GENOTYPIC AND ALLELIC FREQUENCIES OF ABO AND Rh-D BLOOD GROUPS AMONG SECONDARY AND PREPARATORY SCHOOL STUDENTS OF MESELA, WESTERN HARARGHE, OROMIA, ETHIOPIA

MSc. THESIS

ABRAHIM MOHAMMED

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ABRAHIM MOHAMMED

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HARAMAYA UNIVERSITY

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I hereby certify that I have read and evaluated this Thesis entitled "Genotypic and Allelic Frequencies of ABO and Rh Blood Groups among Students of Mesela Secondary and Preparatory School at Mesela Western Hararge, Oromia, Ethiopia" prepared under my guidance by Abrahim Mohammed Ahmed. I recommend that it be submitted as fulfilling the Thesis requirement.

Yohannes Petros(PhD) _____ _____

Major Advisor Signature Date

Tamru Oljira(PhD) _____ _____

Co-advisor Signature Date

As members of the Board of Examiners of the M.Sc Thesis Open Defense Examination, I certify that I have read and evaluated the Thesis prepared by Abrahim Mohammed Ahmed and examined the candidate. I recommend that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Biology

_____ _____ _____

Chairperson Signature Date

_____ _____ _____

Internal Examiner Signature Date

_____ _____ _____

External Examiner Signature Date

DEDICATION

I dedicate this thesis manuscript to my wife **Sado Ahmed**, my Mother **Hawa Omare** and Father **Mohammed Ahmed** for their advice and support have given me the strength to take on the challenges that life presents.

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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Name: Abrahim Mohammed Ahmed Signature: _____

Date:_____

School/Department: Biological Sciences and Biotechnology

BIOGRAPHICAL SKETCH

The author was born in 1973 in Mesela distric*t*, at a particular place called Goliya from his father Mohammed Ahmed and his mother Hawa Omare. He completed his elementary education at Goliya Primary School and secondary education at Hirna Secondary School from 1990 to 1994. Then, he joined Jimma University in 1995 and graduated with Dipiloma in Biology in June 1996.

Soon after graduation, he was employed by Oromia Regional Educational Bureau, as biology teacher at Mesela Secondary School, Western Hararge. Then, he joined Addis Ababa University in 1997 and graduated with B.Ed in Biology in September 2001. He joined the Postgraduate Program Directorate of Haramaya University in July 2015 to pursue his M.Sc study in Biology.

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ACRONYMS AND ABBREVIATIONS

HDN	Hemolytic Disease of the New Born
Ig	Immunoglobulin
MWEP	Mesela *Woreda* Enviromental Profile
ISBT	International Society of Blood Transfusion.
RBC	Red blood cells
Rh	Rhesus

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Genotypic and Allelic Frequencies Of ABO and Rh Blood Groups Among Secondary and Preparatory School Students of Mesela, Western Hararghe, Oromia, Ethiopia.

ABSTRACT

The ABO and Rh blood groups are the most important blood group systems despite the long list of several other blood groups discovered so far. The frequencies of blood types of ABO and Rh blood groups vary worldwide and are not found in equal numbers even

among the same ethnic groups. Therefore, this study was aimed at having information on the allelic and genotypic frequencies of ABO and Rh blood groups among students of Mesela Secondary and Preparatory School, Western Hararge, Oromia, Ethiopia. A total of 400 students were voluntarily selected among students of Mesela. Blood samples were obtained from each student who participated voluntarily in the study. The blood samples were typed by open slide test method between February and March 2017. A drop of each anti-serum anti- A, anti- B, and anti- D was added and observed for agglutination. There were differences in frequency of ABO blood type alleles among students. Blood type O and Rh-positive have the highest frequency while blood type AB and Rh-negative have the lowest frequencies among students .In this study, the frequency distribution of blood type O was 0.4375; followed by blood type A, 0.2750, and blood type B 0.2400, and the least frequency was that of blood type AB which was 0.0475, Whereas, the Rh blood type phenotypic frequencies were 0.95 for Rh positive and 0.05 for Rh negative. The frequencies of ABO blood type alleles were found to be 0.6662, 0.1774, and 0.1564 for I^O, I^A *and* $I,^B$ *respectively. Among the ABO blood group genotypes, the most frequent were* I^OI^O, I^AI^O *and* I^BI^O *occurring with frequencies of 0.4438, 0.3264 and 0.2084, respectively. In the Rh blood group system, allele D occurred with a frequency of 0.7764 and d with a frequency of 0.2236. Homozygous Rh blood group genotype DD was the most predominant of the three Rh genotypic classes and occurred with a frequency of 0.6028.* The *implication of this finding is that blood group O is the most readily available blood group in the study area which is more advantageous for the population in the event of blood transfusion.*

Key words*:* Antisera, observed frequency, expected frequency *Hard-weinberg* blood transfusion

1. INTRODUCTION

Blood is the most important body fluid. This is responsible for circulation of important nutrients, enzymes and hormones all across the body, besides the most crucial substance, oxygen. Blood group or blood type is based on the presence or absence of inherited antigenic substances; glycolipids and glycoproteins on the surface of red blood cells that can be determined by specific antibodies. More than 600 surface antigens have been found on red blood cells (Waters, 1995) and several of these antigens that stem from one gene or very closely linked genes collectively form a blood group system (Waite, 2009). Blood group antigens are hereditary characters that appear early during fetal development and remain unchanged throughout one's life (Firkin *et al*., 1989).

The ABO and Rh blood groups are among the most important blood groups (Seeley *et al.* 1998). Karl Landsteiner first described the ABO blood group in1900. Blood group testing plays a key role in medical treatment prior to blood transfusion and child birth. The blood group of a person does not change within one's own life time and so it is considered as a unique genetic marker for research. The blood group is determined by the genetic make-up of the alleles of a system (Gupta, 1999). Furthermore, the presence of Rhesus system was recognized in 1939 and it was confirmed within few years (Landsteiner and Weiner, 1940). The study of blood grouping is important as it plays an important role in genetics, blood transfusion and forensic biology.

The need for blood group allelic frequency and prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Jolly, 2000). Blood group antigens are not only important in relation to blood transfusion and organ transplantation, but also can be utilized in genetic research, anthropology and tracing ancestral relation of human (Khurshid *et al*.,2000).

Estimates of gene frequencies provide very valuable information on the genetic similarity of different populations and to some extent on their ancestral genetic linkage, despite the cultural and religious differences of the populations (Meade *et al*., 1998).

ABO and Rh blood group antigens are hereditary characters and are useful in population genetic studies, researching population migration patterns, as well as resolving certain medico legal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice. The ABO and Rh antigens are recognized as the major clinically significant blood group antigens (Malison, 1979). Certain inherent characteristics, such as the blood groups are transmitted to the embryo within about 4 to 6 weeks after fertilization during which time the formation of primary blood cells begins, when it is possible to prove the presence of antigen of the ABO and Rh systems (Markovic, 1994).

