FREQUENCY OF ABO AND Rh BLOOD GROUP ALLELES, PHENOTYPES AND GENOTYPES AMONG STUDENTS OF AMHARA AND OROMO ETHNIC GROUPS IN SHENO SECONDARY AND PREPARATORY SCHOOL, NORTH SHEWA, ETHIOPIA

M.Sc. THESIS

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November 2018

HARAMAYA UNIVERSITY, ETHIOIPIA

Frequency of ABO and Rh Blood Group Alleles, Phenotypes and Genotypes among Students of Amhara and Oromo Ethnic Groups in Sheno Secondary and Preparatory School, North Shewa, Ethiopia

A Thesis Submitted to the School of Biological Sciences and Biotechnology
Postgraduate Programs Directorate
HARAMAYA UNIVERSITY

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

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November 2018

Haramaya University, Haramaya, Ethiopia

APPROVAL SHEET
HARAMAYA UNIVERSITY

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As a thesis Research advisors, we hereby certify that we have read and evaluated this Thesis, prepared, under my guidance by Almaz Gebru, entitled: Frequency of ABO and Rh Blood Group Alleles and Genotypes among Students of Amhara and Oromo Ethnic Groups in Sheno Secondary and Preparatory Schools, North Shewa, Ethiopia. I recommend that it be submitted as fulfilling the thesis requirement.

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As member of the Board of Examiners of the M.Sc. Thesis Open Defense examination, we certify that we have read and evaluated the Thesis prepared by Almaz Gebru and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of Master of Science in field of Genetics

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DEDICATION

This piece of work is dedicated to my beloved family: my Father Gebru Dinku, my mother Tayitu Lema, all my sisters and brothers.

BIOGRAPHICAL SKETCH

The author, Almaz Gebru, was born from her father Gebru Dinku and her mother Tayitu Lemma in November 10, 1993 in Kumdingay kebele, Kimbibit Woreda, North Shewa Zone, Oromia Regional State, Ethiopia. She attended her elementary school at Kumdingay Elementary School from 2002 to 2009 then she attended her Secondary and Preparatory School from 2010 to 2013 at Sheno Secondary and Preparatory school. After completion of her Preparatory at Sheno School in 2013, she joined Wolkite University in 2014. She graduated, in July 2016 with B.Sc. degree in Biology. After graduating she joined the School of Graduate Studies at Haramaya University as a candidate for Master of Science in Genetics.

ACKNOWLEDGEMENTS

First of all I would like to thanks my most profound gratitude my major advisor Dr. Yohannes Petros and co-advisor Dr. Tamiru Oljira for their valuable comments, suggestions, support and professional guidance for the completion of this thesis work. I am also indebted to Sheno Health Center, High school and Preparatory Schools and all the study participants for their kind cooperation. Moreover, thanks to the Ministry of Education (MoE) for its budget support and Department of Biological Sciences and Biotechnology, Haramaya University as well for hosting me as a student. My deepest appreciation goes to all my brothers, sisters, and partners for their constant encouragement on my work.

LIST OF ACRONYMS AND ABBREVIATIONS

DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FMC	Flinders Medical Center
HDN	Hemolytic Disease of the Newborn
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
PCR	Polymerase Chain Reaction
Rh	Rhesus

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ABSTRACT

The Frequencies of Blood types, alleles and genotypes of the ABO and Rh blood group system varies worldwide. Therefore, this study was conducted to get information on the frequencies of alleles, phenotypes and genotypes of ABO and Rh D blood group systems among the students of Amhara and Oromo ethnic groups at Sheno Secondary and Preparatory School, North Shewa Zone, Oromia Region. A total sample of 600 students from two ethnic groups were randomly selected and tested for ABO and Rh blood groups. Blood samples were taken from finger tip and slide method of testing was followed. A drop of each of the antisera, anti-A, anti-B and anti-D was added and mixed with each blood sample and rocked gently for about 60 seconds to observe agglutination. As a result, frequency distributions of the ABO blood groups were recorded for the two ethnic groups. In Amhara ethnic group, the frequency showed O (0.347), A (0.316), B (0.26) and AB (0.077). In Oromo ethnic group, the frequency was O (0.38), A (0.30), B (0.253) and AB (0.067). The more frequent blood type was O in the two ethnic groups. The frequency distribution of Rh-positive blood group in Amhara and Oromo were obtained as (0.923) and (0.953) respectively. The frequency of Rh-negative blood group were (0.077) and (0.047) in Amhara and Oromo ethnic groups respectively. The allelic frequencies of ABO blood groups for Amhara ethnic group were I^O, 0.5925, I^A, 0.2214 and I^B, 0.1861 and I^O, 0.6196, I^A, 0.2048 and I^B, 0.1756 for Oromo ethnic group. An allelic frequency for Rh blood group in overall ethnic groups were 0.7516, I^D and 0.2484, I^d. The distributions of ABO and Rh blood groups in this study have similar trends with the data from the previous studies in Ethiopia. The sample size used to conduct this study was small and only focused on the two ethnic groups therefore; to study further on all ethnic groups was mainly recommended

Key words: ABO, Alleles, Blood group, Ethnic Group, Frequency, Genotypes, Rhesus factor.

1. INTRODUCTION

Blood is the most important body fluid. This is responsible for circulation of important nutrients, enzymes, and hormones all across the body, apart from carrying the most critical substance, oxygen. The human red blood cell membrane contains different types of polysaccharide antigens, called agglutinogen. (Ganong,1995). The antigenic substances are capable of inducing a specific immune response so that specific response results in the production of plasma cells that produce antibodies (Novak, 1995). The Blood group is determined by alleles of a system (Gupta, 1999).

According to Mourant *et al. (*1976), the ABO blood group and Rhesus (Rh) D blood group antigens are the most frequently studied genetic markers in a large number of populations worldwide. The ABO and Rh blood group alleles vary worldwide and are not found in equal numbers even among the same ethnic groups. For example, Among African-Americans the frequency of ABO blood group was O (46%), type A, (27%); type B, (20%); and type AB; (7%). Among Caucasians in the United States, the frequency of type O was (47%); A, (41%); B, (9%), AB, (3%). Also, among Western Europeans, the frequency of O, (46%); A, (42%); B, (9%); and AB, (3%) (Garratty,2000). Rh-positive is documented as 95% among African-Americans. Rh negative is 5.5% in South India, 5% in Nairobi, 7.3% in Lahore, 4.8% in Nigeria (Iyiola *et al.*, 2011; Abraham *et al*., *2012*).

The discovery of ABO and Rh blood groups has contributed immensely (great extent) to blood banking services and transfusion medicine in order to avoid morbidity and mortality in both adults and children (Adeyemo and Sedoyejo, 2006). ABO and Rh systems have major clinical significance and they are determined by the nature of different carbohydrates present on the surface of red blood cells. The antigens of the ABO system are an integral part of the red blood cell membrane and they are also found in plasma and other body fluids. Red blood cells contain a series of glycoprotein and glycolipids on their surface which constitute the blood group antigens. Production of these antigens is genetically controlled (Srikumari *et al.*, 1987).

All human populations share the same blood systems, although they differ in the frequencies of specific types. The distribution patterns of ABO and Rh systems are complex around the world. Some variation may even occur in different areas within one small country (Enosolease , 2008). The blood group distribution also shows variety according to races (Kaya *et al*., 1999). ABO and Rhesus (Rh) blood group antigens are hereditary characters and are useful in population genetic studies, researching population migration patterns, as well as resolving certain medicolegal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice. The need for blood group frequency studies is multipurpose, as besides their relation to disease and environment is being increasingly sought in modern medicine (Green *et al.,* 1995).

ABO and Rh blood group systems in humans are two important genetic markers that are routinely analyzed prior to blood transfusion and medical treatment. The *ABO* blood group system is governed by a single gene with three alleles (I^A, I^B and I^O), of which I^A and I^B alleles are co-dominant but both of them are dominant over the recessive allele I^O in intra-allelic interaction in diploid condition. The gene for ABO blood group is located on chromosome 9 of human genome whereas that of Rh is located on the short arm of chromosome 1 (Murphy *et al.*, 2003).

Blood grouping has improved with the advent of monoclonal antibodies and the automation of tests. Although different advanced techniques, such as micro plate method, PCR based typing, Flinders Medical Centers based typing, mini sequencing analysis, fluorescent immuno micro plate technique, sandwich ELISA method, etc., are available for ABO genotyping, the manual method has its own significance not only in blood typing but also measuring its genotypic frequency by Hardy-Weinberg Law,(Rai *et al*., 2009). Importance of knowing your blood types becomes very essential when you need a blood transfusion or are planning to donate blood. Not all blood types are compatible, so it's important to know your blood types. Receiving blood that's incompatible with your blood type could trigger a dangerous immune response, (Westhoff, 2004).

This study was conducted between Amhara and Oromo ethnic groups, because there is the large number of two ethnic groups in the study area and the schools were located in the Oromia region. Moreover, no similar study was reported in the literature regarding the frequencies of ABO and Rh D blood group alleles and genotypes in the population and different ethnic groups of north Shewa zone, Sheno town. Therefore, this study aims to investigate the Phenotypic, allelic and genotypic frequency of ABO and Rh D blood groups among students of Amhara and Oromo ethnic groups in Sheno Secondary and Preparatory School.

General objective

- To investigate the Phenotypic, allelic and genotypic frequencies of ABO and Rh blood groups among the two ethnic groups.

Specific Objectives

- To determine the frequency of the ABO and Rh blood group phenotypes and genotypes among the students of Amhara and Oromo ethnic group in Sheno Secondary and Preparatory schools.
- To estimate the frequency of the ABO and Rh blood group alleles among the students of the two ethnic groups.
- To test the significance difference between the different Blood group phenotypes.

