PHENOTYPIC, GENOTYPIC AND ALLELIC FREQUENCIES OF ABO AND Rh(D) BLOOD GROUPS AMONG STUDENTS BELONGING TO SOME SELECTED ETHNIC GROUPS IN GERESU DUKI PREPARATORY SCHOOL IN WOLISO TOWN, OROMIA, ETHIOPIA

MSC THESIS

TESFAYE DUGASSA

MAY 2017

HARAMAYA UNIVERSITY, HARAMAYA

Phenotypic, Genotypic and Allelic Frequencies of ABO and Rh(D) Blood Groups among Students Belonging to Some Selected Ethnic Groups in Geresu Duki Preparatory School in Woliso Town, Oromia,Ethiopia

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

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

Tesfaye Dugassa

May 2017
Haramaya University, Haramaya

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

As thesis research advisors we hereby certify that we have read and evaluated this thesis entitled **Phenotypic, Genotypic and Allelic Frequencies of ABO and Rh(D) Blood Groups among Students Belonging to Some Selected Ethnic Groups in Geresu Duki Preparatory School in Woliso Town, Oromia, Ethiopia ,prepared under our guidance by Tesfaye Dugassa.** We recommend that it be submitted as fulfilling the thesis requirements.

Yohannes Petros(PhD)_____ _____

Major advisor Signature Date

Tamiru Oljira(PhD) _____ _____

Co-Advisor Signature Date

As members of the Board of Examiners of the MSc Thesis Open Defense Examination, we certify that we have read and evaluated the Thesis prepared by Tesfaye Dugassa and examined the candidate. We recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Biology.

_____ _____ _____

Chairman 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 Studies (CGS) through the candidate's department or School graduate committee (DGC or SGC).

DEDICATION

I dedicate this Thesis manuscript to my mother Age Lame and My Father Dugassa Feyissa who laid the foundation to all my life since my early stage of childhood and devoted much in nursing me with special affection and love throughout my life. Also my wife W/o Soreti Baba and My Son Beka and Oli Tesfaye for their love and moral support.

STATEMENT OF THE AUTHOR

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 completion 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 requirements for an MSc 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 quotations from this Thesis may be made without special permission provided that accurate and complete acknowledgement of the sources 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 School or the Department when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author of the Thesis.

Name: Tesfaye Dugassa Feyissa

Date of submission: _____ Signature: _____

Department: Biology

BIOGRAPHICAL SKETCH

Tesfaye Dugassa was born on August 25, 1980 in Gida Kiremu Woreda, Kiremu 01 kebele, East Wollega Zone, Oromia Regional State from his Father Dugassa Feyissa and Mother Age Lame.

He attended his elementary school education at Kiremu Primary and Junior School (1-8) in 1987-1995, and secondary school education at Gida Ayana Secondary School (9-12) in 1996-2000. He joined Jimma Teachers College in 2001 and received his Diploma with Major Biology and Minor Chemistry in 2003. He was employed by the Ministry of Education as Biology teacher at Uraga Secondary School at Guji Zone for two years and then joined Summer program at Dilla University in 2006 and graduated with Bachelor of Education with Biology in 2011. In 2012 he was transferred from Guji Zone to South West Shoa and he worked as Biology teacher at Geresu Duki Preparatory School in Woliso town currently. He then joined Haramaya University in 2014 to pursue MSc in Biology.

ACKNOWLEDGMENTS

I am truly grateful for the support, inspiration, and encouragement I have received from many people in my educational pursuit. My greatest appreciation and respect is extended to my major advisor Dr. Yohannes Petros and Co-advisor Dr. Tamiru Oljira. for their unlimited constructive advice, comments, scientific criticisms and continuous evaluations throughout my study.

I would like to acknowledge the Postgraduate Program Directorate and the Department of Biology, Haramaya University, for the kind assistance provided in many ways.

I also extend my appreciation to Geresu Duki Preparatory School students for their kindness in participating in blood test, Principal of Geresu Duki Preparatory School Mr. Muleta Kebebe, his moral and material support, Yeshi Chibsa and Etenesh Zegeye as Laboratory technician and others for their help in testing blood to generating quality data, Woliso Health center who allow lab technicians for facilitating the process. Furthermore, I want to thank my family members, my friends and all the study participants for their kind cooperation, who have directly or indirectly contributed towards the completion of this study. I am very much indebted to my wife W/o Soreti Baba for her moral support and encouragements in the course of my study.

ACRONYMS AND ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Assay
FMC	Flinders medical center
HDN	Hemolytic Disease of the Newborn
Ig M	Immunoglobin M
HWP	Hardy-Weinberg Principles
HWE	Hardy-Weinberg Equilibrium
ISBT	International Society of Blood Transfusion
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
Rh	Rhesus
WF	Willebrand Factor
WTA	Woliso Town Authority

TABLE OF CONTENTS

DEDICATION iii

STATEMENT OF THE AUTHOR iv

BIOGRAPHICAL SKETCH v

ACKNOWLEDGMENTS vi

ACRONYMS AND ABBREVIATIONS vii

LIST OF TABLES xi

LIST OF TABLES IN THE APPENDIX xii

LIST OF FIGURE IN THE APPENDIX xiii

ABSTRACT xiv

1. INTRODUCTION 1

2. LITERATURE REVIEW 5

2.1. Discovery of ABO and Rh(D) Blood Group System 5

2.1.1.Blood Group Systems 6

2.1.2. The ABO Blood Group System 7

2.1.3. Various Applications of ABO Blood Groups 8

2.1.4. The ABO Blood Group Antibodies 9

2.1.5. Red Blood Cells Compatibility 9

2.1.6. Frequencies of ABO Phenotypes in Different Populations 10

2.2. The Rh Blood Group System 12

2.2.1. Rh (D) Blood Grouping 12

2.2.2. Frequencies of RhD Blood Group Phenotypes in Different Populations 13

2.3. Clinical Significance of Blood Groups 14

2.3.1. Blood Transfusion 14

2.3.2. Hemolytic Diseases of the New Born (HDN) 15

2.4. Methods of Blood Typing 16

2.5. Genes in a Population 17

2.6. Significance of the Distribution 17

2.7. The Hardy-Weinberg Principle 18

2.8. The Chi-square Test to Calculate Observed and Expected ABO and Rh Blood Group Distribution 20
3. MATERIALS AND METHODS 21
3.1. Description of The Study Area 21
3.2. Study Participants 22
3.3. Blood Sample Collection Procedure and Typing 22
3.4. Methods of Data Analysis 23
3.4.1. Phenotypic Frequency Determination of ABO and RhD Blood Groups 23
3.4.2. Estimation of Allele Frequency 23
3.4.3. Genotype Frequency of ABO and Rh(D) Blood groups 24
3.4.4. Chi-square Test 24
3.5. Ethical Considerations 25
4. RESULTS AND DISCUSSIONS 26
4.1. Frequency Distribution of ABO and Rh(D) Phenotype Among the Three Ethnic Groups 26
4.1.1. Frequency of ABO Blood Group Phenotypes Among Students of the Three Ethnic Groups 26
4.1.2. Frequency Distribution of Rh (D) Blood Group Phenotypes among the three Ethnic Groups 29
4.1.3. Frequency Distribution of ABO Phenotypes Based on Rh (D) Blood Groups 30
4.2. Allele and Genotype Frequency Distribution of ABO and Rh(D) Blood Groups Among Students of the Three Ethnic Groups 31
4.2.1. Allele and Genotype Frequencies of ABO Blood Groups of the Three Ethnics Groups 32
4.2.2. Allele and Genotype Ffrequency of Rh(D) Blood Groups Among the Three Ethnics Groups 34
4.3. The Chi-Squared Test of Deviation Between Observed and Expected Phenotype Frequencies of ABO and Rh(D) Blood Groups 35
4.3.1. Chi-Square Test in ABO Blood Group Among the Three Ethnic Groups 35
4.3.2. The Chi- Square Test in Rh (D) Blood Group in the Three Ethnic Groups 36

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 38

5.1. Summary 38

5.2. Conclusion 40

5.3. Recommendations 41

6. REFERENCES 42

7. APPENDIX 49

LIST OF TABLES

Table **Page**

1. ABO blood groups, antigens and antibodies 9
2. Frequencies of ABO blood groups studied in different populations across the world 11
3. Frequency of Rh blood groups in different populations across the world 13
4. Agglutination reactions of the RBC by ABO blood-typing sera (ISBT, 2008) 17
5. Frequency of ABO blood group phenotype among students of three ethnic groups 26
6. Phenotypic frequency of Rh (D) blood groups among the three ethnic groups 29
7. Distributions of the combined ABO and RhD blood group phenotypes among students of three ethnic groups 30
8. Allele Frequency of ABO blood groups among students of Oromo, Amhara and Gurage ethnic groups. 32
9. Allele Frequency of Rh (D) blood groups among students of Oromo, Amhara and Gurage ethnic groups 34
10. Chi-square test in ABO blood groups among students of Oromo, Amhara and Gurage ethnic groups 35
11. Chi-square test in Rh(D) blood group among students of Oromo, Amhara and Gurage 36

LIST OF TABLES IN THE APPENDIX

Appendix Table	Page
1. ABO and Rh-D blood group Phenotypes recording sheet	49
2. Probability Values for Chi-Square Analysis	50

LIST OF FIGURE IN THE APPENDIX

Appendix Figure **Page**

1. Pictures of agglutination reaction and blood type determination 51

Phenotypic, Genotypic and Allelic Frequencies of ABO and Rh(D Blood Groups among Students Belonging to Some Selected Ethnic Groups in Geresu Duki Preparatory School in Woliso Town, Oromia, Ethiopia

ABSTRACT

The ABO and Rh blood groups are the most important blood groups despite the long list of several other blood groups discovered so far. The ABO and Rh blood groups varies worldwide and are not found in equal numbers even among ethnic groups. Therefore, the objective of the study was to estimate phenotypic, genotypic and allelic frequencies of ABO and Rh blood groups among students of Oromo, Amhara and Gurage ethnic groups in Geresu Duki preparatory school in Woliso town, Oromia, Ethiopia. The study was conducted on 470 (48%) out of 985 students in Geresu Duki preparatory school who were enrolled in 2016/17. These were purposively selected and the sample were divided into three ethnic groups, Oromo ,Amhara and Gurage and stratified along ethnic lines. The frequencies of ABO and Rh(D) blood group phenotypes were determined and Hardy-Weinberg Law was used to determine allelic and genotypic frequencies. In the overall sample, the O, A, B, and AB blood group percentages were 40%, 30.64%, 21.70% and 7.66%, respectively. The most prevalent blood group was type O followed by A, B, and AB. Blood group O had the highest frequency while blood group AB had the least distribution. The Rhesus positive frequency was 96.38%, while Rhesus negative was 3.62% in the overall sample. The order of ABO blood group allele frequencies was $I^O > I^A > I^B$ in the overall samples and in each of the three ethnic groups. The overall allelic frequencies of the population as they were calculated using the extension of the Hardy–Weinberg law were 0.63, 0.21 and 0.16 for I^O, I^A and I^B alleles, respectively. Also on the Rhesus status, the allelic frequencies were 0.81 and 0.19 for D and d alleles, respectively. The Chi-square test was done to compare observed phenotypic frequency distribution of ABO and Rh (D) blood groups with that expected .Overall results from this study show that phenotypic, genotypic and allelic frequencies of ABO and Rh blood group were varied in different ethnic groups.