The knowledge of distribution of ABO and Rhesus (Rh) blood groups at local and regional levels is helpful in the effective management of blood banks (Patel et *al)* 2012. There is no report in the literature about research conducted in Mesela *district* to determine the distribution of phenotypic, allelic and genotypic frequencies of the ABO and Rh blood groups. The presence of such a gap in the information regarding blood groups calls for a need to carry out the present study to determine the frequencies of the phenotypes, alleles and genotypes of these two very important blood group systems in the study area.

General objective

Thus, the general objective of the study is to estimate the Phenotypic, allelic and genotypic frequencies of ABO and Rh blood group systems among Mesela Secondary and Preparatory School students.

Specific Objectives of the Study:

- To determine the frequencies of the ABO and Rh blood group phenotypes among the students.
- To determine allelic and genotypic frequencies for the ABO and Rh blood group system among the students.
- To examine the distribution of Rh phenotypes within each of the ABO blood groups.

2. LITERATURE REVIEW

2.1. Historical Overview of ABO and Rh Blood Group

The ABO blood group system was discovered by Karl Landsteiner in 1901. Rh blood group system is the fourth system to be discovered and yet it is the second most important group from the point of view blood of transfusion. The ABO and Rh antigens are recognized as the major clinically significant blood group antigens (Molison. 1979). The discovery of the ABO blood group caused great excitement, all blood had been assumed to be the same before that and the often tragic consequences of blood transfusions were not understood. The understanding of the ABO group has made blood transfusion become a great deal safer and enabled scientists to learn the inheritability of traits in humans.

2.2. Inheritance of ABO and Rh Blood Groups

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three alleles: I^O, I^A, and I^B. The gene encodes a glycosyl transferase that modifies the carbohydrate content of the red blood cell. The gene is located on the long arm of the ninth chromosome. The IA allele gives type A blood, I^B gives type B, and I^O gives type O blood. As both I^A and IB are dominant over I^O, only $I^O I^O$ people have type O blood. Individuals with $I^A I^A$ or $I^A I^O$ have type A blood, and individuals with $I^B I^B$ or $I^B I^O$ have type B. $I^A I^B$ people have both phenotypes, because A and B express a special dominance known as : co dominance, which means that type A and B parents can have an AB child. A type A and a type B couple can also have a type O child if they are both heterozygous

($I^BI^OI^AI^O$) The cis-AB phenotype has a single enzyme that creates both A and B antigens(Yazer *et al*.,2006).

The Rh blood group is classified according to the presence or absence of a second erythrocyte antigen identified as Rh. Although dozens of Rh antigens have been identified, only one, designated D, is clinically important. Those who have the Rh D antigen present on their erythrocytes described as Rh positive (Rh^+) and those who lack it are Rh negative (Rh^-).
Note that the Rh group is distinct from the ABO group, so any individual, no matter their ABO blood type, may have or lack this Rh antigen. When identifying a patient's blood type, the Rh group is designated by adding the word positive or negative to the ABO type. For example, A positive (A^+) means ABO group A blood with the Rh antigen present, and A negative (A^-) means ABO group A blood without the Rh antigen (American Red Cross.,2013).

The ABO blood group and the Rhesus factor or Rh blood group are two of the most notable blood type groups in humans due to their importance in blood transfusion (Khattak, *et al*., 2008). The Rh blood group system is commonly denoted by the terms Rh^- and Rh^+ based on the presence or absence of the D antigen, the Rh blood group system is special because the D antigen is used to determine the risk of hemolytic disease of the new born (HDN) (Reid and Lomas,2004,). The mode of inheritance of the ABO blood group follows the multiple allelic mode of inheritance and is quite stable to be used to exclude paternity in paternity issues. The Rh antigen is named after the rhesus monkey, Macaca mulatta (Zimmerman) where it was initially detected. The inheritance of these antigens is complex, and there are two theoretical models that explain the pattern of inheritance. The Wiener system postulates a single gene locus with a series of at least ten alleles. The Fisher system assumes the existence of at least three closely linked loci designated as C, D, and E. Both are currently in use and are still being studied.

However, only the presence of the D antigen in the Fisher system serves as the basis for classification of the Rh blood group; this way, the mode of inheritance is simply single gene

inheritance with accompanying dominance. The most notable medical importance of this blood group system is the occurrence of Rh incompatibility between mother and fetus, which is a major factor in the development of fetal erythroblastosis which is a hemolytic disease of the newborn child (Dennis, *et al*., 1998).

2.3. Description of Blood Groups

2.3.1 ABO Blood Group System

ABO blood groups are defined by the presence of two antigens (antigen A and B) on red blood cells and two antibodies (antibody A and B) circulating in the blood plasma. In the ABO blood system, there are four blood types, known as blood types A, B, AB and O Blood type A has antigen A, blood type B has antigen B, blood type AB has both antigen and blood type O has neither antigens on their RBCs. Similarly, an individual with type B blood has pre-formed anti-A antibodies. Individuals with type AB blood, which has both antigens, do not have preformed antibodies to either of these. People with type O blood lack antigens A and B on their erythrocytes, but both anti-A and anti-B antibodies circulate in their blood plasma (American Red Cross., 2013).

1.1.2. 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 blood cell surface.

While many blood systems are known besides the ABO system, the Rh blood group system is of special importance. Rh blood group system is defined by the presence or absence of a single RBCs antigen.

Rh antigens are highly immunogenic and till now 49 Rh antigens are identified. D antigen is most significant and D negative individuals produce anti-D if they encounter the D antigen through transfusion or pregnancy and causes hemolytic transfusion reaction, or hemolytic disease of fetus and newborn (disorder causing agglutination and hemolysis in an Rh^+ fetus or newborn of an Rh^- mother). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers (Bethesda, 2005).

Table 11. Allelic Frequency of Rh blood groups studied in different populations across the world.

Population	Rh^+	Rh^-	References
Ethiopia	0.94644	0.05356	Seifu and Kifle, 1985
Germany	0.9500	0.05000	Akbas *et al*; 2003
Kenya	0.8030	0.1970	Lyko *et al*, 1992
Lagos (Nigeria)	0.9400	0.0600	Adeyemo and Sabayeso, 2006
Mandi Bahauddin (Pakistan)	0.91400	0.0860	Anees *et al*, 2007
Nigeria	0.9430	0.0570	Falusi *et al*, 2000
Ogbomoso (Nigeria)	0.9670	0.0330	Bakare *et al*, 2006
Port Harcourt (Nigeria)	0.9677	0.0323	Jeremiah, 2006
Red Indians (USA)	1.00	0	Reddy *et al*, 2008

Saudi Arabia	0.9300	0.0700	Khattak *et al*, 2008
USA	0.8500	0.1500	Khattak *et al*, 2008

Source: ISBT, 2008(http://www.bloodbook.com/world-abo.html

1.4. Distribution of ABO and Rh Blood Group Phenotypes

Several studies reported that there are variations in the distribution of ABO blood groups among ethnic groups (Falusi *et al*; 2000). The genetic structure of a population is determined by the total of all alleles (the gene pool). In the case of sexually interbreeding individuals, the structure is also characterized by the distribution of alleles into genotypes. The genetic structure can be described in terms of allelic and genotypic frequencies (Russell, 2005).

