ALLELIC AND GENOTYPIC FREQUENCIES OF ABO AND RHESUS (Rh-D) BLOOD GROUPS AMONG STUDENTS OF AMHARA , OROMO AND WOLAYITA ETHNIC GROUPS IN ARSI UNIVERSITY, ETHIOPIA

M Sc. Thesis

Submitted to:

Department of Biology

College of Natural and Computational Sciences

School of Graduate Studies

MADDA WALABU UNIVERSITY

In Partial Fulfillment of the Requirements for

The Master of Science Degree in BIOLOGY

Submitted by: **Suleyman Deko**

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June, 2019

Bale- Robe, Ethiopia

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DEDICATION

I dedicated this Thesis manuscript to my father teacher Deko Abebe and my lovely mother W/r Misra Dekebo and as well as to my four brothers for nursing me with love, incredible support, advice and their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

As MSc student I declare and affirm that this thesis is the result of my own work and that all sources or materials used for this thesis. I have followed all technical principles and ethical in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in thesis has been given recognition through citation. This thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at Madda Walabu University and is deposited at the university library. I confidently declare that this thesis has not been submitted to any other institutions anywhere for the award of any academic certificate, diploma and degree. Quotations from this thesis may be used without special permission provided that accurate and appropriate information of the source is made. Requests for permission for extended quotations from, or reproduction of, this thesis in whole or in part may be granted by the Dean of the School of Graduate Studies when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

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BIOGRAPHICAL SKETCH

Suleyman Deko Abebe was born in Gedeb Asasa Woreda, in West Arsi Zone of the Oromia Region from his father Teacher Deko Abebe and his mother Misra Dekebo on May 19, in 1985. He attended his Elementary School in Mamuda Tuse, Gedeb Asasa Junior School and High Secondary in Dodola Junior Schools and Secondary School Education in Dodola Town, Oromia. After completion of secondary school education, he joined Adama Teachers Teaching Collage Graduated in Diploma in 2006 and he was employed in teaching program in Tiyo Woreda Educational Bureau and served for four years went Haramaya University to attend Degree program in Biology and graduated in 2010. After this much time service he adjusted himself in July 2015 to join Madda Walabu University, College of Natural and Computational Sciences, Department of Biology to pursue his M. Sc. degree in Biology.

ACKNOWLEDGMENTS

First of all, I would like to thank my almighty Allah. I would like to express my heartfelt thanks and sincere appreciation to my advisor, Temegen Bedassa (*Assistant Professor*) for his valuable professional guidance, constructive comments, genuine cooperation, and experience sharing in directing me as far as the research concerned.

My special gratitude may goes to my father teacher Deko Abebe and my mother W/r Misra Dekebo and my wife Mrs Zahara Mohammed, my daughter Hasenet Suleyman for their unlimited support and making all my life bright and new in giving their ever long last love. I am fortunate to have been surrounded by set of my brothers as back bone Muzeyen Deko, Mohammed Deko, Tahir Deko, Mubarek Deko is really unforgettable and I have a deep admiration, with your help it becomes fruitful. I thank all my friend's and staff members of Ketar secondary school and Tiyo woreda educational bureau for their genuine comments, guiding ,encourage and support me during the whole process of my efforts to realize this thesis.

I deeply thank Arsi University students for their kindness in participating voluntarily during blood test and forward my special thanks to laboratory technicians Mr. Gosaye Ketema and Mrs. Tsige Abayneh for their Professional support throughout my laboratory session work by giving time for my work kind cooperation. I would like also special thanks to Adem Kedir (PhD) *Associate Professor*, Gebi Huseen chief Academic Research and Community Service Director, Tullu Kurki (PhD) *Associate Professor of Surgery*, Abinet Kebede, Hailu Fekadu (MPH) Dean For Research and Community Service of Asella Specialised Referral Hospital for their assistance on my research work. Similarly, I would like to express my gratitude to Madda Walabu University, all management and staff members Biology Department for giving the chance to pursue my M Sc and for finical support.