2. LITERATURE REVIEW

2.1. History of ABO Blood Group

The two most significant blood group systems ABO and Rh were discovered by Karl Landsteiner during early experiments with blood transfusion: the ABO blood group in 1901 and in co-operation with Alexander S. Wiener, the Rhesus group in 1937(Landsteiner,2000). Development of the Coombs test in 1945, the advent of transfusion medicine, and the understanding of ABO hemolytic disease of the newborn led to the discovery of more blood groups, and now 35 human blood group systems are recognized by the International Society of Blood Transfusion (ISBT), and across the 35 blood groups, over 700 different blood group antigens have been found many of these are very rare or are mainly found in certain ethnic groups (Anstee, 2009).

The history of blood group antigens is characterized by important landmarks. At the beginning of the 20^{th} century an Austrian scientist Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals (Giri, 2011). 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. Agglutination occurred when the RBC antigens were bound by the antibodies in the serum. Landsteiner in 1901 named the first 2 blood groups antigens A and B, using the first two letters of the alphabet while red blood cells (RBCs) not reacting with anti-A and anti-B were called type C. 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. In 1902, Von Decastello and Sturli described RBCs reacting with both anti-A and anti-B, but did not give these types a name, but continued calling RBCs that did not react with anti-A and Anti-B type C. (Garratty, 2000).

The discovery of the ABO blood groups by Karl Landsteiner is an important achievement in the history of blood groups. The vast majorities of these antigens are inherited in a simple Mendelian fashion and are stable characteristics which are useful in paternity testing (Hillier, 2008). Karl Landsteiner has been credited for the discovery of ABO blood group system in 1900. His extensive research on serology based on simple but strong scientific reasoning led to

identification of major blood types such as O, A, and B types, compatibility testing, and subsequent transfusion practices. He was awarded Noble Prize in 1930 for this discovery (Landsteiner, 2000). 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 and Reid, 2000).

2.2. Blood Group Systems

Humans contain a series of glycoproteins 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, MN and Rh blood group systems, etc, (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 most important in clinical practice, (Mandal, 2002)

Blood group refers to the entire blood group system comprising red blood cell (RBC) antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. Blood types refers to a specific pattern of reaction to testing antisera within a given system. Over a period of time, our understandings of blood groups have evolved to encompass not only transfusion-related problems but also specific disease association with RBC surface antigens.

Later, Jan Jansky described classification of human blood groups into four types. At present, 35 blood group systems representing over 700 antigens are listed by the International Society of Blood Transfusion (Lögdberg *et al* ., 2004). Most of the genes have been cloned and sequenced. The genes of these blood group systems are autosomal, except XG and XK which are X-borne, and MIC2 which is present on both X and Y chromosomes. The antigens can be integral proteins where polymorphisms lie in the variation of amino acid sequence as in the Rhesus antigen, glycoproteins or glycolipids as in the ABO (Lögdberg *et al.*, 2010).

2.3. ABO Blood Group System

Among the 35 blood group systems, ABO remains the most important in transfusion and transplantation since any person above the age of 6 months possess clinically significant anti-A and/or anti-B antibodies in their serum. The ABO blood group system is the most important blood-group system in human-blood transfusion. The associated anti-A and anti-B antibodies are usually Immunoglobulin *M*, abbreviated IgM, antibodies. ABO IgM antibodies are produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses. The *O* in ABO system is often called *0* (*zero*, or *null*) in other languages (Khurshid *et al.,* 2008). Blood group A contains antibody against blood group B in serum and vice-versa, while blood group O contains no A/B antigen but both their antibodies in serum.

2.4. Inheritance of ABO Blood Group

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three alleles: I^O, I^A, and I^B. The gene encodes a glycosyltransferase that modifies the carbohydrate content of the red blood cell, (Yazer *et al.,* 2006). The gene is located on the long arm of the ninth chromosome. The I^A allele give type A blood, I^B gives type B, and I^O gives type O blood. As both I^A and I^B are dominant over I^O, only I^OI^O people have type O blood. Individuals with I^AI^A or I^AI^O have type A blood, and individuals with I^BI^B or I^BI^O have type B. I^AI^B people have both phenotypes, because A and B express co dominance, in which both alleles are expressed in the heterozygous state.

2.5. The Genetics of ABO Blood group systems

Blood Group System may be defined as a genetically discrete group of antigens controlled by a single gene or by a cluster of two or more closely linked homologous genes with virtually no recombination occurring between them. The classification of blood groups into type A, B, AB and O in ABO system, Rh- positive and Rh-negative in Rh system is based on the presence or absence of inherited antigenic substances on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoproteins, glycolipids depending on the blood group system (Hasna *et al.,* 2010). A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type in one of the many possible

combinations of blood-group antigens. Across the thirty blood groups, over 700 different blood group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups (Seltsam *et al.,* 2003). ABO system consists of four main groups, A, B, AB and O which is determined on the basis of presence or absence of A and B antigens. These antigens are under control of three allelic genes, namely I^A, I^B and I^O which determine blood groups. I^A produces A antigen, I^B produces B antigen whereas I^O produces neither. I^A and I^B are mutant alleles and show co dominances with each other but, both are dominant over the wild type allele I^O (Table 1). The three alleles can produce six genotypes and four phenotypes of blood groups which are listed below.

Table.1. Phenotypes and Genotypes of the ABO Blood Groups

Phenotypes	Genotypes
O	I^OI^O
A	I^AI^A/I^AI^O
B	I^BI^B/I^BI^O
AB	I^{AB}

Source: Rai and Kumar (2011)

2.6. Antibodies of ABO blood group

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. 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) (Avent and Raid, 2000). 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 2).

Table. 2. ABO blood groups, antigens and antibodies

Blood Group phenotypes	ABO antigens present on the red cell surface	ABO antibodies present in the plasma
A	A antigen	anti-B
B	B antigen	anti-A
AB	A and B antigens	-
O	Neither A nor B	anti-A and anti-B

Source: ISBT (2008)

2.7. Genotypes of the ABO Blood group system

In addition to the current practices of serologic testing of blood types, the progress in molecular diagnostics allows the increasing use of blood group genotyping. In contrast to serologic tests reporting a direct blood phenotype, genotyping allows the prediction of a phenotype based on the knowledge of the molecular basis of the currently known antigens. This allows a more detailed determination of the blood type and therefore a better match for transfusion, which can be crucial in particular for patients with needs for many transfusions to prevent alloimmunization (Anstee, 2009).

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

2.8. Distribution of ABO Blood Group System in different population

The O blood type is very common around the world; about 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of central and South America where it approaches 100%. The lowest frequency of (O) is found in Eastern Europe and central Asia, where B is common, (Kumar *et al.*, 2009).

The ABO (Table 3) blood group distribution varies among the different racial and ethnic groups all over the world. For example, blood group B has its highest frequency in northern India and neighboring Central Asia. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian, Aborigine populations and the black foot Indians of Montana (ISBT, 2006).

Table .3. Distributions of the ABO blood types along racial and ethnic lines.

People	O	A	B	AB
Aborigines	61	39	0	0
Abyssinians	43	27	25	5
Ainu (Japan)	17	32	32	18
Albanians	38	43	13	6
Grand Andamanese	9	60	23	9
Arabs	34	31	29	6
Armenians	31	50	13	6
Asian	40	28	27	5
Austrians	36	44	13	6
Bantus	46	30	19	5
Basques	51	44	4	1
Belgians	47	42	8	3
Blackfoot	17	82	0	1
Bororo (Brazil)	100	0	0	0
Brazilians	47	41	9	3
Bulgarians	32	44	15	8
Burmese	36	24	33	7
Buryats (Siberia)	33	21	38	8
Bushmen	56	34	9	2
Chinese-Canton	46	23	25	6

Dutch	45	43	9	3
Egyptians	33	36	24	8
English	47	42	9	3
Eskimos (Alaska)	38	44	13	5
Eskimos(Greenland)	54	36	23	8
Estonians	34	36	23	8
Fijians	44	34	17	6
Finns	34	41	18	7
French	43	47	7	3
Georgians	46	37	12	4
Germans	41	43	11	5
Greeks	40	42	14	5
Gypsies (Hungary)	29	27	35	10
Hawaiians	37	61	2	1
Hindus (Bombay)	32	29	28	11
Hungarians	36	43	16	5

Source: ISBT (2006)

2.9. Rh Blood Group System

Rhesus-system is the second most important blood group system after ABO. Currently, the Rh-system consists of 50 defined blood group antigens out of which only five are important, (Westhoff, 2004). Red Blood Cell (RBC) surface of an individual may or may not have a Rh factor or immunogenic D-antigen. Accordingly, the status is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). In contrast to the ABO system, anti-Rh

antibodies are, normally, not present in the blood of individuals with D-negative RBCs, unless the circulatory system of these individuals has been exposed to D-positive RBCs. These immune antibodies are immunoglobulin G (IgG) in nature and hence, can cross the placenta. Prophylaxis is given against Rh immunization using anti-D Ig for pregnant Rh-negative mothers who have given birth to Rh-positive child.

2.10. Inheritance of Rh Blood Group System

The Rh factor genetic information is also inherited from the parents, but it is inherited independently of the ABO blood type alleles. The Rh blood group is one of the most complex blood groups known in humans. Clinically, it is the most important blood group system after *ABO*. There are 2 different Phenotype for the Rh factor known as Rh^{+} (D), positive and Rh^{-}(d), negative. A mother who is Rh- negative can only pass an Rh– negative allele to her son or daughter. A father who is Rh-positive could pass either an Rh- positive or Rh- negative allele to his son or daughter. This couple could have Rh-positive children (Rh- negative from mother and Rh- positive from father) or Rh- negative children (Rh- negative from mother and Rh-positive from father), (Reid and Lomas, 2004).