All human populations share the same blood group systems, differing only in the frequencies of specific types. The incidence of ABO and Rh groups varies in different parts of the world (Khattak *et al.,* 2008). Table 2 shows the distribution of the ABO blood groups along ethnic lines. Blood type B has its highest frequency in Northern India and neighboring Central Asia, an its frequency diminishes both towards the west and the east, falling to single digit percentages among the Swiss. It is believed to have been entirely absent from Native American and Australian Aboriginal populations prior to the arrival of Europeans in those areas. Blood group A has high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigine populations and the Blackfoot Indians of Montana, (Encyclopedia Britannica, 2002).

Table 2 2. Ethnic distribution of ABO (without Rh) blood types

Population	O (%)	A (%)	B (%)	AB (%)
Ethiopian	43	27	25	5
Ainu(Japan)	17	32	32	18
Albanians	38	43	13	6
Grand(Andamasene)	9	60	23	9
Arabs	34	31	29	6
Armenians	31	50	13	6
Asia(in USA general)	40	28	27	5
Austrians	36	44	13	6
South Africans	45	40	11	4
Basques	51	44	4	1
Belgians	47	42	8	3
Black foot(N.India)	17	82	0	1
Bororo(Brazil)	100	0	0	0
Sudanese	62	16	21	0
Bulgarians	32	44	15	8
Burmese	36	24	33	7

Buryals(Siberia)	33	21	38	8
Bushmen	56	34	9	2
Chinese-Canton	46	23	25	6
Kukuyu(Kenya)	60	19	20	1

Source: (ISBT, 2008C: http:\www.bloodbook.com\world-abo.html)

It has also been reported in several studies that there are variation in ABO blood group among different ethnic groups (Falusi *et al*., 2000). Many other studies have shown that blood type O was the most common blood type and blood type AB was the least common blood type in some ethnic groups (Nwauche and Ejele, 2004). For instance, in African-American ABO blood group, the distribution is type O, 46%; type A, 27%; type B, 20%; and type AB; 7%. Among the Caucasians in the United State, the distribution is type O, 47%; type A, 41%; type B, 9%; type AB, 3%. In addition, among Western Europeans, type O is 46%; type A, 42%; type B, 9%; and type AB, 3% (Adeyemo and Soboyejo, 2006).

The O blood type is very common around the world; about 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of central and South America where it approached 100%. The lowest frequency of (O) is found in Eastern Europe and central Asia, where B is common (Khan *et al*., 2004). Of the Rhesus blood group system, the allele D that gives rhesus positive status is at its lowest in Europe. It increases in frequency eastward and southward to approximately 80% over almost all of Africa south of the Sahara. In eastern Asia, Australia and Indonesia; it often attains 100%. The same holds for American indigenous population in many of whom the D frequency is 100 % (Neil, 2006).

The distribution of O-blood type was 36.73%, 40.14% and 46.26% among the Sodo, Silte and Meskan ethnic populations respectively (Kasahun *et al*., 2015). The percentage of Rh positive was 91.16%, 93.19%, and 91.84% in Sodo, Silte and Meskan ethnic groups respectively. While the percentage of Rh negative was 8.84%, 6.81%, 8.16% in Sodo, Site and Meskan ethnic groups, respectively. The frequency of ABO blood groups in both Rh positive and

negative subjects among the three ethnic groups of the Silte Zone, Ethiopia was O > A > B > AB, except in the Sodo ethnic group where the blood group A was the commonest among Rh negative subjects (Kassahun *et al.*, 2015).

1.5. Clinical Importance of Studying Blood Groups

1.5.1. Blood Transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor in a similar way as medicine. Much of the routine work of a blood bank involve testing blood from both donors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between a donor and recipient, a severe acute hemolytic reaction with RBC destruction, renal failure and shock is likely to occur, and death is a possibility. Antibodies can be highly active and can attack RBCs and bind components of the complement system to cause massive hemolysis of the transfused blood. Patients should ideally receive their own blood or type specific blood products to minimize the chance of a transfusion reaction. Risks can be further reduced by cross matching blood. But this may be skipped when blood is required for an emergency (Bruce, 2002).

2.5.2. Hemolytic Disease of the New Born (HDN)

People are Rh positive if they have a certain Rh antigen (the D antigen) on the surface of their erythrocytes, and people are Rh negative if they do not have this Rh antigen. Rh incompatibility can pose a major problem in some pregnancies when the mother is Rh negative and the fetus is Rh positive (Avent., 1999). If fetal blood leaks through the placenta and mixes with the mother's blood, the mother becomes sensitized to the Rh antigen. The mother produces Rh antibodies that cross the placenta and cause agglutination and Hemolysis of foetal

erythrocytes. This disorder is called Hemolytic disease of the newborn (HDN), or erythroblastosis foetalis, and it may be fatal to the fetus (Dennis *et al*., 1998).

2.5.3. Universal Donors and Universal Recipients

With regard to transfusion of packed red blood cells, individuals with type O Rh negative blood are often called universal donors, and those with type AB Rh Positive blood are called universal recipients; however, these terms are only generally true with respect to possible reaction of the recipient's anti-A and anti-B antibodies to transfused red blood cells. Blood donors with particularly strong anti-A, anti-B or any atypical blood group antibody are excluded from blood donation. The possible reaction of anti-A and anti-B antibodies present in the transfused blood to the recipients RBCs need not be considered, because a relatively small volume of plasma containing antibodies is transfused (Fauci, 1998).

2.5.4. 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).

2.5.5. Blood Products

A blood product is any component of the blood which is collected from a donor for use in a blood transfusion. Whole blood is uncommonly used in transfusion medicine at present; blood products may also be called blood- based products to differ from blood substitute which generally refer to artificially produced products. Whole blood may be classified as a blood product or as a separate entity. (Henrik *et al.,* 2012). Blood group AB individuals have both A and B antigens on the surface of their RBCs, and their blood -plasma do not contain any antibodies against either A or B antigen. Therefore, an individual with type AB blood can receive blood from any group but cannot donate blood to either A, O or B group. They are known as universal recipients.

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

In General assessing blood group frequency distribution is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought out in modern medicine (Khattak *et al.,* 2008). Blood group antigens are not only crucial in the medical field, but can also be utilized in genetic research, anthropology, and tracing ancestral relation of humans (Khan *et al.,* 2004*)*.

1.6 The Hardy-Weinberg Genetic Equilibrium

The Hardy-Weinberg principle provides the solution to how variation is maintained in a population with Mendelian inheritance. According to this principle, the frequencies of alleles (variations in a gene) will remain constant in the absence of selection, mutation, migration and genetic drift. The Hardy-Weinberg "equilibrium" refers to this stability of allele frequencies over time (James, 1999).