Keywords: ABO, Allelic frequency, Genotype, Phenotype, Rhesus factor

1. INTRODUCTION

Blood is the most important body fluid, which is responsible for circulation of important nutrients, enzymes, and hormones all across the body, besides the most critical substance, oxygen. The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900. He described A,B and O blood groups for which he was awarded the Nobel Prize in 1930. Alfred Von Decastello and Adriano Sturli discovered the fourth type AB, in 1902.

The differences in human blood are due to the presence or absence of certain protein and carbohydrate 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. 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 (Giri *et al*., 2011).

The ABO and Rh(D) blood groups are the hereditary characters and are useful in population genetic studies, in resolving medico-legal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice and ABO antigens are found on platelet and body secretions like saliva, sweat, tear, urine, semen, serum, etc., and are used in forensic investigations. The ABO blood types are also present in some other animals, for example rodent and apes, such as chimpanzees and gorilla (Alimba *et al.*, 2010).

Several of RBC surface antigens that stem from one gene (or very closely linked genes) collectively form a blood group system. Blood groups are genetically determined and exhibit polymorphism in different populations. Percentages of people belonging to these blood groups are different in different communities. Distribution of these blood groups is also different in different race. A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT, 2008).

In clinical practice, ABO and Rh blood groups are the most important among the 30 blood groups (Jaf, 2010). ABO blood group system was the first human blood group system, while Rhesus blood group system was the fourth system out of the 15 most important blood group systems discovered and yet it is the second most important blood group from the point of view of transfusion (Khan *et al.*, 2006).

The ABO blood group system consists of four main blood groups: A, B, AB and O which is determined on the basis of the presence or absence of two antigens: A and B antigen (Landsteiner, 1994).

These antigens may be carbohydrates, glycoprotein, and glycolipids depending on the blood group system and some of these antigens are also present on the surface of other types of cells and are under the control of three allelic genes, namely I^A, I^B, and I^O. The I^A allele produces the A antigen, the I^B produces antigen B, and I^O produces neither. The I^A and I^B alleles are co-dominant but both of them are dominant over the recessive allele I^O (Povey *et al.*, 1978).

Not all blood groups are compatible with each other, mixing incompatible blood groups leads to blood clumping or agglutination, which is dangerous for individuals. For a blood transfusion to be successful, ABO and Rh blood groups must be compatible between the donor blood and the recipient blood. If they are not, the red blood cells from the donated blood will clump or agglutinate. The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The agglutinated red blood cells also crack and their contents leak out in the body (Anstee, 2009).

Many people also have a so called Rh factor on the red blood cells surface, those who have it are called Rh^+, those who don't have are called Rh^-.The Rh^+ and Rh^- in Rh (D) blood group system are based on the presence or absence of inherited antigenic substances (D) which are determined by two alleles: D and d on the surface of the red blood cells. Identification of Rh (D) blood group system is important to prevent the erythroblastsis fetal is; which commonly arises when an Rh^- mother carries an Rh^+ fetus. The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh^- can bear an Rh^+ child if the father is Rh^+ (either homozygous or heterozygous).

Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy (Urbaniac *et al*., 2000).

A person with Rh^- blood does not have Rh antibodies naturally in the blood plasma; as one can have A or B antibodies, for instance. But a person with Rh^- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh^+ blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh^+ blood can receive blood from a person with Rh^- blood without any problems (Eweidah, 2011).

Blood grouping has improved with the advent of monoclonal antibodies and the automation tests. In addition to the advanced techniques, such as micro plate method, polymerase chain reaction (PCR) based typing, flinders medical center (FMC) based typing, mini sequence analysis, fluorescent immune plate technique, sandwich enzyme linked immunosorbent assay (ELISA) method, etc, the manual method is also used in blood typing and measuring its genotypic frequency using Hardy-Weinberg Law (Khan *et al.*, 2006).

The need for blood group prevalence studies is multipurpose; as besides their importance in evolution and their relation to disease is being increasingly sought in modern medicine. Various studies on ABO incompatibility have produced a very high frequency of prenatal death among incompatible mating. Red blood cells have a series of glycoprotein's and glycolipids on their surfaces which constitute the blood group antigens (Srikumeri *et al*., 1987).

The study of blood grouping is very important as it plays an important role in genetics, blood transfusion, forensic study, blood bank, organ transplantation, paternity test and some groups may have association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh incompatibility and ABO incompatibility of newborn (Rehman *et al*., 2005). It is, therefore, imperative to have information on the distribution of these blood groups in any population group that comprise different ethnic groups (Kumar *et al*., 2009).

In Ethiopia, distributions of ABO and Rh (D) blood group system studies have been conducted in different parts of the country including colleges and Universities. However, no investigations have been conducted in the population of woliso town ever before.

Therefore, the aim of this study is to investigate the distribution of phenotypes, genotypes and the allelic frequencies of ABO and Rh(D) blood groups as well as to generate data to be used as a reference in the future for different purposes by health planners and other researchers.

General objective

The general objective of this study was:
To investigate the phenotypic, genotypic and allelic frequencies of ABO and Rh(D) blood group system among students of three ethnic groups (Oromo, Amhara and Gurage) in Geresu Duki Preparatory School at Woliso town, Oromia regional state, Ethiopia.

The specific objectives were:

- To determine the distribution of ABO and Rh (D) blood group systems phenotype among the three ethnic groups.
- To estimate the allelic frequencies of ABO and Rh(D) blood groups for each ethnic group and for the whole population from the phenotypes of respective blood groups assuming Hardy-Weinberg equilibrium.
- To estimate the genotypic frequencies for each group and for the whole population.

2. LITERATURE REVIEW

2.1. Discovery of ABO and Rh(D) Blood Group System

At the beginning of the 20th century an Austrian scientist, Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals. He made a note of the patterns of agglutination and showed that blood could be divided into groups. This marked the discovery of the first blood group system ABO and earned Landsteiner a Nobel Prize. Landsteiner explained that the reactions between the RBCs and serum were related to the presence of markers (antigens) on the RBCs and antibodies in the serum (Giri *et al.*, 2011).

Agglutination occurred when the RBC antigens were bound by the antibodies in the serum. He called the antigens A and B, and depending upon which antigen the RBC expressed, blood either belonged to blood group A or blood group B. A third blood group contained RBCs that reacted as if they lacked the properties of A and B, and this group was later called "O" after the German word "Ohne", which means "without". The following year the fourth blood group AB was added to the ABO blood group system. These RBCs expressed both A and B antigens,(Avent, 2000).

In 1910, scientists proved that the RBCs antigens were inherited and that the A and B antigens were inherited co dominantly over O. There was initially some confusion over how a person's blood type was determined, but the puzzle was solved in 1924 by Bernstein's "three allele model". The ABO blood group antigens are encoded by one genetic locus, the ABO locus, which has three alternative (allelic) forms A, B, and O (Avent, 2000). In 1911,Von Dungern and Hirszfeld (Griffithis *et al*, 2008) 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 (Mollision,1994).

The Rh(D) blood group is named after the Rhesus monkey, *Macacu mulatta* (Zimmerman) in which the Rh antigens were discovered in 1940. This group is determined by a genes called D which has two alleles: D, d. Whatever other alleles a

person may have, anyone with genotype DD or Dd has D antigens on his or her RBCs and is classified as Rh positive (Rh^+). In Rh^-negative (Rh^-) people, the D antigen is lacking. The Rh blood type is tested by using an anti-D reagent. In the ABO blood group, anti-D antibodies are not normally present in the blood. They form only in Rh^- individuals who are exposed to Rh^+ blood. If an Rh^- person receives an Rh^+ transfusion, the recipient produces anti-D. A related condition sometimes occurs when an Rh^- woman conceive an Rh^+ fetus (Alimba *et al*., 2010)

The first pregnancy is likely to be uneventful because the placenta normally prevents maternal and fetal blood from mixing. However, at the time of birth, or if a miscarriage occurs, placental tearing exposes the mother to Rh^+ fetal blood. She then begins to produce anti-D antibodies. If she becomes pregnant again with an Rh^+ fetus, her anti-D antibodies may pass through the placenta and agglutinate the fetal erythrocytes. Agglutinated RBCs hemolyze, and the baby is born with a severe anemia called hemolytic disease of the newborn (HDN), or erythroblastosis fetal (Saladin, 2003).

2.1.1.Blood Group Systems

Humans contain a series of glycoprotein and glycolipids on the surface of RBCs which constitute the blood group antigens. According to the presence or absence of antigens human blood can be classified into different blood group systems, example ABO blood group, Rh blood group systems, etc. All blood groups in human are under genetic control, each series of blood groups being under the control of genes at a single locus or of genes that are closely linked and behave in heredity as though they were at a single locus (Jaff, 2010).

The human blood groups have been studied extensively for their involvement in incompatibility reactions. There are many blood group systems on the basis of different blood group antigens. ABO and Rh systems are important in clinical practice (Mandal, 2002).

2.1.2. The ABO Blood Group System

The ABO blood group system consists of A antigens, B antigens, and antibodies against these antigens. Landsteiner discovered the ABO system in 1900. As opposed to many other blood group systems such as the Rh system, in this system the presence of "naturally occurring" antibodies against A and B antigens in individuals who do not express those antigens (Landsteiner"s Law) causes an adverse and occasionally fatal outcome at the first mismatched transfusion. 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. 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 donor's type (Matsushita *et al*., 1983).

The ABO system is the most important blood group system in human blood transfusion. The associated anti-A and anti-B antibodies are usually immunoglobulinM, abbreviated IgM, antibodies. ABO IgM antibodies are produced in the first years of life by sensitization to environmental substance such as food, bacteria and viruses. The O in ABO is often called 0 (zero or null) in other languages (Khurshid *et al*., 1992).

The classification of blood groups into A, B, AB, and O in ABO blood group system is based on the presence or absence of inherited antigenic substances on the surface of red blood cells. 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 North India and neighboring central Asia and its incidence diminishes both towards the West and the East, falling to single percentages in Swiss. It is believed to have been entirely absent from native America and Austria aboriginal population prior to the arrival of Europeans in these areas.

Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although their highest frequencies occur in some Austrian aborigine population and the black foot India of Montana (ISBT,2008).

The antigens may be carbohydrates, glycoprotein and glycolipids depending on the blood group system (Hasna *et al.*, 2010).