LIST OF ABBREVIATIONS AND ACRONOMYS

AU	Arsi University
CSA	Central Statistical Agency
DNA	Deoxy Ribonucleic acid
ETB	Ethiopian Birr
HDN	Hemolytic Disease of the Newborn
HWE	Hardy-Weinberg Equilibrium
HWP	Hardy-Weinberg Principles
Ig	Immunoglobulin
Ig A	Immunoglobulin A
Ig G	Immunoglobulin G
Ig M	Immunoglobulin M
ISBT	International Society of Blood Transfusion
MoSHE	Ministry of Science and Higher Education
RBC	Red Blood Cells
Rh	Rhesus
SPSS	*Statistical Package for Social Science*
WF	Willebrand Factors
WHO	World Health Organization
USA	United States of America

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ABSTRACT

Blood is specialized connective tissues with complete and unique identity in an individuals. The frequency ABO and Rh-D blood groups varies worldwide, ethnically and in a population are not found in equal numbers of frequency even among ethnic groups. Therefore, the objective of the study was to estimate allelic and genotypic frequencies of ABO and Rh-D blood groups among students of Amhara, Oromo and Wolayita ethnic groups in Arsi University, Oromia, Ethiopia in 2018 . The present study was conducted on 384 randomly selected volunteer University Students among which 219 (57.03%) were males and 165 (42.97%) were females with their ages 18-35 years old. Blood grouping were done using slide where a drop of blood sample from a left ring finger pricks were placed in three different places on clean slide followed by a drop of blood grouping using anti-sera anti- A, anti -B and anti- D which were added and mixed using open slide method for 60 sec to checked agglutination. The frequencies of ABO and Rh-D blood groups phenotypes were expressed in percentages and the modified Hardy-Weinberg Law was used to determine allele and genotype frequencies. In the overall sample, the most prevalent blood group was type O followed by A, B, and AB with calculated frequencies 40.10%, 31.23%, 23.43% and 5.21 , respectively. Blood group O had the highest frequency while blood group AB had the least frequency. The overall Rh+ frequency was 92.45%, while Rh- was 7.55%. The order of allelic frequency in ABO blood group was $I^O > I^B > I^A$ in the overall samples and in each of the three ethnic types were 0.633 I^O, 0.2124I^B and 0.1634 I^A in the total sample. The frequency of allelic Rhesus D and d blood group were 0.8256 and 0.1744 respectively. The calculated overall X^2 , value for studied ethnic groups is 0.02088 with P value is greater than 0.05 at 2 degrees of freedom. Chi-Square value for overall Rh-D in the studied ethnic groups is 3.20074 which has P value less than 0.05 with 2 degree, of freedom. This implies that there is no significant difference between values obtained for total studied ethnic group and the expected value. Therefore, The result of present study confirmed that the frequency of ABO and Rh-D blood group had no significant variation among Arsi University Students in the three ethnic group.

Key Words: ABO, Agglutination, Allele frequency , Arsi University, Blood Sample, Ethnic groups, Frequency, Genotype, Rh-D

1. INTRODUCTION

1.1 Background of the Study

Blood is the essential life line for our existence. The blood plays more roles than one might expect, it is involved in respiration, nutrition, waste elimination, thermo regulation, immune defense, water and acid- base balance and internal communication. Most adults have 4 to 6 L of blood. It consists of Erythrocytes (red blood cells), Leukocytes (white blood cells) and Thrombocyte (Platelets). Erythrocytes have dual principal functions: (1) to pick up oxygen from the lungs and deliver it to tissues elsewhere and (2) to pick up carbon dioxide from other tissues and unload it in the lungs. An Erythrocyte is a disc-shaped cell with a thick rim and a thin sunken center where the nucleus used to be originated. It is about 7.5µm in diameter and 2.0µm thick at rim (Saladin, 2003; Mindaye Regassa and Temesgen Bedassa Gudeta, 2019).

The ABO blood group system was the first human blood group system to be discovered by Australian Scientist karl Landsteiner in 1900.The ABO and Rh blood groups are the most important blood groups before the long list of several other blood groups discovered so far. The two significant blood group systems were discovered during early experiments with blood transfusions, the ABO group in 1901 and the rhesus Rh-D group in 1939 (Landsteiner and Weiner, 1940; Worlledge *et al*.,1974). The ABO blood group system is the only system in which antibodies are consistently and predictably present in the serum of normal individuals whose red cells lack the antigens. Apart from differences amongst species, differences between the individuals of the same species have also been demonstrated (Lasky *et al.*, 2002; Fekadu, 2015).

