FREQUENCIES OF ABO AND RH-D BLOOD GROUP ALLELES AMONG STUDENTS OF FOUR ETHNIC GROUPS IN MECHARA SECONDARY AND PREPARATORY SCHOOL,WEST HARARGHE, OROMIA

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DEDICATION

I dedicate this Thesis to my lovely wife Asha Adem and lovely son Huzeyfa Seid .

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 compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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ACRONYMS AND ABBREVIATIONS

HDN	Hemolytic Disease of the Newborn
HWE	Hardy-Weinberg Equilibrium
HWP	Hardy-Weinberg Principle
IgG	Immunoglubulin G
IgM	Immunoglubulin M
ISBT	International Society of Blood Transfusion
MN	M and N blood group
MoH	Ministry of Health
RBC	Red Blood Cell
Rh	Rhesus
vWF	Willebrand Factor
WHO	World Health Organization

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ABSTRACT

Frequencies of ABO and Rh-D blood groups vary worldwide and are not found in equal numbers even among different ethnic groups. Some variations may even occur within one ethnic group in different geographical areas. Therefore, this study was conducted to determine the frequencies of ABO and Rh-D blood group phenotypes, alleles and genotypes among Students of Oromo, Amhara, Argoba and Somali ethnic groups in Mechara Secondary and Preparatory School,West Hararghe, Oromia. A total of 449 students were purposely selected among school Students and tested for ABO and Rh-D blood groups antigens. Blood groupings were done using open slide method. The frequencies of ABO and Rh-D blood groups phenotypes were expressed in percentages and the modified Hardy-Weinberg Law was used to determine allele and genotype frequencies whereas Chi-square test was used to compare observed phenotypic frequency distribution of ABO and Rh-D blood groups with that expected under the Hardy-Weinberg law . In the overall sample, the observed O, A, B, and AB blood group percentages were 42.1%, 29.2%, 20.48% and 8.24%, respectively. The Rh-D positive incidence was 98.84%, while Rh-D negative was 1.34% in the overall sample. The order of ABO blood group allele frequencies were $I^O > I^A > I^B$ in the overall samples and in each of the four ethnic groups. The allele frequencies of I^O, I^A, and I^B in the total sample were found to be 0.64, 0.21 and 0.16 respectively. The allele frequencies were 0.64 I^O, 0.22 I^A and 0.14 I^B for Oromo, 0.66 I^O, 0.19 I^A and 0.15 I^B for Amhara, 0.61 I^O, 0.20 I^A and 0.19 I^B for Argoba and 0.63 I^O,0.22I^A,0.15 I^B for Somali ethnic groups. The Rhesus blood group allele frequencies of the total sample were 0.884 D and 0.116 d.. There were no significant differences among ethnic groups in blood type frequencies.

Key words: ABO, Antibody, Antigen, Genotype, incompitability, Rhesus

1. INTRODUCTION

The ABO and Rh-D blood group systems are the most commonly utilized grouping systems. The most common blood group classification system is the ABO system which is discovered by an Austrian scientist, Karl Landsteiner in the early1900s (Landsteiner, 1900). He found three different blood groups (A, B, and O) from serological differences in blood. In 1902, von Decastello and Sturli discovered the fourth blood group, AB (von Decastello and Sturli, 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 (RBCs) and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules. Some of the antigens are also present on the surface of other types of cells and body secretions like saliva, sweat, semen, serum, tears, urine etc, which are used in forensic investigations. Several of these RBC surface antigens that stem from one allele or very closely linked genes collectively form a blood group system. Blood groups are genetically determined and exhibit polymorphism in different populations (Daniels, 2005). 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 group systems (Jaf, 2010).

According to the ABO blood group system, there are four different kinds of blood groups: A, B, AB and O. Blood group A has A antigens on the surface of RBCs and anti-B antibodies in blood plasma; blood group B has B antigens on the surface of RBCs and anti-A antibodies in blood plasma; blood group AB has both A and B antigens on the surface of RBCs and no A and B antibodies at all in blood plasma, and blood group O has neither A nor B antigens on the surface of RBCs but it has both anti-A and anti-B antibodies in blood plasma (Daniels, 2005). Many people also have a so called Rh factor on the red blood cells surface. This is also an antigen and those who have it are called Rh positive (Rh^{+}) (Eweidah, 2011).

Those who haven't are called Rh negative (Rh^-). A person with Rh positive blood does not have Rh antibodies naturally in the blood plasma. But a person with Rh negative blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh positive blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh positive blood can receive blood from a person with Rh negative blood without any problems (Eweidah, 2011**).** The Rh is genetically complex but it is simply described in terms of a single pair of alleles, *D* and *d*. Rh positive persons are *DD* or *Dd*, and Rh negative are *dd*. The Rh blood group is determined together with ABO groups because of their relation to hemolytic disease of the newborn (HDN) and their importance in blood transfusion (Khan *et al*., 2009**).** However, 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 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 its contents leak out in the body (Anstee and Tanner, 2009**).** The red blood cells contain hemoglobin which becomes toxic when outside the cell. This can have fatal consequences for the patient. If the donor blood and the recipient blood are not compatible, the RBCs will be linked together, like bunches of grapes, by the antibodies and this clumping could lead to death (Daniels, 2005).

Frequencies of ABO and Rh-D blood groups vary worldwide and are not found in equal numbers even among different ethnic groups. For example, Among African-Americans, the frequency of type O, is 46%; type A, 27%; type B, 20%; and type AB; 7%. In Caucasians of the United State, the frequency is type O, 47%; type A, 41%; type B, 9%; type AB, 3%. Also, among Western Europeans, type O, is 46%; type A, 42%; type B, 9%; and type AB, 3%. Moreover, 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*).

Among Ethiopians, the distribution is that type O, is 42%; type A, is 30%; type B, is 22%; and type AB, is 6 %. Among Ethiopian blood donors, the frequency of type O is 40%; type A, is 31%; type B, is 23%; and type AB, is 6 % (Tibebu, 1998). In population of south west Ethiopia, at Gilgel Gibe Field Research Center, the frequency of O, A, B and AB phenotypes are 42%, 31%, 21% and 6% respectively among a total of 1965 study participants (Abraham *et al*., 2012*)*. The phenotypic frequency of O, A, B, and AB blood groups of Sidama ethnic group was found to be 51.3%, 23.5%, 21.9% and 3.3% , respectively (Tewodros *et al*., 2011).

The study of blood grouping is very important as it plays an important role in genetics, blood transfusion, and 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*., 2004). The need for blood group prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease is being increasingly sought in modern medicine. It is, therefore, imperative to have information on the distribution of these blood groups in any population that comprise different ethnic groups (kumar *et al*., 2009). However, there was no similar study done on the frequency distribution of ABO and Rh-D blood group phenotypes, genotypes and alleles in the mechara town. There have been no sufficient documented data on the distribution pattern and frequencies of the ABO and Rh blood group phenotypes, genotypes and alleles of the Oromo, Amhara, Argoba and Somali ethnic groups in the study area.

Therefore, the aim of this study was to investigate the phenotype, genotype and allele frequencies of ABO and Rh-D blood group systems among students of Oromo, Amhara, Argoba and Somali ethnic groups, who were enrolled in the academic year 2016/17 into Mechara Secondary and Preparatory Schools.

The General objective of this study was:

- To investigate the phenotypic, genotypic and allelic frequencies of the ABO and Rh-D blood group systems among students of Oromo, Amhara, Argoba and Somali ethnic groups in Mechara Secondary and Preparatory School.

The specific objectives of this study were:

- To determine the frequency distribution of the ABO and Rh-D blood group phenotypes among students of the four (Oromo. Amhara, Argoba and Somali) ethnic groups in the study area.
- To estimate the allelic and genotypic frequencies of the ABO and Rh-D blood groups in the populations from the phenotypic frequencies.
- To compare the four populations in terms of phenotypic frequencies of the blood groups.