2.1.3. Various Applications of ABO Blood Groups

The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations (Avent, 2000).

The ABO blood group antigens remain of prime importance in transfusion medicine and they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused.

The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations, suggesting that a particular blood type conferred a selection advantage (example; resistance against an infectious disease.). People with the common blood type O express neither the A nor B antigen, and they are perfectly healthy. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease (Garratty *et al.*, 2000)

For example, the ABO phenotype has been linked with stomach ulcers (more common in group O individuals) and gastric cancer (more common in group A individuals). Another observation is that individuals with blood type O tend to have lower levels of the Von Willebrand Factor (WF), which is a protein involved in blood clotting (Laura, 2005).

2.1.4. The ABO Blood Group Antibodies

ABO antibodies are naturally occurring antibodies that occur without exposure to red cells containing the antigen. There is some evidence that similar antigens found in certain bacteria, like *Escherichia coli*, stimulate antibody production in individuals who lack the specific A and B antigens. They are absent at birth and start to appear around 3-6 months as result of stimulus by bacterial polysaccharides (Avent,2000).

Normal healthy individuals produce antibodies against A or B antigens that are not expressed in their own cells. These naturally occurring antibodies are mainly immunoglobulin M (IgM). They attack and rapidly destroy red cells carrying the corresponding antigen. For example, anti-A attacks red cells of Group A or AB. Anti-B attacks red cells of Group B or AB. Table 1 shows ABO blood groups antigens and their corresponding antibodies (ISBT, 2008).

Table 1. ABO blood groups, antigens and antibodies

Blood Group phenotypes	Antigens present on the red cell surface	Antibodies present in plasma	Can received blood group from	Can give blood group to
O	Neither A nor B	anti-A and anti-B	O	O,A,B,AB
A	A antigen	anti-B	O,A	A,AB
B	B antigen	anti-A	O,B	B,AB
AB	A and B antigens	Neither anti-A nor anti-B	O,A,B,AB	AB

2.1.5. Red Blood Cells Compatibility

Blood group A individuals have the A antigen on the surface of their red blood cells and blood serum containing IgM antibodies against the B antigen. Therefore, a group A individual can receive blood only from individual of A or O (with A being preferable). and can donate blood to individual with type A or AB (Simpkins and Williams, 1997).

Blood groups B individuals have the B antigen on the surface of their red blood cells and blood serum containing IgM antibodies against the A antigen. Therefore, a group B individual can receive blood only from individuals of groups B or O (with B being preferable). And can donate blood to individuals with type B or AB (Bruce, 2002).

Blood group AB individual have both A and B antigens on the surface of their red blood cells and their blood serum does not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood group can receive blood from any group (with AB being preferable). But can donate blood only to another AB individual (Greenwalt, 1997).

Blood group O individual (whose red cells bear neither antigen) is classically referred to as a universal donor, since this blood can fairly safely be given to a recipient with any ABO type. But their blood serum contains IgM anti-A antibodies and anti-B antibodies against the A and B blood group antigens. Therefore, a group O individual can receive blood only from a group O individual (Griffithis *et al.*, 2008).

2.1.6. Frequencies of ABO Phenotypes in Different Populations

The ABO blood group phenotypes are not found in equal numbers in different populations. The distribution of the blood groups A, B, O and AB varies across the world according to the population. The A blood allele is somewhat more common around the world than B. About 21% of all people share the A allele. The highest frequencies of A are found in small, unrelated populations, especially the Blackfoot Indians of Montana (30-35%), the Australian Aborigines (many groups are 40-53%), and the Lapps or Saami people of Northern Scandinavia (50-90%). The A allele apparently was absent among Central and South American Indians (Al-Arrayed *et al*., 2001).

For example, in Caucasians in the United States, the distribution is type O 47%, type A 41%, type B 9% and type AB 3%. Among African Americans, the distribution of type O is 46%, type A 27%, type B 20% and type AB 7%. Among Western Europeans, 42% are group A, 9% group B, 3% group AB and the remaining 46% group O (Laura, 2005).

Among Ethiopians, the distribution is type O 40%, type A 31%, type B 23% and type AB 6% (Misganaw, 2004). Table 2 shows the frequency of ABO blood groups studied in different populations across the world.

Table 2. Frequencies of ABO blood groups studied in different populations across the world

Populations	A	B	AB	O	References
Ethiopia	0.2700	0.2500	0.5000	0.4300	Bloodbook.com
Britain	0.4170	0.0860	0.0300	0.4670	Khattak *et al*., 2008
Saudi Arabia	0.2400	0.1700	0.0400	0.5200	Khattak et *al*., 2008
India	0.1885	0.3250	0.0990	0.3875	Khattak *et al*., 2008
Turkey	0.1220	0.1213	0.0085	0.7398	Akbas *et al*., 2003.
Hungary	0.2766	0.1218	0.0423	0.5593	Tuaszik, 1995
Kuwait	0.1608	0.1400	0.0265	0.6678	Al-Bustan *et al*.,2002
Nairobi ,Kenya	0.1580	0.1261	0.029	0.600	Lyko *et al*., 1992
Cameroon	0.2541	0.2212	0.0421	0.4900	Hamed *et al*., (2012)
Egypt	0.3025	0.2418	0.0821	0.3324	Tagny *et al*., (2012)
Sudan	0.1814	0.1235	0.0268	0.6683	Khalil *et al*., 1989
Pakistan	0.1740	0.2229	0.0435	0.5596	Anees *et al*., 2005
Ogbomosho Nigeria	0.2290	0.2130	0.0590	0.5000	Bakare *et al*., 2006

2.2. The Rh Blood Group System

The rhesus blood type named after the rhesus monkey was first discovered in 1937 by Karl Landsteiner and Alexander S.Wiener. The significance of the discovery was not immediately apparent and was only realized in 1940, after subsequent findings by Philip Levine and Rufus Stetson. This serum that led to the discovery was produced by immunizing rabbits with red blood cells from a rhesus macaque. The antigen that induced this immunization was designated by them as Rh factor to indicate that rhesus blood had been used for the production of the serum (Avent, 2000).

In 1939, HDN was first described by Levine and Stetson. The cause of hemolytic disease was not specifically identified but maternal antibody was suspected. A year later, in 1940 Karl Landsteiner and Alexander Wiener injected animals with Rhesus monkey cells and produced an antibody which reacted with the red blood cells of 85% of humans, which they named anti-Rh. Within a year, Levine made connection between maternal antibodies causing HDN and anti-Rh. Between 1943 and 1945; the common antigens of the Rh system were identified. For many years, the exact inheritance of the Rh factors was debated, Weiner promoting Rh terminology and Fisher-Race utilizing D, C, c, E and e for the various Rh antigens (Daniels, 2005).

2.2.1. Rh (D) Blood Grouping

The Rh (D) blood group is one of the complex blood groups known in human. It is one of thirty current human blood group systems. Clinically it is the most important blood group system after ABO at present. The Rh (D) blood group systems consist of 50 defined blood group antigens, among which the five antigens D, C, c E, and e are the most important (Daniels, 2005).

The commonly used terms Rh factor, Rh positive and Rh negative refer to the D antigen only. Beside its role in blood transfusion, the Rh (D) blood group system specially, the D antigens is used to determine the risk of hemolytic disease of the newborn (HDN) (Reid and Lomas,2004).

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 positive (Rh^+ does have the D antigen) or Rh negative (Rh^- does not have the D antigen) as suffix to the ABO blood type. However, other antigens of this blood group system are also clinically relevant. Unlike the anti-A and anti-B antibodies, anti-D antibodies are only seen if a patient lacking D antigens is exposed to D^+ cells. The exposure of D^+ cells usually occurs through pregnancy or transfusion. 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, 2005).

2.2.2. Frequencies of RhD Blood Group Phenotypes in Different Populations

Rh blood group distribution varies worldwide. Rh negative blood group is documented as 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria, 7.3% in Lahore, 7.7% in Rawalpindi. About 95% of African-Americans are Rh-positive, (Chavhan *et al.*, 2010).

Table 3. Frequency of Rh blood groups in different populations across the world

Population	**Rh $^+$**	**Rh $^-$**	**References**
Britain	0.9140	0.0860	Khattak *et al*., (2008
U.S.A	0.8500	0.1500	Khattak *et al*., (2008
Kenya	0.8030	0.1970	Lyko *et al*., (1992)
Cameroon	0.9620	0.03800	Hamed *et al*., (2012)
Egypt	0.9624	0.0376	Tagny *et al*., (2012)
Ethiopia	0.9330	0.0670	Bloodbook.com
Saudi Arabia	0.9300	0.0700	Khattak *et al*., (2008)
Germany	0.9500	0.0500	Khattak *et al*., (2008)
India	0.9445	0.0550	Das *et al*., (2001)
Lagos(Nigeria)	0.9400	0.0600	Bakare *et al*., 2006
Ogbomosho (Nigeria)	0.9670	0.0330	Bakare *et al*., 2006

2.3. Clinical Significance of Blood Groups

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 within one's own life time and so it is considered as a unique genetic marker for research. The blood group is determined by the alleles of a system (Gupta, 1999).

2.3.1. Blood Transfusion

Clinical complications result from RBC destruction due to the interaction of antibody with RBCs carrying the corresponding antigen. The D antigen is highly immunogenic and induces an immune response in 80% of D-negative persons when transfused with 200 mL of D-positive blood. For this reason, in most countries D typing is performed routinely on every blood donor and transfusion recipient so that D-negative patients receive D-negative RBC products. Consequently, clinical complications due to mismatched transfusions are infrequent. In contrast, despite the use of immunosuppressive therapy with anti-D immunoglobulin prophylaxis, D immunization in pregnancy still occurs, (Mollison ,1994).

Transfusion is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of blood bank to provide transfusion services for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor in a similar way as a medicine. Much of the routine work of the blood bank involves 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 transfused between a donor and recipient severe hemolytic reaction with hemolytic (RBC destruction), renal failure and shock is likely to occur and death is a possibility. Antibodies can be highly active and can attach to 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 transfusion reaction. Risks can be further reduced by a 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 donors and shacking 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 (Bruce, 2002).

In addition to the ABO system, the Rh blood group system can affect transfusion compatibility. An individual is either positive or negative for the Rh factor; this is denoted by a '+' or '-' after their ABO type. Blood that is Rh-negative can be transfused into a person who is Rh-positive, but an Rh-negative individual can create antibodies for Rh-positive RBCs. Because of this, the AB+ blood type is referred to as the "universal recipient", as it possesses neither Anti-B nor Anti-A antibodies in its plasma, and can receive both Rh-positive and Rh-negative blood. Similarly, the O- blood type is called the "universal donor" since its red blood cells have no A or B antigens and are Rh negative, no other blood type will reject it. (Bruce, 2002)

2.3.2. Hemolytic Diseases of the New Born (HDN)

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

Hemolytic disease of the newborn (HDN), 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. Every blood group antibody that can occur as IgG can cause HDN. 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 utero 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).