Blood grouping in human are due to the presence or absence of certain protein and carbohydrate molecules called antigens and antibodies. The antigens are found on the surface of the red blood cells and the antibodies are in the blood plasma. Human beings have different types and combinations of these molecules. The classification of blood groups into type A, B, AB and O in ABO system, Rh positive and Rh-negative in Rh system is based on the presence or absence of inherited antigenic substances on the surface of the red blood cells (Giri *et al*., 2011).

During the World wars, it was discovered for the first time that the frequency of ABO and Rh (D) blood types were varies in persons native to different parts of the world. categorize have been made to classify the racial groups of mankind according to the incidence of known blood groups (Lasky *et al.,* 2002). Human beings are naturally out breeding organisms and reproduce sexually. The transmission of genes (DNA) from parents to offspring in a generation takes place biologically through the gametes. The genotype frequencies for a certain gene(s) in a population depend on the gene frequency. The proportions of various alleles of a gene in Mendelian inheritance are known as gene frequency (Singh, 1990).

All human populations share the same blood systems, although they differ in the frequencies of specific types. The allelic and genotypic frequencies of ABO and Rh-D systems are complex globally. Some variation may even occur in different areas within one small country (Enosolease, 2008). The Rh-D is genetically complex but it is only expressed in terms of a single pair of alleles, D and d. Rhesus positive (+) persons are either homogeneous DD or heterogeneous Dd and Rhesus (-) negative are homogeneous recessive dd. The first discovery that the frequencies of the alleles differed from one population to another was made in the early 20th century. Subsequent results from practically all countries of the world have corroborated this, and have also shown that frequency figures are valid only for the specific population from which they are derived (Mourant *et al.,* 1999). The allelic and genotypic frequencies in blood groups system also shows certain variation according to races (Kaya *et al*., 1999). Various populations intermarriage throughout the world with relative freedom, resulting in uniform distribution of different blood groups. However, a significant regional heterogeneity is also found in ABO and Rh-D blood group allele frequencies (Bhasin and Chahal, 1996).

The knowledge of the allelic and genotypic frequencies of ABO and Rh-D blood groups is essential for effective management of blood banks inventory, each individual blood group known and written on his/her ID and also can be used to facilitate a smaller local blood transfusion service (clinic) or a regional or national blood transfusion service. The ABO blood groups and Rh-D blood group antigens are the commonly frequently investigated genetic markers in a large group of people. In addition to importance in blood transfusion service, the ABO and Rh-D blood groups are useful in the study of population genetic

studies, researching population migration patterns as well as resolving certain medico-legal issues, particularly of disputed parentage (Worlledge *et al.*, 1974).

The ABO blood type system is governed by a single gene with three alleles (I^A, I^B and I^O) of which I^A and I^B alleles are co-dominant but both of them are dominant over the recessive allele I^O in intra-allelic interaction in diploid condition. The gene for ABO blood group is located on chromosome 9 of human genome where as that of Rh-D is located on the short arm of chromosome one (1) (Murphy *et. al*, 2003).

Comparatively with that of ABO system, the Rh blood group system can also affect transfusion compatibility. An individual is either positive or negative for the Rh factor; this is denoted by a '+ve' or '-ve' after their ABO type. Blood that is Rh-negative can be transfused into a person who is Rh-positive, but an Rh-negative individual can create antibodies for Rh-positive RBCs. Due to this, the AB+ blood type is referred to as the "universal recipient", as it possesses neither Anti-B nor Anti-A antibodies in its plasma, and can receive both Rh-positive and Rh-negative blood. In addition, the O- blood type is called the "universal donor", since its red blood cells have no A or B antigens and are Rh-negative, no other blood type will reject it. Determination of ABO and Rh gene frequencies among human populations has significant benefits in transfusion medicine, transplantation and disease risk (Akinnuga *et al.*, 2011).