2. LITERATURE REVIEW

2.1. The ABO blood group system

The classification of blood groups into type A, B, AB, and O in ABO system, Rh-positive and Rh-negative in the Rh system is based on the presence or absence of inherited antigenic substance on the RBC depending on the blood group system (Hasna *et al*., 2010). 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 blood group, MN 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).

The four phenotypes A, B, O, and AB are present in most populations, but their frequencies differ substantially throughout the world. The second most popular blood group system is the Rh factor system. This one refers to whether an Rh antigen is or is not present on surface of the red blood cells. People who are rhesus positive (Rh^+) have a protein known as D antigen on the surface of their red blood cells, and they are said to be Rh-D positive. People who do not have the D antigen are known as Rh-D negative (Rh^-). Rh-positive is the predominant among people but this varies with race; 85% of Caucasians, 94% of Africans, and about 90% of Asians are Rh-positive (Ganong, 2005).

2.1.1. History of ABO blood grouping system

The history of the studies of blood groups dates back to early 20th century. In 1900, Landsteiner described the blood groups A, B, and O, and the presence of Rhesus system was recognized by him in 1939 (Giri, 2011). 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, 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 and Reid, 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 and Reid, 2000).

2.1.2. Various applications of ABO blood groups

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 and Reid, 2000). The ABO blood group antigens remain of prime importance in transfusion medicine; they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is an 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) (Avent and Reid, 2000)

2.1.3. The genetics of ABO blood group system

The ABO locus is located on chromosome 9 at 9p34.1-q34.2 and has three main allelic forms: I^A,I^B and I^O. ABO gene spans about 18-20 kilo bases organized into seven exons. Exons 6 and 7 contain 77% of the full coding region and encode the domain responsible for catalytic activity. Exon 7 contains most of the largest coding sequence. Exon 6 contains the deletion found in most *O* alleles. The exons range in size from 28 to 691 base pair (Daniel and Elizabeth, 2009). The ABO gene codes for the glycosyltransferase that transfers specific sugar residues to H substance, resulting in the formation of A and B antigens. *A* and *B* alleles have seven nucleotide substitutions each.

The antigens A, B and their variants result from functional glycosyltransferase genes capable of transferring N-acetyl-D-galactosamine or D-galactose to the non reducing ends of suitable oligosaccharide chains found on red cell membrane glycoprotein and glycolipids. The red cell phenotype denoted O occurs because the glycosyltransferase gene that generates A or B antigens is inactive (Daniel and Elizabeth, 2009).

The ABO blood type is controlled by a single gene (the ABO gene) with three types of alleles inferred from classical genetics*: i,* I^A, and I^B. I^A and I^B alles are co-dominant, giving the AB phenotype. The gene encodes a glycosyltransferase that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome (9q34). The I^A allele gives type *A*, I^B gives type B, and *i* gives type O. As both I^A and I^B are dominant over i, only ii people have type O blood. Individuals with I^AI^A or I^{Ai} have type A blood, and individuals with I^BI^B or I^{Bi} have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship: co dominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi, I^Ai). The cis-AB phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A1 or B red blood cells, which can help solve the problem of an apparently genetically impossible blood group (Yazer *et al*., 2006).

2.1.4. Blood group antigens and antibodies

The ABO blood groups are defined by the presence of two alternative antigens called A and B on red blood cells, determined by three alternative alleles at a single genetic locus. RBCs of type A have the A antigen on their surface, those of type B have antigen B, type AB red cells bear both antigens, while type O cells bear neither antigen. The blood group substances A and B represent ABO blood group antigens are attached to oligosaccharide chains that project above the RBC surface. two modified forms of a "stem" carbohydrate present on red blood cells and other tissues. These chains are attached to proteins and lipids that lie in the RBC membrane. ABO antigens are glycolipids in nature, meaning they are oligosaccharides attached directly to lipids on red cell membrane (Laura, 2005). The precursor to the ABO blood group antigens present in people of all common blood type is called the H antigen. Individuals with the rare Bombay phenotype (hh) do not express antigen H on their red blood cells. As the H antigen serves as a precursor for producing A and B antigens, the absence of the H antigen means that the individuals also lack A or B antigens as well (similar to O blood group. However, unlike O group, the H antigen is absent; hence the individuals produce isoantibodies to antigen H as well as to both A and B antigens. If they receive blood from someone with O blood group, the anti-H antibodies will bind to the H antigen on the red blood cells ('RBC') of the donor blood and destroy the RBCs by complement-mediated lyses. Therefore, people with Bombay phenotype can receive blood only from other hh donors (although they can donate as though they were type O) (Benjamin and Pierce, 2008). Some individuals with the blood group A may also be able to produce anti-H antibodies due to the complete conversion of the entire H antigen to A1 antigen. Production of the H antigen, or its deficiency in the Bombay phenotype, is controlled at the H locus on chromosome 19.

The H locus is not the same gene as the ABO locus, but it is epistatic to the ABO locus, providing the substrate for the I^A and I^B alleles to modify (Benjamin and Pierce, 2008).
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 *Eiscercia 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 and Raid, 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. The following table shows ABO blood groups antigens and their corresponding antibodies (ISBT, 2008).

Table 1. ABO blood groups antigens and antibodies.

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

2.1.5. ABO incompatibility

If maternal anti-Rh antibodies to fetal red blood cells can damage the RBC of the developing fetus, why is incompatibility for ABO blood groups not as dangerous as Rh negative incompatibility, particularly since ABO isoagglutinins normally exist in mothers which could potentially damage the infant even during a first pregnancy? The answer lies in the isotype of antibody produced in the two cases. Anti-Rh-antibodies are mainly IgG which are capable of crossing the placenta and entering the fetal circulation. The natural antibodies (isoagglutinins) to A and B blood group substances, however, are mostly of the IgM class (typical of anti-carbohydrate responses) and therefore do not cross the placenta. IgG antibodies against the A and B blood group antigens may develop in some individuals, and the resulting ABO incompatibility actually accounts for about two thirds of all discernable cases of HDN. Such cases, however, are generally very mild and

require little or no treatment. Thus, while ABO incompatibility is actually much more common than Rh incompatibility, it is much less likely to cause significant disease (Daniel and Elizabeth, 2009).

2.1.6. Methods of blood typing

Blood typing is performed with anti sera blood serum that contains specific antibodies. For ABO blood typing antibodies against A and B antigen (these antibodies are also called anti-A and anti B antibodies) are used. If clumping or clotting occurs in the test blood upon exposure to the A antibodies (anti-A serum) the blood contains the 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 sera) the type is AB and if no clumping occurs with either serum type, the type is O (Avent, 2009).

Table 2. Agglutination reactions of the RBC ABO blood-typing sera

Reaction		Blood types
A antibody (anti A Serum)	B anti body (anti B Serum)	
Clumping	No Clumping	Type A
No Clumping	Clumping	Type B
Clumping	Clumping	Type AB
No Clumping	No Clumping	Type O

2.1.7. Frequencies of ABO phenotypes in different populations

The ABO 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 anti bodies. 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.*, 2008).

The ABO Blood Group distribution varies among the difference 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 East, falling to single percentage in Swiss. It is believed to have been entirely absent from native America and Austrians Aboriginal population prior to the arrival of Europeans in those areas. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and central Europe, although their highest frequencies occur in some Austrian Aborigine population and the black foot India of Montana (ISBT, 2006).

Among Ethiopians, the distribution is that type O, is 42%; type A, is 30%; type B, is 22%; and type AB, is 6 %. Among Ethiopian blood donors, the frequency of type O is 40%; type A, is 31%; type B, is 23%; and type AB, is 6 % (Tibebu, 1998). In population of south west Ethiopia, at Gilgel Gibe Field Research Center, the frequency of O, A, B and AB phenotypes are 42%, 31%, 21% and 6% respectively among a total of 1965 study participants (Abraham *et al*., 2012*)*. The phenotypic frequency of O, A, B, and AB blood groups of Sidama ethnic group was found to be 51.3%, 23.5%, 21.9% and 3.3% , respectively (Tewodros *et al*., 2011).

