FREQUENCIES OF ABO AND Rh-D BLOOD GROUPS AMONG STUDENTS OF LEMEN SECONDARY AND PREPARATORY SCHOOL, SOUTH-WEST SHAWA, OROMIA, ETHIOPIA

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Frequencies of ABO and Rh-D Blood Groups among Students of Lemen Secondary and Preparatory School, South-West Shawa, Oromia, Ethiopia

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We hereby certify that we have read and evaluated this Thesis Entitled "**Frequencies of ABO and Rh-D Blood Groups among Students of Lemen Secondary and Preparatory School, South-West Shawa, Oromia, Ethiopia**" prepared under our guidance by Mekonnen Sime Dadi. We recommend that it be submitted as fulfilling the Thesis requirement.

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DEDICATION

I dedicate this thesis to my lovely daughter; Simbo Mekonnen and to all my families.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is the result of 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 and that all sources or materials used for this thesis have been duly acknowledged.

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BIOGRAPHICAL SKETCH

The author was born on September 24, 1987 in a village called Jalisa Lutu, in Kuyu District, North Shawa Zone, from his father Sime Dadi and his mother Tsige Gadafa. He attended his Elementary School education at Amuma Wachale Elementary School from 1994-1998 and Gebra Gurracha no 2 from 1999-2003 and Secondary School education at Gebra Gurracha Secondary and Preparatory School from 2004 -2006. He joined Ambo University in 2007 and graduated with Bachelor of Education degree in Biology in July 2009. He was employed by the Oromia Education Bureau as a teacher at Tare Secondary School in 2012. After two years of service he was transferred to Lemen Secondary School. He joined Haramaya University, School of Biological Sciences and Biotechnology in 2014 to pursue his Master of Science study in Biology.

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academic achievement. My special sincere and profound gratitude goes to my wife, Nibret Amde for her encouragement and continuous support during my study.

LIST OF ABBREVIATIONS AND ACRONYMS

CSA	Central Statistical Agency
ELISA	Enzyme Linked Immune sorbent Assay
FMC	Flinders Medical Center
HDN	Hemolytic Disease of the New Born
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
MN	M and N Blood groups
PCR	Polymerase Chain Reaction
Rh	Rhesus

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Frequencies of ABO and Rh-D Blood Groups among Students of Lemen Secondary and Preparatory School, South-West Shawa, Oromia, Ethiopia.

ABSTRACT

Frequencies of ABO and Rhesus (Rh-D) blood groups phenotypes, alleles, and genotypes vary geographically, ethnically and from one population to another. Some variations may even occur within one ethnic group and within one small country. Therefore, the aim of this study was to determine the frequencies of ABO and Rh-D Blood Groups phenotypes, alleles and genotypes among students of Lemen Secondary and Preparatory School, South-West Shawa, Zone, Oromia, Ethiopia. A total of 384 students (male=276 and female=108) voluntarily participated in this study. Each student was typed for ABO and Rh-D blood groups antigens using agglutination reactions. In the sample, the O, A, B, and AB blood group systems phenotype percentages were 39.6%, 32.3%, 22.92% and 5.2%, respectively. The Rhesus positive incidence was 95.8%, while Rhesus negative was 4.2%.The genotypic frequencies of ABO blood group of the sample was 0.0443(4.43%) I^AI^A, 0.267(26.71%) I^AI^O, 0.0234(2.34%) I^BI^B, 0.1942(19.42%) I^BI^O, 0.064(6.4%) I^AI^B and 0.4031(40.31%) I^OI^O and genotypic frequencies for Rh-D blood group systems are 0.6336(63.36%) for DD, 0.3246(32.46%) for Dd and 0.0415(4.15%) for dd. The order of ABO blood group allele frequencies were $I^O > I^A > I^B$ in the samples. The allele frequencies of I^O, I^A, and I^B in the sample were found to be 0.6349, 0.2104, and 0.1529 respectively. The Rhesus blood group allele frequencies of the sample were 0.7961D and 0.2039 d. The result of the present study implies that blood type O is the most frequently occurred blood type in the study area.

Key words: Antibody, Antigens, Alleles, Genotype, Phenotype and Rhesus

1. INTRODUCTION

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, The presence of Rhesus system was recognized in 1939 by Levine and Stetson (Giri, 2011). Humans contain a series of glycoprotein and glycolipid on the surface of RBCs which constitutes the blood group antigens. A blood type is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). 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, Rhesus (Rh) blood group systems, etc.) These antigens may be proteins, carbohydrates, glycoprotein, or glycolipids. Individuals have different types and combinations of these molecules. Several of these red blood cell surface antigens can stem from one allele (or an alternative version of a gene) and collectively form a blood group system (Maton *et al*., 1993).

ABO and Rh systems are important in clinical practice (Mandal, 2002) blood groups are genetically determined and exhibit polymorphism in different populations. A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT, 2008). In clinical practice, ABO and Rh blood groups are the most important among the 30 blood groups (Jaff, 2010). Although about 400 blood grouping antigens have been reported, the ABO and Rh are recognized as the major (clinically significant) blood group antigens. Antigen- A and antigen -B determine ABO blood group systems while antigen - D determine Rh-D blood group. ABO blood group system was the first human blood group system, while Rhesus blood group system was the fourth system, out of 15 most important systems discovered and yet it is the second most important blood group from the point of view of transfusion (Khan *et al*., 2004).

For the ABO blood group system, there are four different kinds of blood types: Type-A, Type-B, Type-AB and Type-O. Individuals with blood type-A, has A antigen on the surface of RBCs and anti-B antibodies in blood plasma; blood those with type-B, have B antigens on the surface of RBCs and anti-A antibodies in blood plasma; those with blood type-AB, have both A and B antigens on the surface of RBCs and no anti-A or anti-B

antibodies at all in blood plasma, and blood type-O, has neither A or 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+). Those who lack are called Rh-negative (Rh-). A person with Rh- blood does not have Rh antibodies naturally in the blood plasma; as one can have anti-A or anti-B antibodies, for instance. But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh+ blood can receive blood from a person with Rh- blood without any problems (Eweidah, 2011).

The Rh system is one of the most polymorphic of the human blood groups. More than 40 different antigens have been identified; five are common and known as D, C, c, E and e(Daniels, 2002).The Rh is genetically complex but it is simply described in terms of a single pair of alleles, *D* and *d*. Rh positive (Rh+) persons are DD and Dd and Rh negative (Rh-) are dd.The Rh blood groups rank with ABO groups in clinical importance because of their relation to hemolytic disease of the newborn (HDN) and their importance in blood transfusion (Khan *et al*., 2009).

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

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

The need for blood group prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine. Frequency of ABO and Rh blood groups vary worldwide and are not found in equal numbers even among different ethnic groups. It is, therefore, imperative to have information on the distribution of these blood groups in any population group that comprise different ethnic group (kumar *et al*., 2009).

The present study was undertaken to estimate the existing frequencies of ABO and Rh-D blood groups system phenotype, allele, and genotype frequencies among students of Lemen Secondary and Preparatory School.

Objective of the study

General objective

The general objective of the study was to determine the frequencies of phenotypes, alleles and genotypes of the ABO and the Rh-D blood group systems among students of Lemen Secondary and Preparatory School.

Specific objectives

The specific objectives of this study were:

- To determine the frequencies of ABO and Rh-D blood group phenotypes among students of Lemen Secondary and Preparatory School.
- To estimate the allele and genotype frequencies for the two blood group systems among the students.

2. LITERATURE REVIEW

2.1. Blood Group Systems

Humans contain a series of glycoprotein's 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).

2.1.1. Discovery of ABO blood group

At the beginning of the 20th century an Austrian scientist, Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals. He made a note of the patterns of agglutination and showed that blood could be divided into groups. This marked the discovery of the first blood group system, ABO, and earned Landsteiner a Nobel Prize. Landsteiner explained that the reactions between the RBCs and serum were related to the presence of markers (antigens) on the RBCs and antibodies in the serum (Giri, 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. 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". One genetic locus, the ABO locus, which has three alternative (allelic) forms A, B, and O (Avent and Reid, 2000) encode the ABO blood group antigens.