Rh blood type is determined by a pair of genes, one inherited from each parent. Blood is either Rh-positive or Rh-negative, depending on whether or not certain molecules are present. A person who is Rh-negative will experience a severe immune system reaction if Rh-positive blood gets into their bloodstream. This can happen during pregnancy if an Rh-negative woman carries an Rh positive baby. If blood cells from the baby travel across the placenta, the woman's immune system will regard the Rh-positive cells as a threat. Specialized white blood cells will make antibodies designed to kill Rh-positive blood cells. If the woman later conceives another Rh-positive baby, her immune system will flood the fetus with antibodies. These antibodies then destroy the baby's red blood cells. If left untreated, this can result in severe anemia or even death. This is called hemolytic disease of the newborn (Bakare *et al*., 2006).

2.4. Methods of Blood Typing

Blood typing involves identifying substances called antigens present on RBCs membranes. Many different antibodies exist on human RBCs but those of clinical importance include only the ABO and Rh groups. Blood typing is performed with antisera. For ABO blood typing, antibodies against A and B antigen are used. If clumping or clotting occurs in the test blood upon exposure to the A antibody, the blood contains the A antigen. If clumping occurs in the test blood upon exposure to the B antibody (anti-B serum), the blood contains the B antigen. If clotting occurs with both A and B antibodies (anti-A and anti-B), the blood type is AB, and if no clumping occurs with either serum type, the blood type is O (Rai and Lomas, 2004). Table 4 below shows agglutination reactions with ABO blood typing sera (ISBT, 2008).

In addition to the current practice of serological testing of blood types, the progress in molecular diagnostic allows increasing use of blood group genotyping. In contrast to serological tests reporting a direct blood group type phenotype, genotyping allows the

prediction of phenotype based on knowledge of molecular basis of the currently known antigen. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for the patients with needs for much transfusion to prevent all immunization (Anstee, 2000).

Table 4. Agglutination reactions of the RBC by ABO blood-typing sera (ISBT, 2008)

Reaction		**Blood type**
A antibody (anti-A serum)	**B antibody (anti-B serum)**	
clumping	no clumping	Type A
no clumping	clumping	Type B
clumping	clumping	Type AB
no clumping	no clumping	Type O

2.5. Genes in a Population

A gene is a unit of hereditary transmission. Different forms of the same gene are known as alleles. Alleles may be combined in genotypes which may or may not have distinct phenotypes. The relative proportion of each allele in a population is called its allele frequency, similarly the relative proportion of each genotype is its genotypic frequency and the relative proportion of each phenotype is the phenotypic frequency. Genotypic frequencies always determine the allelic frequencies, the reverse is not necessarily true, and that is, we cannot always calculate the genotypic frequencies from the allelic. Given some assumptions, random union of gametes, very large population size, absence of selection, migration, etc. one can calculate the genotypic frequencies from the allelic frequencies using the Hardy-Weinberg principle (Sarhan *et al.*, 2009)

2.6. Significance of the Distribution

The knowledge of distribution of ABO and Rh blood groups at local and regional levels are helpful in the effective management of blood banks and safe blood transfusion services. Identification of ABO and Rh (D) blood group distribution among populations

has various benefits in transfusion medicine, transplantation and disease risk assessment. 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.

For a blood donor and recipient to be ABO-compatible for a transfusion, the recipient must not have Anti-A or Anti-B antibodies that correspond to the A or B antigens on the surface of the donor's red blood cells (since the red blood cells are isolated from whole blood before transfusion, it is unimportant whether the donor blood has antibodies in its plasma). If the antibodies of the recipient's blood and the antigens on the donor's red blood cells do correspond, the donor blood is rejected (Fareed *et al*., 2014).

2.7. The Hardy-Weinberg Principle

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 (Mollisi.,1994). Violation of any of these assumptions can result in evolutionary change in terms of allelic frequency distribution (Mayo, 2008).

In a large population where there is no genetic drift, and in the absence of selection, migration and mutation, the allelic frequencies remain constant from generation to generation. If mating is random, the genotypic frequencies are related to the allelic frequencies by the square expansion of allelic frequencies. Thus, for autosomal genes in diploid organisms in which there are two alleles with frequencies p and q, the frequencies of the three genotypes are predicted by the formula $(p+q)^2 = p^2 + 2pq + q^2$. Furthermore, for autosomal genes at equilibrium genotypic frequencies at any given locus are attained in a single generation providing there is no overlapping of generations (Bryant, 1994).

Modified Hardy- Weinberg equation will be used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies .When two alleles, for example, p and q are present at a locus, based on the Hardy-Weinberg principle at equilibrium the frequencies of the genotypes become $p^2 + 2pq + q^2 = 1$, which is the square of the allelic frequencies $(p + q)^2$.

This is a simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are inherited two at a time into a diploid zygote. The three alleles of ABO blood group which are I^A, I^B and I^O are represented as p, q and r, respectively in which p is the frequency of allele A, q is the frequency of allele B and r is the frequency of allele O. Therefore the genotypic frequencies are represented by trinomial expansion as $(P+q+r)^2 = p^2 + 2pq + q^2 + 2pr + 2qr + r^2 = 1$(Hanania *et a*l.,2007).

Where: 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^O

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

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, and r denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O). A correction factor (θ) will be calculated according to $\theta = 1 - p - q - r$. The final allele frequencies were then calculated as follows: $p^1 = p\,(1 + \theta/2)$, $q^1 = q\,(1 + \theta/2)$ and $r^1 = (r + \theta/2)\,(1 + \theta/2)$. Where p^1, q^1 and r^1 denote corrected allele frequencies. The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test to check whether population was at Hardy-Weinberg genetic equilibrium or not (Chakraborty, 2011).RhD allele frequencies were calculated according to the Hardy-Weinberg equation (Al-Arrayed *et al*., 2001). Frequencies of RhD blood group alleles D and d are represented as p and q respectively in which p is frequency of allele D and q is frequency of allele d. Using Hardy- Weinberg equation, at equilibrium the frequencies of the genotype were represented as $(p + q)\,2 = p2 + 2pq + q2 = 1$,

where p2 is frequency of genotype DD, 2pq is frequency of genotype Dd and q2 is frequency of genotype dd, (Dar *et al.*, 2010).

2.8. The Chi-square Test to Calculate Observed and Expected ABO and Rh Blood Group Distribution

The observed and expected genotype frequencies in Hardy-Weinberg were calculated on the basis of allele frequency and Chi-square tests was done to test the independence and the goodness of fit for genotype frequencies. Chi-square test was used to compare observed genotypic frequency distributions of the blood group ABO and Rh anti-gens to that expected under the Hardy–Weinberg equilibrium (Chakraborty, 2010).

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

3. MATERIALS AND METHODS

3.1. Description of The Study Area

The study was conducted in Geresu Duki Preparatory School in Woliso town, South West Shewa Zone, Oromia Regional State, Ethiopia. The school was established in 2005 on about a total area of 7700.540 m^2 and located in 02 kebele of the town along south-west direction which is about 200m from the main road to Jimma.

Woliso is located at a distance of 114Km south west direction from the capital of the country Addis Ababa and 225 Km away from Jimma town along Addis Ababa–Jimma main road. Geographically, the town is located at 8.31^0 60" north latitude and 37.58^0 $60^{"}$ east longitude. The elevation of the town ranges from 1900 to 2000 meters above sea level. The mean temperature of the town is 22.5^0C and the mean annual rainfall is 1200mm.

Woliso town is one of the towns found in Oromia Regional State of Ethiopia, which was established in 1926 (WTA). However, the town was officially recognized in 1930 as municipal town after 4 years of its establishment. In 2003, the town was put under reform by urban proclamation 65/2003 of the regional state. The town has a big strategic vision to become the centre of trade, conference and tourism. Currently, the town is serving as a seat for Woliso *Wereda*/district/ administration and the capital city of South West Showa Zone divided into 4 *kebeles*(WTA).

According to the 2007 population census of Central Statistical Agency (CSA) report, the population size of Woliso town was 37, 868 and in the year 2015/16 this number was projected to reach about 49,947.892 out of which population size the Oromo account for 34,963, Amhara is 4,995 and that of Gurage population is 9,989 assuming that the population grows at the rate of 2.9 percent per annum (Source: Woliso Town Authority, 2015, annual report).

3.2. Study Participants

Geresu Duki Preparatory School has 985 students who were registered for the academic year 2016/2017 (Source: Gereu Duki preparatory Recorded office). Among them 502(50.96%) are males and 483(49.04%) are females. The study was carried on a total of 470 (48%) students in the school. Voluntary students were selected purposively among the students and the sample was divided into three ethnic groups (267 from Oromo, 108 from Amhara and 95 from Gurage) and stratified along ethnic lines. These sample Size were small that to represent their corresponding population, for example in case of Gurage ethnic group even though their population size were next to Oromo ethnic groups in woliso town but number of Gurage students in the school were less than Amhara and Oromo students. However other ethnic groups are not included because their numbers are insignificant. Participation was voluntary after the objective and procedure of the study were explained. The information about the students' ethnic group was obtained from the students themselves, i.e self declared.

3.3. Blood Sample Collection Procedure and Typing

Blood sample was collected from each participants. The blood sample was taken from finger pricks by scrubbing the middle finger with a piece of cotton saturated with 70% alcohol and piercing by sterile packed lancet. Then, placed on a single clean slide in three places and a drop of each of the Anti-sera that is Anti A, Anti B, and Anti D were added to each of an individual's blood samples and mixed using a glass road. The blood samples were collected by qualified laboratory technicians to test ABO blood types and Rh (D) factor at Woliso Health Center using the standard clinical procedure with sterilized packed lancet, slides, and chemicals like Anti- A, Anti-B and Anti-D. The blood group was determined on the basis of agglutination and recorded, as blood group A^+, B^+, AB^+, O^+ or A^-, B^-, AB^- and O-.

3.4. Methods of Data Analysis

In this study, the phenotypic distribution of blood groups among students was expressed in simple percentages and frequencies. Allele frequencies were calculated from phenotypic frequencies by considering two alleles at the same locus for Rh system and three alleles at the same locus for ABO system using Hardy-Weinberg equilibrium equations.