A second component of the Hardy-Weinberg principle concerns the effects of a single generation of random mating. In this case, the genotype frequencies can be predicted from the allele frequencies. For example, in the simplest case of a single locus with two alleles: the dominant allele is denoted A and the recessive allele a and their frequencies are denoted by *p* and *q*; frequency (A) = p; frequency (a) = q; p + q = 1. If the genotype frequencies are in Hardy-Weinberg proportions resulting from random mating, then we will have frequency (AA) = p2 for the AA homozygote in the population, frequency (aa) = q2 for the a homozygote, and frequency (Aa) = 2pq for the heterozygote (Russell, 2005).

$$P^2 \text{ (AA)}: \ 2pq \text{ (Aa)}: \ q2 \text{ (aa)}$$

1.6.1 Estimation of Genotypic and Allelic Frequency Distribution

An important application of the Hardy-Weinberg law is estimating the heterozygous frequencies in a population. The majority of the deleterious recessive genes in human population are carried in heterozygous condition. To calculate the frequency of individuals who have heterozygous recessive traits, it is usually begun by counting the number of homozygous recessive individuals.

These homozygous individuals can be distinguished from the rest of the population by clinical symptoms that indicate the defects. By using the Hardy-Weinberg law we can calculate the frequency of the heterozygous condition (Cummings, 2000). For this study, the frequencies of

the ABO blood group genotypes and alleles were calculated or estimated using the extension of the Hardy-Weinberg law as employed by Griffith *et al., (* 2008).

In other words , when you add up the frequency of the A, B and O alleles, you have accounted for 100% of the alleles for this gene that are present in the population .The genotypic frequencies are given by the following equation, when the frequencies are p, q and r for the alleles I^A, I^B and I^O respectively. Thus, $(p + q + r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) (Griffith *et al.,* 2008).

The frequencies of the genotype at equilibrium will be computed by the square of the allelic frequencies. In this system, the alleles I^A and I^B are co-dominant and both are dominant to O. This system has six possible genotypic combinations but only four phenotypic blood groups. Homozygous I^AI^A individuals and heterozygous I^AI^O individuals are phenotypic ally identical, as are I^BI^B and I^BI^O individuals. This results in four phenotypic combinations, known as blood types A, B, AB, and O. The frequency of I^AI^A genotype is predicted to be p^2, I^AI^B individuals 2pq, I^AI^O individuals 2pr, I^BI^B individuals' q^2, I^BI^O individuals 2qr, and I^OI^O individual's r^2.

1.6.2 Extension of the Hardy – Weinberg Law to Loci with More Than Two Alleles

When two alleles are present at a locus (with the frequency of p and q), the Hardy-Weinberg law tells us that at equilibrium the frequencies of the genotype is p2 + 2pq + q2, which is the square of allelic frequencies (p + q) 2. This is the simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are sampled two at a time into a diploid zygote (Daniel and Clark, 2007).

Table 33. Punnet Square showing Hardy-Weinberg frequencies of three autosomal alleles

Male gamete

Allele	I^A	I^B	I^O
Frequency	p	q	r

I^A I^A P^2	I^A I^B Pq	I^A I^O Pr
I^A I^B Pq	I^B I^B $q2$	I^B I^O qr
I^A I^O Pr	I^O I^B rq	I^O I^O r^2

Female gamete

Allele	Frequency
I^A	p
I^B	q
I^O	r

Extension of the Hardy-Weinberg principle to multiple alleles of a single autosomal gene can be illustrated by a three-allele case. Table 3, shows the results of random mating in which three alleles are considered. The alleles are designated I^A, I^B, and I^O, where the uppercase letter

represents the gene and the subscript designates the particular allele. The allele frequencies are *p*, *q*, and *r*, respectively. With three alleles (as with any number of alleles), the allele frequencies of all alleles must sum up to 1; in this case, $p + q + r, = 1$. As in Table 3, the entry in each square is obtained by multiplying the frequencies of the alleles at the corresponding margins; any homozygote {such as $I^A I^A$} has a random-mating frequency equal to the square of the corresponding allele frequency (in this case, p^2). Any heterozygote {such as $I^A I^O$} has a random-mating frequency equal to twice the product of the corresponding allele frequencies (in this case, *2pr*). The extension to any number of alleles is straightforward. 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 genotyped (Dar *et al*., 2010).

3. MATERIALS AND METHODS

1.1. Description of the Study Area

Mesela is one of the 14 districts of Western Hararghe Zone, Oromia Regional State. It has a total area of 654.4sq.km.The district shares boundary with Melkabelo *Woreda* in east and southeast, Tullo *woreda* in north and northeast, Chiro *woreda* in northwest and Gemechis *woreda* in west, south and southwest (MWEP,2012). According to CSA (2007) report the total population of Mesela *woreda* is 151,698 of which male and female comprises 76,864 and 72,412, respectively.