At present, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important. The commonly-used terms Rh factor, Rh positive and Rh negative refer to the *D* antigen only. Besides its importance in blood transfusion, the Rh blood group system specifically, the D antigen, is used to determine the risk of hemolytic disease of the newborn (HDN). The Rh system is the second most significant blood-group system in human-blood transfusion. The most significant Rh antigen is the D antigen, because it is the most likely to provoke an immune system response of the five main Rh antigens. It is common for D-negative individuals not to have any anti-D IgG or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following a sensitizing event: possibly a feto maternal transfusion of blood from a fetus in pregnancy or occasionally blood transfusion with D positive RBCs. Rh diseases develop in these cases (Moise, 2008).

2.10.1. Frequency of Rh Blood Groups in different population

Rh negative blood types are much less in proportion of Asian populations (0.3%) than they are in White (15%). Rhesus blood group system, the gene *D* which gives rhesus positive status is at its lowest in Europe (Reddy *e t al*., 2008). In eastern Asia, Australia and Indonesia; it often attains 100%. The same holds for American indigenous populations in many of whom the *D* frequency is100 % (Table .4 & 5).

Table.4. Allele frequencies of Rh blood groups studied in different population across the world

Population	Rh+	Rh-
Ethiopia	0.94644	0.05356
German	0.95	0.05
Kenya	0.803	0.197
Lagos (Nigeria)	0.94	0.06
Mandi Bahauddin	0.914	0.086
Nigeria	0.943	0.057
Ogbomoso (Nigeria)	0.967	0.033
Port Hargourt	0.9677	0.0323
Red Indians (USA)	1	0
Saudi Arabia	0.93	0.077
U.S.A	0.85	0.015

Source: Reddy *e t al*.,(2008)

Table. 5. Frequencies of Rh blood groups studied in different areas of Nigeria

Population	Rh+	Rh-	Reference
Lagos (Nigeria)	0.94	0.06	Adeyemo and Soboyejo, 2006
Ogbomoso (Nigeria)	0.967	0.033	Bakare *et al*., 2006
Benin (Nigeria)	0.9388	0.0603	Enosolease and Bazuaye, 2008
Adamawa (Nigeria)	0.974	0.026	Abdulazeez *et al*., 2008
Portharcourt (Nigeria)	0.9677	0.0323	Jeremiah, 2006
Ibadan (Nigeria)	0.95	0.048	Omotade *et al*., 1999
Nigeria	0.943	0.057	Falusi *et al*.,2000

Source : World Journal (2011)

2.11. Blood transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide transfusion services for blood and other blood products, (Daniels *et al*., 2006). Across the world, blood products must be prescribed by a medical doctor (licensed physician or surgeon) in a similar way as medicines. The ABO blood group system is the most important in human blood transfusion. A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type is one of the many possible combinations of blood group antigens. Across the 35 blood groups, over 700 different blood-group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups (ISBT., 2008). Much of the routine work of a 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, a severe acute hemolytic reaction with hemolysis (RBC destruction), renal failure and shock is likely to occur, and death is a possibility. Patients should ideally receive their own blood or type-specific blood products to minimize the chance of a transfusion reaction. Risks can be further

reduced by cross-matching blood, but this may be skipped when blood is required for an emergency. Cross-matching involves mixing a sample of the recipient's serum with a sample of the donor's red blood cells and checking if the mixture agglutinates, or forms clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination with a microscope. If agglutination occurs, that particular donor's blood cannot be transfused to that particular recipient. In a blood bank it is vital that all blood specimens are correctly identified, so labeling has been standardized using a barcode system known as ISBT 128 (Bruce *et.al.* 2002)

2.11.1. Hemolytic disease of the newborn

Hemolytic Disease of the Newborn (HDN),also known as erythroblastosis fetalis,iso immunization, or blood group incompatibility, occurs when fetal red blood cells (RBCs), which possess an antigen that the mother lacks, cross the placenta into the maternal circulation, where they stimulate antibody Production, (Louise, 1995). The antibodies return to the fetal circulation and result in RBC destruction. Hemolytics is the break down or rupture of the red cell membrane by specific antibody (hemolysin) through the activation of complement with the release of hemoglobin, and the librated hemoglobin can easily be observed staining the supernatant fluid.

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

Hemolytic disease of the newborn due to anti-Rh D occurs when mother and infant are always incompatible with respect to the Rh factor: The mother Rh D negative, and the infant Rh (D) positive (inherited the D factor from the father), (Bakare *et al*., 2006). ABO incompatibility between the mother and fetus reduces the chance of maternal immunization to the D antigen. 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. The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh-negative can bear an Rh- positive child if the father is Rh-negative (either homozygous or heterozygous). Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy (Daniel and Elizabeth, 2009).

2.11.3. Hemolytic Diseases of the Newborn due to ABO Blood Group Incompatibility

Hemolytic disease of the newborn due to ABO blood grouping usually occurs when the mother is invariably group O (posses IgG anti-A and B), the infant group A or B and when the mother and infant are Rh compatible, (Benjamini *et al*., 2000). The fetal red cells cross the placenta into the maternal circulation stimulating the existing anti-A and B to high titters; the "immune" anti A and B stimulated is largely IgG. An ABO hemolytic disease of the new born occurs in the first pregnancy because anti-A and anti-B are always present and therefore readily stimulated.

2.12. The Hardy-Weinberg Genetic Equilibrium

The Hardy-Weinberg principle provides the solution to how variation is maintained in a population with Mendelian inheritance. According to this principle, the frequencies of alleles will remain constant in the absence of selection, mutation, migration and genetic drift.

The Hardy-Weinberg equilibrium refers to this stability of allele frequencies over time (James, 1999). A second component of the Hardy-Weinberg principle concerns the effects of a single generation of random mating. In this case, the genotype frequencies can be predicted from the allele frequencies. For example, in the simplest case of a single locus with two alleles: the dominant allele is denoted A and the recessive allele a and their frequencies are denoted by *p* and *q*; frequency (A) = p; frequency (a) = q p + q = 1.

= (p +q + r) 2
= p2 (I^AI^A) + 2pq (I^AI^B) + q2 (I^BI^B) + 2pr (I^AI^O) + 2qr (I^BI^O) + r2 (I^OI^O) (Griffiths *et al*., 2008).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in the Sheno Secondary and Preparatory School Sheno town, Kembibit woreda, North Shewa zone, Oromia region. Sheno is a town in Central Ethiopia Located in the North Shewa Zone of the Oromia Region; it has a latitude and longitude of 9°20′N 39°18′E with an elevation of 2918 meters above sea level.

3.2. Study Design

The study was cross-sectional survey at Sheno Secondary and Preparatory Schools, on frequency of ABO and Rh blood groups among students of Amhara and Oromo ethnic groups. The study was carried out from December 2017 to February 2018 in the Schools.

3.3. Study Population

Sheno Secondary and Preparatory School had a total of about 2537 students. Sheno Preparatory school had 600 students and Secondary School had 1937 students. Study participants were selected from both schools.

3.4. Sample Size Determination

Since there was no previous investigation conducted on the same title in the study area, p value of 0.5 was taken to ensure that the sample size was large enough to satisfy the precision and confidence constraints. By taking this into consideration, the sample size for the population was calculated based on the 95% confidence limits and 5% sampling error by using a formula described in Hassan (1991)

$$n = \frac{Z^2 P(1-p)}{d^2}$$

Where,

n = sample size

Z=Statistic for a level of confidence

d=Precision

P=Expected prevalence or proportion

Based on the above formula, the sample size (n) is calculated as follows:

$$n = \frac{(1.96)^2 (0.5) (0.5)}{(0.05)^2}$$

$$= 384$$

To ensure that the sample size was large enough to satisfy the precision and confidence constraints, the sample size was made up to 600 (Hassan, 1991). This allowed to take 300 samples from each of the two ethnic groups. However, had there been enough evidence to believe that p differ from 0.5, the sample size may be significantly reduced (Hassan, 1991).

3.5. Blood Sample Collection

Blood samples from 600 individuals of both sexes were collected from willing individuals of the two ethnic groups. Blood samples were taken from finger pricks, and open slide method of testing ABO blood types and Rh (D) factor was followed (Bhasin and Chahal, 1996). The ABO and Rh blood group test was performed by sterilized lancet, to obtain a drop of blood from a sterilized finger. The blood samples was collected by qualified laboratory technicians using the standard clinical procedure with sterilized lancet blade, slides, sticks, cotton and anti- A, Anti-B and Anti-D. The ABO blood grouping and Rh factor typing was determined by glass slide method. The glass slide method was based on antibody antigen agglutination and the blood group was determined based on agglutination. Blood sample was mixed with respective antibodies against type A, B and D blood, and the sample was checked to see whether or not the blood cells stick together (agglutinate). If blood cells stick together, it means the blood reacted with one of the antibodies. Blood group was determined on the basis presence or absence of reaction and recorded, as blood type A^+, B^+, AB^+ O^+ or A^-, B^-, AB^- and O^-.

3.6. Inclusion and Exclusion Criteria

Subjects considered for this study were students of Sheno Secondary and Preparatory Schools belonging to Amhara and Oromo ethnic groups those who were willing and anemic and those who were not willing and anemic were excluded.

3.7. Methods of Data Collection

The genetic structure can be described in terms of phenotypic, allelic and genotypic frequencies (Russell, 2005). For the present study, the frequency of the blood group phenotypes was used to calculate the frequency of the ABO blood group alleles by using the extension of Hardy-Weinberg principle as employed by (Griffith *et al.,* 2008). For this study three alleles frequency was computed (A, B and O), with frequencies equal to p, q and r, respectively. The frequencies of the genotypes at equilibrium was computed by trinomial expansion.

3.7.1 Calculation of Blood Group Phenotype Frequencies

The frequency of the blood types phenotypes was calculated as the number of individuals belonging to the phenotypic ethnic groups divided by the total number.

$$\text{Phenotypic frequency} = \frac{\text{Number of individuals}}{\text{Total number of individuals}}$$

3.7.2 Allelic frequency determination of ABO and Rh-D blood groups

Frequency of the three ABO blood group alleles (p, q, and r) was determined as follows:

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

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

$r = \sqrt{O}$ (p, q, r denote allele frequencies and A, B, O denote observed frequencies of blood groups *A, B* and *O.*

A correction factor (d) was calculated according to $d = 1 – p – q – r$. The final allele frequencies were then calculated as follows:

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

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

$r1 = (r + d/2) (1 + d/2)$, where p1, q1, and r1 denote corrected allele frequencies.