3.4.1. Phenotypic Frequency Determination of ABO and RhD Blood Groups

Observed percentage = $\frac{observed \quad number}{Total \quad number} x100$

Observed frequency = $\frac{Observed\ number}{Total\ Number}$

3.4.2. Estimation of Allele Frequency

The genetic structure can be described in terms of allelic and genotypic frequencies (Chavhan, 2010). Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium. Frequency of the three ABO blood group alleles (p, q, and r) were determined as follows

$r = \sqrt{O}$

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

$q = 1 - \sqrt{A+O}$, Where r =O, p=A and q=B

Frequency of the two Rh(D) blood group alleles (p and q) were determined as follows

$q = \sqrt{Rh-}$

$P = 1 – q$, Where p=Rh+and q=Rh^-

3.4.3. Genotype Frequency of ABO and Rh(D) Blood groups

The genotypic frequencies of ABO blood groups was calculated as follows

Genotypic frequency= $(P+q+r)^2 = p^2 + 2pq + q^2 + 2pr + 2qr + r^2=1$

Genotype $I^{AA} = p^2$

Genotype $I^{AO} = 2pr$

Genotype $I^{BB} = q^2$

Genotype $I^{BO} = 2qr$

Genotype $I^{AB} = 2pq$

Genotype $I^{OO} = r^2$

The genotypic frequencies of RhD blood groups were calculated as follows:

Genotype $DD = p^2$

Genotype $Dd = 2pq$

Genotype $dd = q^2$

3.4.4. Chi-square Test

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test to check whether population was at Hardy-Weinberg genetic equilibrium or not. The differences in the phenotypic and allelic frequency of ABO and RhD blood groups among the three groups were also statistically tested using the contingency chi-square test (Chakraborty, 2010).

$$\text{Chi-square } \chi 2 = \sum \frac{(O-E)^2}{E}$$

Where:- O=observed frequency and E= Expected Frequency

Expected phenotypic frequencies were calculated as:

E_f = Genotypic frequency X number of total sample

For A blood group E_f = frequency of (AA + AO) X number of total sample

For B blood group E_f = frequency of (BB + BO) X number of total sample

For AB blood group E_f= frequency of AB X number of total sample

For O blood group E_f = frequency of OO X number of total sample

3.5. Ethical Considerations

Data collection was conducted in the study area after obtaining informed consent from the concerned office (i.e Woliso Woreda Health Office, all have given their written consent for the study. All the information that was obtained about the subjects were kept confidential.

4. RESULTS AND DISCUSSIONS

4.1. Frequency Distribution of ABO and Rh(D) Phenotype Among the Three Ethnic Groups

4.1.1. Frequency of ABO Blood Group Phenotypes Among Students of the Three Ethnic Groups

The frequency distribution of ABO blood groups among the three ethnic groups are shown in Table 5. Phenotypic frequencies of ABO and RhD blood groups of 470 students (307 male and 163 Females) who were purposively selected from among a total of 985 students in Geresu Duki preparatory school are presented in the table. There are differences in frequency distribution of the blood group (ABO) among the ethnic groups of the students.

Table 5. Frequency of ABO blood group phenotype among students of three ethnic groups

Ethnic Group	**Distribution of ABO Blood Groups Count (%)**								**Total**	χ^2
	A		**B**		**AB**		**O**			
	No	**%**	**N0**	**%**	**No**	**%**	**No**	**%**		
Oromo	87	32.58	45	16.85	19	7.12	116	43.45	267	4.01
Amhara	27	25.00	35	32.41	8	7.41	38	35.19	108	5.035
Gurage	30	31.58	22	23.16	9	9.45	34	35.79	95	1.075
Total	144	30.64	102	21.70	36	7.66	188	40.00	470	3.37

There are differences among phenotypic percentage distribution of the ABO blood group in three ethnic groups of the study area. Over all Blood groups "AB" has the lowest frequency while Blood group "O" has the highest frequency (Table 5). In the study area, blood group "O" was 43.45%, 35.19% and 35.79% in Oromo, Amhara and Gurage respectively, and followed by blood group "A",32.58%, 25% and 31.58% and blood group "B" 16.85%, 32.41% and 23.16% in Oromo, Amhara and Gurage respectively.

When one compare the persentage distribution of ABO blood phenotypes among the three ethnic groups, blood types O have highest frequency in Oromo (43.45%) than Amhara (35.19%) and Gurage(35.79%) ethnic groups. Blood group A has the highest frequency in Oromo (32.58%) than in Amhara (25.00%) and Gurage (31.58%) ethnic groups. Blood group B has the highest frequency in Amhara (32.41%) than in Gurage (23.16%) and Oromo (16.85%) ethnic groups (Table 5). The highest percentage of type-AB was observed in Gurage (9.45%) whereas the least was observed in both Oromo(7.12%) and Amhara (7.41%) ethnic groups (Table 5). But the differences in blood type percentage in each ethnic groups and in the over all sampled population are not significant (χ^2=3.37, P<0.05). This indicates that the population is homogenous which might be due to random intermarriage among the three ethnic groups and small sample sizes used.

The order of dominance of percentage of ABO blood group phenotype in the three ethnic groups there were similar pattern among Oromo And Gurage ethnic groups in which blood group “O” was dominant and followed by blood group A,B and AB blood groups; that was (O>A>B>AB). But the order of dominance of ABO blood groups phenotype in Amhara ethnic group were different from Oromo and Gurage ethnic groups, that is blood group” O” was dominant and followed by blood group B,A and AB blood groups (O>B>A>AB). However, blood group “O” were dominant and “AB” blood groups were the least in the three ethnic groups and follow similar patterns.

The whole percentage of ABO blood group phenotypes observed in the three ethnic groups were 0.40 O, 0.3064 A, 0.2170 B, and 0.0766 AB respectively. It has also been reported in several studies that there are variation in "ABO" blood group among different tribes (Falusi et al., 2000).

Many other studies have shown that blood group O was the most common blood group and blood group AB was the least common blood group in different populations and ethnic groups (Bakare *et al*., 2006).For example, among Ethiopians, the distribution was type O, 42%; type A, 30%; type B, 22%; and type AB, 6 % (Abraham *et al*., 2012).

The study carried out by Tibebu (1998) showed that the distribution of type O was 40%; type A was 31%; type B was 23%; and type AB was 6% among Ethiopian blood donors. In population of south west Ethiopia (at Gilgel Gibe Field Research Center), the distribution of type O, was 42%; type A, was 31%; type B, was 21%; and type was AB, 6% (Abraham *et al.*, 2012). Among Sidama ethnic group (Ethiopia), the distribution is type O, 51.3%; type A, 23.5%; type B, 21.9%; and type AB, 3.3% (Tewodros *et al*., 2011). Therefore, the results of this study are in agreement with the data from previous studies in Ethiopian populations.

Similarly among Egyptian population, O was, 33%, A was, 30%, B was 24%, AB 8% (Hamed *et al*., 2012) had reported that students were 27% A blood type, 16% B blood type, 4% AB blood type and 53% O blood type in Nigerian population which is in agreement with this study (Bakare etal., 2006).

However, the results of this study do not agree with the results from some Asian countries where blood group B has the highest frequency in some and blood group A in the others. For example Chavhan *et al*.,(2010); Girri *et al*., (2011); Kumar (2009); and Kumar *et al*., (2010) reported high frequencies of B blood group in Indian population. Khan *et a*l. (2006) and also reported high frequencies of B blood group in Pakistan populations. The highest frequency of A blood group was documented in Jordanian populations (Hanania *et al*., 2007); and Saudi Arabian populations (Khan *et al.*, 2006).

4.1.2. Frequency Distribution of Rh (D) Blood Group Phenotypes among the three Ethnic Groups

Table 6. Phenotypic percentage of Rh (D) Blood Groups among the three Ethnic Groups.

Ethnic groups	Distribution of Rh(D) Blood groups				Total	χ^2	
	Rh^+		Rh^-			Rh^+	Rh^-
	No	%	No	%			
Oromo	258	96.63	9	3.37	267 (1.00)	$3.9x10^{-3}$	0.1
Amhara	105	97.22	3	2.78	108 (1.00)	$9.6x10^{-3}$	0.25
Gurage	90	94.74	5	5.26	95 (1.00)	$2.2x10^{-2}$	0.67
Total	453	96.38	17	3.62	470 (1.00)	0.0352	1.02

The phenotype distribution of Rh(D) blood group among each ethnic groups are shown in Table 6. This study has shown that Rh- positive has the higher percentage while Rh negative has the lower percentage in the total sample as well as in each of the three ethnic groups. The phenotype distribution of Rh-positive (+) was higher in Amhara (97.22%) than in Oromo (96.38%) and Gurage (94.74%) respectively, these might be due to high number Positive Rh-D phenotpe compared to their total sample of their corresponding ethnic group and the Rh-negative(-) was high frequent in Gurage(5.26%) than Oromo (3.37%) and Amhara ethnic groups (2.78%) as a result of high number of negative Rh-D phenotype in the sample population Table 6. The percentage of Rh blood group in the overall sample were 96.38% Rh positive and 3.62% Rh negative (Table 6).

The variations in the percentage distribution of Rh+ and Rh- among the three ethnic groups followed the same pattern as shown in Table 6. The difference in the frequencies of Rh-D positive and negative phenotypes in each of the ethnic groups and among the three ethnic groups there was no significant variation,(χ^2=0.0352 Rh^+ and χ^2 =1.02 Rh^-, df=2, P>0.08), this indicates that populations are at Hardy Weinberg equilibrium.

These results are consistent with previous findings of Ethiopian populations and South Ethiopian Populations (Abraham *et al.,* 2012). Again, the findings of this study are in agreement with report from previous similar studies in different parts of the world where the RhD positive was found to be higher in the population sampled than the RhD negative (Bakare *et a*l., 2006, Iyiola *et al*., 2011). RhD negative blood group was documented as 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria, 7.3% in Lahore and 7.7% in Rawalpindi. About 95% of African-Americans are Rh-positive (Abraham *et al.,* 2012 and Chavhan *et al.,* 2010)

.4.1.3. Frequency Distribution of ABO Phenotypes Based on Rh (D) Blood Groups

Table 7. Distributions of the combined ABO and RhD blood group phenotypes among students of three ethnic groups.

Blood groups		Ethnic Groups						Total		χ^2
		Oromo		Amhara		Gurage				
ABO	RhD	No	%	No	%	No	%	No	%	
O	RhD^{+ve}	110	41.19	36	33.33	30	31.58	176	37.45	0.77
	RhD^{-ve}	6	2.25	2	1.85	4	4.21	12	2.55	2.6×10^{-2}
A	RhD^{+ve}	86	32.21	26	24.07	30	31.58	142	30.21	0.64
	RhD^{-ve}	1	0.38	1	0.93	0	0	2	0.43	0.74
B	RhD^{+ve}	43	16.11	35	32.45	22	23.16	100	21.28	3.28
	RhD^{-ve}	2	0.75	0	0	0	0	2	0.75	0.59
AB	RhD^{+ve}	19	7.12	8	7.41	8	8.42	35	7.45	6.3×10^{-2}
	RhD^{-ve}	0	0	0	0	1	1.05	1	1.05	0.81
Total	RhD^{+ve}	258	96.63	105	97.22	90	94.74	453	96.38	4.753
	RhD^{-ve}	9	3.37	3	2.78	5	5.26	17	3.62	2.166

Table 7 above shows the distribution of the combined ABO and Rh-D blood groups of the three ethnic groups. In all of the three ethnic groups ABO positive phenotype based on Rh-D has the highest frequency which is about 96.38% and ABO negative phenotype based on Rh-D has the least frequency that was 3.62%.