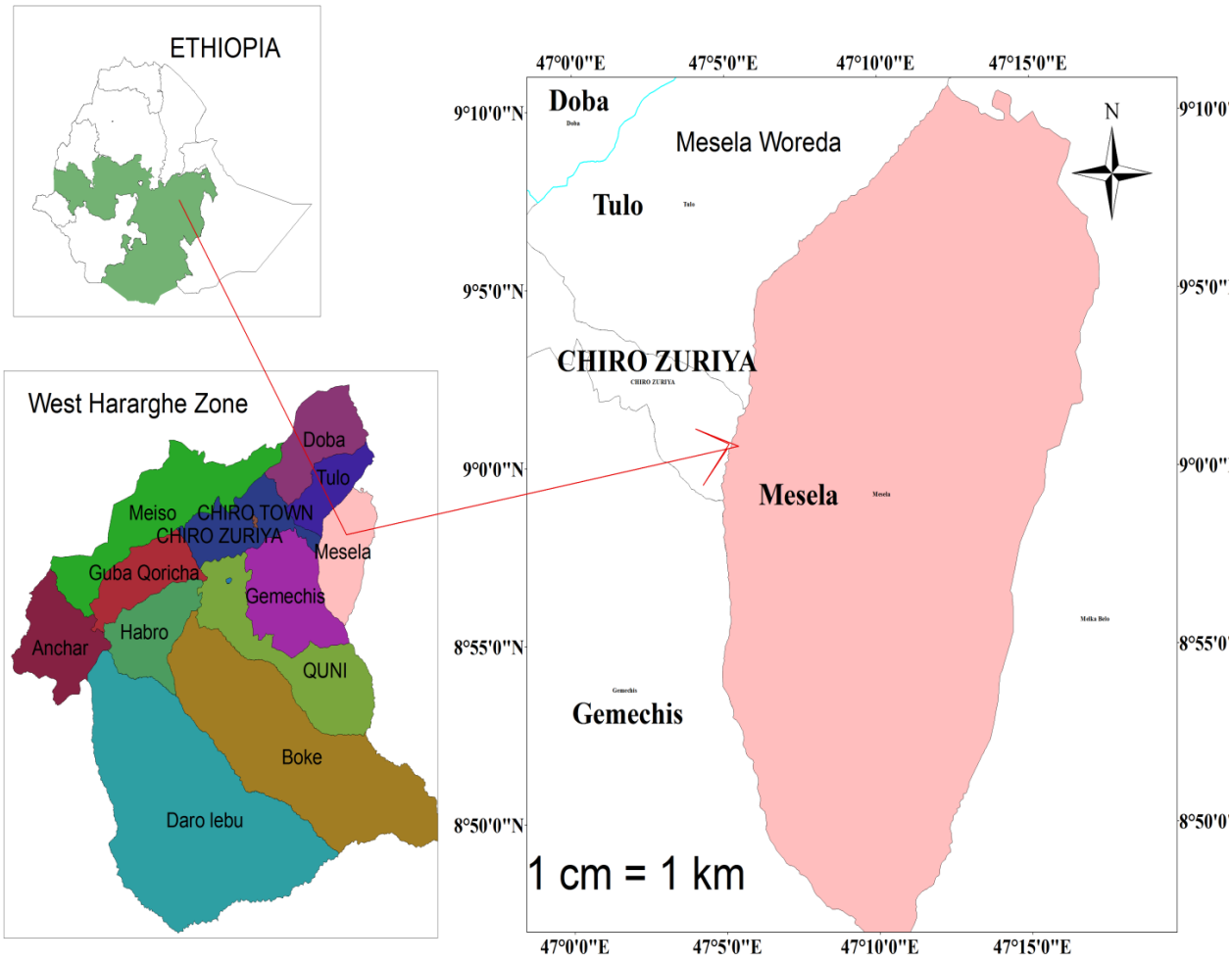


Figure 1. Map of Mesela district

3.2. Sample Size

A total of 1400 students of Mesela Secondary and Preparatory School, the study was conducted on 400 sample students purposively selected comprising approximately 29 % of the student population in the school in 2009. Therefore, a total of 400 voluntary students from the School participated in the study. The reason why I targeting at Secondary and Preparatory School students is that since Secondary and Preparatory School students came from all kebele's of the woreda they can represent the wareda.

3.3. Blood Sample Collection and Typing Procedures

Blood sample was collected from each study subjects after their agreement to participate in the research process and signed the agreement form. The ABO and Rh blood group test was performed by using sterilized needles to obtain drops of blood from sterilized finger pricks by appropriate technicians. The blood samples were collected by qualified laboratory technicians using standard clinical procedures with sterilized slides needles and chemicals. Blood samples were taken from finger pricks, and open slide method of testing ABO blood types and Rh(D) factor was used following (Bhasin and Chahel 1996). Then, the blood was placed on a clean slide in three places and a drop of one of the Anti-seraA, Anti B and Anti D that obtained from Mesela health center was added to each of an individual's blood samples and mixed using a glass rod. Blood group was determined on the basis of agglutination and recorded as blood group A^+ , B^+ ,AB^+ ,O^+ or A^-,B^-,AB^- and O^-.

3.4. Methods of Data Analysis

The genetic structure can be described in terms of phenotypic, allelic and genotypic frequencies (Russell, 2005). For the present study, the frequency of the blood group phenotypes was used to calculate the frequencies of the ABO and Rh blood group alleles by using the extension of Hardy- Weinberg principle as employed by Griffith *et al*., (2008). For the ABO blood groups, three alleles were used (I^A, I^B and I^O), with frequencies equal to p,

q and r respectively. The frequencies of the genotypes at equilibrium were computed by trinomial extension $(p+q+r)^2$

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

The following formulae were used to calculate blood type, allelic, genotypic and expected phenotypic frequencies

I. Formula for the calculation of blood type frequency

Number of individuals/ Total sample

II. Formula for the calculation of allelic frequency

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

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

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

A correction factor (d) was calculated according to $d = 1 – p – q –r$, and the final corrected frequencies p', q' and r' for the I^A, I^B and I^O alleles, respectively, were calculated as follows-

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

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

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

III. Formula for the calculation of genotypic frequency

$$I^AI^A = p\,2, \quad I^AI^O = 2pr$$

$$I^B I^B = q2, \quad I^BI^O = 2qr$$

$$I^AI^B = 2pq \quad I^O = r^2$$

IV. Formula for the calculation of expected phenotypic frequency that is the number of individuals belonging to different phenotypic classes for each blood group

Expected number of A = (p2 +2pr)x total sample size

Expected number of B = (q2 +2qr) x total sample size

Expected number of AB =(2pq) x total sample size

Expected number of O =(r2) x total sample size

3.5. Statistical Method to Test the Goodness of Fit of the Observed and Expected Phenotypic Frequencies

Chi-square test was used to test whether there was significant variation between the observed number of individuals in each of the phenotypic classes in the ABO and the Rh blood group systems and those expected using the Hardy-Weinberg equation (chakraborty, 2010). The chi-square test statistic is,

3.6. Ethical Considerations

An authorization to carry out the study was obtained from Health Center of the Mesela *woreda* by using a letter of support or cooperation obtained from Department of Biology Haramaya University. All the information that was obtained about the participants was kept confidential.

4. RESULTS AND DISCUSSION

4.1. Frequency of ABO and Rh Blood Group Phenotypes

For this study, four hundred individuals were selected randomly. The phenotypic frequency of ABO blood is calculated in Table 4 and that of the Rh blood group is in Table 5.

Table 44. Phenotypic Frequency of ABO blood group phenotypes among secondary and preparatory school students of Mesela

Blood Type	Number of Students	Frequency	Frequency(%
O	175	0.4375	44%
A	110	0.2750	28%
B	96	0.2400	24%
AB	19	0.0475	4%
Total	400	1.00	100%

There are differences in the frequency distribution of the ABO blood group phenotypes a0mong individual of students at Mesela. Blood type O has the highest frequency (0.4375) while blood type AB has the lowest frequency (0.0475). Blood type B has the frequency of (0.24) whereas, blood type A has the frequency of (0.275).

Many studies have shown that blood type O is the most common blood group and blood type AB is the least common blood group in different ethnic group (Nwauch and Ejele, 2004). 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. For example, 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 Amahara, 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 Amahara, Oromo and Afar respectively. However, the present result obtained for the Mesela students population different from results from Swat district in Pakistan where the percentage frequency A=27.9%, B=32.40%, O=29.1% and AB= 10.58% in which B>O>A>AB (Khattak *et al*., 2008). It also was also not consistent with ABO phenotypic frequency of Bororo (Brazil) in where 100% of the population presented O Blood group (ISBT, 2006).