2.1.2. Applications of ABO blood groups

The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed the same, and the often-tragic consequences of blood transfusions were not understood. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations (Avent 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 a clerical error in which an incompatible type of ABO blood is transfused. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations, suggesting that a particular blood type conferred a selection advantage (example; resistance against an infectious disease.). However, despite their obvious clinical importance, the physiological functions of ABO blood group antigens remain a mystery. People with the common blood type O express neither the A nor B antigen, and they are perfectly healthy. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease. For example, the ABO phenotype has been linked with stomach ulcers (more common in group O individuals) and gastric cancer (more common in group A individuals). Another observation is that individuals with blood type O tend to have lower levels of the von Wille brand Factor (vWF), which is a protein involved in blood clotting (Laura, 2005).

2.1.3. Frequencies of ABO phenotypes in different populations

The ABO blood group phenotypes are not found in equal numbers in different populations. For example, in Caucasians in the United States, the distribution is type O,

47%; type A, 41%; type B, 9%; and type AB, 3%. Among African American, the distribution is type O, 46%; type A, 27%; type B, 20%; and type AB; 7%. Among Western Europeans, 42% have group A, 9% group B, 3% group AB and the remaining 46% group O (Laura, 2005).

Among Ethiopians, the distribution is that type O, is 42%; type A, is 30%; type B, is 22%; and type AB, is 6 %, (http://rhesusnegative.net). 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). Table 1 shows frequency of ABO and Rh blood type distribution by country populations across the world.

Table 1: ABO and Rh-D blood type distribution by country (population averages**)**

		Percentage Frequencies of ABO and Rh-D Blood Type							
Country	Population	O^+ %	A^+ %	B+ %	AB+ %	O- %	A- %	B- %	AB- %
Argentina	41,343,201	45.4	34.26	8.59	2.64	8.4	0.44	0.21	0.06
Australia	21,262,641	40.0	31.0	8.0	2.0	9.0	7.0	2.0	1.0
Austria	8,210,281	30.0	33.0	12.0	6.0	7.0	8.0	3.0	1.0

Bahrain	1,234,571	48.48	19.35	22.61	3.67	3.27	1.33	1.04	0.25
Bangladesh	161,083,804	31.18	21.44	34.58	8.85	1.39	0.96	0.96	0.64
Belgium	10,414,336	38.0	34.0	8.5	4.1	7.0	6.0	1.5	0.8
Bolivia	10,088,108	51.62	29,45	10.11	1,15	4,39	2,73	0,5	0.1
Brazil	198,739,269	36.0	34.0	8.0	2.5	9.0	8.0	2.0	0.5
Canada	33,487,208	39.0	36.0	7.6	2.5	7.0	6.0	1.4	0.5
Cambodia	14,952,665	46.7	27.2	18.5	4.9	1.3	0.8	0.5	0.1
Cameroon	19,958,000	42.8	38.8	12.0	3.3	1.4	1.2	0.4	0.1
Chile	17,114,000	85.6	8.7	3.35	0.99	1.2	0.1	0.05	0.1
China	1,339,724,852	47.7	27.8	18.9	5.0	0.3	0.2	0.1	0.03
Czech	10,532,770	27.0	36.0	15.0	7.0	5.0	6.0	3.0	1.0
Ethiopia	84,320,987	39.0	28.0	21.0	5.0	3.0	2.0	1.0	1.0

Source: http://www.rhesusnegative.net/themission/bloodtypefrequencies/

2.1.4. The Genetics of ABO blood system

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 (Rai and Kumar, 2011). The three alleles can produce six genotypes and four phenotypes of blood groups which are shown below in table 2.

Table2: show six genotype and four phenotypes of blood groups.

Phenotype	Genotype

A	$I^A I^A$, $I^A I^O$
B	$I^B I^B$, $I^B I^O$
AB	$I^A I^B$
O	$I^O I^O$

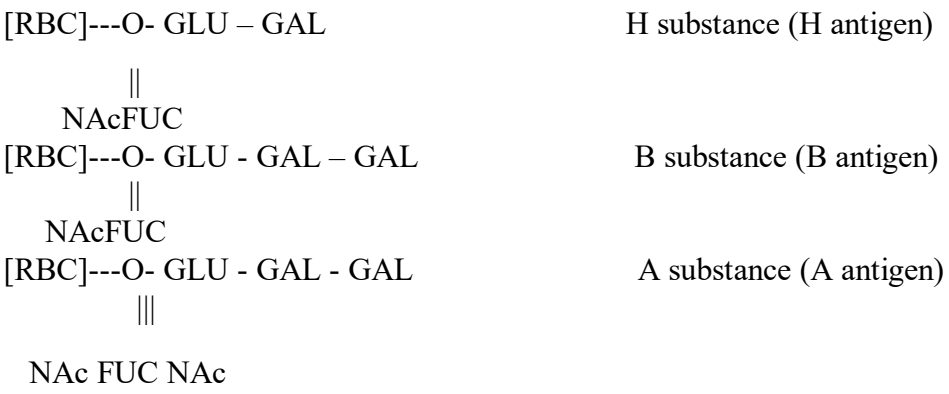
Source: Mandal, 2002

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 glycosyl transferase 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. Four nucleotide substitutions are translated into different amino acid substitution. The antigens A, B and their variants result from functional glycosyl transferase 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 glycosyl transferase gene that generates A or B antigens is inactive (Daniel and Elizabeth, 2009).

2.1.5. The ABO blood group antigens

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 two modified forms of a "stem" carbohydrate present on red blood cells and other tissues. Their structures are shown below, where GLU is glucosamine, GAL is galactose or galactosamine, FUC is frucose, and NAc represents an N acetyl group. The H gene (HH/Hh) encodes for an enzyme, which converts the

precursor substance in red cells in to H substance (H antigen). A and B genes encode specific transferase enzymes which convert H substance in to A and B red cell antigens. Some H substance remains unconverted (the H substance is partly converted). O gene encodes for an inactive enzyme, which results in no conversion of the substance in-group O red cells. This indicates group O individual contains the greatest concentration of H antigen. Persons who do not inherit H gene (very rare hh genotype) are unable to produce H substance and therefore even when A and B genes are inherited, A and B antigens cannot be formed. This rare group is referred to as Oh (Bombay group)(Bryant, 1994).



These carbohydrates are also a common component of many foods we eat and many microorganisms in our intestinal tract. The immune system is therefore constantly exposed to these antigens, and responds by making an effective humeral response. Since the immune system does not in general respond to antigens which are a normal part of a type B individual does not make antibodies to the B blood group substance, although the response to the type A antigens is robust. The net result is the production of antibodies, mostly of the IgM class, to which ever of these substances is not present on an individual's red blood cells (Avent and Reid, 2000).The ABO blood group antigens are attached to oligosaccharide chains that project above the RBC surface. 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).

2.1.6. Antibodies of ABO blood groups

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 E.*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 immune globulinM (IgM). They attack and rapidly destroy red cells carrying the corresponding antigen.

For example, anti A attacks red cells of Group A or AB. Anti-B attacks red cells of Group B or AB. Table 3 shows ABO blood groups antigens and their corresponding antibodies.

Table 3: ABO blood groups, antigens and antibodies

Blood Group Phenotypes	ABO antigens present on the red cell surface	AB 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

Source : ISBT, 2008.

2.1.7. Mode of inheritance and medico-legal application of ABO blood groups

If both of the parents in a given family are of O blood group, all the children must have O group blood like their parents. If on the other hand, both of the parents are A group, and both happened to be heterozygous, then they may have some children with O blood group.

Therefore, in this way, if we know the blood group of a child and his or her mother then, we can legitimately claim or test the probable blood group of the child's father. Table 3 below shows the summarized form of medico legal application of the ABO blood groups in the case of disputed paternity (Mandal, 2002).