The following Table 3 shows the frequency of ABO blood types studied in different populations across the world.

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

Population	A	B	AB	O
Swat, Pakistan	0.1583	0.2832	0.0448	0.5522
Britian	0.4170	0.0860	0.0300	0.4670
Saudi Arabia	0.2400	0.1700+	0.0400	0.5200
India	0.1885	0.3250	0.0990	0.3875
Turkey	0.1220	0.1213	0.0085	0.7398
Hungary	0.2760	0.1218	0.0423	0.5593
Kuwait	0.1608	0.1400	0.02365	0.6678
Nirobi, Kenya	0.1580	0.1261	0.0265	0.6678
Sudan	0.1814	0.1235	0.0268	0.6683
Nigeria	0.2443	0.2443	0.0275	0.4894

2.2. The Rh-D blood group system

The Rh-D blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to ABO, is the most clinically important in transfusion medicine. The ability to clone complementary DNA (cDNA) and sequencing of genes encoding the Rh proteins have led to an understanding of the molecular bases associated with some of the Rh antigens. Serologic detection of polymorphic blood group antigens and of phenotypes provides a valuable source of appropriate blood samples for study at the molecular level (Avent and Reid, 2000). In Rh system, blood groups are designated as Rh-positive or Rh-negative on the basis of presence or absence of Rh antigens on red cell surface.

2.2.1. The discovery of Rh-D blood group

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 T (Landsteiner, 1940). His 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 (Landsteiner K and Wiener, 1941). In 1939, Phillip Levine and Rufus Stetson published in a first case report the clinical consequences of non-recognized Rh fact*or*, hemolytic transfusion reaction and hemolytic disease of the newborn in its most severe form. It was recognized that the serum of the reported woman agglutinated with red blood cells of about 80% of the people although the then known blood groups, in particular ABO were matched. No name was given to this agglutinin when described. In 1940, Karl Landsteiner and Alexander S. Wien*er* made the connection to their earlier discovery, reporting a serum that also reacted with about 85% of different human red blood cells (Landsteiner K and Wiener, 1940).

Based on the serologic similarities Rh factor was later also used for antigens, and anti-Rh for antibodies, found in humans such as the previously described by Levine and Stetson. Although differences between these two sera were shown already in 1942 and clearly demonstrated in 1963, the already widely used term "Rh" was kept for the clinically described human antibodies which are different from the ones related to the rhesus monkey. This Rh factor found in rhesus macaque was classified in the Landsteiner-Wiener antigen system (antigen LW, antibody anti-LW) in honor of the discoverers (Scott ML, 2004). It was recognized that the Rh factor was just one in a system of various antigens. Based on different models of genetic inheritance, two different terminologies were developed; both of them are still in use.

The clinical significance of this highly immunizing D antigen (i.e. Rh factor) was soon realized. Some keystones were to recognize its importance for blood transfusion including reliable diagnostic tests, and hemolytic disease of the newborn including exchange transfusion and very importantly the prevention of it by screening and prophylaxis.

2.2.2. Frequencies of Rh-D blood group phenotypes in different populations

Rh-D 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 4. Frequency of Rh-D blood groups studied in different population across the world.

Population	Rh-positive	Rh-negative	Reference
Britain	0.8300	0.1700	Khattak *et al.,* (2008)
USA	0.8500	0.1500	Khattak *et al*.,(2008)
Kenya	0.8030	0.1970	Lyko *et al*(1992)
Saudi Arabia	0.9300	0.0700	Khattak *et al.,* (2008)
Germany	0.9500	0.0500	Akbase *et al.,* (2008)
India	0.9445	0.0555	Khattak *et al* (2008)

2.2.3. The antigens and antibodies of Rh-D blood group system

The proteins which carry the Rh antigens are transmembrane proteins, whose structure suggest that they are ion channels (Rai *et al*., 2009). The main antigens are D, C, E, c and e, which are encoded by two adjacent gene loci, the *Rh-D* gene which encodes the Rh-D protein with the *D* antigen and variants and the *RhCE* gene which encodes the RhCE protein with the C, E, c and e antigens and variants (Daniels, 2002).There is no d antigen. Lowercase "*d*" indicates the absence of the *D* antigen (the gene is usually deleted or otherwise nonfunctional).

Unlike the anti-A and anti-B antibodies, anti-D antibodies are only seen if a patient lacking *D* antigen is exposed to *D*+ cells. The exposure to D+ cells usually occurs through pregnancy or transfusion. Rh positive cells infused into an Rh negative recipient can give rise to a strong antibody response, mainly of the IgG class, which can result in

dangerous reactions to subsequent transfusions. Blood typing and cross matching are therefore important to ensure compatibility for the Rh factor as well as ABO.

However, unlike the A and B antigens, the Rh antigens are present only on red blood cells. Therefore, while they are important for blood transfusion, they do not normally play a role in organ transplantation, and Rh typing of organ donors and recipients therefore not a significant consideration (Laura, 2005). Rh phenotypes are readily identified by identifying the presence or absence of the Rh surface antigens. Most of the Rh phenotypes can be produced by several different Rh genotypes. The exact genotype of any individual can only be identified by DNA analysis. Regarding patient treatment, only the phenotype is usually of any clinical significance to ensure a patient is not exposed to an antigen they are likely to develop antibodies against. A probable genotype may be speculated on, based on the statistical distributions of genotypes in the patient's place of origin (Laura, 2005).

Rh antibodies are IgG antibodies which are acquired through exposure to Rh-positive blood (generally either through pregnancy or transfusion of blood products). The *D* antigen is the most immunogenic of all the non-ABO antigens. Approximately 80% of individuals who are D-negative and exposed to a single D-positive unit will produce an anti-D antibody. The percentage of alloimmunization is significantly reduced in patients who are actively exsanguinating (some say to approx 15%) (Brecher.ME, 2005).

All Rh antibodies *except D* display dosage (antibody reacts more strongly with red cells homozygous for an antigen than cells heterozygous for the antigen (*EE* stronger reaction Vs *Ee*).

If anti-E is detected, the presence of anti-c should be strongly suspected (due to combined genetic inheritance). It is therefore common to select c-negative and E-negative blood for transfusion patients who have an anti-E. Anti-c is a common cause of delayed hemolytic transfusion reactions (Mais, 2009)

2.3. The biological significance of blood group polymorphism

Very little is known about the biological significance of the polymorphisms that make blood groups alloantigen. In any polymorphism one of the alleles is likely to have, or at least to have had in the past, a selective advantage in order to achieve a significant frequency in a large population, though genetic drift and founder effects may also have played a part. Glycoprotein and glycolipid carrying blood group activity are often exploited by pathogenic micro - organisms as receptors for attachment to the cells and subsequent invasion; surviving certain parasite possibly being the most significant force affecting blood group expression. In some cases, however, selection may have nothing to do with red cells; the target for the parasite could be other cells that carry the protein. It is likely that most blood group polymorphism is a relic of the selective balances that can result from mutations making cell surface structures less suitable as pathogen receptors and resultant adaptation of the parasite in response to these selective pressures. It is important to remember that whilst blood group polymorphism undoubtedly arose from the effects of selective pressures, these factors may have disappeared long ago, so that little hope remains of ever identifying (Anstee, 2010).

Despite, there obvious clinical and transfusion medicine ABO blood group has great importance in physiology, people with a blood type O express neither A nor B antigen, nor they are perfectly healthy. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease. For example, stomach ulcer more common in group O individuals, gastric cancer in group A individuals. Another observation is that individuals with blood type O tend to have lower levels of the von Willebrand Factor (vWF), which is a protein involved in blood clotting (Laura, 2005)

2.4. 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, as you can guess, 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., however, the genotypic frequencies eventually take a form that depends only on the allele frequencies (Sarhan *et al*., 2009).