Table 55. Frequency of Rh blood group phenotypes among secondary and preparatory school students of Mesela

Rh phenotype	Numberof individuals	Frequency	%
Rh positive	380	0.95	95%
Rh negative	20	0.05	5%
Total	400	1.00	100%

As shown in Table 5, the frequency distribution of Rh phenotypes among individuals of people varies. The frequency of Rh positive is 0.95 and Rh negative is 0.05.Thus, the frequency distribution of Rh positive blood group is higher than the frequency distribution of Rh negative among different blood groups among the student population at Mesela secondary and preparatory schools. This result was similar with the study in Germany where the frequency of Rh positive was 0.9500 and Rh negative was 0.0500 (Akbas et al.,2003). The results were agree 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 66. Frequency distribution of the Rh phenotypes in each of the ABO blood groups

Rh Phenotypes	Number	Frequency	(%)
ARh^+	101	0.2525	25%
ARh^-	9	0.0225	2%
BRh^+	90	0.225	22%
BRh^-	6	0.015	2%
$ABRh^+$	18	0.045	5%
$ABRh^-$	1	0.0025	-
ORh^+	171	0.4275	43%
ORh^-	4	0.01	1%
Total	400	1.00	100%

The frequency distribution of Rh Negative is very small or rare in the ABO blood grouping system. Blood group B Rh^+ was found to be 0.225, blood group AB Rh^+ was found to be 0.045, and blood group O Rh^+ was found to be 0.4275 and blood group A Rh^+ was found to be 0.2525. The blood group A Rh^- was 0.0225, BRh^- 0.015, ORh^- which is 0.01, and AB^- is 0.0025 which is the smallest frequency distribution in the ABO blood grouping system as well as in the Rh system. From this study, blood group O Rh positive was found to be the highest with the frequency of 0.4275, while the lowest was blood group AB Rh positive with the frequency of 0.045. Similarly, blood group A Rh Negative was found to be the highest with the frequency of 0.0225, while blood group AB Rh Negative was the lowest with the frequency of 0.0025. Similar pattern of frequency was also observed in Pakistan where Rh positive was 0.9140 and Rh negative was 0.0860 (Anees *et al.,* 2007). Thus, there is a need to have information on these blood group systems in any population of individuals. The relevance of

having knowledge about the blood group systems among individuals in any population is enormous. The types of information obtained from the findings are useful for genetic information, genetic counseling, medical diagnosis and general and physiological wellbeing of individuals in a population.

4.1.1. Frequency of ABO Blood Group Alleles and Genotypes

By using the extension of the Hardy-Weinberg law employed by Griffith *et al., (*2008), the genotypic and the allelic frequency distribution among Mesela secondary and preparatory school students is presented in Table 7.

Table 77. Allelic, genotypic, and phenotypic frequencies of ABO and Rh blood groups

A l l e l e Frequency	Allele	Genotype	Genotype frequency	Phenotype	Phenotype Frequency(%)
0.6662	I^O	I^OI^O	0.4438	O	44.38
0.1564	I^B	I^BI^B	0.0244	B	2.44
		I^BI^O	0.2084	B	20.84
0.1774	I^A	I^AI^A	0.0385	A	3.15
		I^AI^O	0.2365	A	23.64
		I^AI^B	0.0555	AB	5.55
0.7764	D	DD	0.6028	Rh+ve	60.28
		Dd	0.3472	Rh+ve	34.72
0.2236	D	dd	0.0500	Rh-ve	5

In Table 7, allelic frequencies show a high frequency of the allele I^O over I^A and I^B using the extension of the Hard-Weinberg law employed by Griffith *el al*, (2008). The genotypic and the allelic frequency distribution among individuals is calculated and listed in Table 7. The allelic frequencies for ABO blood group alleles were obtained in the order of (I^O=0.6662, I^A=0.1774 and I^B=0.1564).These results are in accord with the other previous studies in the northern part of Iraq which stated that the allele frequencies were in the order

of $I^O > I^A > I^B$, where I^O = 0.647, I^A = 0.194, I^B =0.172 in Nianwa (Sadiq, 1989). The genotypic frequencies of the homozygous O blood group (I^OI^O) was obtained as 0.4438 whereas, that of genotype I^AI^A is 0.0385, genotype I^AI^O was 0.2365, genotype I^BI^B obtained as 0.0244 whereas genotype I^BI^O was 0.2084 and genotype I^AI^B 0.0555.

Most of the A and B Blood types were heterozygous dominant I^AI^O and I^BI^O, respectively, in the population studied. This agrees with Bakare *et al*. (2008), who suggested that the predominance of I^O allele may be as a result of the fact that many A's and B's may have been heterozygous carrying I^O allele silently thereby maintaining I^O allele in the heterozygous population. With respect to Rhesus blood group system, of the total four hundred samples, 380 were Rh positive, while 20 were Rh negative with the frequency of 0.6028 for allele D and 0.0500 for allele d. The frequency of heterozygous (Dd) obtained for this study is 0.3472.

4.1.2. Chi-square test for examining difference between the observed and expected frequencies of ABO and Rh blood Groups.

This section presents the observed versus expected number of ABO and Rh blood group among students of Mesela secondary and preparatory schools.

Table 88. Observed versus expected frequency of ABO blood groups

Blood group	Observed	Expected			
A	110	108.16	1.84	3.3856	0.0313
B	96	96.16	-0.16	0.0256	0.003
AB	19	23.64	-4.64	21.5296	0.9107
O	175	171.76	3.24	10.4976	0.0611

Σ	400	399.72	**X^2=1.0034**

In Table 8, the calculated Chi-square value is 1.0043, which has the P value between 0.90 and 0.80 with 3 degrees of freedom. This means that there is no significant difference between the observed number of individuals in each of the four classes of the ABO blood group phenotypes and those expected in each of the phenotypic classes. The results indicate that the study population is stable regarding the different ABO blood groups phenotypes. Because the population is stable, it can safely be asserted that in the next generation, the frequency of the ABO will be the ones obtained in the present study.

Table 99. Observed versus expected frequency of Rh blood groups

Rh blood group	Observed	Expected			
Rh+	380	380	0	0	0
Rh-	20	19.96	0.04	0.0016	0.00008016
Σ	400	399.96			X^2=0.0000806

In Table 9, the calculated chi-square test in Rh blood groups were 0.00008, which has the p value between 0.50 and 0.30 with 1 degree of freedom for Rh blood groups. This means that there is no significant difference between the observed Rh blood group phenotypes and ones expected assuming the Hardy-Weinberg genetic equilibrium.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

There has been no known data on the distribution patterns and frequency of ABO and Rh blood group phenotypes, genotypes and alleles in the population of Mesela town. This study aims at providing information on the distribution of the phenotypes and allelic and genotypic frequencies among students of Mesela secondary and preparatory schools with a view of contributing to existing knowledge and subject matter.

A total of 400 individuals were selected purposively among students of Mesela secondary and preparatory schools. In the study, the frequency distribution of blood group O was the highest with frequency of (0.4375), followed by blood group A (0.2750) and blood group B (0.2400) and the least frequency was although blood group AB (0.0475) in the studied sample. Moreover, this study confirmed that Rh (D) positive has the highest percentage frequency while Rh negative has the lowest percentage frequency as observed among students. In the

overall sample population of the students the blood group O (0.4375)> A (0,2750)>B (0.2400)> AB (0.0475). With respects to allelic frequency of the ABO blood group, allele I^O records the highest frequency (0.6662), followed by allele I^A (0.1774) while allele I^B records the least frequency (0.1564). In the case of Rhesus factor, allele D has a frequency distribution far higher than d allele which is Rh(D) (0.7764)>Rh(d) (0.2236).