Frequencies of RhD blood group alleles D and d were represented as p and q respectively

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

$q = \sqrt{Rh-}$

$p = 1 - q$

3.7.3. Genotypic frequency determination of ABO and Rh-D blood groups

The genotypic frequencies of ABO blood group was calculated as follows

p^2 is the frequency of genotype I^AI^A (homozygous for blood type A)

q^2 is the frequency of genotype I^BI^B (homozygous for blood type B)

2pq is frequency of genotype I^AI^B (heterozygous for blood type AB)

2pr is frequency of genotype I^AI^O (heterozygous for blood type A)

2qr is the frequency of genotype I^BI^O (heterozygous for blood type B)

r^2 is the frequency of genotype I^OI^O(homozygous for blood type O)

The genotypic frequencies of Rh-D blood group was calculated as follows

Genotype DD = p^2

Genotype Dd = 2pq

Genotype dd = q^2

3.7.4. Goodness-of-fit test between observed and expected phenotype frequencies

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test.

Where , Oi and Ei was observed and expected frequencies respectively. P -value < 0.05 was considered as statistically significant.

Expected phenotypic frequencies ABO and Rh-D blood groups were calculated as: Expected frequency (Ef) = Genotypic frequency X number of total sample

A blood type Ef = frequency of (AA + AO) X number of total sample

B blood type Ef = frequency of (BB + BO) X number of total sample

AB blood type Ef = frequency of AB X number of total sample

O blood type Ef = frequency of OO X number of total sample

Rh+ = frequency of (DD + Dd) X number of total sample

Rh- = frequency of dd X number of total sample

3.8. Ethical Clearance

A permission to carry out the study was obtained from Kimbibit Woreda Health Office by using the supportive letter prepared by Haramaya University, School of Biological Sciences and Biotechnology; they gave me an agreement letter. All the information that was obtained about the subjects kept confidential. The objective and procedures of the study was explained to the participants before giving consent to participate. Participation was voluntary-based at any stage of the data collection.

4. RESULTS AND DISCUSSION

4.1. Phenotypic Percentage of ABO and Rh Blood groups among students of Amhara and Oromo Ethnic group

For this study, six hundred (600) individuals were selected randomly from the two ethnic groups, which consist of 297(49.5%) females and 303(50.5%) males.

There was the difference in frequency distribution of the ABO blood group among students of the two ethnic groups. Blood type O has the highest frequency while blood type AB has the lowest frequency. In this study, the phenotypes of 300 blood samples from each ethnic groups were O (34.7%), A (31.7%), B (26%), and AB (7.7%) for Amhara ethnic groups and O (38%), A (30%), B (25.3%), AB (6.7%) for Oromo ethnic groups. The overall frequency of blood group ABO in both ethnic group showed O (36.3%), A (30.8%), B (25.7%) and AB (7.2%), (Table 6).

The data revealed that the ABO blood group phenotypic percentage distribution in Amhara ethnic group was found in the order O > A > B > AB (34.7%, 31.7%, 26% and 7.7%) respectively and O > A > B > AB (38%, 30%, 25.3%, and 6.7% respectively for Oromo ethnic groups. Many studies have shown that blood type O was the most common blood type and blood type AB was the least common blood group in different populations and ethnic groups (Nwauche and Ejele, 2004).

When compared with other reports from similar studies, the results of two ethnic groups were consistent with previous findings in Ethiopia. For example, among Ethiopians, the frequency of distribution blood type O, 42%; A, 30%; B, 22%; and AB, 6 (www.rhesusnegative.net, 2012). The present study was in agreement with previous findings from Ethiopia. For example, the study conducted in population of south west Ethiopia (at Gilgel Gibe Field Research Center) showed that the distribution of blood type O, 42%; blood type A, 31%; blood type B, 21%; and blood type AB, 6% (Abraham *et al.,* 2012). The Study carried out by Tibebu (1998) showed that the distribution of blood type O were 40%; blood type A, 31%; blood type B, 23%; and blood type AB, 6% in Ethiopian blood donors.

The present study also show similarity with the study at Dilla University Distribution of ABO and Rh Blood Groups among Students of Some Ethnic Groups where blood type O was the most frequent blood type (44.49 %) followed by A (26.32 %), B (26.32 %), and AB (2.87 %), (Fekadu, 2015). The present study was also in agreement with the distribution of ABO blood group in Oromo, Amhara and Wolayita ethnic groups where blood type O, 42 %, 43% and 44.5% followed by blood type A, 28%, 29% and 27% and blood type B, 25%, 23% and 24% in Oromo, Amhara and Wolayita respectively, and the least percentage frequency was that of blood type AB in the three ethnic groups which was 5%, 5% and 4.5% in Oromo, Amhara and Wolayita respectively,(Nigusu, 2013).

The Study carried out by Kassahun *et al.,* (2014), also found that blood type O was the most common blood type and blood type AB was the least common blood type in Silte Zone. The frequency of blood type AB was 5.21%, 5.48%, 5% and 5.5% in, Tigrean, Kunama, Saho and Blen Ethnic people respectively. Blood type B has the frequency of 24.78%, 26.03%, 24% and 25.5% where as blood type A had the frequency of 26.51, 23.9%, 27%, and 27.5% respectively in Tigrean Kunama Saho and Blen ethnic people. The highest frequency of blood group phenotype for this study were blood type O which has 43.47 %, in Tigrean, 41.09 in Kunama, 44% Saho and 41.5% in Blen, reported by Zelalem, (2014**)** also in agreement with present study.

However, the present finding does not agree with the relative frequency of blood types determined to some population. For instance, ABO blood group antigens from Ainu (Japan) where ABO blood group frequency occurred in the order A = B (32%) > AB (18%) > O(17%) (ISBT, 2006). Frequency of ABO blood group among the Banjara Population of Akola District, Maharashtra, India where ABO blood group frequency occurred in the order of B> O>A>AB (37.45%, 27.64%, 22.91%, and 12% respectively), (Chavhan *et al.,* 2012). In Ogbomoso, South-west Nigeria, phenotypic frequencies of 50% for O, 22.9% for A, 21.3% for B and 5.9% for AB was reported by (Bakare *et al*., 2006).

It also seem not to agree with the results obtained from Swat district in Pakistan where the percentage frequencies were A, 27.92%, B, 32.40 %, O, 29.10% and AB, 10.58% in which B > O > A > AB (Khattak *et al.*, 2008). It is also not consistent with ABO phenotypic frequency of Bororo (Brazil) in which 100% of the population are O blood groups (ISBT, 2006). The finding of this Study seem to deviate from the results obtained by Khan and his colleagues on the genotype frequencies of blood group antigens from Bannu region in Pakistan where ABO blood group frequency occurred in the order B > A > O > AB (Khan et al., 2009).

Table. 6. Numbers and Percentages of ABO Blood Group phenotypes among Amhara and Oromo ethnic groups in sheno secondary and preparatory schools (2018).

Ethnic Group	ABO Blood Grouping							
	O		A		B		AB	
	Number	percent	Number	Percent	Number	percent	Number	Percent
Amhara	104	(34.7%)	95	(31.7)%	78	(26)%	23	(7.7)%
Oromo	114	(38%)	90	(30)%	76	(25.3)%	20	(6.7)%
Total	218	(36.3%)	185	(30.8%)	154	(25.7)%	43	(7.2)%

Regarding to Rhesus blood grouping system, the frequency of Rh D positive blood group was 92.3%, while the frequency of Rh negative was 7.7% in Amhara ethnic groups and the frequency of Rh D positive blood group was 95.3% while the frequency of Rh negative was 4.7% in Oromo ethnic groups. In the overall of 600 study population, 93.8% were Rh (*D*) positive while 6.2% were Rh (*d)* negative). These study shows that in the two ethnic groups the proportion of Rh-d negative is far lower than for Rh D positive, (Table 7).

These results were consistent with previous findings of Ethiopian populations (Abraham *et al.*, 2012; Tewodros *et al.*, 2011; and Nigusu, 2013). The findings were consistent with reports from previous similar studies among different sets of Nigerian population where the Rh positive was found to be higher in the population sampled than the Rh negative (Table 5) (Kulkarni *et al*., 1985; Ahmed and Obi, 1998; Omotade *et al*., 1999; Ahmed *et al*., 2004; Ahmed *et al*., 2007; Jeremiah and Odumody, 2005, Bakare *et al*., 2006, Akhigbe *et al*., 2009, and Adeyemo and Soboyejo, 2006).

The results, however; differ from the work reported by Yousaf and colleagues where the population sampled among Bahawalpur division of Pakistan population were all Rh positive (Yousaf *et al*., 1988). It also disagrees with that of Salmon *et al*., (1988) and Njoku *et al*., (1996) who reported rhesus positive values of 100% for Eastern Highlands of Papua New Guinea and Nigeria. In addition, it is dissimilar to that in Indians with preponderance of the Rh(*d*) of 89.7% over the Rh(*D*) gene of 10.3% (Thangaraj *et al*., 1992).

Table .7. Numbers and Percentages of Rh Blood Group phenotypes among Amhara and Oromo Ethnic groups in sheno secondary and preparatory schools (2018).