In all ethnic groups AB phenotype has the least frequency. AB- phenotype was observed only in Gurage ethnic group and B- observed only in Oromo ethnic group but O- was observed in all ethnic groups.

The ABO blood group distribution based on Rh (D) in Oromo; blood group A with Rh+ is 32.21% while in Gurage ethnic group blood group A with Rh+ is 31.58% but in Amhara the percentage of blood group A Rh+ is reduced to 24.07% of the total population. As it is indicated in Table 7, blood group AB with Rh positive has a small percentage distribution in the three ethnic groups than blood group A and B. Blood group O with Rh positive of Oromo was 41.19 % and that of the Amhara was 33.33% and 31.58% for the Gurage ethnic group. So, blood group O with Rh positive is dominant in the Amhara ethnic group. However, the percentage distribution of Rh negative is very rare in the three ethnic groups. The combined distribution of ABO and Rh-D positive and negative blood group percentage in each of the three ethnic groups where(χ^2 =0.77, 0.64, 3.28, 0.063) for blood group O^+,A^+,B^+ and AB^+ respectively and (χ^2 = 0.026, 0.74, 0.59 and 0.81) for negative blood groups respectively as well as in the over all sample population, (χ^2 = 4.753) positive and (χ2=2.166) negative ABO combined frequency shows no significant variation in the study area..

4.2. Allele and Genotype Frequency Distribution of ABO and Rh(D) Blood Groups Among Students of the Three Ethnic Groups

The genotypic and allelic frequencies for ABO and RhD blood groups of 470 students were calculated based on estimated allele frequencies according to the Hardy-Weinberg Law. Table 8 and 9 presents the frequencies of the various genotypes and allele frequencies in the ABO and Rh-D blood group system of the three ethnic groups (Oromo, Amhara and Gurage). The main application of the Hardy-Weinberg law is to estimate the heterozygous and homozygous genotype distribution in the population studied. The genotypic and allelic frequency distribution among the students of three ethnic groups was calculated and the results are shown in Table 8 and 9 respectively.

4.2.1. Allele and Genotype Frequencies of ABO Blood Groups of the Three Ethnics Groups

Table 8. Allele Frequency of ABO blood groups among students of Oromo, Amhara and Gurage ethnic groups.

Ethnic groups	Allele	Frequency	Phenotype	Frequency	Genotype	Frequency
Oromo	I^O	0.6592	O	0.4345	I^OI^O	0.4345
	I^A	0.2128	A	0.0453	I^AI^A	0.0453
	I^B	0.1280	A	0.2806	I^AI^O	0.2806
			B	0.0164	I^BI^B	0.0164
			B	0.1688	I^BI^O	0.1688
			AB	0.0545	I^AI^B	0.0545
Amhara	I^O	0.5932	O	0.3519	I^OI^O	0.3519
	I^A	0.2243	A	0.0503	I^AI^A	0.0503
	I^B	0.1826	A	0.2661	I^AI^O	0.2661
			B	0.0333	I^BI^B	0.0333
			B	0.2166	I^BI^O	0.2166
			AB	0.0819	I^AI^B	0.0819
Gurage	I^O	0.5983	O	0.3579	I^OI^O	0.3583
	I^A	0.2226	A	0.0496	I^AI^A	0.0496
	I^B	0.1791	A	0.2664	I^AI^O	0.2664
			B	0.0321	I^BI^B	0.0321
			B	0.2143	I^BI^O	0.2143
			AB	0.0797	I^AI^B	0.0797

As indicated in Table 8 above, most of A and B blood types are heterozygous in each of the three ethnic groups. The frequency of genotype I^AI^O makes up 0.2806, 0.2661 and 0.2664 in Oromo, Amhara and Gurage, respectively. While I^BI^O makes up 0.1688, 0.2166 and 0.2143 in Oromo, Amhara and Gurage, respectively. The results of this study agree with the suggestion of Bakare *et al.,* (2006) that the predominance of O allele may also be as a result of the fact that many A's and B's may have been heterozygous carrying O allele silently thereby maintaining O allele in the heterozygous population. Homozygous blood group A of the three ethnic groups was calculated to be 0.0453, 0.0503 and 0.0496 in Oromo, Amhara and Gurage,, respectively. Whereas the homozygous blood group B obtained for the three ethnic groups was 0.0164, 0.0333 and 0.0321 in Oromo, Amhara and Gurage, respectively. Similar results were reported by Iyiola *et al*., (2011), Hanania *et al*., (2007), and Bakare *et al*., (2006). The allele frequency in each ethnic groups of the study area follow the same patterns, ($I^O>I^A>I^B$) where 0.6592 I^O ,0.2128 I^A and 0.128 I^B in Oromo and 0.5932 I^O, 0.2243 I^A, 0.1826 I^B in Amhara and 0.5983 I^O, 0.2226 I^A, 0.1791 I^B in Gurage ethnic groups respectively.

The overall allelic frequencies of three ethnic groups of the populations as it was calculated using the extension of the Hardy–Weinberg law were 0.63, 0.21 and 0.16 for I^O, I^A and I^B alleles, respectively.

4.2.2. Allele and Genotype Ffrequency of Rh(D) Blood Groups Among the Three Ethnics Groups

Table 9. Allele Frequency of Rh (D) blood groups among students of Oromo, Amhara and Gurage ethnic groups

Ethnic groups	Allele	Frequency	Phenotype	Frequency	Genotype	Frequency
Oromo	D	0.8164	RhD+ve	0.6665	I^DI^D	0.6665
			RhD+ve	0.2998	I^DI^d	0.2998
	d	0.1836	RhD-ve	0.0337	I^dI^d	0.0337
Amhara	D	0.8333	RhD+ve	0.6944	I^DI^D	0.6944
			RhD+ve	0.2778	I^DI^d	0.2778
	d	0.1667	RhD-ve	0.0278	I^dI^d	0.0278
Gurage	D	0.7706	RhD+ve	0.5938	I^DI^D	0.5938
			RhD+ve	0.3536	I^DI^d	0.3536
	d	0.2294	RhD-ve	0.0526	I^dI^d	0.0526

In the case of Rh (D) blood grouping system, of the 470(48%) , 0.9663 of population sampled were Rh^+ and 0.0337 is Rh^- as indicated in Table.8, the heterozygous Dd positive was 0.2998, 0.2778 and 0.3536 in Oromo, Amhara and Gurage respectively. Homozygous DD positive genotype of the three ethnic group calculated by using Hardy-Weinberg equilibrium were 0.6665 in Oromo, 0.6944 in Amhara and 0.5935 in Gurage. So, the homozygous DD positive is more distributed in the three ethnic groups. Also the homozygous recessive genotype dd negative was calculated as 0.0337 in Oromo, 0.0278 in Amhara and 0.0526 in Gurage which show homozygous genotype dd negative is the least frequent among the three ethnic groups. The overall Rhesus status, the allelic frequencies were 0.81 and 0.19 for D and d alleles respectively.

4.3. The Chi-Squared Test of Deviation Between Observed and Expected Phenotype Frequencies of ABO and Rh(D) Blood Groups

The chi- square test for each ethnic group was calculated by using the result obtained from Table.5 and the ABO and Rh (D) frequency distribution to know whether the population of each ethnic group is at Hardy- Weinberg equilibrium or not. In this study the observed ABO blood group distribution from each ethnic group was compared under Hardy- Weinberg equilibrium with the expected value.

4.3.1. Chi-Square Test in ABO Blood Group Among the Three Ethnic Groups

Table 10. Chi-square test in ABO blood groups among students of Oromo, Amhara and Gurage ethnic groups

Ethnic Groups	**ABO blood System**					
	Blood Groups	Observed No (O)	Expected No(E)	Differences D=(O-E)	D^2	D^2/E
Oromo	O	116	116.0115	-0.0115	1.32×10^{-4}	1.93×10^{-6}
	A	87	87.0153	-0.0153	2.34×10^{-4}	2.69×10^{-6}
	B	45	49.4484	-4.4484	19.79	0.4
	AB	19	14.55	-4.4485	19.79	1.36
			267			$\chi^2=1.76$
Amhara	O	38	38.0052	-0.0052	2.7×10^{-5}	7.12×10^{-7}
	A	35	34.1496	0.1496	2.24×10^{-2}	6.55×10^{-4}
	B	27	26.99	0.0108	1.16×10^{-4}	4.32×10^{-6}
	AB	8	8.85	-0.85	0.7225	0.0816
		108				$\chi^2=0.0823$
Gurage	O	34	34.0005	-0.0005	2.5×10^{-7}	7.35×10^{-9}
	A	30	30.02	-0.02	4×10^{-4}	1.3×10^{-5}
	B	22	23.408	-1.408	1.9825	0.0847
	AB	9	7.5715	1.4285	2.0406	0.2695
		95				$\chi^2=0.3542$

Table 10 shows observed versus the expected values of ABO blood group phenotypes in the three ethnic groups. The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square. The distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy- Weinberg equilibrium. This shows that the population is at genetic equilibrium.

As indicated in Table 10 calculated chi-square test of ABO blood groups in the three ethnic groups above was,1.76, 0.0823, 0.3542 in Oromo, Amhara and Gurage respectively, which have the $p < 0.05$ with 3 degree of freedom. The result shows that the ABO blood group distribution between observed and expected in each ethnic group did not show significant difference.

4.3.2. The Chi- Square Test in Rh (D) Blood Group in the Three Ethnic Groups

Table 11. Chi-square test in Rh(D) blood group among students of Oromo, Amhara and Gurage ethnic groups

Ethnic Groups	**Rh(D) blood System**					
	Blood Groups	Observed No (O)	Expected No(E)	Differences D=(OE)	D^2	D^2/E
Oromo	Rh(D)+ve	258	258.0021	-0.0021	4.41×10^{-6}	1.71×10^{-8}
	Rh(d)-ve	9	8.99	0.01	0.00011	1.11×10^{-5}
		267				$\chi^2=1.1\times10^{-5}$
Amhara	Rh(D)+ve	105	104.99	0.01	0.0001	9.5×10^{-7}
	Rh(d)-ve	3	3.0024	-0.0024	05.76×10^{-6}	1.92×10^{-6}
		108				$\chi^2=2.86\times10^{-6}$
Gurage	Rh(D)+ve	90	90.003	-0.003	9×10^{-6}	1×10^{-7}
	Rh(d)-ve	5	4.993	0.007	4.9×10^{-5}	9.8×10^{-6}
		95				$\chi^2=9.9\times10^{-6}$

The chi-square test for the three ethnic groups are compared with observed and expected number to test whether the population in each ethnic groups are in Hardy- Weinberg equilibrium or not.