Presence of rhesus (Rh-D) factor on the erythrocyte surface," $Rh^{+"}$, lack of it is called "$Rh^{-"}$. The Rh^+ and Rh^- in Rh (D) blood group system determination are based on the presence or absence of two alleles inherited antigenic substances (D): D and d on the surface of the erythrocyte. Identification of Rh (D) blood type system is essential to prevent the hemolytic disease of the new born (HDN) is; which usually arises when an Rh^- mother carries an Rh^+ fetus. The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh^- can bear an Rh^+ child if the father is Rh^+ (either homozygous or heterozygous). while there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy (Urbaniac *et al*., 2000).

As an individual with Rh^- blood does not have Rh antibodies naturally in the blood plasma; as one can have A or B antibodies. 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 which develop in to Hemolytic Disease of Newborn (HDN). A person with Rh^+ blood can receive blood from a person with Rh^- blood without any problems (Eweidah, 2011).

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

1.2 Statement of the Problem

Various countries of the world have had well organized documents on the frequency of alleles, phenotypes and genotypes of ABO and Rh (D) blood groups among their blood donors. Among Caucasians in the United States, the distribution of type O is 47%; type A, 41%; type B, 9%; and type AB, 3%. Also, among Western Europeans, type O is 46%; type A, 42%; type B, 9% and type AB, 3% (Adeyemo and Soboyejo, 2006). As a result, they made the information available for the purpose of blood transfusion and other blood related activities and hence reduced problems with respect to blood transfusion and HDN. The frequency of the ABO and Rh-D blood group different figures markedly in different races and ethnic groups in different parts of the world. Apart from the spatial and ethnic/racial variations, the ABO and Rh-D blood group frequencies may change temporally in a single population. Variation of ABO gene distribution with socio-economic status was also demonstrated in England. It is, therefore, imperative to have a reliable and up-to-date data on the distribution of these blood groups in various populations (Iyiola, 2012; Mindaye Regassa and Temesgen Bedassa, 2019).

Scholars in different years with numerous studies have carried out study on the genetic composition of different individual or ethnic groups around the world Similarly in Ethiopia, frequency of ABO and Rh-D blood group system studies have been conducted in different parts of the country. However, in Arsi University, where not less than eighty (80) ethnic groups are found (AU Registrar office Alumni Directorate 2018), allelic and genetic

frequency of ABO and Rh-D blood group system among those ethnic groups was not studied yet. Among the existing students' ethnic groups in Arsi University, Amhara, Oromo and Wolayita ethnic groups are the most dominant as my preliminary survey from students council indicated. So that the need for the current study is to examine the frequencies of ABO and Rh-D blood type among the Amhara, Oromo and Wolayita ethnic groups of AU students. The present study attempts to provide initial data on the genetic composition of blood group in the student ethnic groups in AU, using ABO blood system. Therefore, their degree of awareness of the blood type system and in blood transfusion as well as its risk factors in pregnancy is important to assess the opinion of AU student, which can make the University student more responsive to people's needs and expectations. Finally, the aim of this study is to determine the allelic and genotypic frequencies of ABO and Rh-D blood type system as well as to generate immunohematology data to be used as an initial reference in the future for blood transfusion by identifying areas of incompatibility blood types as it will cause an unexpected impact on the health system for people.

1.3 Objective of the study

1.3.1 General objective

The general objective of this study is to investigate the allelic and genotypic frequencies of ABO and Rh-D blood types among the students of Amhara, Oromo and Wolayita ethnic group in Arsi University, Ethiopia.

1.3.2 Specific objectives

The specific objectives of this study were to:

- ✓ To estimate the allelic frequency of ABO and Rh-D blood types among Amhara, Oromo and Wolayita ethnic groups of AU.
- ✓ To identify genotypic frequency of the ABO and Rh-D blood groups among students of AU.
- ✓ To verify if the population are at Hardy Weinberg law of genetic equilibrium.

1.4 Significance of the study

The result of the study is hoped to provide valuable information that would be advantageous to students and researchers. First, the results of the study give valuable inform about the allelic, phenotypic and genotypic frequencies of ABO and Rh-D blood groups which help them to have the concepts in order to teach their students in ease way. Second, the finding of the study would help students to know their blood type that enhance them to estimate their allelic, phenotypic and genotypic of ABO and Rh-D blood groups. Third, to know about the risks and its possible solution of ABO and Rh-D blood complication in pregnancy and blood transfusion in Ethiopia and elsewhere and to see the usual trend of the prevalence rate of various blood type among the school students. Finally, the results of this study is hoped to stimulate possible researchers to those identified gaps in order to put more clear directions to conduct further studies, to undertake different measures, to serve as a reference material and as an initial work.