2.5. The Hardy-Weinberg Principle and its Assumptions

The principle of HWE believe that, if there is infinite population, discrete generations ,random mating, no selection, no migration, no mutation and equal initial genotype frequencies in the two sexes the population is homogenous or at HWE. Hence

Modified Hardy- Weinberg equation is used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies (Strickberger, 1976). 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 $p2 + 2pq + q2 = 1$, 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 were represented by trinomial expansion as $(P+q+r)\ 2 = p2 + 2pq + q2 + 2pr + 2qr + r2 = 1$ (Hanania *et al.*, 2007), where:

P2 is the frequency of genotype I^AI^A

*q2 i*s 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

r2 is the frequency of genotype I^OI^O.

ABO allele frequencies was estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates was

calculated as: $p = 1 - \sqrt{B+O}$, $q = 1 - \sqrt{A+O}$, $r = \sqrt{O}$, where (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) was calculated according to $\theta = 1 - p - q - r$. The final allele frequencies was then be calculated as follows: $p1 = p(1 + \theta/2)$; $q1 = q(1 + \theta/2)$; $r1 = (r + \theta/2)(1 + \theta/2)$ where *p1*, *q1*, and *r1* denote corrected allele frequencies. Rh-D allele frequencies were calculated according to the Hardy-Weinberg equation (Al-Arrayed *et a*l., 2001).

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium was tested using chi-square test to check whether population was at Hardy-Weinberg genetic equilibrium or not (Chakraboty, 2011).

Frequencies of Rh-D 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 will be represened as $(p + q)^2 = p2 + 2pq + q2 = 1$, where *p2* is frequency of genotype *DD*, 2*pq* is frequency of genotype *Dd* and *q2* is frequency of genotype *dd* (Dar *et al.,* 2010).

The assumptions of HWE behind the principle is that in the presence of infinite population, discrete generations ,random mating and in the absence of selection, migration and mutation populations are assumed to be at HWE.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Mechara Secondary and Preparatory School at Mechara town, which is one of the schools in West Hararghe zone of Oromia Regional State in Darolabu werede.

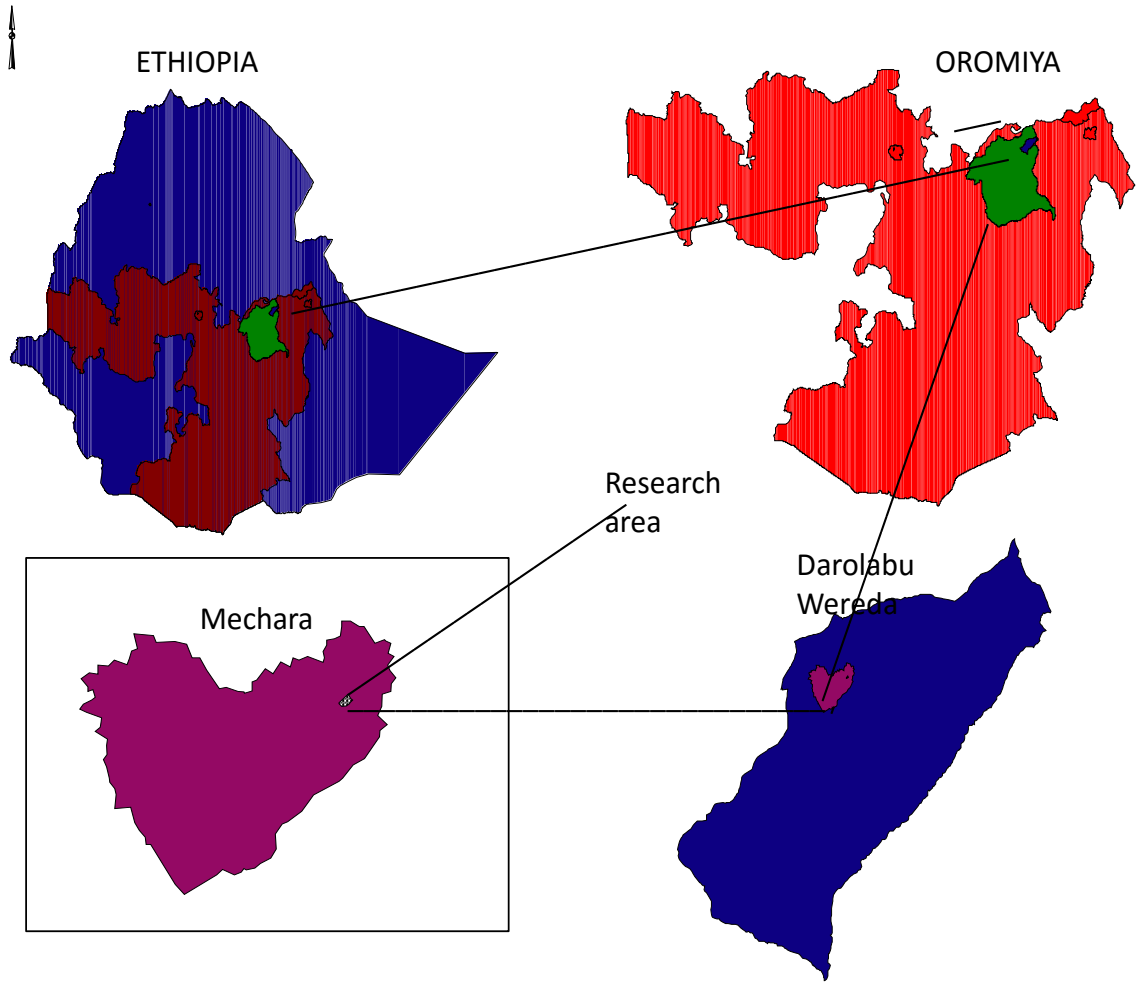

Figure 1: Map of the study area

Mechara town is found at a distance of 436kms and 114kms to the east of the capital city of the country, Addis Abeba and capital city of West Hararghe Zone, Chiro, respectively.

Mechara town is located astronomically at latitude of 8° 15′00″N- 8° 43′00″N and longitude of 40°17′00″E-40°45′00″E and has an altitude that ranges from 1147-1230m above Sea level with an average minimum and maximum temperature of 18.28°C and 31°C, respectively. The average annual rainfall of the town is 950mm.The current (2017) population size estimate for the town, based on the 2.9% (for Oromia) increase per year (CSA, 2007) is around 26,467, of whom 13860 (52.36) were females and 12,607 (47.64%) were males. The town is (2017) primarily (65%) inhibited by Oromo people, whereas other of the inhabitants are identified as Amhara (15%), Argoba (10%), and Somali (8%) and the rest (2%) are classified as "Other Ethiopian National Groups" according to the information from the Communication Bureau of the district.

All ethnic groups live together by respecting each other's language, religion ,culture and other differences. There were free mate selection (intermarriage) among four ethnic groups in the study area. There has been unity in diversity in the town.

3.2. The study Population

The study population was the students of Mechara secondary and preparatory school who were registered for the academic year 2016/17. A total of 897 students have been enrolled during the beginning of the academic year; of which 478 (53.28%) were males and 419 (46.71%) were females. The study was carried out with a total of 449 students, of whom 173 students were from Oromo, 115 students were from Amhara, 91 students were from Argoba and 70 students were Somali ethnic groups and most of the students were from Mechara town.

3.3. Inclusion and Exclusion criteria

Inclusion criteria

Any volunteer students of all age and sexes belonging to any of the four ethnic groups (Oromo, Amhara, Argoba and Somali), until the sample size reaches the desired numbers.

Exclusion criteria

Students of other ethnic groups were excluded by informing them about the objectives of the study, because they are not significant in numbers.

3.4. Study Participants and Selection method

The study involved a total of 449 (50%) voluntary students belonging to the four ethnic groups (173, 115, 91 and 70 from Oromo, Amhara, Argoba and Somali ethnic groups, respectively). Participants were selected purposely from the four ethnic groups based on their willingness to participate in the study. The researcher explained the purpose and procedures of the study to the students before asking them to participate. Then voluntary students gave their ethnic information (orally) for inclusion or exclusion.They were instructed also to withdraw from participation at any time from the study in case they want to do so.