Ethnic Group	Rh(Rhesus Blood Grouping)			
	Rh positive		Rh negative	
	Number	Percent	Number	percent
Amhara	277	(92.3)%	23	(7.7)%
Oromo	286	(95.3)%	20	(4.7)%
Total	563	(93.8%)	43	(6.2%)

Table 8 presents the combined frequency distributions of ABO and Rh blood group phenotypes. The frequency of the ABO phenotypes linked with Rh positive phenotypes were

O^+ (32.3%), followed by A^+ (28%), B^+ (25%), and AB^+ (7%) while Rh negative were O^- (2.33%), A^-(3.67%), B^-(1%) and AB^- (2.33%) in Amhara ethnic groups. The frequency of the ABO phenotypes linked with Rh positive phenotypes were O^+(33%)), followed by A^+ (29%), B^+ (23.3%), and AB^+ (6.67%) while Rh negative were O^- (5%), A^-(1%), B^-(2%) and there were no individuals with blood type AB^- who were Rh-d negative in Oromo ethnic.The present study was in agreement with the study conducted in south west Ethiopia, (Jimma town blood bank) where blood type O has highest frequency in both Rh-D positive and Rh-d negative individuals followed by blood type A, B and AB blood type has the least frequency in both the Rh-D positive and Rh-D negative subjects (Teklu and Shiferaw, 2016).

Table.8. Distribution of Rh blood group alleles among the ABO blood group phenotype

Ethnic groups	Rh Blood group	ABO Blood types							
		A		B		AB		O	
		No	%	No	%	No	%	No	%
Amhara	Positive	84	28%	75	25%	21	7%	97	32.3%
	Negative	11	3.6%	1	1%	2	0.6%	6	2.33%
Oromo	Positive	87	29%	70	23.%	20	6.7%	99	33%
	Negative	3	1%	6	2%	0	0%	15	5%
Overall	Positive	171	28.5%	145	24.2%	41	6.8%	196	32.7%
	Negative	14	2.33%	9	1.5%	2	0.33	22	3.6%

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

This section represents the allelic and genotypic frequencies of ABO and Rh blood groups among two ethnic groups in which 300 study participants were randomly selected from each ethnic group. The allelic frequencies of ABO blood groups for Amhara ethnic group were I^O, 0.5925, I^A, 0.2214 and I^B, 0.1861. The allelic frequencies of ABO blood group for the Oromo

ethnic group were I^O, 0.6196, I^A, 0.2048 and I^B,' 0.1756. The allelic frequencies of ABO blood group for the overall students of two ethnic groups were population was I^O, 0.6053, I^A, 0.2136 and I^B, 0.1811. The result shows that a high frequency of the allele I^O over I^A and I^B alleles in the order of ($I^O > I^A > I^B$) in each ethnic groups in (Table 9). It shows similar patterns of allelic frequencies with those documented from earlier studies among various segments of the population Ethiopia. For instance in Ethiopia, the study reported by Nigusu, (2013) found allelic frequencies of I^O= 0.6540 >I^A = 0.1821 >I^B= 0.1639 in Oromo ethnic group; I^O = 0.6600 >I^A = 0.1881 >I^B= 0.1519 in Amhara ethnic group; and a frequency of I^O = 0.6772 >I^A = 0.1729 >I^B= 0.1549 for Wolayita ethnic groups. Allele frequencies shows a high frequency of the allele I^O over I^A and I^B in each ethnic groups, where in order of I^O, 0.659, I^A, 0.174 and I^B, 0.157, I^O, 0.718, I^A , 0.157 and I^B, 0.147 and I^O,0.71, I^A, 0.143 and I^B, 0.1403 in Amhara, Oromo and Afar respectively, (Mohammed, 2013). Allelic frequency of ABO blood group were I^O,0.6160, I^A, 0.2118, and I^B,0.1722 in Amhara, I^O, 0.6509 I^A, 0.1941, and I^B, 0.1550, in Oromo I^O, 0.7033, *IA*, 0.1671 and I^B, 0.1296, in Tigray ethnic groups,(Mosisa, 2018).

Table . 9. Allelic and Genotypic frequencies of ABO blood groups for the Amhara and Oromo ethnic groups in sheno secondary and preparatory schools (2018).

Ethnic Group	Allele	Allelic fre.	Genotype	Genotypic Freq.
Amhara	I^O	0.5925	I^OI^O	0.3511
	I^A	0.2214	I^AI^A	0.049
			I^AI^O	0.2624
	I^B	0.1861	I^BI^B	0.0346
			I^BI^O	0.2205
			I^AI^B	0.0824
Oromo	I^O	0.6196	I^OI^O	0.3356
	I^A	0.2048	I^AI^A	0.0419
			I^AI^O	0.2538
	I^B	0.1756	I^BI^B	0.0308
			I^BI^O	0.2176
			I^AI^B	0.0719
over all	I^O	0.6053	I^OI^O	0.3664
	I^A	0.2136	I^AI^A	0.0456
			I^AI^O	0.2586
	I^B	0.1811	I^BI^B	0.0328
			I^BI^O	0.2192
			I^AI^B	0.0774

The genotypes frequencies were I^OI^O, 0.3511, I^AI^A, 0.0490, I^AI^O, 0.2624, I^AI^B, 0.0824, I^BI^B, 0.0346 and I^BI^O 0.2205 in Amhara ethnic group and I^OI^O, 0.3839, I^AI^A, 0.0419, I^AI^O, 0.2538, I^AI^B, 0.0719, I^BI^B, 0.0308 and I^BI^O, 0.2176 in Oromo ethnic groups. As it is shown in (Table 9) in the two ethnic groups the genotypic frequencies of ABO blood group occurred in the order $I^O > I^A > I^B$.

Most of the A and B blood types are heterozygous (dominant) in two the ethnic groups. The genotypes I^AI^O makes *26*.24% and 25.38 % in Amhara and Oromo respectively. Whereas, genotypes of I^BI^O were 22.05%, and 21.76% in Amhara and Oromo respectively. The predominance of O allele may also be due to the fact that many A and B blood types may have been heterozygous carrying the *O* allele silently thereby maintaining *O* allele in the heterozygous population, (Bakare *et al*., 2006). Homozygous blood type A (I^AI^A) of the two ethnic groups were calculated to be 4.9% and 4.19 % in Amhara and Oromo respectively.

Previous studies among various segments of the Ethiopia population have documented similar pattern of genotypic frequencies. ABO blood group genotypic frequency of the three ethnic groups at Arsi University were I^AI^A, 0.0449, I^AI^O, 0.2609, $I^BI,^B$ 0.0296, I^BI^O, 0.2121, I^AI^B, 0.0729 and I^OI^O, 0.3796 in Amhara (Mosisa, 2018). Similar results also were reported by Iyiola *et al.* (2011), Saleh *et al.* (2016), Bakare *et al.,* (2006).

With respect to Rhesus blood group system, the allelic frequency of Amhara ethnic groups was 0.7225, I^D and 0.2775, I^d. And the allelic frequency was found to be 0.7832, I^D and 0.2168, I^d for Oromo ethnic groups in (Table 10). The allelic frequencies in the overall ethnic groups were 0.7516, I^D and 0.2484, I^d. The frequency of heterozygous Rh positive (*Dd*) was also calculated using the Hardy-Weinberg law and presented in Table 10. The heterozygous Rh positive (*Dd*) in each ethnic group were found to be 40.1% and 33.96% in Amhara and Oromo respectively. The genotypes frequency of Rh blood group in Amhara ethnic group were 0.5220, I^DI^D, 0.401, I^DI^d and 0.0770, I^dI^d and 0.6134, I^DI^D, 0.3396, I^DI^d and 0.0470, I^dI^d in Oromo ethnic groups. The genotypic frequencies in the overall ethnic groups were 0.5649,

I^DI^D, 0.3734, I^DI^d and 0.061, I^dI^d. Among Rhesus factor, allele *D* is far higher in frequency than allele d in the two ethnic groups and in the overall study population.

Table .10. Allelic and Genotypic frequencies of Rh blood groups for the Amhara and Oromo ethnic groups in Sheno Secondary and Preparatory schools (2018).

Ethnic Group	Allele	Allelic Freq.	Genotype	Genotypic Freq.
Amhara	I^D	0.7225	I^DI^D	0.522
			I^DI^d	0.401
	I^d	0.2775	I^dI^d	0.077
Oromo	I^D	0.7832	I^DI^D	0.6134
			I^DI^d	0.3396
	I^d	0.2168	I^dI^d	0.047
Over all	ID	0.7516	I^DI^D	0.5649
			I^DI^d	0.3734
	I^d	0.2484	I^dI^d	0.061

4.3. Chi-square Test for the Goodness of Fit of ABO and Rh Blood Group Distribution

As it is shown in Table 11 the application of extended Hardy-Weinberg principle for two or more alleles yields little variation in the observed and expected genotypic frequencies which serves as a base in determining the chi-square ($\chi 2$) values that further used in determining the goodness-of- fit. Table 11 shows that observed versus the expected values of ABO blood group phenotypes in the total sample. The variation of distribution of the overall observed frequencies of ABO blood group phenotypes from those expected under Hardy-Weinberg equilibrium were insignificant (Goodness of-fit $\chi 2 = 0.301$, df=1, P-value <0.05) in the Amhara and Oromo ethnic groups.

Table .11. Observed versus Expected proportions of ABO blood Groups frequency in Amhara and Oromo Ethnic group in Sheno Secondary and Preparatory schools(2018).