As indicated in table above the calculated chi-square of Rh(D) in Oromo was 1.1×10^{-5}, Amhara, 2.86×10^{-6} and in Gurage was 9.9×10^{-6}, which has the $p < 0.05$ with 1 degree freedom each. This means that there is statistically insignificant difference between observed and expected value in the three ethnic groups.

The chi-square test value of ABO and Rh (D) blood groups for the three ethnic groups calculated above are insignificant, this shows that populations of the three ethnic groups are at Hardy-Weinberg equilibrium. It is predicted that there was random mating or inter-marriage among the ethnic group, no migration and might be small sample size of the population, etc and thus the allelic frequency will not change from one generation to the next.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

The ABO and Rh (D) blood groups are hereditary characters and are useful in population genetic studies. There are many blood group systems on the basis of different blood group antigens, but only ABO and Rh (D) blood groups are the most important in clinical practice. The study was carried out in Geresu Duki Preparatory School at Woliso town, South West Shewa Zone, Oromia Regional State, Ethiopia. Oromo population size is 34,963, Amhara is 4,995 and that of Gurage population is 9,989 out of the total population in the town.

A total of 470 sample students from 985 students were selected purposively from three ethnic groups, Oromo ,Amhara and Gurage students who enrolled in 2016/17 and were involved in the research to determine the distribution of ABO and Rh blood group phenotypes, alleles and genotypes and compare the results with similar data of previous studies in Ethiopian population. The sample was consisting of 267 students from Oromo, 108 from Amhara and 95 from Gurage students. Blood sample was taken from finger pricks of students by qualified medical laboratory technicians, using the standard clinical procedure, with disposable lancet. ABO blood grouping was carried out using monoclonal ABO blood grouping reagents while Rh blood group was determined using Anti-D monoclonal blood grouping reagents (antibodies).

The frequencies of ABO and RhD blood groups phenotypes were expressed in simple percentages and Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies from phenotypic frequencies.

In each of the three ethnic and in the total sample, blood group O has the highest frequency and blood group AB has the lowest frequency. Similarly RhD positive blood group has the highest frequency while RhD negative blood the lowest frequency.

The frequency of ABO blood groups phenotypes of students in the overall sample were 0.40 O, 0.3064 A, 0.2170 B and 0.0766 AB. The frequencies of RhD blood groups were 0.9638 RhD positive and 0.0362 RhD negative.

In the three ethnic groups in the sample the order of ABO blood group alleles is $I^O > I^A > I^B$. The allele frequencies of ABO blood group of Oromo ethnic were 0.6592 I^O, 0.2128 I^A and 0.1280 I^B, Amhara ethnic were 0.5932 I^O, 0.2242 I^A and 0.1826 I^B, and that of Gurage ethnic were 0.5983 I^O, 0.2226 I^A and 0.1791 I^B. The frequency of allele D and d of RhD blood group in each three ethnic groups were 0.8164, 0.8333 and 0.7706 respectively. The frequency of allele d in each ethnic groups were 0.1836, 0.1667 and 0.2294 respectively.

5.2. Conclusion

The distribution of ABO and RhD blood groups of this study has similar trends with the data from previous studies in Ethiopian populations and with most populations of the world. In ABO blood group system in each of the three ethnic groups as well as in the total sample blood group O has the highest frequency and blood group AB has the lowest frequency. In the RhD blood group system RhD positive blood group has the highest frequency while RhD negative blood the lowest frequency. In all of the three ethnic groups in the total sample the order of the frequencies of ABO blood group alleles is $I^O > I^A > I^B$. In RhD system frequency of allele D is higher than frequency of d allele. The chi-square test shows that the population is at the Genetic equilibrium.

5.3. Recommendations

From the research findings the following recommendations were drawn.

- The data generated in this study would be helpful as a base for researchers who are interested to conduct similar type of study.
- The sample size used to conduct this study from each of the three ethnic was small and may not represent the number of population in the study area. Therefore it is advisable to use larger sample size from each of the three ethnic group to obtain more accurate data regarding the pattern of distribution of these blood groups.
- Conducting similar well designed study using large sample size, which represent the whole population is necessary to obtain sufficient serologic data of ethnic group.

6. REFERENCES

Abraham Haileamlak, Ayalew T. Muluneh, Fessahaye Alemseged, Fasil Tessema, Kifle Woldemichael, Makonnen Asefa, Yoseph Mamo, Solomon Tamiru, and Gemeda Abebe. 2012. Hematoimmunological profile at Gilgel Gibe Field Research Center, south west Ethiopia. *Ethiopian Journal of Health Sc*ience, 22(1): 39_50

Al-Arrayed,S.,Shome,D.K. and Hafadh, N. 2001. ABO blood group and RhD phenotypes in Bahrain: results of screening school children and blood donors. *Bahrain Medical Bulletin*; 23(3):112–115

Al-Bustan, S.,M. El-Zawahri, D. Al-Azmi and Al-Bashir A.A. 2002. Allele frequencies and molecular genotyping of the ABO blood group system in a Kuwaiti population. *Int. J. Hematol.*75: 147-153.

Alimba,C.G., Adekoya, K.O. and Oboh, B.O. 2010. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood groups and hemoglobin Variants among a Nigerian population. Egypt J Med Hum Genet;11:153–8.

Akabas, F., M. Aydin and Cenani. 2003. ABO sub blood group allele frequencies in the Turkish population. *Anthropol. Anz*. 61:257-60.

Al-Arrayed,S.,Shome,D.K. and Hafadh, N. 2001. ABO blood group and RhD phenotypes in Bahrain: results of screening school children and blood donors. *Bahrain Medical Bulletin*; 23(3):112–115.

Anees M, Shabir Mirza M. 2005.Distribution of ABO and Rh blood group alleles in Gujrat region of Punjab, Pakistan. *Proc Pakistan Acad Sci;* 42(4):233–8

Anstee, D.J.2009. Red blood cell genotyping and the feature of parents fusion testing.*Blood* 114(2):248-56.

Avent,N.D.2000. Large scale blood group genotyping: clinical implication.*Br.JHeamatol l* 44 (1):3.

Bakare, A.A., Azeez, M.A. and Agbolade, J.O.2006. Gene frequencies ABO and Rhesus blood groups and heamoglobin variants in Ogbomosho, south east Nigeria. *Afr.J. Biotechnol*.5:224-229.

Bloodbook.com. Retrieved. 2010. Racial and ethnic distribution of ABO blood types. 0801

Bhasin,M.K. and Chahal, S.M.1996. *Immunology a short course*. 3rd edition. Wiley's liss. NeYork,P484.

Bruce,M.G.2002. Members chairman's annual report.2001. *The blood care foundation.* http://www. Blood care. Org. uk/html/resources chairman. Accessed on June.

Bryant, N.J.1994. *An Introduction to Immunohematology*, 3rd edition,Philadelphia, WB Saunders. 1233_1287

Chakraborty,S.2010. Genetic analysis on frequency of alleles for Rh and ABO blood groupsystems in the Barak Valley Populations of Assam .*Science Biological* 2 (2), 31-34.

Chavhan,A.,Pawar,S.and Baig,M.2010. Allelic frequency of ABO and Rh D blood groupamong the Banjara backward caste of Yavatmal District, Maharashtra, India, *Nature Proceedings:5(1):65_76*

Daniel,G.2005. The molecular genetics of blood group polymorphism.*Transplant immune*. 14 (3-4):143-153.

Dar,N.J., Srivastava, A. and Dar, F.A.2010. Distribution of ABO blood groups and Rh (D) factor among the Brahmin and Kushwaha populations of Jhansi District(U.P); *Anthropologist:11(4): 305-306.*

Das, P.K., Nair, S.C., Harris, V.K., Rose, D., Mammen, J.J., Bose, Y.N. and Sudarsanam, A. 2001. Distribution of ABO and Rh-D blood groups among blood donors in a tertiary care centre in South India. *Tropical Doctor, 31, 47.*

Enosolease, M.E., Bazuaye, G.N.2008. Distribution of ABO and Rh-D blood groups in the Benin area of Niger-Delta: Implication for regional blood transfusion. *Asian J TransfusSci 2: 3-5.*

Eweidah, M.S. 2011. Distribution of ABO and Rhesus (RHD) blood groups in Al-Jouf Province of the Saudi Arabia, *Anthropologist*, 13(2): 99-102.

Falusi,A.G.. O.G. Ademowo,,C.A. Okeke,, P.O.Olatunji., T.O.onyekwere., et al 2006. Distribution of ABO and Rh genes in Nigeria. *Afr. J. Med. Sci. 29:23-6*

Fareed, M.,Hussain,R.,Shah,A. and Afzal,M., 2014.Frequencies among six population of Jammu and Kashmir,India.*Transfusion and Apheresis science* 50(2)247.

Garratty,G.W.,Dzink.P.D., Issitt,D.M., Lublin Reid And Zelinski,T. 2000. Termology for blood group antigens and genes-historical origins and guide line in the new millennium.*Transfusion*; 40; 477-489.

Giri, P.A., Yadav, Parhar,,G.S. and Phalke,D.B.2011. Frequency of ABO and Rhesus blood groups: A study from a Rural Tertiary Care Teaching Hospital in India. *International Journal of Biological Medical Re*search; 2(4): 988 -990.

Greenwalt,T.J.1997. A short history of transfusion medicine.*Transfusion*;37(5):550=563.

Griffthis,A.F., Wessler,R.C.,Lewontin,S.R andCarroll,S.B., 2008.*Introduction to genetic analysis*.9th edition.W.H.Freeman and company. New york.p838.

Gupta, P. K. 1999. Genetics. Rastogi Publications, Meerut, India.

Hamed, C.T., Bollahi, M.A., Abdelhamid, I., Med Mahmoud, M.A., Ba, B., Ghaber, S., Habti, N. & Houmeida, A. (2012). Frequencies and ethnic distribution of ABO and Rh (D) blood groups in Mauritania: results of first nationwide study. *International Journal of Immunogenetics*, 39, 151

Hanania,S., Hassawi, D. and Irshaid, N. 2007. Allele frequency and molecular genotypes of ABO blood group system in a Jordanian population. *Journal of Medical Science*;7:51–58.

Hasna,A.M.,2010. A study of ABO blood group and Rh factor distribution. *Journal of Basrah researches (science) vol*. (36) A /No (5). 15.

ISBT (International Society of Blood Transfusion) URL accessed on 2008. 11-14.

Iyiola,O.A.,Igunnugbemi,O.O.,Raheem,U.A. and Anifowoshe,A.T.2011. Gene frequencies of ABO and Rh (D) blood group alleles in Ilorin, North-Central Nigeria, *World Journal ofBiological Research*, 4(2): 633_743

Jaff, M.S.2010. ABO and rhesus blood group distribution, in Kurds. *Journal of Blood Medicie*,1:143–146Khan, M.S., Bakhshi,A., Akhtar, M.S. and Amin-ud-Din, M. 2004. Distribution of ABO and RhD blood groups in the population of Poonch District, Azad Jammu and Kashmir; *E.Mediterranean Health J*ournal 15(3):717-721.