3.5. Blood Sample Collection and Typing

Blood sample was collected by pricking the finger-tip of participants with sterile disposable blood lancet (one for each participant) to identify blood groups of the students. The ABO and Rhesus blood grouping were done using the open slide method. A drop of blood was placed on each of three places on a single clean dry glass slide for each participant. Then a drop of anti sera (anti-A or anti-B or anti-D) was added to each blood drop in that order and each was mixed with the antiserum with the aid of a plastic stick. Blood groups were determined on the basis of agglutination reaction. Each of which blood types were classified as negative or positive, which was the reference to the blood's Rhesus factor (Theresa *et al*., 2004). Blood type were scored as A, B, AB, and O blood types and each blood type were further classified as Rh negative or positive based on the formation of agglutination with anti-A,anti -B and anti-D to the three drops of blood. Blood collection and typing was done by a trained Laboratory Technician.

3.6. Methods of data analysis

In this study, first, the distribution of blood types among students of the four ethnic groups in Mechara Secondary and Preparatory School were expressed in sample percentage and frequencies. Modified Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies of ABO and Rh-D blood groups from phenotypic frequencies (Strickberger, 2003).

Allele frequencies were calculated by considering two alleles at the same locus for Rh system and three alleles at the same locus for ABO using standard formulae of quantitative genetics. ABO alleles were estimated according to a published method which yields results that are closer to maximum likelihood estimate (Strickberger, 2003). Preliminary estimates were calculated as: p=1-√B+O, q=1-√A+O, r=√O where p, q, r denote allele frequencies for I^A, I^B, and $I,^O$ respectively and A, B, O denote observed frequencies of blood types A, B, and O. A correction factor (ɵ) was calculated as ɵ=1-p-q-r. The final allele frequencies were then calculated as follows*:* $p_1=p$ (1+ ɵ/2); $q_1=q$(1+ ɵ/2); r_1=(r+ ɵ/2) (1+ ɵ/2),where p_1,q_1,and $r_1$denote corrected allele frequencies(Al-Arrayad *et al.,* 2001). Frequency of the two Rh-D blood group alleles (p and q) were determined as follows*:* $q = \sqrt{Rh^-}$, $P = 1 - q$, where Rh- is the blood's Rhesus factor. From the allele frequencies genotype frequencies were calculated assuming the populations are in Hardy-Weinberg Equilibrium (Chakraborty, 2010).

Expected phenotype frequencies were calculated from the genotype frequencies where genotypes I^{AA},I^{AO}, I^{BB},I^{BO},I^{AB} and I^{OO} are classified as homozygous or heterozygous type. Chi-square tests (goodness of fit) were done to test the goodness–of fit between the observed and expected phenotype frequencies (Chakraborty, 2010).

The genotypic frequencies of ABO blood groups are calculated as follows:

- Genotype $I^{AA} = p2$
- Genotype $I^{AO} = 2pr$
- Genotype $I^{BB} = q2$
- Genotype $I^{BO} = 2qr$

- Genotype $I^{AB} = 2pq$
- Genotype $I^{OO} = r2$

The genotypic frequencies of Rh-D blood groups are calculated as follows

- Genotype $DD = p2$
- Genotype $Dd = 2pq$
- Genotype $dd = q2$

The deviations between the observed and Hardy-Weinberg equilibrium expected values were tested using chi-square test (goodness of fit) to check whether population was at Hardy- Weinberg genetic equilibrium or not.

The differences in the phenotypic frequencies of ABO and Rh-D blood groups among the four ethnic groups were statistically tested using the contingency chi-squared formula as follows:

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

Where O=Observed phenotypic count

E= Expected phenotypic count (calculated by multiplying genotype frequency with the total sample size in each case).

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

- For B blood group E = frequency of (*BB* + *BO*) X number of total sample
- For AB blood group E = frequency of *AB* X number of total sample
- For A blood group E = frequency of *OO* X number of total sample

The observed phenotype frequencies were calculated as

- Observed frequency = Observed number/Total number
- Observed percentage = Observed number x 100/Total number

3.7. Ethical consideration

An authorization to carry out the study was obtained from the Health office of the district

All information that was obtained about the subjects were kept confidential.

4. RESULTS AND DISCUSSIONS

4.1. ABO and Rh-D Phenotype Frequency

The phenotype frequencies of the ABO and Rh-D blood group systems for the whole sample (n=449) and for each of the four ethnic groups in this study are presented in the following section.

4.1.1. ABO Blood Group Phenotype Frequency

Table 5. Frequencies of ABO blood group phenotypes among the students of Oromo, Amhara, Argoba and Somali ethnic groups

	ABO Phenotypes					
Ethinic Groups	Count of type-O (%)	Count of type-A (%)	Count of type-B (%)	Count of type-AB (%)	$\chi 2$	P- value
Oromo (n=173)	73 (42.19)	55 (31.80)	32 (18.50)	13 (7.51)	0.835	0.05
Amhara (n=115)	52 (45.22)	31 (26.96)	23 (20.00)	9 (7.83)	0.481	0.05
Argoba (n=91)	37 (40.66)	22 (24.18)	21(23.08)	11 (12.09)	3.054	0.05
Somali (n=70)	27 (38.57)	23 (32.86)	16 (22.86)	4 (5.71)	1.222	0.05
Total (N=449)	**189 (42.10)**	**131 (29.18)**	**92 (20.49)**	**37 (8.24)**	**5.560**	0.05
$\chi 2$	**0.506**	**1.098**	**1.121**	**2.383**		
P- Value	**0.08**	**0.05**	**0.04**	**0.01**		

In all of the four ethnic groups as well as in the total sample, blood group O has the highest frequency while blood group AB has the least frequency (Table 5). Several studies have also revealed 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.For example, 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% in 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 6% type-AB (Abraham *et al.,* 2012). Among Sidama ethnic group (Ethiopia), the distribution was type-O, 51.3%; type-A, 23.5%; type-B, 21.9%; and type-AB, 3.3% (Tewodros *et al*., 2011). Therefore the result of this study was in accordance with the data from previous studies of most of the Ethiopian populations (Tewodros *et al*., 2011). .

Other reports from similar studies in different parts of the world also show consistent results with the results of the this study in that O>A>B>AB. For example, in Ogbomoso, South-west Nigeria, phenotypic frequencies of ABO blood groups were 50% for O; 22.9% for A; 21.3% for B and 5.9% for AB among 7653 individuals sampled (Bakare *et al.,* 2006). In Britain (Anees, 2007) the frequencies of the ABO blood group were 41.7% , 8.6% , 3% and 46.7% for A, B , AB and O blood groups, respectively. Frequencies of 55.3%, 25.3%, 16.7 % and 2.7% for O, A, B, and AB, respectively were also obtained among 150 students of Cell Biology and Genetics at the University of Lagos, Nigeria (Adeyemo and Soboyejo, 2006). Among Western Europeans, 46% have group O , 42% group A, 9% group B, and the remaining 3% group AB (Iyiola *et al*., 2011). Among the Caucasians in the United States of America, the frequency of blood type-O, -A, -B and -AB are 47%, 41%, 9% and 3%, respectively (Adeyemo and Soboyejo, 2006). Among African Americans, the distribution is type-O, 46%; type-A, 27%; type-B, 20%; and type-AB; 7% (Iyiola *et al*., 2011).