Ethnic group	Blood group	Obs. (O	Exp. (E)	O – E	$(O - E)^2$	(O-E)2/E	x^2
Amhara	O	104	105.33	1.33	1.7689	0.0168	
	A	95	93.42	1.58	2.4964	0.0267	
	B	78	76.53	1.47	2.1609	0.02802	0.1914
	AB	23	24.72	1.72	2. 9584	0.1197	
Oromo	O	114	115.17	1.17	1.3689	0.119	
	A	90	88.71	1.29	1.661	0.0187	
	B	76	74.52	1.48	2.1904	0.0294	0.1743
	AB	20	21.57	1.57	2.4649	0.1143	
Overall	O	218	219.84	1.84	3.3	0.015	0.301
	A	185	182.5	2.5	6.25	0.034	
	B	154	151.2	2.8	7.84	0.052	
	AB	43	46.44	3.44	1.8	0.2	

Table.12 presents the observed proportions of Rh individuals in the studied population when compared with expected proportions. It also shows the chi-square ($\chi 2$) and probability (p) value for the two ethnic groups separately and for the overall student population sampled in the study. As it is shown in Table 12, the application of extended Hardy-Weinberg principle for two or more alleles yields little variation in the observed and expected genotypic frequencies and numbers which serves as a base in determining the chi-square ($\chi 2$) values that further used in determining the goodness-of- fit. Table 12 shows observed versus the expected values of Rh-D blood group phenotypes in the total sample. The variation of distribution of the overall observed frequencies of Rh-D blood group phenotypes from those expected under

Hardy-Weinberg equilibrium were significant (Goodness of-fit $\chi 2 = 1.7\times10^{-3}$ df= 1, P-value < 0.05).in the two ethnic groups.

Table .12. Expected and Observed frequencies ABO blood group among the Amhara and Oromo ethnic groups in sheno secondary and preparatory schools (2018).

Ethnic group	Blood group	Obs.(O)	Exp.(E)	O – E	$(O - E)^2$	(O-E)2/E	x^2
Amhara	Rh+	277	276.9	0.1	0.01	3.6×10-5	0.00466
	Rh-	23	23.1	0.1	0.01	4.3×10-4	
Oromo	Rh+	286	285.9	0.1	0.01	3.5×10-5	0.001435
	Rh-	14	14.1	0.1	0.02	1.4×10-3	
Overall	Rh+	563	563.98	0.98	0.9604	1.7×10-3	0.001712
	Rh-	37	32.02	0.02	0.0004	1.2×10^{-5}	

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

The frequency of blood type O was high with percentage frequency of 34.7% and 38% in Amhara and Oromo ethnic groups respectively, followed by blood type A (31.6 % and 30%) ,blood type B (26% and 25.3%), and the least percentage frequency was that of blood type AB (7.7% and 6.7%) in the two ethnic groups respectively. With regard to Rh-D blood group system, 93.8 % of the total samples were Rh-positive and 6.2 were Rh-negative. The frequencies of I^O, I^A and I^B alleles were 0.5925, 0.2214 and 0.1861 respectively, in Amhara ethnic groups and I^O, I^A and I^B alleles were 0.6196, 0.2048 and 0.1756 respectively, in Oromo ethnic groups. The allelic frequency of Rh-D blood group in Amhara ethnic groups was 0.7225, I^D and 0.2775, I^d. and 0.7832, I^D and 0.2168, I^d were for Oromo ethnic groups. The genotypes frequencies of ABO blood group were I^OI^O , 0.3511, I^AI^A, 0.0490, I^AI^O, 0.2624, I^AI^B , 0.0824, I^BI^B, 0.0346 and I^BI^O 0.2205 in Amhara ethnic group and I^OI^O, 0.3839, I^AI^A, 0.0419, I^AI^O, 0.2538, I^AI^B, 0.0719, I^BI^B, 0.0308 and I^BI^O, 0.2176 in Oromo ethnic groups. The genotypes frequency of Rh blood group in Amhara ethnic group were 0.5220, I^DI^D, 0.401, I^DI^d and 0.0770, I^dI^d and 0.6134, I^DI^D, 0.3396, I^DI^d and 0.0470, I^dI^d in Oromo ethnic groups. The distribution of ABO and Rh-D blood group systems in the students did not differ significantly from those expected under the hardy Weinberg law (P-value< 0.05).

5.2. Conclusion

The distributions of ABO and Rh blood groups in this study have similar trends with the data from the previous studies in Ethiopia. In ABO blood group system, blood type O has the highest frequency and blood type AB has the lowest frequency. In the Rh-D blood group system, blood type Rh-D positive has the highest frequency while Rh-d negative blood type has the lowest frequency. The order of the frequencies of ABO blood group alleles is $I^O>I^A>I^B$. In Rh-D system, frequency of allele *D* is higher than frequency of *d* allele.

This information would be useful to the population geneticists and to the clinicians, especially in the planning of blood transfusion program. This study was vital for blood banks and transfusion services that contribute to patient's health care. The data generated would be

helpful as a support for researchers who are interested to conduct blood frequency related studies type of study in Amhara and Oromo ethnic Groups. The knowledge of frequencies and distribution of the different blood groups is very important for blood banks and transfusion. To conclude that, this study provides information on the phenotypic, genotypic and allelic frequencies of ABO and Rh-D blood group systems of the student in Sheno Secondary and Preparatory Schools.

5.3. Recommendation

- ✓ The sample size used to conduct this study was small and only focused on the two ethnic groups therefore; to study further on all ethnic groups was mainly recommended
- ✓ The sample size used to conduct this study was small and may not represent the number of population in the two ethnic groups. Therefore it is advisable to use larger sample size to obtain more accurate data regarding the pattern of distribution on these blood groups.
- ✓ Further study at molecular level would definitely reveal the degree of genetic proximity

6. REFERENCES

Abraham Haileamlak, Ayalew T. Muluneh, Fessahaye Alemseged, Fasil Tessema, Kifle. 2012. Hematoimmunological Profile at Gilgel Gibe Field Research Center, Southwest Ethiopia. *Ethiopian Journal of Health Sciences,* 22: 39-50.

Adeyemo, O.A. and Soboyejo, O.B. 2006. Frequency distribution of ABO, RH blood groups and blood genotypes among the cell biology and genetics students of University of Lagos, Nigeria. *African journal of biotechnology, 5*(22).

Ahmed, S.G. and Obi, S.O. 1998. The incidence of ABO and rhesus-D blood groups in northeast Nigeria. *Niger journal Medicine, 7,* 68-70.

Ahmed, S.G., Ibrahim, U.A. and Hassan, A.W. 2007. Adequacy and pattern of blood donations in north–eastern Nigeria: the implications for blood safety. *Annals of Tropical Medicine & Parasitology, 101(8):725-731*

Ahmed, S.G., Kangu, M.B. and Abjah, U.A.M. 2009. The role of du testing in scaling down the burden of Rhesus-D negative transfusion in Northern Nigeria. *International journal Third World Medical .8* (2)

Akbase, F., Aydin, M. and Cenani, A. 2003. ABO blood subgroup allele frequencies in the Turkish population. *Anthropologischer Anzeiger,* 257-260.

Akhigbe, R.E., Ige, S.F., Afolabi, A.O., Azeez, O.M., Adegunlola, G.J. and Bamidele, J.O. 2009. Prevalence of haemoglobin variants, ABO and rhesus blood groups in ladoke akintola University of Technology, Ogbomoso, Nigeria. *Trends Medicinal Research, 4* .24-9.

Anees, M. and Mirza, M.S . 2005. Distribution of ABO and Rh blood group alleles in Gujrat region of Punjab, Pakistan. *Proceeding-Pakistan Academy of Sciences, 42*(4): 233.

Anstee, D.J . 2009. Red cell genotyping and the future of pretransfusion testing. *Blood, 114*(2), 248-256.

Avent, N.D. and Reid, M.E.2000. The Rh blood group system: a review. *Blood, 95*(2): 375-387.

Bakare, A.A., Azeez, M.A. and Agbolade, J.O. 2006. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South–West Nigeria. *African Journal of Biotechnology, 5*(3):224-229.

Benjamini E, Coico R, Sunshine G. 2000. *Immunology A short Course, 4th ed. Wiley Liss*.

Bhasin, M.K. and Chahal, S.M.S. 1996. *A laboratory manual for human blood analysis*. Delhi: Kamla-Raj Enterprises.

Bruce, M.G., 2002. BCF–*Members–Chairman's Annual Report.* The Blood Care Foundation.

Chakraborty, D.P . 2010. Prediction accuracy of a sample-size estimation method for ROC studies. *Academic radiology*, 17(5): 628-638.

Chandra, T. and Gupta, A. 2012. Frequency of ABO and rhesus blood groups in blood donors. *Asian journal of transfusion science*, 6(1) : 52.

Chavhan, A., Jadhao, R., &Pawar, S. 2012. The study of Allelic Frequency of ABO and Rh-D Blood Group among the Banjara Population of Akola District, Maharashtra, India. *Antrocom Online Journal of Anthropology*, 8(2): 71-73.

Cummings. M.R. 2008 *Human Heredity; Principles and Issues. 5th edition.* University of Illionis. *478*.

Daniel, L.H.,and Elizabeth, W. J. 2009. *Genetics: analysis of genes and genomes.* 17th*Edition*. Jones and Bartlett publishers, Canada; 989-121.

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

Dar, N.J., Srivastava, A. and Dar, F.A. 2011. Distribution of ABO blood groups and Rh (D) factor among the Brahmin and Kushwaha populations of Jhansi District (UP). *Anthropologis*t, 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(1) : 47-48.

Enosolease, M.E. and Bazuaye, G.N. 2008. Distribution of ABO and Rh-D blood groups in the Benin area of Niger-Delta: Iplication for regional blood transfusion. *Asian journal of Transfusion sciences*, 2(1) : 3.

Falusi AG, Ademowo OG, Latunji CA, Okeke AC, Olatunji PO, Onyekwere TO, Jimmy EO, Raji Y, Hedo CC, Otukonyong EE, Itata EO. 2000. Distribution of ABO and Rh genes in Nigeria. *African Journal Medicinal Sciences*, 29:23–6.

Fekadu Alemu. 2015 Distribution of ABO and Rh Blood Groups among Students of Some Ethnic Groups at Dilla University, Ethiopia. International Journal of Genetics and Genome . 3: 8-19.

. Ganong, WF. 1995. *Review of Medical Physiology.17th edition.* Prentice Hall International Inc. London: 487-9.

