_____

Title of the proposal " Genotype and allelic frequencies of the ABO and Rh-D blood groups among primary and secondary school students of Hirna, west Hararghe Zone of Oromia."

To the Post Graduate Program Directorates.

The IRERC has reviewed the aforementioned project proposal with special emphasis on the following point:

1. Are all the ethical principles considered?

1.1 Respect of person: yes ☐ No ☐

1.2 Beneficence Yes ☐ No ☐

1.3 Justice: Yes ☐ No ☐

2. Are the objectives of the study ethically achievable? Yes ☐ No ☐

3. Are/is the method ethically Yes ☐ No ☐

He the above-mentioned ethical assessment the institutional research ethical review committee has

A. Approved proposal for: 1. Regional National review ☐

2. Implementation ☐

Expiry Date of review:

_____/_____/2016

Date Month Year

B. Conditionally approved ☐

iv. Red blood cell compatibility

	Donor							
Recipient	O−	O+	A−	A+	B−	B+	AB−	AB+
O−	✓	✗	✗	✗	✗	✗	✗	✗
O+	✓	✓	✗	✗	✗	✗	✗	✗
A−	✓	✗	✓	✗	✗	✗	✗	✗
A+	✓	✓	✓	✓	✗	✗	✗	✗
B−	✓	✗	✗	✗	✓	✗	✗	✗
B+	✓	✓	✗	✗	✓	✓	✗	✗
AB−	✓	✗	✓	✗	✓	✗	✓	✗
AB+	✓	✓	✓	✓	✓	✓	✓	✓

i. Plasma compatibility table

	Donor			
Recipient	O	A	B	AB
O	✓	✓	✓	✓
A	✗	✓	✗	✓
B	✗	✗	✓	✓
AB	✗	✗	✗	✓

Source: Blood component ABO compatibility chart Red blood cells and plasma blood bank labsite University of Michigan. Retrieved December 2014.