2 LITERATURE REVIEW

2.1 . History of ABO and Rh (D) Blood Grouping

Blood is specialized connective tissues with complete and common identity. The two most significant blood group systems were discovered by karl Landsteiner during early experiments with blood transfusion. The ABO blood group, in 1901 and in cooperation with Alexander Swiener the Rhesus (Rh) blood group, in 1939. Development of the combs test in 1945, the advent of transfusion medicine and the understanding of ABO hemolytic disease of the newborn led to discovery of more blood groups, and now 30 human blood group systems are recognized by the International Society of Blood Transfusion (ISBT), and cross the 30 blood groups, over 600 different blood group antigens have been found many of these are very rare or are mainly found in certain ethnic groups (Astee, 2009).

The Landsteiner's in the field of immunohematology, blood transfusion among humans irrespective of their natives, complication in pregnancy, legal medicine, anthropology and the discovery of additional blood group systems. The discovery of the ABO blood groups was an important achievement in the history of blood determined. Mendelian fashion of genetic inheritance which shows stable characteristics and are useful in paternity testing Anti-A and anti-B antibodies (called isohaemagglutinins), which are not present in the newborn, appear in the first years of life. They are isoantibodies, that is, they are produced by an individual against antigens produced by members of the same species (isoantigens). Due to Anti-A and anti-B, antibodies are usually IgM type, which are not able to pass through the placenta to the fetal blood circulation. O-type individuals can produce IgG-type ABO antibodies (Hillier, 2008).

Classification of the blood group was based on the observation of the agglutination reaction hematology between an antigen on erythrocytes and antibodies present in the serum of individuals directed against these antigens. When agglutination will occurred, either antigens or the antibody will missing from the mixture. In 1902 von Decastello and Sturil described RBCs reacting with both anti-A and anti-B but did not give these type a identity, but continued calling RBCs that did not agglutinate with anti-A and anti-B type (Garrattey *et al*; 2000). Blood type have been used in forensic science and were definitely used to determine impossibility of paternity (example, a type AB man cannot be the father of a type

O infant), but both of these uses are being replaced by genetic finger printing which confidently provides greater certainty (Johnson, 2003).

2.2 ABO Blood Group system

The ABO blood group system is the most important blood type system (blood group) in human blood transfusion and blood bank. The associated anti-A and anti B antibodies are usually IgM antibodies, which are mostly produced in the first years of life by sensitization to environmental substance such as food, bacteria and virus. ABO blood type are also present in some other animals, for example apes such as chimpanzees and gorillas which portrays our recent common ancestry (Maton *et al*., 1993).The labeling of blood groups in type A, B, AB and O in ABO system, which is determined on the unique feature of presence or absence of A and B antigens. The antigens may be proteins, carbohydrates, glycol proteins and glycol lipids depending on the blood group system (Hasna *et al*., 2010).

A complete blood type would describe a full set of 30 substance on the surface of RBCs and an individual's blood type in one of many possible combinations of blood group antigens. These include the ABO, Rhesus, Kell, Duffy, Lutheran, Hh, Kidd (JK) , Diego , MNS ,etc. Across the 30 blood groups, over 600 different blood group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups (ISBT, 2008; Mandal, 2002; Mindaye Regassa and Temesgen Bedassa Gudeta, 2019).

Usually an individual has the same blood group for life, but very rarely an individual's blood type change through many possible combinations of blood-group of antigen in infection, malignancy, or autoimmune disease. Another more common in blood type change in bone marrow transplant. Bone marrow transplants are performed for many leukemia's and lymphomas, among other diseases. If a person receivers bone marrow from someone who is different ABO type (example. type A Patient receives a type O bone morrow). The patient's blood type will eventually convert to the donor's type (Masushita *et al*., 1983).