5.2. Conclusion

From this study, it is evident that the proportion and allele frequencies of individuals who belong to blood group O of the studied students was most predominant. The implication of this finding is that blood group O is the most readily available blood group in the study area which is more advantageous for the population in the event of blood transfusion.

In general this study provides information for Mesela secondary and preparatory school students to know their own blood types.

5.3. Recommendations

The following recommendations are drawn from the study:

- The sample studied may not represent the population in the study area since; the sample size used to conduct this study was small. Therefore, it is advisable to use larger sample size to obtain more accurate information about the distribution of ABO and Rh blood group alleles.
- The information obtained from this study will be used in the planning of blood transfusion programs in the study area.

6. REFERENCES

Abraham Haileamlak, Ayalew T. Muluneh, Fessahaye Alemseged, Fasil Tessema, Kifle Woldemichael, Makonnen Asefa, Yoseph Mamo, Solomon Tamiru, and Gemed a Abebe. 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.

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

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.

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

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

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

Avent, N.D., Reid, M.E. 2000. "The Rh blood group system: a review". Blood, 95 (2):37- 387.

Bakare, A.A.,M.A.Azeez and J.O.Agbolade,2008. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomosho, South-West *Nigeria. African Journal of Biotechnology, 5(1): 224-229.*

Bethesda D.L.,2005. Blood Groups and Red Cell Antigens. In: The Rh blood group. USA: National Center for Biotechnology Information. p.1-6. (Retrieved on 23 April 2013 from http://www.ncbi.nlm.nih.gov/books/NBK2269/pdf/ch07Rf.

Bhasin,M.K., and S.M.Chahal. 1996. *A* Laboratory *Manual for Human Blood Analysis*. Delhi: Kamla-Raj Enterprise.

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

Chakraborty, S. 2010. Genetic analysis on frequency of alleles for Rh and ABO blood

group system in the Barak vally 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.

Cummings, M.R., 2000. Human Heredity Principles and Issues 5th edition. University of Illinois, Chicago.478.

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

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

Dennis, Y.M., Hylem, N.M., Fidler, C, Sargent, I.L, Murphy, M.F, Chamberlain P.F .1998. Prenatal diagnosis fo fetal RhD status by molecular analysis of material plasma. New Engl. J. Med. 337:1734–1738.

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

Fauci ,A. S., Eugene Braunwald., kurt. J. Isselbacher, Jean .D. Wilson, Joseph.B. Martin., Dennis.Kasper, Stephen L. Longo. 1998. *Harrison's Principals of Internal Medicine.* New York: M.c Graw-Hill. 719.

Firkin F, Chesterman C, Penington D, Rush B 1989. Blood groups; blood transfusion; acquired immune deficiency syndrome. In: *Haque International Journal of Pharmacy. Issue 3, 814 816* 816 de Gruchy's clinical hematology in medical practice. 5th ed,New Delhi, Oxford University Press. 475-96.

Flusi, 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 ,29(1):23-26.* glycol biology". Transfuse Medicine Revision, 20(3): 207-17.

Griffiths. A.F, S.R, Wesser, R.C, Lewontin., and S.BCarrol., 2008. Introduction to Genetic Analysis. 9t h edition . W.H. Freeman and company. New York. 838.

Gupta, P.K., 1999. Genetics, Rastogics Publications, Meerut, India. *International Society of Blood Transfusion.*

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

Henrik, B. Fredericck M, and Linette S. 2012. Forum on Blood safety and Blood

Availability, Institute of Medicine.

Hillier, C.D Shaz BH, winkler AM, Reid M. 2008. Department of pathology and Laboratory Medicine,EmoryUniversity,Atlant.

ISBT, 2006." Table of blood group systems;" *International Society of Blood Transfusion.* URL;http;//ibgrl.blood.co./isbt, accessed March 5, 2008.

ISBT, 2008." Table of blood group systems;" *International Society of Blood Transfusion.* URL;http;//ibgrl.blood.co./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.

James, F., 1999. "Hardy, Weinberg and language impediments". *Genetics*152 (3): 821–825.

Johnson, P., R. Williams, and P. Martin, 2003. "Genetics and Forensics: Making the National DNA Database". *Science Studies,*16 (2): 22–37.

Jolly, J.G.,2000. Medico legal significance of human blood groups. *Journal of Indian Medicine Association*,98:340-1.

Kassahun Tesfaye, Yohannes Petros,Mebeasselassie.2015. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. *Egyptian Journal of Medical Human Genetics*,16(1):71-76.

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

Khattak, I.D., T.M.Khan, P.Syed, A.M.Shah, S.T.Khattak and A.Ali, 2008. Frequency of ABO and rhesus blood groups in district Swat, Pakistan. *Journal of Ayub Medicine, 20(4).*

Khurshid, B., M.Naz, M. Hassan, S.F.Mabood, 2003. Frequency of ABO and Rh (D) blood groups in district Swabi, NWFP, Pakistan. *Journal of Science Tech University Peshawar*, 16:5-6.

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

Laura Dean, M.D.2005. *Blood group and red cell antigens*; National Center for Biotechnology information , United States Government,861-967.

Markovic, M. S.1994. The ABO system of blood groups and the Rh factor in 360 pairs of twins and in 11 triplets from Belgrade. Adam Mickiewicz University, Faculty of Biology, Pozna *Variability and Evolution*. 4(1): 97–102.

Meade, T.W., J.A.Cooper, Y.Stirling, D.J.Howarth, V. Ruddock, and G. J. Miller, 1998. Factor VIII, ABO blood group and the incidence of ischemic heart disease. *Bratian Journal of Haematology,88:601–7.*

Mohamed Saied,2013. Allelic frequency of ABO and Rh-D blood groups among patients belonging to three Ethnic groups between Februry to March. 2013 at Desse Referal Hospital Ethiopia.

Molison, P.L. Blood transfusion in clinical medicine. 6th ed. Oxford UK: Blackwell Scientific

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

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

Patel, P. Sangeeta, S. Jigesh, O. Haren.2012. Frequency and distribution of blood groups in donors in Western Ahmedabad-A hospital based study *National Journal of Medicine* , 2(2) 202– 206.

Reddy, V.M., M. Daniel, E. Bright, S.R. Broad and A.A. Moir, 2008. "Is there an association between blood group O and epistaxis". Journal Laryngology and Otology, 122: 366-368.

Reid, M.E. and C. Lomas-Francis, 2004. The Blood Group Antigen Facts Book. Second ed. New York: Elsevier Academic Press.

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.

Russel, J.P.2005. Ggenetics: A.Mendilian Approach, Pearson Benjamin Cumming. New York. 842. S.M, editors. Practical Haematology. 8th ed. London: Churchill Livingstone. . 44564.