ii. Blood group system showing antigen on surface and antibodies in plasma

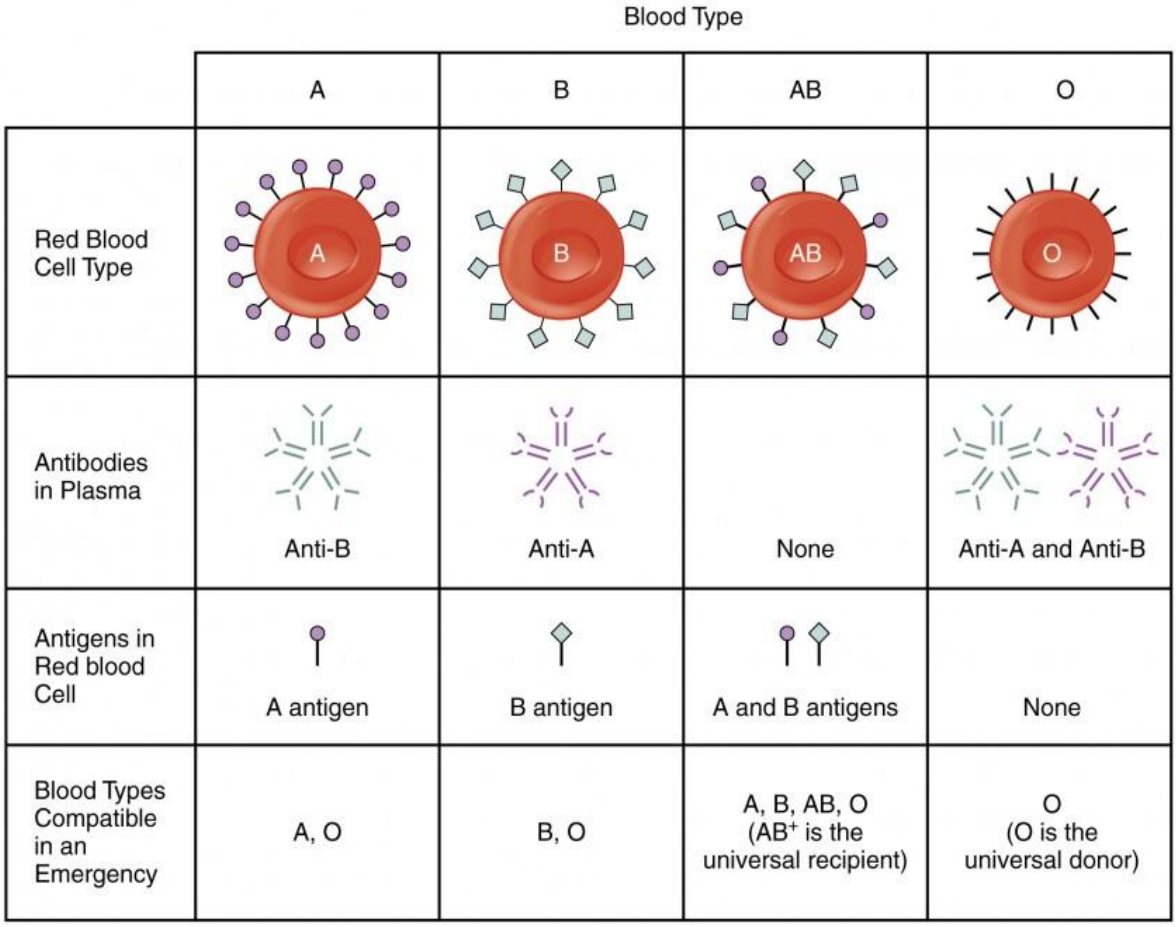
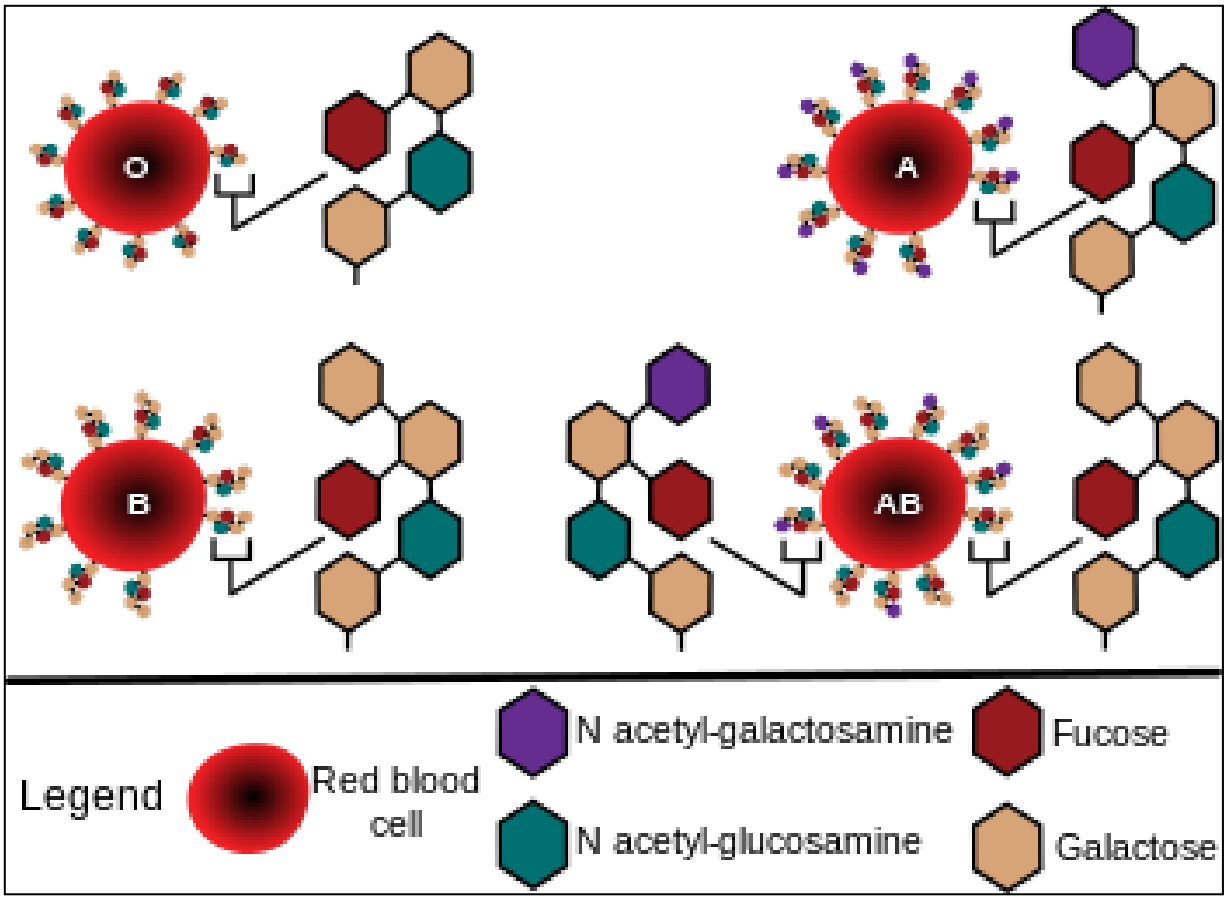


Figure 2

American Red Cross (US). Blood types [Internet]. c2013 [cited 2013 Apr 3]. Available from:http://www.redcrossblood.org/learn-about-blood/blood-types 2013

iii. ABO blood group system: Diagram showing the carbohydrate chains that determine the ABO blood group.



https://en.wikipedia.org/wiki/FileABObloodgroupdiagram.svg

Figure 3