However, the results of this study are different from the results of some Asian countries where blood type-B has the highest frequency in some and blood type-A in the others. For example, the highest frequency of A blood type was documented in Jordan populations (Hanania *et al*., 2007); and Saudi Arabian populations (Khan *et al.*, 2006). Highest frequency of type-B blood was also reported in Pakistani populations (Khan *et al*., 2009; Rahman and Lodhi, 2004; Khan *et al*., 2004 and 2006) and Mahamood *et al*., 2005) and in Indian population (Giri *et al*., 2011; Warghat *et al*., 2011, and Rai *et al*.,2009). When one compare the frequency distribution of ABO blood phenotypes among the four ethnic groups, blood types O has highest frequency in Amhara (45.21%) and Oromo (42.19%) ethnic groups than in Argoba (40.65%) and Somali (38.57%) ethnic groups. Blood group A has the highest frequency in Somali (32.85%) and Oromo (31.79%) than in Amhara (26.95%) and Argoba (24.17%) ethnic groups. Blood group B has the highest frequency in Argoba (23.07%) and Somali (22.85%) than in Amhara (20%) and Oromo (18.49%) ethnic groups (Table 5). The highest percentage of type-AB was observed in Argoba (12.08%) whereas the least was observed in Somali group (5.71%) (Table 5). But the differences in blood type frequencies among the ethnic groups are not significant (χ^2=0.835 for oromo, 0.4811 for Amhara, 3.054 for Argoba and 1.222 for Somali with df=3, P=0.05) (Table 5). This indicates that the population is homogenous which might be due to random intermarriage among the four ethnic groups and small sample sizes used.

4.1.2. Rh-D blood group phenotype Frequency

The frequency distribution of Rh-D blood group phenotypes of the four ethnic groups are shown in the following Table 6.

Table 6. Frequencies of Rh-D blood group phenotypes among the students of Oromo, Amhara, Argoba and Somali ethnic groups

Ethnic groups	Rh-D phenotypes		Total	$\chi 2$	P- Value
	$Rh\text{-}D^+$	$Rh\text{-}D^-$			
Oromo	171 (98.84%)	2(1.15%)	173	0.042	0.05
Amhara	114 (99.13%)	1(0.87%)	115	0.192	0.05
Argoba	91(100%)	0(0%)	91	1.226	0.05
Sumale	67(95.71%)	3(4.28%)	70	0.723	0.05
Total	**443 (98.42%)**	**6 (6.3%)**	**449**	**2.01**	**0.05**
$\chi 2$	0.0991	0.521			
P-value	0.05	0.05			

The above table 6 shows that Rh-positive blood has the higher percentage frequencies (444) (98.66%) than Rh negative (6) (1.34%) in the total sample as well as in each of the four ethnic groups ranging from 95.714% in Somali to 100% in Argoba (X^2 =0.042 for Oromo, 0.192 for Amhara, 1.226 for Argoba, 0.723 for Somali, df=2, p=0.05) (Table 6). The variations in the frequency distribution of Rh+ and Rh^- among the four ethnic groups followed the same pattern in that Rh+ follow same pattern with O and Rh^- follow same pattern with AB blood group as shown in Table 6. These results agreed with previous study of 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 Rh-D positive was found to be higher in the population sampled than the Rh-D negative (Bakare *et a*l., 2006). Rh-D-negative blood type was recorded as 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria, 7.3% in Lahore, 7.7% in Rawalpindi. About 95 African-Americans are Rh-positive (Chavhan *et al.,* 2010). The difference in the frequencies of Rh-D phenotypes among the four ethnic groups was not significant (χ^2=2.01, df=2, P>0.05%).

4.1.3. The Combined distributions of the ABO and Rh-D blood group phenotypes

The following Table 7 describes combined distributions of the ABO and Rh-D blood group phenotypes among students of Oromo, Amhara, Argoba and Somali ethnic groups

Table 7. The Combined distributions of the ABO and Rh-D blood group phenotypes of the four ethnic groups

Blood groups		Ethnic groups			
		Oromo	Amhara	Argoba	Somali
ABO	Rh-D	Count (%)	Count (%)	Count (%)	Count (%)
O	RhD^+	71 (41.04)	52 (45.22)	37 (40.65)	26 (37.14)
	RhD^-	2 (1.15)	0 (0)	0 (0)	1 (1.42)
A	RhD^+	55 (31.79)	31 (26.95)	22 (24.2)	21 (30)
	RhD^-	0 (0)	0 (0)	0 (0)	2 (2.86)
B	RhD^+	32 (18.49)	22 (19.13)	21 (23.07)	16 (22.86)
	RhD^-	0 (0)	1 (0.86)	0 (0)	0 (0)
AB	RhD^+	13 (7.51)	9 (7.82)	11 (12.08)	4 (5.71)
	RhD^-	0 (0)	0 (0)	0 (0)	0 (0)
Total		**173 (100)**	**115 (100)**	**91 (100)**	**70 (100)**

The above Table 7 shows the distributions of the combined ABO and Rh-D blood groups of the four ethnic groups. In all of the four ethnic groups (with varied percentage), blood type O^+ (71 in Oromo,52 in Amhara, 37 in Argoba and 26 in Somali) phenotype has the highest frequency whereas the AB^- phenotype was not observed in all of the four ethnic groups. In Amhara and Argoba ethnic groups there were no O^- phenotypes. The highest frequency of O^- phenotypes (2) (1.15%) were observed in Oromo ethnic group. The A^- phenotype (2) (2.86%) were seen only in Somali ethnic group while B^- (1) (0.86%) was seen only in Amhara ethnic group. AB^- phenotype was not observed in all of the four ethnic groups.

The percentage distribution of Rh negative is very rare in the four ethnic groups .These might be attributed to small size of each sample used from the four ethnic groups.

The following table 8 shows distributions of the combined ABO and Rh-D blood group phenotypes in the overall sample.

Table 8. The combined frequency distributions of ABO and Rh-D blood group phenotypes in the overall sample

ABO Blood Group	Rh-D Blood Group	
	Count Rh-D^{+ve} (%)	Count Rh-D^{-v}e (%)
O	186 (41.42)	3 (0.668)
A	129 (28.73)	2 (0.445)
B	91 (20.26)	1 (0.222)
AB	37 (8.24)	0 (0)
Total	**443 (98.66)**	**6 (1.34)**

The prevalence of the ABO blood groups with respect to Rh positive phenotypes in the overall sample were 186 (41.42%), 129 (28.73%), 91(20.26%), and 37 (8.24%) for O^+, A^+, B^+ and AB^+ blood phenotypes respectively (Table 8). For Rh negative phenotypes, the frequencies were 3 (.668%), 2 (0.445%), 1 (0.222 %), and 0 (0 %) for O^-, A^-, B^- and $AB,^-$ respectively (Table 8). In both Rh positive and Rh negative, phenotypes O blood group has the highest prevalence while AB blood group has the lowest prevalence.

This illustrates that Rh-D Positive and Rh-D negative incidences were recorded highest in O blood group, followed by A, B and AB (Bakare *et a*l., 2006).

4.2. Allele And Genotype Frequencies of ABO and Rh-D Blood Groups

4.2.1. Allele And Genotype Frequencies of ABO blood groups

Allele and Genotype frequencies of ABO blood groups among the Students of the four ethnic groups in this study are Presented in the following Table 9.

Table 9. Allele and Genotypic frequency of ABO blood groups among the students of Oromo, Amhara, Argoba and Somali ethnic groups

Ethnic groups	Allele frequency			Genotypic frequency					
	P (I^A)	*q* (I^B)	*r* (I^O)	(p_2) $I^A I^A$	(2pr) $I^A I^O$	(q_2) $I^B I^B$	(2qr) $I^B I^O$	(2pq) $I^A I^B$	(r2) $I^O I^O$
Oromo	0.22	0.14	0.64	0.048	0.281	0.019	0.179	0.061	0.409
Amhara	0.19	0.15	0.66	0.036	0.251	0.022	0.17	0.057	0.435
Argoba	0.20	0.19	0.61	0.04	0.244	0.036	0.231	0.076	0.372
Somali	0.22	0.15	0.63	0.048	0.277	0.022	0.189	0.066	0.396
Total	**0.21**	**0.16**	**0.64**	**0.044**	**0.256**	**0.025**	**0.202**	**0.067**	**0.409**

The frequencies of alleles I^A, I^B, and I^O were calculated according to the modified Hardy - Weinberg Law of equilibrium based on data presented in Table 9.