Table 4: Inheritance and Medico-legal application of ABO blood groups

Blood group of child	Blood group of mother	Blood group which the father cannot have
O	O	AB
O	A	AB
O	B	AB
A	O	O,B
A	B	O,B
B	O	O,A
B	A	O,A

A B	A	O,A
A B	A	BO
AB	B	O,B

Source: Mandal, 2002

2.1.8. The Secretor trait

It has been found that some individuals have A or B antigens in their body secretions such as from eyes, nose, salivary gland and mammary gland and are known as secretors. Persons who are secretors have water-soluble antigen, which can pass out of the red blood corpuscles, and be present in the body secretions. Nevertheless, in the case of non-secretors, antigens are only alcohol-soluble and cannot be dissolved out in the secretions. So, the secretors can be identified by test done on the blood as well as on the body secretions. This secretor trait is inherited as a dominant trait 'S' while the non-secretor trait is recessive (Mandal, 2002).

2.2. The Rh Blood Group System

The Rh 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(Rai *et al*., 2009)..

2.2.1. The Discovery of Rh blood group

While many blood group systems are known other than the ABO system, the Rh system is of special importance. This was originally defined by a rabbit antibody directed against the red blood cells of Rhesus monkeys, an antibody which turned out to be capable of distinguishing between the red blood cells of different human individuals (Avent and Reid, 2000). In 1939, HDN was first described by Levine and Stetson. The cause of hemolytic disease was not specifically identified but maternal antibody was suspected. A year later, in 1940 Karl Landsteiner and Alexander Wiener injected animals with Rhesus monkey cells and produced an antibody which reacted with the red blood cells of 85% of humans, which they named anti-Rh. Within a year, Levine made connection between maternal antibodies causing HDN and anti-Rh. Between 1943 and 1945; the other common antigens of the Rh system were identified. For many years, the exact inheritance of the Rh factors was debated, Weiner promoting Rh and hr terminology and Fisher-Race utilizing D,C,c,E,e for the various Rh antigens (Daniels, 2005).

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

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

Table5: Rh-D phenotypes frequencies in different studies

Population	Rh+(D) %	Rh-(d)%	Source
Britain	83	13	Mahapatra *et al*.,2014
USA	85	15	Frances ,2002
Nigeria	95.2	4.8	Falusi *et al*.,2000
Ethiopia	92.06	7.94	Kassahun *et al*.,2015
Guinea	95.9	4.1	Loua *et al.,*2007
Saudi Arabia	93	7	Bashwari *et al*.,2001

Pakistan	93	7	Rahman and Lordhi,2004
Nepal	96.7	3.3	Pramanik *et al*.,2000
India	95.36	4.64	Purushottam *et al*.,2011

Source: http://www.rhesusnegative.net/themission/bloodtypefrequencies/

2.2.3. The antigens of Rh blood group system

Rh antigens are determined by three pairs of closely linked allelic genes located on chromosome one (Rai *et al*., 2009).There are 5 Rh antigens that may be found in most individuals. They are D, C, E, c and e Daniels, 2002). Rh or D is the most important antigen after A and B antigens. Natural antibodies to Rh do not exist in humans, as they do for the AB antigens. 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 of D+ cells usually occurs through pregnancy or transfusion. Rh+ 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).

2.2.4. Hemolytic diseases of the new born (HDN)

2.2.4.1. Rh incompatibility

Rh blood type is determined by a pair of genes, one inherited from each parent. Blood is either Rh-positive or Rh-negative, depending on whether or not certain molecules are present. A person who is Rh-negative will experience a severe immune system reaction if Rh-positive blood gets into their bloodstream. This can happen during pregnancy if an Rh-negative woman carries an Rh-positive baby. If blood cells from the baby travel across the placenta, the woman's immune system will regard the Rh-positive cells as a threat. Specialized white blood cells will make antibodies designed to kill Rh-positive blood cells. If the woman later conceives another Rh-positive baby, her immune system will flood the

fetus with antibodies. These antibodies then destroy the baby's red blood cells. If left untreated, this can result in severe anemia or even death. This is called hemolytic disease of the newborn (Bakare *et al*., 2006).The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh-can bear an Rh+ child if the father is Rh+ (either homozygous or heterozygous). Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy (Daniel and Elizabeth, 2009).

At the time of birth, however, tissue damage resulting from the separation of the placenta from the uterine wall can result in a significant amount of fetal blood entering the maternal circulation; which may stimulate a strong immune globulinG (IgG) anti-Rh response in the mother. If the same mother then bears a second Rh+ child, the existing anti-Rh antibodies can cross the placenta during the pregnancy and destroy fetal red blood cells. The ensuing damage to various organs results in the potentially dangerous condition erythroblastolis fetalis also known as hemolytic disease of the new born. This can be diagnosed prenatally by carrying out amniocentesis and examining the amniotic fluid for the presence of free hemoglobin and its degradation products (Laura; 2005).Various approaches can be used during and after birth to rescue the infant, including exchange transfusion, complete replacement of the infant's blood to remove the anti-Rh antibodies and provide undamaged RBCs. However, the production of anti-Rh antibodies in an Rh- mother can often be prevented by administering anti-Rh immune globulin into the mother, typically at around 28 weeks of gestation and again within 72 hours of the birth of her Rh+ baby. By mechanisms which are still not fully understood, these antibodies greatly reduce the likelihood of sensitization of the mother's immune system by the Rh+ erythrocytes. If this procedure, developed in the1960's, is successfully carried out during each Rh+ pregnancy, anti-Rh antibodies are not produced by the mother, and subsequent pregnancies will not be at risk. While Rh incompatibility is of considerable clinical significance, it should be noted that not all untreated incompatible pregnancies result in disease. Only a small fraction of incompatible pregnancies actually result in the production of maternal anti-Rh antibodies

and in only a fraction of these cases is there significant damage to the newborn (Daniel and Elizabeth, 2009).

2.2.4.2. 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-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).

2.3. Methods of Blood Typing

Blood typing involves identifying substances called antigens present on RBCs membranes. Many different antibodies exist on human RBCs but those of clinical importance include only the ABO and Rh groups. Blood typing is performed with antiserum, 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 up on exposure to the A antibody, the blood contains the A antigen. If clumping occurs in the test blood upon exposure to the B antibody (anti-B serum), the blood contains the B antigen. If clotting occurs with both A and B antibodies (anti-A and anti-B sera), the blood type is AB, and if no clumping occurs with either

serum type, the blood type is O (Rai and Kumar, 2010).Table 6 below shows agglutination reactions with ABO blood typing sera.

Table:6 Agglutination reactions of the RBCs in ABO blood-type sera**.**

Reaction		Blood Type
A-antibody	B-antibody	
Clumping	no clumping	A
no clumping	Clumping	B
Clumping	Clumping	AB
no clumping	no clumping	O

Source: ISBT, 2008.

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 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).

2.4.1. The Hardy-Weinberg principle

In a large population where there is no genetic drift, and in the absence of selection, migration and mutation, the allelic frequencies remain constant from generation to generation. If mating is random, the genotypic frequencies are related to the allelic frequencies by the square expansion of allelic frequencies. Thus, for autosomal genes in diploid organisms in which there are two alleles with frequencies p and q, the frequencies

of the three genotypes are predicted by the formula $(p + q)2 = p2 + 2pq + q2$. Furthermore, for autosomal genes the equilibrium genotypic frequencies at any given locus are attained in a single generation providing there is no overlapping of generations (Bryant, 1994).The Hardy-Weinberg equilibrium is a neutral equilibrium. This means that the allelic and genotypic frequencies do not change because of random mating, but if some other force, such as selection or migration, changes the frequencies of the alleles to new values, the genotypic frequencies automatically shift according to the formula $p2 + 2pq + q2$. Thus, the genotypic frequencies do not return to their previous values but are defined by the new allelic frequencies. If no other force is applied, the population will remain at this new equilibrium, (Dar *et al*., 2010).