Garratty, G., Dzik, W., Issitt, P.D., Lublin, D.M., Reid, M.E. and Zelinski, T. 2000. Terminology for blood group antigens and genes—historical origins and guidelines in the new millennium. *Transfusion*, 40(4) : 477-489.

Green, D., Jarrett, O., Ruth, K.J., Folsom, A.R. and Liu, K. 1995. Relationship among Lewis phenotype, clotting factors, and other cardiovascular risk factors in young adults. *Journal of Laboratory and Clinical Medicine*, 125(3) : 334-339.

Griffiths.,A.F, S.R,Wessler, R.C,Lewontin., and S.B.Carroll. 2008. *Introduction to Genetic Analysis. 9th Edition*. W.H. Freeman and Company. New York. 838.

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

Hasna Amer Mouhaus . 2010. A study of ABO blood group and Rhesus factor distribution *Journal of Basrah Researches Sciences*, 36(5): 15.

Hillier CD Shaz BH, Winkler AM, Reid M. 2008. *Transfusion medicine Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA 30321, USA.*

ISBT (International Society of Blood Transfusion) . 2006. Table of blood group systems.

ISBT (International Society of Blood Transfusion) . 2008. Table of blood group systems. accessed july 5-14.

Iyiola, O.A., O.O. Igunnugbemi., U.A .Raheem. and A.T. Anifowoshe . 2011. Gene frequencies of ABO and Rh (D) blood group alleles in Ilorin, North-Central Nigeria. *University of Ilorin, Ilorin Kwara state, Nigeria.*

Jaff, M.S . 2010. ABO and rhesus blood group distribution, in Kurds. *Journal of Blood Medicine,* 1 : 143–146.

Jame . 1999. Hardy, Weinberg and language impediments. *Genetics*, 152(3) : 821-825.

Jeremiah, Z.A. and Odumody, C .2005. Rh antigens and phenotype frequencies of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria. *Immunohematology*, 21(1) : 21-24.

Kassahun Tesfaye, Yohannes Petros and MebeaselassieAndargie. 2015. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. *The Egyptian Journal of Medical Human Genetics,* 16 : 71–76.

Kaya, H., Gündodu, M., Akarsu, E., Kiki, I. and Tekin, B . 1999. The distribution of blood groups in Erzurum. *Medicinal Journal Atatürk University,31*: 20-22.

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

Khurshid, B., Naz, M., Hassan, M. and Mabood, S.F. 1992. Frequency of ABO and Rh (D) blood groups in district Swabi NWFP (Pakistan). *Journal Science Technology University. Peshawar*, *16* : 5-6.

Kulkarni, A.G., Peter, B., Ibazebo, R., Dash, B. and Fleming, A.F. 1985. The ABO and Rhesus groups in the north of Nigeria. *Annals of Tropical Medicine & Parasitology*, 79(1) : 83-88.

Landsteiner, R . 2000. Karl Landsteiner and the first human marker locus. *Genetics*, 155(3): 995-998.

Laura, D . 2005. Blood groups and red cell antigens. *Bethesda (MD): National Center for Biotechnology Information, US URL http://www. ncbi. nlm. nih. gov/books/NBK2277*.

Lögdberg, L., Reid, M.E. and Zelinski, T . 2011. Human blood group genes chromosomal locations and cloning strategies revisited. *Transfusion Medicine Reviews*, 25(1) : 36-46.

Lögdberg, L., Reid, M.E., Lamont, R.E. and Zelinski, T . 2005. Human blood group genes . 2004: chromosomal locations and cloning strategies. *Transfusion Medicine Reviews*, 19(1) : 45-57.

Lyko, J., Gaertner, H., Kaviti, J.N., Karithi, M.W. and Akoto, B . 1992. The blood groups antigens ABO and Rh in Kenyans. *Hamdard Medicus,* 35(3) : 59-67.

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

Mohamed Saied . 2013 Allelic frequency of ABO and Rh blood groups belonging to three Ethnic groups at Desse Referal Hospital.

Moise Jr, K.J . 2008. Management of rhesus alloimmunization in pregnancy. *Obstetrics and Gynecology*, 112(1):164-176.

Mosisa Bedasa . 2018 Frequency of ABO and Rh Blood Group Alleles and Genotypes among Students of Arsi University Belonging to Amhara, Oromo and Tigray.

Mouhaus, H.A., Abbas, S.H., Musa, A.S. and Mahawi, H.K. 2010. A study of ABO blood group and Rhesus factor distribution among sample of Missan province population. *Journal of Basrah Researches*, 36: 48-53.

Mourant,A.E., AC.Kopec, K.Domaniewska-Sobczak . 1976. *The Distribution of the Human Blood Groups and Other Polymorphisms.* London: Oxford University Press.

Murphy, W.J., Frönicke, L., O'Brien, S.J. and Stanyon, R . 2003. The origin of human chromosome 1 and its homologs in placental mammals. *Genome research*, 13(8): 1880-1888.

Nanu, A. and Thapliyal, R.M . 1997. Blood group gene frequency in a selected north Indian population. *The Indian Journal of Medical Research, 106* : 242-246.

Neil, D.O. 2006. Modern Human variation: Distribution of blood types. *Htm online. PDF*.

Nigusu Girma . 2013.Frequency of ABO and Rh Blood Group Alleles among Oromo, Amhara And Wolayita Ethnic Group Students in Robe Secondary and Preparatory and Zebela Primary School, Bale Zone, Ethiopia.Haramaya University, Ethiopia

Njoku, O.U., Ononogbu, I.C., Alumunan, E.O. and Nwanjoh, J . 1996. Serum lipids, ABO blood group and sickle cell trait. *Indian journal of physiology and pharmacology, 40* (2) : 171-174.

Novak, P.D . 1995. Dorland's pocket medical dictionary. *WB Saunders Company*.

Nwauche, C.A. and Ejele, O.A . 2004. ABO and rhesus antigens in a cosmopolitan Nigeria population. *Nigerian journal of medicine: journal of the National Association of Resident Doctors of Nigeria*, *13*(3) : 263-266.

Omotade, O.O., Adeyemo, A.A., Kayode, C.M., Falade, S.L. and Ikpeme, S . 1999. Gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, Nigeria. *West African Journal of Medicine*, 18(4) : 294-297.

Periyavan, S., Sangeetha, S., Marimuthu, P., Manjunath, B. and Seema, D . 2010. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. *Asian Journal of Transfusion Science*, 4(1) : 41.

Reddy, K.S.N. and Sudha, G . 2009. ABO and Rh (D) blood groups among the Desuri Reddis of Chittoor district, Andhra Pradesh. *The Anthropologist*, 11(3): 237-238.

Reid, M.E. and Lomas-Francis, C . 2004. *The Blood Group Antigen Facts Book. Second.*

Rubeai, M.A . 1975. Taste sensitivity to phenylthiocarbamide and blood groups in the Iraqi population (sample from Baghdad). *Bulletun College Sciences*, 16(2) : 205-215.

Russell. J.P . 2005. *Genetics: A Mendelian Approach. Pearson Benjamin Cummings*. New York. 842.

Saleh, S.M. and Abood, A.S . 2016. ABO and Rh (D) Blood Groups: Distribution and Gene Frequencies in North Baghdad Population–Iraq. *International Journal of Scientific and Engineering Research*, 7(8) : ISSN 2229-5518

Salmon, D., Godelier, M., Halle, L., Lemonnier, P., Lory, J.L., Rouger, P., Ruffie, J. and Salmon, C . 1988. Blood groups in Papua New Guinea Eastern Highlands. *Gene geography: a computerized bulletin on human gene frequencies*, 2(2-3) : 89-98.

Seltsam, A., Hallensleben, M., Kollmann, A. and Blasczyk, R. 2003. The nature of diversity and diversification at the ABO locus. *Blood*, 102(8): 3035-3042.

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

Sultana, R., Rahman, Z., Helali, A.M., Yousuf, R., Mustafa, S., Salam, A. and Haque, M . 2013. Study of ABO and RH-D Blood Groups among the common people of capital city of Bangladesh. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3) : 814-816.

Teklu Zerihun and Shiferaw Bekele . 2016. Pattern of ABO and Rhesus Blood Groups Distribution of Five Years Survey in Jimma Town Blood Bank, South West Ethiopia. Journal of Health *Education Research Development*, 4:176.

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

Thangaraj, K., Srikumari, C.R. and Ramesh, A . 1992. The genetic composition of an endogamous Adi-Dravidar population of Tamil Nadu. *Gene geography: a computerized bulletin on human gene frequencies*, 6(1-2) : 27-30

Urbaniak, S.J. and Greiss, M.A . 2000. RhD haemolytic disease of the fetus and the newborn. *Blood reviews*, 14(1) : 44-61.

Westhoff, C.M . 2004. The Rh blood group system in review: a new face for the next decade. *Transfusion*, 44(11) : 1663-1673.

Yamamoto, F . 2000. Molecular genetics of ABO. *Vox sanguinis, 78* : 91-103.

Yamamoto, F.I., Clausen, H., White, T., Marken, J. and Hakomori, S.I . 1990. Molecular genetic basis of the histo-blood group ABO system. *Nature*, 345(6272) : 229.

Yassin Woliye . 2013. Frequency of ABO and Rhesus(D) Blood Group Alleles among Student of Oromo Ethnic Group Belonging to Arsi,Guji and Borena Clans in Robe.

Yazdanbakhsh, K . 2001. Molecular mechanisms underlying defective expression of blood group antigens. *Transfusion medicine reviews*, 15(1) : 53-66.

Yazer, M.H., Olsson, M.L. and Palcic, M.M . 2006. The cis-AB blood group phenotype: fundamental lessons in glycobiology. *Transfusion medicine reviews*, 20(3) :207-217. .

Yousaf, M., Yousaf, N. and Zahid, A . 1988. Pattern of ABO and Rh (D) Blood groups distribution in Bahawalpur Division. *Pakistan Journal Medicine Research*, 27(1) : 40-1.