As indicated in the above table 9, the frequencies of ABO blood group alleles of the four ethnic groups were 0.22 I^A, 0.14 I^B, and 0.64 I^O in Oromo ethnic group, 0.19 I^A, 0.15 I^B, and 0.66 I^O in Amhara ethnic group, 0.20 I^A, 0.19 I^B, and 0.61 I^O in Argoba ethnic group and 0.22 I^A, 0.15 I^B, 0.63 I^O in Somali ethnic group. The order of allele frequencies of ABO blood group in each of the ethnic groups as well as in the overall sample were $I^O > I^A > I^B$. The allele frequencies of the ABO blood groups in the overall sample were 0.21I^A, 0.16 I^B, and 0.64 I^O (Table 9).

These results are in accordance with the other previous studies in the southern and central parts of Iraq which stated that the gene frequencies were (I^A = 0.19 , I^B = 20 and i = 0.61) in Basrah (Ferhan, 1970) and (I^A = 0.187 , I^B = 0.22 and i = 0.59) in Baghdad (Al-Rubeai, 1975) respectively,and also with the results of (Sadik, 1989) in Nianwa , the northern part of Iraq which stated that the allele frequencies were in the order of (i > I^A >I^B), where (i = 0.647 , I^B =0.172, I^A = 0.194).

The estimates of the chi-square test for allele frequencies of ABO blood group system showed no significant differences among the four ethnic groups (X^2=0.32, df=6, P>95%). This might be due to the fact that the four ethnic groups are found within the same geographical area, small size of sample used from each of the ethnic group and there may also intermarriage among them.

The genotypic frequencies for ABO blood groups of 449 students were calculated based on estimated allele frequencies according to the Hardy-Weinberg Law.

The above Table 9 shows the frequencies of the various genotypes in the ABO blood group system of the four ethnic groups.

As seen from the above table 9, the overall frequencies of ABO genotypes in the sample were as follows: 0.044(4.44%) I^AI^A, 0.256 (25.6%) I^AI^O, 0.025 (2.56%) I^BI^B, 0.202 (20.2%) I^BI^O, 0.0672 (6.72%) I^AI^B and 0.396 (40.9%) I^OI^O (Table 8). If we take blood type-A, the frequency of I^AI^A genotype was 0.044 while that of I^AI^O genotype was 0.256. Hence it is possible to deduce that, among those who are blood group A, 15% were homozygous I^AI^A while about 85% were heterozygous I^AI^O. The same deduction was made for B blood group, which was 11% I^BI^B and 89% I^BI^O (Table 9). As shown in table 9, in all of the four ethnic groups and in the overall data, genotype I^OI^O has the highest frequency while genotype I^BI^B has the least frequency (Table 9).

Other studies from different parts of the world also report similar results with this findings. For example, the results reported by Irshaid *et al*., (2002), Iyiola *et al*., (2011), Hanania *et al*., (2007), and Bakare *et al*., (2006) shows similar results to this study.

4.2.2. Allele And Genotype Frequencies of Rh-D blood groups

Allele and Genotype frequencies of the Rh-D blood groups among the Students of the four ethnic groups in this study are Presented in the following Table 10.

Table 10. Allele and Genotype frequencies of the Rh-D blood groups among the Students of the four ethnic groups in the study area

Ethnic groups	Allele frequency		Genotypic frequency		
	D (p)	d (q)	DD (p2)	Dd (2pq)	dd (q2)
Oromo	0.89	0.11	0.792	0.195	0.012
Amhara	0.907	0.093	0.822	0.168	0.008
Argoba	1	0	1	0	0
Somali	0.783	0.207	0.613	0.324	0.042
Total	**0.884**	**0.116**	**0.781**	**0.205**	**0.013**

The allele frequencies of Rh-D blood group were also calculated according to the Hardy-Weinberg equation using the data presented in the above Table 10. The frequencies of allele *D* and *d* are found to be 0.89 and 0.11, 0.907 and 0.093, 1 and 0 and 0.793 and 0.207 in Oromo, Amhara, Argoba and Somali ethnic group respectively (table 10).

The overall Rh-D allele frequencies in the sample also shows similar patterns with *D*=0.884 and *d*=0.116.

This shows that allele *D* (0.884) has higher frequency than allele *d* (0.116). This result also agrees with many studies where Rh positive has higher incidence than Rh negative in different populations and ethnic groups (Nwauche and Ejele, 2004; Bakare *et a*l., 2006). For example, The study carried out in Missan University of Iraq shows the result of Rh factor as 88.6% , 11.3% for Rh^+ and Rh^- respectively in Missan population , with

frequency of 0.6633 for allele *D* and 0.3367 for *d* respectively (Hasna, Amer M. 2010). The difference in allele frequencies of Rh-D blood group among the the four ethnic groups was also insignificant (X^2= 0.047,df=2, P>95%).

Genotypic frequencies of the Rh-D blood group systems were calculated from it's allele frequencies as shown in the above table (Table 10). The frequencies of the genotypes for Rh-D blood group in the overall data were 0.781, 0.205, 0.013 for *DD, Dd* and *dd* genotypes respectively. Similar pattern of distributions (but with different frequencies) were observed in each of the four ethnic groups, in which the frequency of genotype *DD* > *Dd* >*dd* (Table 10).

4.3. Observed Versus Expected Values of ABO And Rh-D Blood Group Phenotypes

Observed versus expected values of ABO and Rh-D blood group phenotypes among the four ethnic groups were presented in the following Table 11 and 12 respectively.

Table 11 . Observed versus expected frequency of ABO blood group phenotypes among the students of Oromo,Amhara,Argoba and Somali ethnic groups

Ethnic Groups	**ABO blood System**				
	Blood Groups	Observed No (O)	Expected No(E)	Differences D=(O-E)	D^2/E
Oromo	A	55	56.744	1.744	0.054
	B	32	34.254	2.254	0.148
	AB	13	10.726	2.274	0.481
	O	73	70.757	2.243	0.072
		173			χ^2 = 0.755
Amhara	A	31	33.000	2.000	0.121
	B	23	23.000	0.000	0.000
	AB	9	6.555	3.555	1.928
	O	52	50.025	2.025	0.082
		115			χ^2 =2.131
Argoba	A	22	25.844	3.844	0.572
	B	21	24.297	3.297	0.447
	AB	11	6.916	5.916	5.061
	O	37	33.852	4.852	0.685
		91			χ^2 =6.765
Somali	A	23	22.751	1.751	0.135
	B	16	14.771	2.771	0.519
	AB	4	4.621	0.621	0.083
	O	27	27.721	0.721	0.018
		70			χ^2 = 0.775

Table 11 above shows observed versus the expected values of ABO blood group phenotypes in the four ethnic groups. The deviations between the distributions of

observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square. The calculated chi-square test of ABO blood groups in the four ethnic groups above were 0.755,2.731,6.765 and 0.775 in Oromo, Amhara and Argoba and Somali ethnic groups respectively ($p < 0.05$,df=3). The result shows that the ABO blood group distribution between observed and expected in each ethnic group did not show significant difference.

The distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy- Weinberg equilibrium (Goodness of fit $\chi^2 = 3.475$, df=3, $p < 0.05$). This shows that the population is at HWE equilibrium.

The following Table 12 shows observed versus expected values of Rh-D blood groups among the students of Oromo,Amhara,Argoba and Somali ethnic groups.

Table 12. Observed versus expected frequency of Rh-D blood groups phenotypes among the students of the four ethnic groups

Ethnic Groups	**Rh(D) blood System**				
	Blood Groups	Observed No (O)	Expected No(E)	Differences D=(O-E)	D^2/E
Oromo	Rh(D)+ve	171	170.75	0.25	3.63×10^{-4}
	Rh(d)-ve	2	2.11	0.11	5.73×10^{-3}
		173			6.09×10^{-3}
Amhara	Rh(D)+ve	114	113.85	0.15	1.97×10^{-4}
	Rh(d)-ve	1	1.15	0.15	1.95×10^{-2}
		115			1.97×10^{-2}
Argoba	Rh(D)+ve	91	91	0	0
	Rh(d)-ve	0	0	0	0
		91			0
Somali	Rh(D)+ve	67	65.59	1.41	3.03×10^{-2}
	Rh(d)-ve	3	2.99	0.01	3.34×10^{-5}
		70			3.30×10^{-2}

As indicated in the above Table 12 the calculated chi-square test for the four ethnic groups were 6.09×10^{-4}, 1.97×10^{-2}, 0 and 3.30×10^{-2} in Oromo,Amhara,Argoba and Somali ethnic groups respectively ($p < 0.05$, df=1)) (Table 12).