Modified Hardy- Weinberg equation was 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:

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

$q2$ is the frequency of genotype I^BI^B

$2pq$ is frequency of genotype I^AI^B

$2pr$ is frequency of genotype I^AI^O

$2qr$ is the frequency of genotype I^BI^O

r2 is the frequency of genotype $I^{O}I^{O}$

ABO allele frequencies were estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates were calculated as: $p = 1 - \sqrt{B+O}$, $q = 1 - \sqrt{A+O}$, $r = \sqrt{O}$ (*p, q*, and *r* denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O). A correction factor (θ) will be calculated according to $\theta = 1 - p - q - r$. The final allele frequencies were then calculated as follows: $p1 = p\ (1 + \theta/2)$; $q1 = q\ (1 + \theta/2)$; $r1 = (r + \theta/2)\ (1 + \theta/2)$ where *p1, q1*, and *r1* denote corrected allele frequencies. Rh-D allele frequencies were calculated according to the Hardy-Weinberg equation (Al-Arrayed *et.al*, 2001). The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test to check whether population was at Hardy-Weinberg genetic equilibrium or not (Chakraborty, 2011).

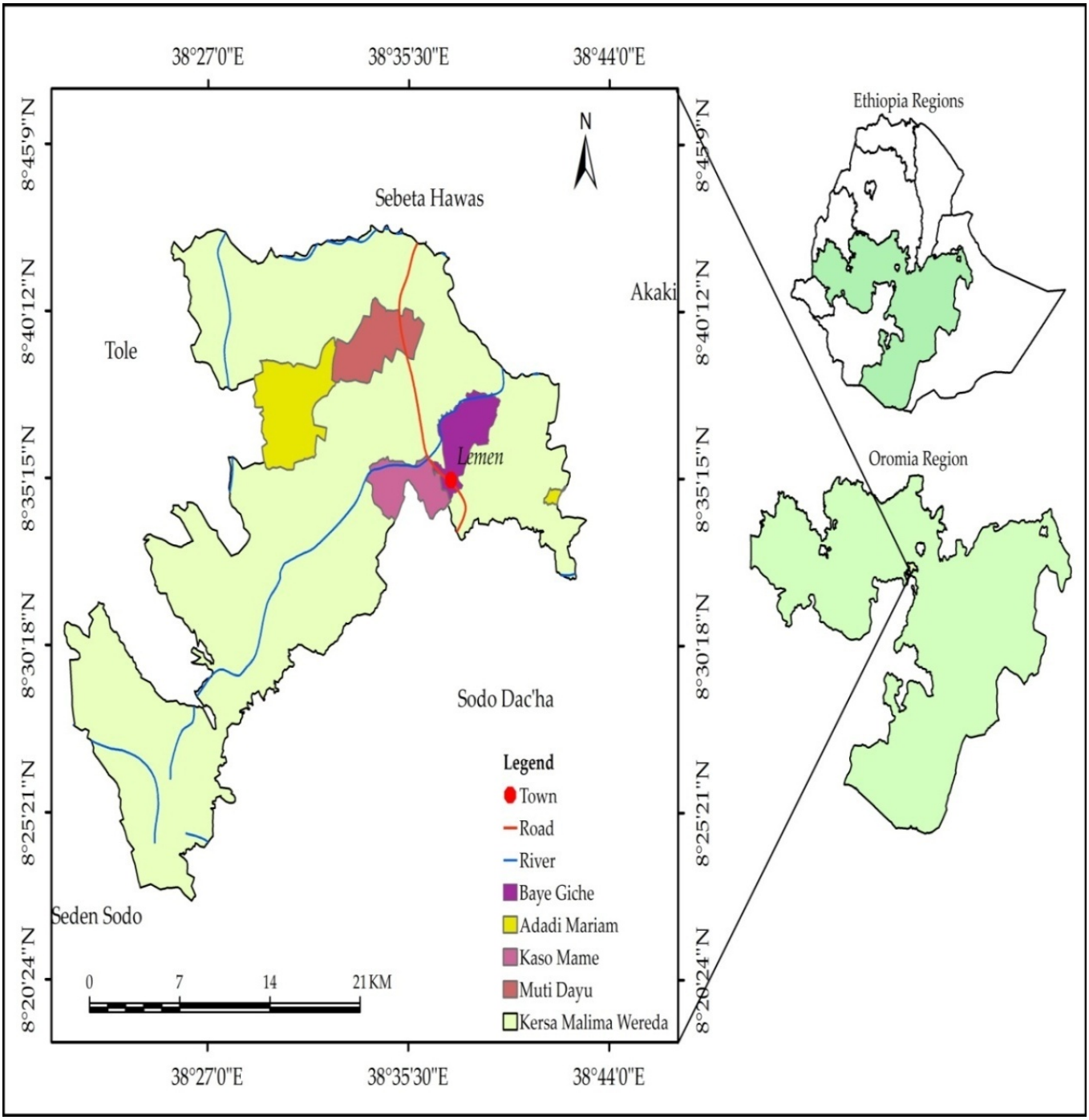
Frequencies of Rh-D blood group alleles D and d are represented as p and q respectively in which p is frequency of allele D and q is frequency of allele d. Using Hardy- Weinberg equation, at equilibrium the frequencies of the genotype were represented as $(p + q)\ 2 = p2 + 2pq + q2 = 1$, where *p2* is frequency of genotype DD, *2pq* is frequency of genotype Dd and *q2* is frequency of genotype d (Dar *et al*., 2010).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Lemen Secondary and Preparatory School in Lemen town, the capital of Kersa Malima district. Lemen is found in South-West Shawa Zone of Oromia Regional State, Ethiopia. Lemen is bordered on the South by the Southern Nations Nationalities and Peoples region, on the West Sadan Sodo, on the Northwest by Tole, on the Northeast by Sebata Hawas, and on the East by East Shawa. This district is

located 60 Km south- west of Addis-Ababa, the capital of Ethiopia. It is found between latitudes of 8^0 20 24 N to 8 40 12 ^{0}N latitude and 38^0 34' E to 38^0 71'E longitude and has altitude of 2230 m above sea level with minimum and maximum temperature 24°C and 38°C respectively. The mean and total annual rainfall was107 and 1284 mm in 2016 respectively. The 2007 national census reported total populations for this district were 81, 015, of whom 41,536 were men and 39,649 were women, 6536 or (8.07%) of its population were urban dwellers and 74,479(91.93%) were rural dwellers .The three largest ethnic groups reported were Oromo (94.01%), the Amhara (3.21%), and the Soddo Gurage (2.44%), all other ethnic groups made up 0.34% of the population CSA (2007). The current population of the district were 2,349,435 ,of whom 1,199,614 men and 1,149,821 women.


38°27'0"E
38°35'30"E
38°44'0"E
8°45'9"N
8°40'12"N
8°35'15"N
8°30'18"N
8°25'21"N
8°20'24"N
N
Sebeta Hawas
Akaki
Tole
Lemen
Sodo Dac'ha
Seden Sodo
0
7
14
21 KM
Legend
Town
Road
River
Baye Giche
Adadi Mariam
Kaso Mame
Muti Dayu
Kersa Malima Wereda
Ethiopia Regions
Oromia Region

(Source: - Ethiopian Geographical Information System of 2016)

Figure 1.Map of Study Area

3.2. Study Design

A cross-sectional design was carried out to investigate the frequency distribution of the ABO and Rh-D blood groups among Students of Lemen Secondary and Preparatory School.

3.3. Study Population

The study population is students of Lemen Secondary and Preparatory School of the 2016/2017 academic year. There were 1,500 students in the school at the beginning of the academic year. Out of these 817(54.5%) were males and 683(45.5%) were females.

3.3.1. Inclusion criteria

Any volunteer students of Lemen Secondary and Preparatory School of any ethnic group as well as all age and both sexes.

3.3.2. Exclusion Criteria

Involuntary students of Lemen Secondary and Preparatory School.

3.4. Sample Size determination and Sampling Method

The sample size for the study was determined by the following formula (Naing *et al*., 2007):-

n=

Where:

n= required sample size=384

Z=standard deviation which is= 1.96

P=prevalence of the issue under study= 0.5

D= confidence limit of prevalence, which =0.05.

The formula gives n=384, therefore 384 out of the 1,500 students were voluntarily participated in the study and randomly selected. Size determination was used during mid-February to mid-April of (2017).