Khalil, I.A., Phrykian S, Farr AD.1989. Blood group distribution in Sudan. Gene Geogr; 3:7–10.

Khan, M.S., Bakhshi,A., Akhtar, M.S. and Amin-ud-Din, M. 2006. Distribution of ABO and RhD blood groups in the population of Poonch District, Azad Jammu and Kashmir; *E.Mediterranean Health J*ournal 15(3):717-721.

Khattak ID, Khan TM, Syed P, Shah AM, Khattak ST, Ali A.2008. Frequency of ABO and Rhesus blood groups in district Swat, Pakistan. *J Ayub Med Coll Abbottabad;* 20 (4):127–9.

Khurshid,B.M.,Hassen,S.F.andMabood.2008. Frequency of ABO and Rh (D) blood groups in direct Swabi N.W.F.P (Pakistan). *J.sc. Tech Univ. Peshwar*: 16;5-6.

Klug, W.S. and Cummings,M.R.2002.Essentials of genetics.4thEd. New Jersey,USA: Prentice Hall; p508

Kumar, P., Singh V. K, and., Rai, V. 2009. Study of ABO and Rh (D) blood groups in Kshatriya (Raj put) of Jaunpur District, Uttar Pradesh; Delhi. *Kamla-Raj Enterprises Bulletin,*11(4): 303-304.

Landsteiner K, Wiener AS *(1941). "Studies on an agglutinogen (Rh) in human blood reacting with anti-rhesus sera and with hu*man iso antibodies*". J Exp Med. **74** (4)*

Laura,M.D.2005. *Blood Groups and Red Cell Antigens*;National Center for Biotechnology Information, United States Government , 861_967

Louise Mary Turgeon. 1995. Fundamentals of Immunohematology, Theory and Technique. 2nd ed. Williams and Wilkins, New York,

Lyko, J., Gaertner H, Kaviti JN, Karithi MW, and Akoto, B. 1992. The blood group antigens ABO and Rh in Kenyans. *Hamdard Medicus*; 35:59–67.

Matsushita S., Imamura T., Mizuta T. and Hanada M. November 1983. Acquired B antigen and poly agglutination in a patient with gastric cancer. *The Japanese Journal of Surgery* 13 (6): 40–52.

Mandal, S.2002.*Fundamentals of Human Genetics*, second edition. New Central Book Agency(P) LTD, New Delhi, India.2nd edition

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

Misganaw Birhaneselassie, 2004. Immuno hematology, Debub University.

Mollision,P.L.1994.The genetic bases of the Rh blood group system. *Transfusion*; 34;539-541.

Nickel, R.G.,Fiedshoff and Robert,L.1999. Determination of Duffy genotypes in three population of America descent using PCR and sequence of specific oligo-nucleotides. *Human immunology* 60(8):738-42.

Povey, S.C., Slaugher, D.F.,Wilson,I.P.,Gormly, Bukton,P.A. Perry and M.Bobrow.1978. Evidence for the assignment of loci AK 3. And AconS to chromosome 9 in man.*Ann Hums Genet* 38:1-5.

Rahman, M and Lodhi, Y.2004. Frequency of ABO and Rhesus blood groups in blood donors in Punjab;*Pakistan Journal of Medical Science*; 20:315-8

Reid, M.E. and Lomas-Francis,C. 2004. *The blood group antigen facts books*. 2nd edition. New York: Elsevier Academic Press.

Saladin,K.2003.Anatomy and Physiology: The Unity of Form and Function, 3rd Edition.

Sarhan, M.A., Saleh, K.A., Bin-Dajem, S.M. 2009. Distribution of ABO blood groups and Rhesus factor in Southwest Saudi Arabia; *Saudi Medical Journal*; 30(1):116–119.

Seely,R.R., Stephans,T.D. and Tate, P.1998. *Anatomy and physiology*.4th edition. The McGraw Hill Companies, Inc. USA. P1098.

Simpkins, J., and Williams,J.I. 1997. *Advanced Biology*, 3rd Edition Collins Education, London. P760 The McGraw-Hill Companies, USA,Pp.679-698.

Srikumari, C.R., Rajanikumari, J.and Rao,T.V.1987. Acuity of selective mechanisms operating on ABO. Rh (D) and MN blood groups. *Am.J.Phy.Anthrop*.72 (1):117-121.

Tagny, C.T., Diarra, A., Yahaya, R., Hakizima, M., Nguessan, A. and Mensa, G. (2009a). Characteristics of blood donors and donated blood in sub-Saharan Francophone Africa. *Transfusion*, 49, 1592.

Tewodros Zerihun, Abraham Degarege and Berhanu Erko. 2011. Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*: 289-294.

Tibebu Mokonnin. 1998. *The Blood Bank Manual*, Ethiopian Red Cross society, National Blood Transfusion Service, Addis Ababa, 54_63.

Urbniac,S.J and Graiss,M.A.2000. RhD hemolytic disease of the fetus and the new born. *Blood Rev*.14:44-61.

7. APPENDIX

Appendix 1. ABO and Rh-D blood group Phenotypes recording sheet

No	Students Name	sex	Blood Groups		Ethnic Groups		
			ABO	Rh(D)	Oromo	Amhara	Gurage
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

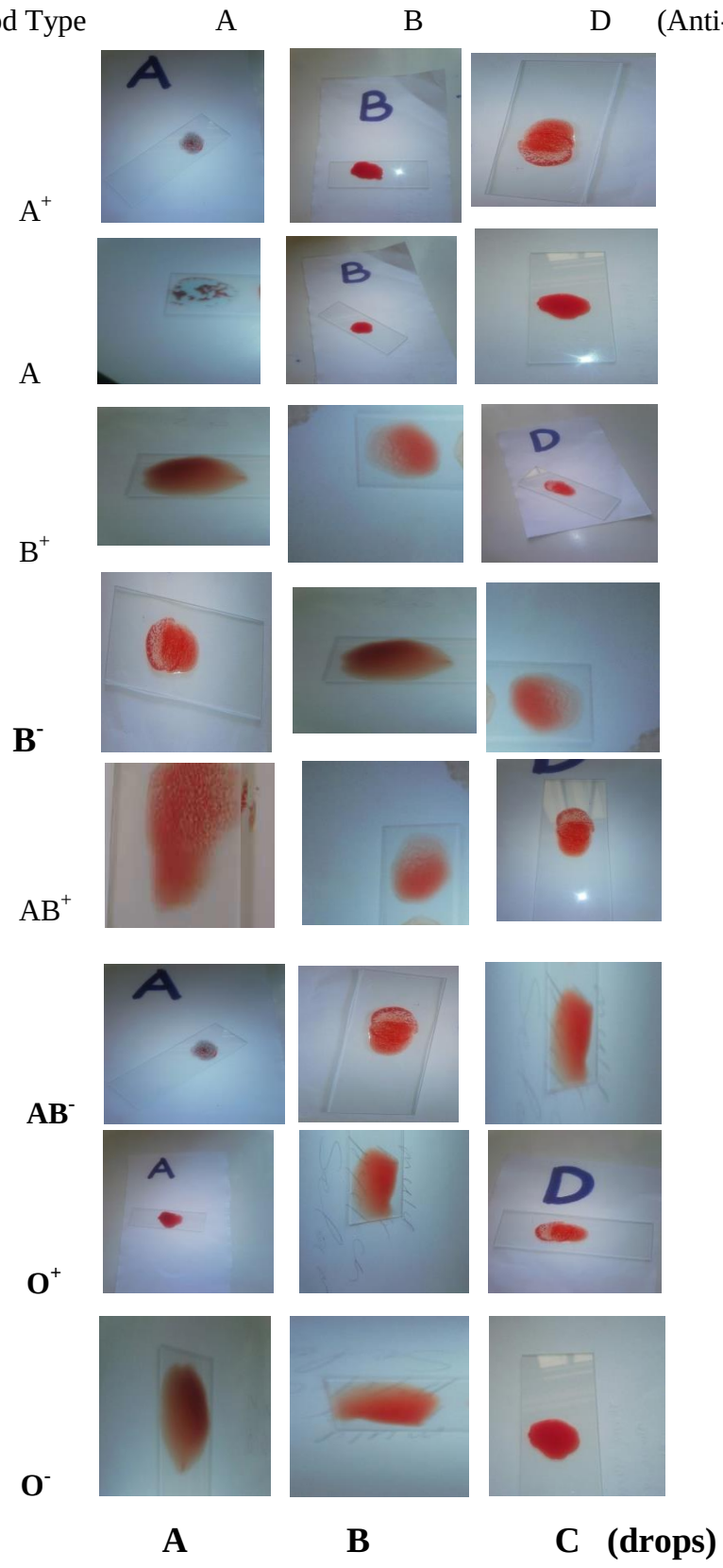
Appendix 2. Probability Values for Chi-Square Analysis

Degrees Of Freedom	Probability		
	0.05*	0.01**	0.001***
1	3.84	6.64	10.83
2	5.99	9.21	13.82
3	7.82	11.35	16.27
4	9.49	13.28	18.47
5	11.07	15.09	20.52

From. MCB142/IB163 Thomson, Mendelian and Population Genetics

Sep.16, 2003

Figure 1. Pictures of agglutination reaction and blood type determination



The above figure 1 shows agglutination reaction with anti-A,anti-B and anti-D anti-sera.

As seen from the above figure1 blood type was determined as follows:

Blood group A^+ :- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure1 above respectively, blood sample on drop A and C form aggregates (agglutinate) but the blood sample found on drop B remain fluid (No aggregates)

Blood group A^- :- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure1 above respectively, blood sample on drop 'A' forms clump (agglutinates) but the two blood sample found on drop B and C remain fluid (No clump).

Blood group B^+ :- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure1 above respectively, blood sample on drop B and C form aggregates (agglutinates) but the blood sample found on drop A remain fluid (No aggregates).

Blood group B^- :- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure1 above respectively, blood sample only on drop B forms aggregates (agglutinates) but the two blood sample found on drop A and C remain fluid (No aggregates).

Blood group AB^+ :- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure1 above respectively, all blood sample found on drop A, B and C forms aggregates (agglutinates).

Blood group AB^- :- on adding anti-A, anti-B and anti-D on slide A,B,C as shown on figure 1 above respectively, the blood sample on drop A and B forms aggregates (agglutinates) but the blood sample found on drop C remain fluid (No aggregates).

Blood group O^+: - on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure 1 above respectively, the blood sample found only on slide C forms aggregates (agglutinates) but the two blood sample found on drop A and B remain fluid (No aggregates).

Blood group O Negative (O^-):- on adding anti-A, anti-B and anti-D on drop A,B,C as shown on figure 1 above respectively, all blood sample found on drop A, B and C remain fluid (No aggregates).