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.

Seeley, R.R, Stephens TD, Tate P 1998. Anatomy and Physiology. 4th edition. The M.c Graw Hill Companies, Inc. USA . 1098.

Waite, G.N. Blood components 2009. In: Rhoades R.A, Bell D.R, editors. Medical physiology.

Waters, A.H .1995. Red cell blood group antigens and antibodies. In: Dacie V.J and Lewis.

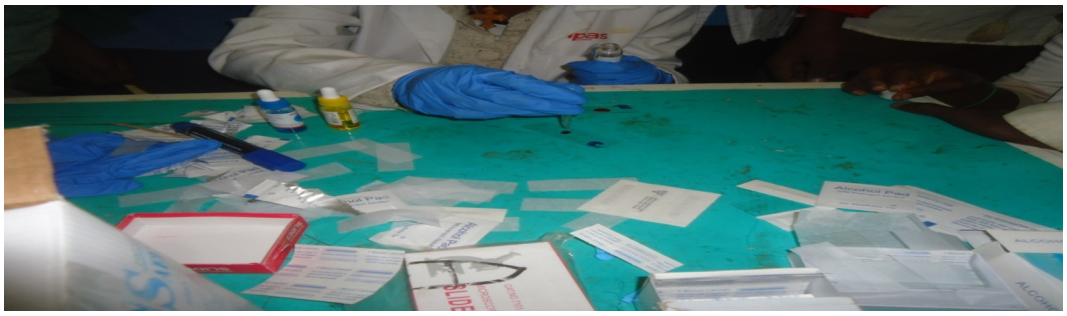
Yazer, M .O lsson M , Palcic M. 2006."the cis-A.B group phenotype: fundamental lesson in glycol biology". Transfuse. Medicine. Revision. 20 (3): 207–17.

7. APPENDIX

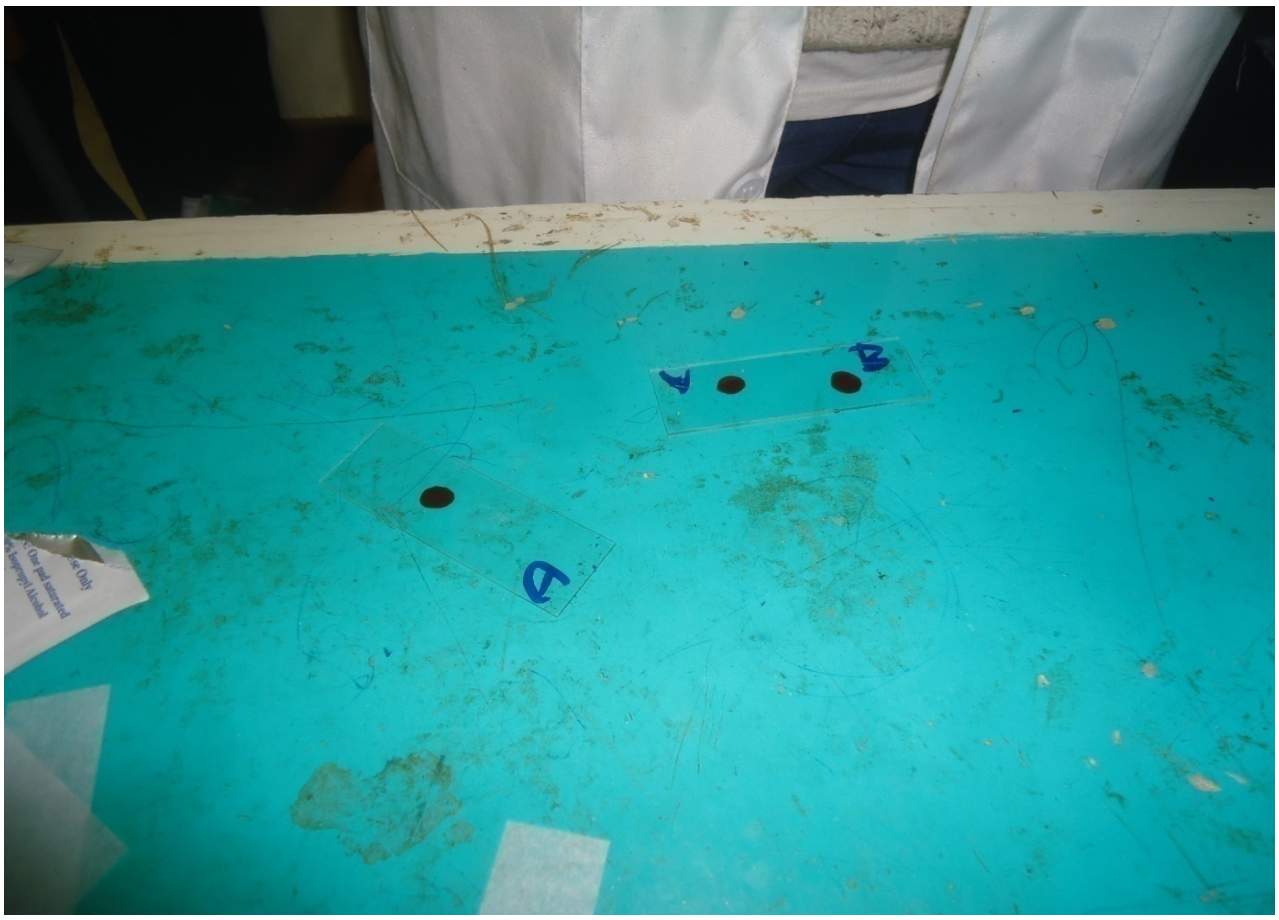
Students during ABO and Rh blood Tests

Appendix figure 11. Sample picture of students taken while they were giving blood for typing


GREEN
SAFE
5



Appendix figure 22. Sample pictures of technicians taken while determining blood types



Appendix figure 33. Sample pictures of slides used for identification of ABO and Rh blood groups

CONSENT FORM

I undersigned have been informed and understand that the purpose of this particular research project is to find out the genotypic and allelic frequencies of ABO and Rh blood group among students of Secondary and Preparatory School of Mesela, Western Hararge zone, Ethiopia. I have also been informed that the information that is obtained from me will be treated confidentially. Furthermore, I have been told that I can refuse to participate in the study. Hence, with this understanding, I hereby agree to participate in this particular research voluntarily.

Name of the student _____

Age _____

Signature _____

Date: _____

Ser no: [illegible]

Date 15/08/2017

Ethical Letter

To: Haramaya University

We are writing this references letter for Mr.Abrahim Mohammed to witness that he has worked his laboratory work on ABO and Rh blood group test on Mesela secondary and preparatory students to diffenciete"**genotypic and allelic frequencies of ABO and Rh blood groups among Mesela secondary and preparatory students**". Therefore we are kindly request that he completed his laboratory activites in our health center with the help of our laboratory technicians.

With best regards!

Abdii Ammee Mahammad

Director PHCU Mesela