3.5. Data Collection Methods

3.5.1. Blood Sample Collection

The ABO and Rh blood test was performed by using sterilized lancets, to obtain a drop of blood from a sterilized finger from each individual by laboratory technicians. Blood samples were taken from finger pricks, and open slide method of testing for ABO blood groups and Rh (D) factor was followed (Bhasin and Chahal, 1996).Then, a drop of blood from each student was placed on a clean slide in three places. A drop of each of the anti-sera, anti A, anti B and anti D was added and mixed with each blood sample, with the aid of stick and shaking machine. Blood was mixed thoroughly with the anti -sera and rocked gently for 60 sec to observe agglutination.

3.5.2. ABO and Rh-D Blood Typing Methods

Blood types were determined on the basis of agglutination reaction. Three drops of blood from each person were placed at three places on a clean dry glass slide. A drop of each of the anti-sera, anti-A, and anti-B and anti-D was added and mixed with each blood sample with the aid of plastic stick.

If clumping or clotting occurs in the test blood upon exposure to the A antibody (anti-A serum), the blood contains the A antigen. If clumping occurs in the test blood upon exposure to the B antibody (anti-B serum), the blood contains the B antigen. If clotting occurs with both A and B antibodies (anti-A and anti-B sera), the blood type was AB, and if no clumping occurs with either serum type, the blood type was O (Rai and Kumar, 2010). Each of those blood types classified as negative or positive, which was the reference of the blood's Rhesus factor. Therefore, the results were recorded as A+, B+, AB+, O+ and A-, B-, AB-, and O- (Alimba *et al.*, 2010).

3.6. Methods of Data Analysis

Modified Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies (Strickberger, 1976). For this study three alleles were computed (A, B and O), with frequencies equal to p, q and r respectively. The frequencies of the genotypes at equilibrium were computed by trinomial

expansion $(p+q+r)^2 = p2$ (AA) + $2pq$ (AB) + $q2$ (BB) + $2p$r (AO) + $2qr$ (BO) + $r2$ (OO) (Griffith *et al*., 2008).

Data was analyzed by using the following formulas.

3.6.1. Calculation of the observed phenotypic blood type frequency

Observed percentage =

Observed frequency =

3.6.2. Calculation of allelic frequency

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

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

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

A correction factor (θ) was calculated as $\theta = 1 - p - q - r$. The final allele frequencies were then calculated as follows: $p_1 = p$ (1 +); $q_1 = q$ (1 +(); r_1 = (1+) (1 +).

Frequency of the two Rh-D blood group alleles (p and q) were determined as follows $q = \sqrt{}$Rh-ve

$P = 1 - q$

3.6.3. Calculation of genotypic frequency

Formula for the calculation of genotypic frequencies of ABO was calculated as $(p+ q+ r)^2 = p^2$ (AA) + $2pq$ (AB) + q^2 (BB) + $2pr$ (AO) + $2qr$ (BO) + r^2 (OO)

$(p1)^2$for homozygote AA

2($p1r1$) for heterozygote AO

$(q1)^2$for homozygote BB

$2(q1r1)$ for heterozygote BO

$(r1^2)$ for homozygote OO

The genotypic frequencies of Rh-D blood group system were calculated as follows:

$(p1)^2$for homozygote DD

2(p1q1) for heterozygote Dd

$(q1)^2$for homozygote dd

The expected phenotype frequencies were calculated from genotype frequencies as follows.

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

B= frequency of (BB + BO) X number of total sample.

AB=frequency of (AB) X number of total sample.

O= frequency of (OO) X number of total sample

Then the observed and expected phenotype frequencies were compared or tested for goodness-of-fit using chi-square test as follows (Chakraborty, 2010).

Data in the text and tables were calculated by using SPSS version 20 software.

Chi-square ($\chi 2$) =

$\chi 2$= chi-square

df = degree of freedom

dev = deviation

dev^2 = deviation square

3.7. Ethical Considerations

An authorization to carry out the study was obtained from Health Office of the Kersa Melima *woreda* by using a letter of support or cooperation obtained from School of Biological Sciences and Biotechnology (Department of Biology) Haramaya University. All the information that was obtained about the participants was kept confidentially.

4. RESULTS AND DISCUSSION

A total of 384 students of Lemen Secondary and Preparatory School were voluntarily involved in the research.

4.1. ABO and Rh-D Blood Group Systems Phenotypic Frequency

The frequency distribution of ABO blood groups among the students (n=384) of Lemen Secondary and Preparatory School were shown in Table 7.

Table:7. Percentage frequency of ABO blood group phenotypes among the students

No	Type-A Frequency(%)	Type-B Frequency (%)	Type-AB Frequency (%)	Type-O Frequency (%)
(n=384)	124(32.3)	88(22.9)	20 (5.2)	152(39.6)

As indicated in table 7 blood type-O has the highest frequency while blood type AB has the least frequency. The frequencies of ABO blood group phenotypes observed in the sample were 39.6% O, 32.3% A, 22.9% B, and 5.2%AB.

Many other studies have shown that blood type-O was the most common blood group and blood type-AB was the least common blood group in different populations and ethnic groups (www.rhesusnegative.net, 2012). For example, among Ethiopians, the distribution is type-O, 42%; type-A, 30%; type-B, 22%; and type-AB, 6 %(www.rhesusnegative.net, 2012). The Study carried out by Tibebu (1998) showed that the distribution of type-O is 40%; type-A is 31%; type-B is 23%; and type-AB is 6% in Ethiopian blood donors. In population of south west Ethiopia (at Gilgel Gibe Field Research Center), the distribution of type-O, is 42%; type-A, is 31%; type-B, is 21%; and type-AB is 6% (Abraham *et al.,* 2012).

The study carried out by Mohammed (2013) showed that distribution of O is 43.3%, 51.7% and 50.6% followed by blood type-A, 27.8%, 21.1% and 23.3% and blood type- B 25%, 19.4% and 22.8% in Amhara, Oromo and Afar respectively and the least percentage frequency is that of blood group AB in the three ethnic groups which is 3.9%, 7.8% and 3.3% in Amhara, Oromo and Afar respectively. Therefore the results of this study are in agreement with the data from previous studies in Ethiopia populations. When compared with other reports from similar studies, the results of this study are also agree with previous findings from other parts of the world. For example 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).

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 among 7653 individuals sampled (Bakare *et al.,* 2006). 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). In Ilorin, Kwara state of Nigeria, the frequency of blood type-O, A, B and AB are 58.1%, 18.7%, 17.6%, and 5.6% respectively. Among African Americans, the distribution is type-O, 46%; type A, 27%; type B, 20%; and type AB; 7%. Among Western Europeans, 42% have group A, 9% group B, 3% group AB and the remaining 46% group O (Iyiola *et al*., 2011).

The results of the study were consistent with the recent study done in Sudan by Abbas(2017) on Frequency of ABO and Rh-D Blood Groups among Sudanese Blood Donors Attending Central Blood Bank in Wad Medani, Gezira State, Sudan , phenotypic frequencies of ABO blood group were type-O (51%), A (30%), B (14%) and AB (5%).

However, the results of this study inconsistent with the results from some Asian countries where blood group B has the highest frequency in some and blood group A in the others. For example (Raja *et al*.,2016)Frequency and Distribution of ABO and Rh blood groups among blood donors in Tertiary Care Hospital of South Gujarat, India ,phenotypic frequencies were blood type-B (34.43%) followed by blood type-O (32.26%), blood type-A (24.35%), while the least prevalent blood type was AB (8.94%).

Ilyas *et al.,*(2013)Frequency of ABO and Rh Blood groups in Gujranwala (Punjab), Pakistan, Phenotypic frequencies were blood type-B was most prevalent (35.36%) followed by blood type-O (32.41%) and blood type-A (22.91%)and Blood type-AB was least prevalent with (9.32%).

The highest frequency of type-A blood was documented in Jordan populations (Hanania *et al*., 2007) the frequencies were (38.62%)blood type-A,(36.62%) blood type-O,(18.04%) blood type-B and (6.98%)blood type-AB. Again the results of the study were inconsistent with ABO phenotypic frequency of Bororo (Brazil) in which 100% of the population were O blood types (ISBT, 2006).