Zelalem Alemu . 2014. Allelic and Genotypic Frequencies of ABO And Rh Blood Groups among Tigrean, Kunama, Saho And Blen Ethnic People Inhabiting Western Tigray.

Zerihun, T., Degarege, A. and Erko, B . 2011. Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pacific journal of tropical biomedicine*, 1(4): 289-294.

7. APPENDICES

Appendix 1: Consent form

I the undersigned have been informed and understand the purpose and objectives of the study that plans to asses Frequency of ABO and Rh Blood Groups Alleles and Genotypes among Students of Amhara and Oromo Ethnic Groups in Sheno Secondary and Preparatory Schools, North Shewa. I have been informed that qualified and experienced laboratory technician would do the blood collection according to the established aseptic procedures sterile disposable lancet was used. I have been also informed that all laboratory results would be kept confidential.

I have been given enough time to think over before I signed this informed consent. Therefore; with full understanding of the situation that I have gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name of the student _____

Age _____ Sex _____ year _____

Signature _____ Date: _____

Appendix 2: Data collection format

Appendix Table 1

Sample	Age	Sex	Place of birth	Parents		Blood group		Ethnicity	
Code				Father	Mother	ABO	Rh	Amhara	Oromo
SH001									
SH002									
SH003									
SH004									
SH005									
SH006									
SH007									
SH008									
SH009									
SH010									
SH011									
SH012									
SH013									
SH014									
SH015									
SH016									

Appendix 3 : Probability Values for Chi-square Analysis

Appendix Table 2

Probability Values for Chi-Square Analysis

Df	0.95	0.9	0.7	0.5	0.3	0.2	0.1	0.05	0.01
1	0.004	0.016	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.1	0.21	0.71	1.39	2.41	3.22	4.61	5.99	9.21
3	0.35	0.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35
4	0.71	1.06	2.2	3.36	4.88	5.99	7.78	9.49	13.28
5	1.15	1.61	3	4.35	6.06	7.29	9.24	11.07	15.09
6	1.64	2.2	3.83	5.35	7.23	8.56	10.65	12.59	16.81
7	2.17	2.83	4.67	6.35	8.38	9.8	12.02	14.07	18.48
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.21
		Non-significant							significant

Appendix 4: Calculation of Allele, Genotype and Expected Frequencies of ABO and Rh-D Blood Groups

Appendix Table 3

Ethnic Group	ABO Blood Grouping System			
	O	A	B	AB
Amhara	104 (34.7%)	95 (31.6%)	78 (26%)	23 (7.7%)
Oromo	114 (38%)	90 (30%)	76 (25.3%)	20 (6.7%)
Both	218 (36.3%)	185(30.8%)	154(25.6%)	43 (7.2%)

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

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

Calculation of the allelic frequencies of *A*, *B* and *O* blood group for Amhara ethnic group.

$r = \sqrt{O}$

$= \sqrt{0.347} = 0.5891$

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

$= 1-\sqrt{0.26+0.346} = 0.2209$

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

$= 1-\sqrt{0.316+0.347} = 0.1857$

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

So, $d= 1-0.2209-0.1857-0.5891= 0.0043$.

The final allele frequencies were then calculated as follows:

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

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

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

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

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

= 0.2209 (1+0.00215) =0.2214

q1 = q (1 + d/2)

=0.1857(1+ 0.00215) =0.1861

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

= (0.5891+0.00215) (1+0.00215) = 0.5925

Therefore, the allelic frequencies of I^A= 0.2214, I^B = 0.1861, I^O = 0.5925

Calculation of the genotypic frequencies and Expected number for Amhara ethnic groups.

- Genotype I^{AA} = p^2= $(0.2214)^2$ = 0.0490.
- Genotype I^{AO} =2pr = 2(0.2214 x 0.5925)=0.2624

Therefore, the expected number of individual belonging to blood type A, 93.42.

- Genotype I^{BB} =q^2 = $(0.1861)^2$ =0.0346
- Genotype I^{BO} =2qr = 2(0.1861 x 0.5925) =0.2205

Therefore, the expected number of individual belonging to blood type B,76.53.

- Genotype I^{AB} =2pq =2(0.2214 x 0.1861) =0.0824

Therefore, the expected number of individual belonging to blood type AB, 24.72.

- Genotype I^{OO}= r^2 = $(0.5925)^2$ =0.3511

Therefore, the expected number of individual belonging to blood type O, 105.33.

$\chi 2_{ABO}$ (Amhara) $= \sum \frac{(Oi - Ei)^2}{Ei}$

$\chi 2_{ABO}$ (Amhara) = $\sum \frac{(104-105.33)^2}{105.33} + \frac{(95-93.42)^2}{93.42} + \frac{(78-76.56)^2}{76.56} + \frac{(23-24.72)^2}{24.72}$ =0.1914

The degree of freedom 4-3 = 1

So, the p value p <0.95

Calculation of the allelic frequencies of *A*, *B* and *O* blood group for Oromo ethnic group

r = √O

= √0.38 = 0.6164

p = 1 - √B+O

= 1-√0.253+0.38 = 0.2044

q = 1 - √A+O

= 1-√0.3+0.38 =0.1753

A correction factor (d) calculated according to

$d = 1 - p - q - r.$

So, d= 1-0.2044-0.1753-0.6164 = 0.0039.

The final allele frequencies were then calculated as follows:

p1 = p (1 + d/2);

q1 = q (1 + d/2);

r1 = (r + d/2) (1 + d/2) where, p1, q1, and r1.

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

p1 = p (1+d/2)

=0.2044 (1+0.00195) = 0.2048

q1 = q (1 + d/2)

=0.1753 (1+ 0.00195) = 0.1756

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

= (0.6164+0.00195) (1+0.00195) = 0.6196

Therefore, the allelic frequencies of I^A = 0.2048, I^B = 0.1756, I^O = 0.6196 for Oromo ethnic groups.

Calculation of the genotypic frequencies and Expected number

- Genotype $I^{AA} = p^2 = (0.2048)^2 = 0.0419$
- Genotype $I^{AO} = 2pr = 2(0.2048 \times 0.6196) = 0.2538$

Therefore, the expected number of individual belonging to blood type A, 88.71

- Genotype $I^{BB} = q^2 = (0.1756)^2 = 0.0308$
- Genotype $^{IBO} = 2qr = 2 (0.1756 \times 0.6196) = 0.2176$

Therefore, the expected number of individual belonging to blood type B, 74.52.

- Genotype $I^{AB} = 2pq = 2(0.2048 \times 0.1756) = 0.0719$

Therefore, the expected number of individual belonging to blood type AB , 21.57

- Genotype $I^{OO}=r^2 = (0.6196)^2 = 0.3839$

Therefore, the expected number of individual belonging to blood type O, 115.17

$\chi 2_{ABO}$ (Oromo) $= \sum (Oi - Ei)^2 / Ei$

$\chi 2_{ABO}$ (Oromo) $= \frac{(114-115.17)^2}{115.17} + \frac{(90-88.71)^2}{88.71} + \frac{(76-74.52)^2}{74.52} + \frac{(20-21.57)^2}{21.57} = 0.1743$

The degree of freedom 4-3 = 1

So the p value is $p < 0.95$.

Calculation of the allelic frequencies of A, B, O, D and d blood group of Overall results for ABO and Rh (D) blood groups

$r = \sqrt{O}$

$= \sqrt{0.363} = 0.6024$

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

$= 1-\sqrt{0.256 + 0.363} = 0.2132$

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

$= 1-\sqrt{0.308+0.363} = 0.1808$

A correction factor (d) was calculated according to

$d = 1 – p – q – r.$

So, d= 1- 0.2132- 0.1808 - 0.6024 = 0.0036.

The final allele frequencies were then calculated as follows:

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

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

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

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

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

$=0.2132 (1+0.0018) = 0.2136$

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

$=0.1808 (1+ 0.0018) = 0.1811$

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

$= (0.6024 + 0.0018) (1+ 0.0018) = 0.6053$ and for Rh(D) blood group

$d = \sqrt{q}$

$d = \sqrt{0.0617} = 0.2484$

$D = 1-q$

$D = 1-0.2484 = 0.7516$

So, the overall allelic frequencies of I^A = 0.2136, I^B = 0.1811, I^O = 0.6053, D = 0.7516 and d=0.2484.

Calculation of the Genotypic frequencies and Expected number

- Genotype I^{AA} = p2 = $(0.2136)^2$ = 0.0456
- Genotype I^{AO} =2(0.2136 x 0.6053) =0.2586

Therefore, the expected number of individual belonging to blood type A, 182.52.

- Genotype I^{BB} =q^2 = $(0.1811)^2$ = 0.03279
- Genotype I^{BO} =2qr =2(0.1811x 0.6053) =0.2192

Therefore, the expected number of individual belonging to blood type B, 151.2

- Genotype I^{AB} =2pq =2(0.2136 x 0.1811) = 0.0774

Therefore, the expected number of individual belonging to blood type AB, 46.44.

- Genotype I^{OO} = r^2 = $(0.6053)^2$ =0.3664

The expected number of blood type O is 219.84.

The Chi-square ($\chi 2$) test statistic was then:

$\chi 2$ABO (Overall) =$\sum \frac{(Oi-Ei)^2}{}$

$\chi 2$ABO (Overall) =$\sum \frac{(218-219.84)^2}{219.84} + \frac{(185-182.52)^2}{182.52} + \frac{(154-151.2)^2}{151.2} + \frac{(43-46.44)^2}{46.44}$ = 0.301

The degree of freedom 4-3 = 1

So the p value is p < 0.95

And the genotypic frequencies and expected number of Rh(D) blood group were:

- Genotype DD = p2 = $(0.7516)^2$ = 0.5649
- Genotype Dd =2pq =2(0.7516*0.2484) = 0.3734

So the expected number of Rh(D) +ve blood group, 563.34.

Genotype dd = q2 = ($0.2484)^2$ =0.0617