The variation of distribution of the overall observed frequencies of Rh-D blood group phenotypes from those expected under Hardy-Weinberg equilibrium were also in significant (Goodness of-fit $X^2= 5.88X10^{-2}$, df=1**,** P>95%) (Table 12).

This means that there is statistically insignificant difference between observed and expected value in the four ethnic groups.

The chi-square test value of ABO and Rh (D) blood groups for the four ethnic groups calculated above are insignificant, this shows that populations of the four 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

A total of 449 voluntery students of Mechara Secondary and Preparatory School from four selected ethnic groups were voluntarily 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 173,115,91, and 70 students from Oromo, Amhara, Argoba and Somali ethnic groups respectively. 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 the ABO antisera blood grouping reagents while Rh blood group was determined using Anti-D monoclonal blood grouping reagents.

The frequencies of ABO and Rh-D blood group phenotypes were expressed in sample percentages and Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies from phenotypic frequencies. In each of the four ethnic groups and in the total sample, blood group O has the highest frequency and blood group AB has the lowest frequency. Similarly Rh-D positive blood group has the highest frequency while Rh-D negative blood has the lowest frequency. The frequencies of ABO blood group phenotypes of students in the overall sample were 42.1% O, 29.2% A, 20.48 % B and 8.24% AB. The frequencies of Rh-D blood groups were 98.66 % Rh-D positive and 1.34% Rh-D negative. Similar patterns of variation were obtained in each of the four ethnic groups. In all of the four ethnic groups and in the total sample the order of ABO blood group alleles were $I^O > I^A > I^B$. The frequencies of allele *D* and *d* for Rh-D blood group were 0.884 and 0.116 respectively in the total sample.

Regarding the genotypic frequencies of ABO blood group in each of the four ethnic groups and in the overall sample genotype I^OI^O has the highest frequency and I^BI^B has the least frequency. In the Rh-D blood group, frequency of genotype *DD, Dd* and *dd* was 0.781, 0.205 and 0.013 respectively in the overall sample.

5.2. Conclusions

The distribution of ABO blood groups and Rh factors among students of mechara secondary and preparatory school were as follows : O blood group record the highest frequency followed by blood groups A, B and AB respectively, while the Rh^+ record the highest rhesus phenotype frequency. In all of the four ethnic groups as well as in the total sample, the order of the frequencies of ABO blood group alleles is $I^O > I^A > I^B$. In Rh-D blood type, frequency of allele *D* is higher than frequency of *d* allele.

The distribution of ABO and Rh-D blood groups of this study has similar trends with the data from previous studies in Ethiopian populations and with most populations of the world. The chi-square test shows that the population is at the Genetic equilibrium.

The researcher believe that data from this study have provided information on the genetic variability and polymorphism of the blood group and Rhesus antigens among population in Mechara Town, WestHararghe,Oromia. This information would be useful to the geneticists and to the clinicians especially in the planning of blood tansfusion programmes since they play an integral role in the genetic profile of the Ethiopian population.

5.3. Recommendations

Based on the findings of the study and conclusions drawn, the following recommendations were forwarded.

- The data generated in this study would be helpful as a base for researchers who are interested to conduct similar type of study in Oromo, Amhara, Argoba and Somali ethnic groups in the study area.
- Moreover, it is in reference to students populations and may not represent the whole population of each ethnic group in the study area.
- Conducting similar, well designed study using large sample size (population of the district),which represent the whole Oromo, Amhara, Argoba and Somali population is necessary to obtain sufficient serologic data of each ethnic group in the district.

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7. APPENDIX

Appendix 1. Consent form

Name of the study participant _____ Age_____Sex_____

Ethnic group _____

I have been informed about the purpose and objectives of the study that plans to determine“ **Frequencies of ABO and Rh-D Blood Group Alleles Among Students of four Ethnic groups in Mechara Secondary and Preparatory School,WestHararghe,Oromia”.**

For the study I have been requested to participate in the study and give a drop of blood from finger. They told me that the qualified and experienced laboratory technician would do the blood collection according to the established aseptic procedures by using sterile disposable lancet. Based on this, I have agreed to participate in the study based on my interest. 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. It is 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 participant_____ Signiture_____ Date_____

Name of investigator_____

Signiture_____Date_____

Name of witness _____ Signiture_____Date___

Appendix 2. Students' ABO and Rh-D blood group Phenotypes recording sheet

No	Code for participants	Sex	Age	Ethnicity	ABO & Rh-Blood phenotype	Remark
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
13						
14						
15						
16						
17						
18						
19						
20						
Total						

Appendix 3. 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

Appendix 4. Chi-square test for the differences of ABO phenotypes among the four ethnic groups

Ethnic groups	Blood phenotypes				**Total**	
	A	B	AB	O		$\chi 2$
Oromo	55(50.41)	32(35.4)	13(14.25)	73(72.8)	**173**	**0.835**
Amhara	31(33.5)	23(22.5)	9(9.4)	52(48.4)	**115**	**0.481**
Argoba	22(26.5)	21(18)	11(7.4)	37(38.3)	**91**	**3.054**
Sumale	23(20.4)	16(14.34)	4(5.7)	27(29.4)	**70**	**1.222**
Total	**131**	**92**	**37**	**189**	**449**	

The expected frequency of each of clans is computed by the formula

Expected frequency = $\frac{(\Sigma \text{ column number})(\Sigma \text{ row number})}{\text{Grand total}}$

Chi-square ($\chi 2$) = $\Sigma \frac{(Of - Ef)^2}{Ef}$ =

0.406+0.32+0.109+0+0.186+0.0111+0.017+0.267+0.76+0.5+1.75+0.044+0.33+0.19+0.507+0.195= 5.56

Appendix 5. Chi-square test for the differences of Rh-D phenotypes among the four ethnic group

Ethnic groups	Rh-D phenotypes		**Total**
	Rh-D^+	Rh-D^-	
Oromo	171(170.68)	2(2.31)	**173**
Amhara	114(113.46)	1(1.5)	**115**
Argoba	91(89.78)	0(1.21)	**91**
Sumale	67(69)	3(1)	**70**
Total	**443**	**6**	**449**

Chi-square ($\chi 2$) $= \Sigma \frac{(Of - Ef)^2}{Ef} =$

0.0006+0.042+0.025+0.1666+0.0165+1.21+0.057+0.666=2.01

Appendix 6. ABO and Rh-D antisera and agglutination reaction

A B

Ethical Clearance Letter

Ref. No- 02/359/EF/09

Date- 24/05/2009

To Mechara Health centre

Mechara

Issue: Giving Ethical clearance and support letter for a Research

Application Title:- Frequency of ABO and Rh-D Blood group alleles among the students of four ethnic groups at Mechara Secondary and Preparatory School, West Hararghe, Oromia

Department: Biology

Programme: Msc in Biology

University: Haramaya University

Research ethics exist to ensure that the principles of justice, respect and avoiding doing harm are upheld by using agreed standards. So, here is an application attached with Research Proposal inquiring research ethical clearance letter and it is revised by Darolabu Health office management committee and has got acceptance. Here by, we feel happy when we recommend **Mr. Seyidu Jemal Ahmed** to get the necessary assistance from any concerned body and our office require to get one copy of the final research result to use it in future health planning.

Sincerely

Zafu Menawa

Ittigaalatamaa Qajeelchaa
Fayyaa Aanaa Daaroo-Labuu

CC

To Seyidu Jemal Ahmed