Table: 8. Percentage frequency of Rh-D blood group phenotypes among the students

Rh-D Blood Group	
Rh-D positive Frequency (%)	Rh-D negative Frequency (%)
368(95.8)	16(4.2)

According to table 8 above Rh- positive has the higher percentage frequency while Rh negative has the lower percentage frequency. The frequency of Rh-D positive blood group was 95.8% while the frequency of Rh negative blood was 4.2%. These results are consistent with previous findings of Ethiopian populations (www.rhesusnegative.net, 2012) and South Ethiopian Populations (Abraham *et al.,* 2012).

According to Nugusu (2013) Rh-positive were higher percentage frequencies while Rh-negative has the lower percentage frequencies in Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory School and Zebela Primary School. The frequencies were 94.17% Rh D +ve while 5.83% were Rh D-ve.

The results were in line with the recent study in India (Raja *et al*., 2016) on frequency and distribution of ABO and Rh blood groups among blood donors in tertiary care hospital of South Gujarat, India phenotypic frequencies of Rh-D blood group in 40732 blood samples, incidence of Rh-D positive were 95.12 % (38746) and Rh-D negative were 04.87 % (1986).

Again, the findings of this study are in accordance 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 (Ahmed *et al*., 2009; Ahmed *et al*., 2007; Bakare *et al*., 2006, Akhigbe *et al*., 2009, Adeyemo and Soboyejo, 2006, Iyiola *et al*., 2011).About 95% of African-Americans are Rh-positive (Chavhan *et al*., 2010 and Abraham *et al*., 2012).

Table:9 Distributions of the combined ABO and Rh-D blood group phenotypes among students (n=384)of Lemen Secondary and Preparatory School.

ABO Blood Type	Rh-D Blood Group	No Observed	% Observed
O	$RhD^{+}ve$	144	37.5
	$RhD^{-}ve$	8	2.08
A	$RhD^{+}ve$	118	30.73
	$RhD^{-}ve$	6	1.56
B	$RhD^{+}ve$	86	22.39
	$RhD^{-}ve$	2	0.52
AB	$RhD^{+}ve$	20	5.21
	$RhD^{-}ve$	0	0
Total		384	100

Table 9 (above) shows the combined frequency distributions of ABO and Rh-D blood group phenotypes of the students. The prevalence of the ABO phenotypes linked with Rh positive phenotypes of the sample were O^{+} (37.5%), followed by A^{+} (30.73%), B^{+}

(22.39%), and AB^{+} (5.210%). In Rh negative phenotypes the frequencies were O- (2.08%), followed by A- (1.56%), B-(0.56 %), and AB- (0%).

In both Rh positive and Rh negative phenotypes blood type-O has the highest prevalence while blood type-AB has the lowest prevalence. This indicates that Rh-D Positive and Rh-D negative frequencies were recorded highest in blood type-O, followed by blood type- A, B and AB.

The results of the study were in line with other study done in Sudan (Abbas,2017)Frequency of ABO and Rh D Blood Groups among Sudanese Blood Donors Attending Central Blood Bank in Wad Medani, Gezira State, Sudan, combined frequencies distributions were 97.3% of blood type-O were Rh-D positive and 2.7% were Rh-D negative, 97.3% of blood type-A were Rh-D positive and 2.7% were Rh-D negative, 97.14% of blood type-B were Rh-D positive and 2.86% were Rh-D negative, 100% of blood type-AB were Rh-D positive and 0% were Rh-D negative.

4.2. Allelic and Genotypic Frequency of ABO and Rh-D Blood Group Systems

Allelic frequencies of ABO blood groups among the students (n=384) of Lemen Secondary and Preparatory School are presented in Table 10 below. The frequencies of alleles I^A, I^B, and I^O were calculated according to the modified Hardy Weinberg Law of equilibrium was shown in below.

Table:10 Allelic and genotypic frequencies of ABO Blood group Systems among Students of (n=384) Lemen Secondary and Preparatory School.

Phenotype	Phenotype frequency	Allele	A l l e l e frequency	Genotype	Genotype frequency
A	0.323	I^A(P)	0.2104	$I^A I^A$(p^2)	0.0443
				$I^A I^O$(2pr)	0.2671
B	0.229	I^B(q)	0.1529	$I^B I^B$ (q^2)	0.0234

				$I^B I^O$ (2qr)	0.1942
O	0.396	I^O (r)	0.6349	$I^O I^O$(r^2)	0.4031
AB	0.052			$I^A I^B$(2pq)	0.064

As indicated in table 10, the frequencies of ABO blood type alleles of the students are 0.2104 I^A, 0.1529 I^B and 0 .6349 I^O. The order of allele frequencies of ABO blood group of the students in the sample were $I^O > I^A > I^B$. The results of the study in line with other previous study in Ethiopia. For example, Study by Nugusu(2013) frequencies of ABO blood type alleles of Oromo, Amhara and Wolayita ethnic groups in Robe Secondary and Preparatory School and Zebela Primary School were 0.6621, 0.1810 and 0.1569 and I^O, I^A and I^B respectively.

Previous studies among various segments of the world population have documented similar pattern of allelic frequencies. For instance, studies by Bakare *et al.*,(2006) in Ogbomoso, South-west Nigeria, Yan *et al*., (2005) on Chinese populations, Hussain *et al*., (2001) among Baluchistan in Pakistan and, Iyiola *et al., (*2011) in Ilorin, Kwara State of Nigeria all found the allelic frequencies to occur in $I^O > I^A > I^B$ order.

The frequencies of ABO genotypes of the sample were 0.0443(4.43%) $I^A I^A$, 0.2671(26.71%) $I^A I^O$, 0.0234(2.34%) $I^B I^B$, 0.1942(19.42%) $I^B I^O$, 0.064(6.4%) $I^A I^B$ and 0.4031(40.31%) $I^O I^O$ (Table 10). If one takes blood type-A, the frequency of $I^A I^A$ genotype was 0.0443 while that of $I^A I^O$ genotype was 0.0267.

Thus, among those who are blood group A, 4.43% were homozygous $I^A I^A$ while about 2.67% was heterozygous $I^A I^O$. Similar deductions can be made for blood type B, O, and AB. As shown in table 10, genotype $I^O I^O$ has the highest frequency while genotype $I^B I^B$

has the least frequency. Similar results were reported by Irshaid *et al*., (2002), Iyiola *et al*., (2011), Hanania *et al*., (2007), and Bakare *et al*., (2006).

Table: 11 Allelic and genotypic frequency of Rh-D Blood Group among students (n=384) of Lemen Secondary and Preparatory School

Phenotype	Phenotype frequency	Allele	Allele frequency	Genotype	Genotype frequency
Rh-D$^+$	0.958	*D(p)*	0.796	*DD*(p^2)	0.6336
				Dd(2pq)	0.3246
Rh-D$^-$	0.042	*d(q)*	0.2039	*dd*(q^2)	0.0415

As indicated in table 11, frequencies of the genotypes for Rh blood group in the sample were 0.6336 for *DD*, 0.3246 for *Dd* and 0.0415 for *dd*. The allele frequencies of Rh-D blood group were calculated according to the Hardy-Weinberg equation using the data presented in (Table 11).The frequency of allele D and d are 0.796 and 0.2039, respectively in the sample. This shows that allele D has higher frequency than allele d.

The results of this study were in line with other study done in Ethiopia. For example, Study by Yassin(2013) on Frequency of ABO and Rhesus(Rh-D) Blood Groups Alleles among Students of Oromo Ethnic Group Belonging to Arsi, Guji, and Borena Clans in Robe College of Teachers Education, Ethiopia were 0.6078 for DD, 0.3432 for Dd and 0.0484 for dd. This 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 al*., 2006). Genotypic frequencies were *DD>Dd>dd.*

Table: 12 Observed versus expected frequency of ABO blood groups phenotypes of students (n=384)

Blood type	Observed	Expected	Dev	Dev^2/Expected
O	152	155	-3	0.02344
A	124	120	4	0.0417
B	88	84	4	0.0417

AB	20	25	5	0.0651

Table 12 above shows observed versus the expected values of ABO blood group phenotypes. The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square. The distribution of the overall observed frequencies of ABO blood group phenotypes does not significantly differ from those expected under Hardy Weinberg equilibrium (at $\chi 2$=0.1794,df=3, P=0.9808%).

Chi-square ($\chi 2$) =

$\chi 2$= chi-square

df = degree of freedom

dev = deviation

dev^2 = deviation square

Table:13 Observed versus expected frequency of Rh-D blood groups phenotypes of students (n=384).

Rh-D Blood Group	Observed	Expected	Dev	Dev^2/Expected
Rh-D+ve	368	367.948	0.052	0.0000073

Rh-D-ve	16	15.936	0.064	0.00001066
Total	384			

Table 13 above shows observed versus the expected values of Rh-D blood group phenotypes. The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square. The variation of distribution of the overall observed frequencies of Rh-D blood group phenotypes from those expected under Hardy-Weinberg equilibrium were also not significant(at $\chi 2=0.00001796$, df=1, P=1) .

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

A total of 384 students of Lemen Secondary and Preparatory School were voluntarily involved in the research. The aim of the present study was to determine the distribution of ABO and Rh-D blood group system phenotypes, alleles and genotypes the students. The sample was consisting of 276 male and 108 female students. Blood sample was taken from finger pricks of students by qualified medical laboratory technicians, using the standard clinical procedure, with lancet. ABO blood grouping was determined using monoclonal ABO blood grouping reagents while Rh-D blood group was using Anti-D monoclonal blood grouping reagents.

The frequencies of ABO and Rh-D blood groups phenotypes were expressed in simple percentages and Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies from phenotypic frequencies. From the sample taken, 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 frequency of ABO blood groups phenotypes of students in the sample were 39.6% O, 32.3% A, 22.92 % B and 5.2% AB. The frequencies of Rh-D blood groups were 95.8 % Rh-D positive and 4.2% Rh-D negative.

The genotypic frequencies of ABO blood group of the sample were 0.0443(4.43%) I^AI^A, 0.2671(26.71%) I^AI^O, 0.0234(2.34%) I^BI^B, 0.1942(19.42%) I^BI^O, 0.064(6.4%) I^AI^B and 0.4031(40.31%) I^OI^O and genotypic frequencies for Rh-D blood group systems are 0.6336(63.36%) for DD, 0.3246(32.46%) for Dd and 0.0415(4.15%) for dd. Similar patterns of variation were obtained in the order of ABO blood group alleles is $I^O>I^A>I^B$. The allele frequencies of ABO blood group were 0.6349 I^O, 0.2104 I^A and 0.1529 I^B in the sample.

The frequency of allele D and d of Rh-D blood group were 0.7961 and 0.2039 respectively, in the sample. Regarding the genotypic frequencies of ABO blood group in the sample phenotype I^OI^O has the highest frequency and I^AI^B has the least frequency. In the Rh-D blood group frequencies of genotype DD, Dd and dd were 0.6336, 0.3246 and 0.01415 respectively.

5.2. Conclusions

In ABO blood group systems, blood group O has the highest frequency and blood group AB has the lowest frequency. In the Rh-D blood group system Rh-D positive blood group has the highest frequency while Rh-D negative blood has the lowest frequency.

The order of the frequencies of ABO blood group alleles are $I^O > I^A > I^B$. In Rh-D system frequency of allele D is higher than frequency of allele d. From the results of the study blood type-O is the most frequent ABO blood group followed by A, B and AB with the predominance of Rh-D positivity. The types of information obtained from the findings are useful for emergency and accidental healthy disorder especially, at deficient blood and again for donating.

5.3. Recommendations

From the research findings the following recommendations were drawn.

- The present study is the first study that documents the phenotypic, genotypic and allelic frequencies of ABO and Rh-D blood groups among the students of Lemen Secondary and Preparatory School, Lemen town. So Data obtained from this study can be used in the planning of blood transfusion and donation programs and reducing HDN in the district and adds to the existing knowledge pool regarding to the prevalence of various blood types in the area and providing important information regarding to rare blood phenotype in the study area .
- Further study at molecular level would useful to see the degree of genetic proximity/relationship/ of the ethnic groups live in the study area.

6. REFERENCES

Abbas, A.A. 2017. Frequency of ABO and RHD Blood Groups among Sudanese Blood Donors Attending Central Blood Bank in Wad Medani ,Gezira State, Sudan. *International Journal of Medical Research Professional* doi:1021276/ijmrp.1-4.2017.3.2.009.

Abraham Haileamlak, Ayalew T. Muluneh, Fessahaye Alemseged, Fasil Tessema, Kifle Woldemichael, Makonnen Asefa, Yoseph Mamo, Solomon Tamiru, and GemedaAbeb e. 2012. Hematoimmunological profile at Gilgel Gibe Field Research Center, south west Ethiopia. *Ethiopian Journal of Health Sc*ience 22(1): 39-50.

Adeyemo, 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 Biotechnolog*y 5(22): 2062-2065.

Ahmed, S.G., Ibrahim, U.A; and Hassan, A.W. 2007. Adequacy and pattern of blood donations in northeast Nigeria: The implications for blood safety. *Annual Tropical Medical Parasitological* 10(1): 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 of Third World Medicine* 8(2): 983-1006.

Akhigbe, R.E., Ige, S.F., Afolabi, A.O., Azeez, O.M. Adegunlola, G.J. and Bamidele, J.O. 2009.Prevalence of hemoglobin variants ABO and rhesus blood groups in LadokeAkintola University of Technology, Ogbomoso, Nigeria.*Trends Medical Research*, 4(3): 24-29.

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

Alimba,C.G., Adekoya, K.O. and Oboh, B.O. 2010. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood gr oups anhemoglobin Variants among a Nigerian population. *Egypt Journal of Medical Human Genetics*11:153-158.

Anees, M., Jawad, A., and Has-hmi, I. 2007. Distribution of ABO and Rh blood group alleles in MandiBahauddin district of Punjab, Pakistan, *Pakistan Academic Science* 44(4):289-294.

Anstee, D.J., and Tanner, M.J. 2009. Biochemical aspects of the blood group Rh (rhesus) antigens. *Baillieres Clinical Hematology*6 (1):401-422.

Avent, N.D., 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 hemoglobin variants in Ogbomosho, South-West Nigeria*, African Journal of Biotechnology*5 (1): 224-229.

Bhasin MK., and Chahal .S.M.S .1995.*A Laboratory Manual for Human Blood Analysis*. Kamla-Raj Enterperises. Delhi

Bryant, N. J., 1994. *An Introduction to Immunohematology, 3rd edition,* WB Saunders Philadelphia. 1233-1287.

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

Chavhan, A; Pawar, S; and Baig, M. 2010. Allelic frequency of ABO and Rh D blood group among the Banjara backward caste of Yavatmal District, Maharashtra, India*, Nature Proceedings* 5(1):65-76.

CSA (Central Statistical Agency) .2007.Population and housing census Atlas of Ethiopia.

Daniel, L.H., and Elizabeth, W. J. 2009.*Genetics*: *analysis of genes and genomes* I 7th edition. Jones and Bartlett publishers Canada, 989-1211.

Daniels, G. 2002. *Human Blood Groups*, Second edition, Blackwell Science. New Central Book Agency (P) LTD, New Delhi, India.2nd edition.

Daniels, G. 2005.The Molecular Genetics of blood group polymorphism, *Transplantation Immunology*14:143-153.

Dar,N.J., Srivastava, A., and Dar, F.A. 2010. Distribution of ABO blood groups and Rh (D) factor among the Brahmin and Kushwaha populations of Jhansi District (U.P); *Anthropologis11(4): 305-306.*

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

Falusi, A. G., Ademowo, O. G., Latunji, C. A., Okeke, A. C., Olatunji, P. O., Onyekwere, T. O., and Itata, E. O. 2000. Distribution of ABO and RH genes in Nigeria. *African journal of medicine and medical sciences*29(1): 23-26.

Frances, T. F. 2002. Blood groups (ABO groups). *common Laboratory and diagnostic tests. 3rd Edition, Philadelphia: Lippincott* 19-25.

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

GIS (Geographical Information System, 2016). Ethiopian Geographical Information Agency. Addis Abeba.

Griffith, A.F, S.R, Wessler, R.C, Leewontin., and S.B. Carroll., 2008. *Introduction to Genetic* Analysis.9thedition.W.H.Freeman and company. New York: 900.

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

Hussain, A., Sheikh, S.A., Haider, M., Rashied, R., and Malik, M.R. 2001. Frequency distribution of ABO and Rhesus blood groups in population of Baluchistan, *Pakistan Armed Forces Medical Journal* 51: 22-26.

Ilyas, M., Iftikhar, M., and Rasheed, U. 2013. Frequency of ABO and Rh Blood groups in Gujranwala (Punjab), Pakistan, *Biology (Pakistan*), 59(1): 107–114.

Irshaid, N., Ramadan, S., Wester, E., and, Olsson, M. 2002. Phenotype prediction by DNA-based typing of clinically significant blood group system in Jordanian blood donors; *Voxsang Medical Journal*83:56-62.

ISBT, 2006."Table of blood group systems"; International Society of Blood Transfusion URL:http://ibgrl.blood.co.uk/isbt, accessed March5, 2008.

ISBT, 2008."Table of blood group systems"; International Society of Blood Transfusion URL:http://ibgrl.blood.co.uk/isbt, accessed June 7-12, 2008.

Iyiola, O.A., Igunnugbemi, O.O., Raheem, U.A., and Anifowoshe, A.T. 2011. Gene frequencies of ABO and Rh (D) blood group alleles in Ilorin, North-Central Nigeria, *World Journal of Biological Research*4(2): 633-743.

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

Kassahun Tesfaye, YohannesPetros, Mebeasselassie Andargie.2015. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia *Egyptian Journal of Medical Genetics*(16):71–76.

Khan.S., Bakhshi, A., Akhtar, M.S., and Amin-ud-Din, M. 2009. Distribution of ABO and Rh D blood groups in the population of Poonch District, Azad Jammu and Kashmir; E. *Mediterranean Health Journal*15(3):717-721.

Khan, M. S., Subhan, F., Tahir, F., Kazi, B. M., Dil, A. S., and Sultan, S. 2004. Prevalence of blood groups and Rh factor in Bannu region NWFP (Pakistan); *Pakistan Journal of Medical Research* 3(1): 8 -10.

Khan, M.S., Najam, F., Nosheen, Q., and Faheem, M. 2006. Trend of blood groups and Rh factor in the twin cities of Rawalpindi and Islamabad; *Journalof Pakistan Medical Association* 56(7):299-301.

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

Laura Dean, M.D. 2005. *Blood Groups and Red Cell Antigens*; National Center for Biotechnology Information, United States Government, 861-967.

Loua, A., Lamah, M. R., Haba, N. Y., and Camara, M. 2007. Frequency of blood groups ABO and rhesus D in the Guinean population. *Transfusion Clinic Biology: Journal of transfusion Society* 14(5): 435-439.

Mahapatra, S., Sahoo, D., and Mishra, S. 2014. Distribution and Prevalence of ABO and Rh Phenotype blood groups in Eastern India.*Journalof Biomedical Science* 04(08):712-714.

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

Maton, Anthea; Jean Hopkins; Charles William McLaughlin; Susan Johnson; MaryannaQuon Warner; David LaHart; Jill and D. Wright 1993.*Human Biology and Health*. Englewood Cliffs NJ: Prentice Hall. ISBN 0-13-981176-1.

Mohammed Seid.2013.Allelic Frequency Of ABO and Rh-D Blood Groups among Patients belonging to the three Ethnic Groups between Februry to March, 2013 at Dessie Referral Hospital, Ethiopia.

Naing, L.T.Winn and B.N. Rusil, 2007. Practical issues in calculating sample size for prevalence studies. *Archives Orofacial science* 1: 9-14.

Nugusu Girma.2013. Frequency of ABO and Rh-D Blood Group Alleles among Oromo, Amhara and Wolayita Ethnic Group Students in Robe Secondary and Preparatory and Zebela primary School, Bale Zone, Ethiopia.

Nwauche, C.A. and O.A. Ejele, 2004. ABO and rhesus antigens in a cosmopolitan Nigeria population; *Niger Journal of Medicine* 13(3): 263-266.

Pramanik, T., and Pramanik, S. 2000. Distribution of ABO and Rh blood groups in Nepalese medical students: a report. *East Mediterranean Health Journal* 1:156-158.

Rahman, M and Lodhi, Y.2004. Frequency of ABO and Rhesus blood groups in blood donors in Punjab; *Pakistan Journal of Medical Science* 20(4):315–8.

Rai, V., and Kumar, P. 2010. The incidence of ABO blood group in Muslim population of UttarPradesh, India; *Journal of Applied Biological science* 36(2):191-195.

Raja, K.A. 2016.Frequency and distribution of ABO and Rh D Blood Groups among Blood Donors in Tertiary Care Hospital of South Guajarat, India. *International Journal of Research Medicine Science* 4(12):5377-5381.

Rehman, A., Khan, M.A., Ashraf, M., Malik, S.A., Saed, M.A., Rafique, A., and Ali, A. 2005.ABO and Rhesus blood groups in Pakistan population; *Professional Medical Journal*12(4): 368-371.

Sarhan, M.A., Saleh, K.A., Bin-Dajem, S.M. 2009. Distribution of ABO blood groups

and Rhesus factor in Southwest Saudi Arabia; *Saudi Medical Journal* 30(1):116-119.

Strickberger, M.W. 1976: *Genetics 2nd Edition* McMillan Publishing Company. Inc., New York, 164-18, 735- 755.

Table of ABO and Rh blood type distribution by country (population averages), www.rhesusnegative.net.2012, *accessed March 5, 2012.*

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* 4(8): 289-294.

Tibebu Mokonnin. 1998. *The Blood Bank Manual*, Ethiopian Red Cross society, National Blood Transfusion Service, Addis Ababa, 54-63.

Yan, L., Zhu, F., Fu, Q., and He, J. 2005. ABO, Rh, MNS, Duffy, Kidd, Yt, scianna, and Colton blood group systems in indigenous Chinese. *Immuno Hematology* 21:10-14.

Yassin Woliye Datu. 2013. Frequency of ABO and Rhesus (RhD) Blood Group Alleles Among Students of Oromo Ethnic Group Belonging to Arsi, Guji, and Borena Clans(Doctoral dissertation, Haramaya University) in Robe College of Teachers Education, Ethiopia .

7. APPENDICES

Appendix 1 **Consent form**

Name of the study participants _____ Age____ Sex_____

I have been informed about the purpose and objectives of the study that plans to determine"**Frequencies of ABO and RhD Blood Groups among Students of Lemen Secondary and Preparatory School, South-West Shawa, Oromia, Ethiopia".** 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 (participants) _____Signature _____Date_____
Name (investigator) _____Signature_____Date_____
Name (witness) _____Signature _____Date_____

Appendix2 Students' ABO and RhD Blood Group Phenotypes recording sheet

Code	Sex	ABO and Rh-D Blood Type							
		A+	B+	AB+	O+	A-	B-	AB-	O-
1									
2									
3									
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Appendix 3

Figure.1.During blood sample were taken from voluntary students

Appendix 4

Figure.2.Anti-A, anti- B, and anti-D Monoclonal used for Blood Group determination

Appendix 5 Ethical Clearance

Ref no -LHC/431/09
Date - 14/10/09

Lemen Health Center

Lemen

Issue:-*Giving Ethical Clearance &Support Letter for Academic Research.*

Application Tittle:-Distribution of ABO and RhD blood Group Systems and Its Association with Anthropometric Measurements among Students of Lemen Secondary& Preparatory School, South West Shawa, Oromia.

Department:-Biology

Program: - M.Sc. in Biology

University: - Haramaya University

Research ethics exist to ensure that the principles of justice, respect and avoiding doing harm are up held by using agreed standard. These basic principles are universal, through there are of course many subtitles &diversities how principles are understood &interpreted can vary from place to place. So, here is an application attached with research proposal inquiring research ethical clearance letter. We feel happy when we recommend Mr. **Mekonnen Sime** get necessary assistance from any concerned body & from our office as a general.

Sincerely

Dearetsara Buufata
Fayyaa Leemman