The ABO blood type is controlled or governed by a single gene (the ABO gene) with three alleles ($I^{A,}$ I^{B} and I^{O}) of which I^{A} and I^{B} alleles are co-dominant but both of them are dominant over the recessive allele I^{O} intera- interaction in diploid condition blood group are

inherited from both parents. (povey *et al*.,1978). The gene encodes a glycosyltransferase that is an enzyme that modified the carbohydrate content of the red- blood cell antigen. The gene is located on the long arm of the ninth chromosome (Aventy, 2009).Some blood types are associated with inheritance of other disease, for example, the kill antigen is sometimes associated with melody syndrome (Kwiatkowski, 2005).

Table 1: ABO blood system as cited in Avnet, 2009

Blood type	Antigens on erythrocytes	Antibodies in plasma	Can received blood from groups	Can give blood groups
A	A	B	O,A	A,AB
B	B	A	O,B	B,AB
AB	A and B	None	O,A,B,AB	AB
O	None	A and B	O	O,A,B,AB

2.3 Frequency of ABO and Rh (D) Blood Group systems

2.3.1 Frequency of ABO Blood Group System

The need for blood group frequency studies is unlimited purposes, as besides their importance in hereditary characteristics, their relation to disease and environment is being increasingly sought in modern medicine (Green *et al*., 1995). Various studies on ABO shows that a very high frequency of prenatal death among parents is due to incompatibility mating. Red blood cells have a series of glycoproteins and glycolipids on their surfaces which constitute the blood group antigens (Srikumeri *et al*., 1987).

Individuals based on RBC agglutination patterns, could be divided into 4 major groups A, B, AB, and O (Saladin, 2003; Suzuki, 2005; Yamamoto *et al.*, 2012). ABO and Rhesus Rh(D) blood type antigens are genetically 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 (Enosolease and Bazuaye, 2008; Reddy and Sudha, 2009; Yamamoto *et al.*, 2012). ABO and Rh(D) genes and phenotypes vary widely across ethnic groups, races and geographical boundaries despite the fact that the antigens

involved are stable throughout life (Bhuvnesh *et al;* 2011; Iyiola *et al.*, 2011; Chandra and Gupta,2012).

The blood type frequency also shows variety according to races (kaya *et a*l., 1999). All human population share the same blood systems, although they differ in the frequencies of specific types. These allelic and genotypic frequencies patterns of ABO and Rh (D) system are complex around the world. Some variation may even occur indifferent areas within one small country (kolmakova and kononova, 1999). The knowledge of the frequency of ABO and Rh(D) blood types is essential for effective management of blood banks inventory, be it a facility of a smaller local transfusion service or international transfusion services. A part from their importance in blood transfusion practice, the ABO and Rh(D) blood type are useful in population genetic studies, researching population migration patterns as well as resolving certain medico legal issues, particularly of disputed parentage. It is therefore, imperative to have information on the frequencies of these blood types in any population group (Wolledge *et al*; 1974).

Blood is significant important body fluid, which is responsible for circulation of important nutrients, enzymes and hormones all across the body besides the most critical substance and oxygen. The hum anted blood cells membrane contains different types of polysaccharide antigens called agglutiongen (Ganon, 1995). The antigenic substance are capable of inducing specific immune response result in the production of cells termed as antibodies (Novak, 1995).

Blood type frequency in various groups of population is important in health care and blood transfusion. Clinically, the two most significant blood group system are ABO and Rh (D) Knowledge of phenotype distribution of ABO system in a given population is important for planning procreate of blood supplement and associated disease with blood group and to know if any significant phenotype dispersion is present when comparing with other population(Jaff and Briain, 1987).

The ABO frequency varies world wide as O blood type is very common around the world; about 63% of humans share it. Type O is uniquely high in frequency among the indigenous populations of central and South America where it approaches 100%. The lowest frequency

of (O) is found in Eastern Europe and central Asia, where B most frequently found (Khan *et al*, 2009).

Blood type-B has its highest frequency is found in Northern India and neighboring Central Asia, and its incidence diminishes both towards the west and the east, contains few observed percentages in Spain. Blood type-A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigine populations and the Blackfoot Indians of Montana (Dean, 2005). It is believed to have been entirely absent from Native American and Australian 7 Aboriginal populations prior to the arrival of Europeans in those areas (Encyclopedia Britannica, 2002).

2.3.2 **Rhesus (Rh) Blood Type System**

The Rhesus (Rh-D) blood group is one of the complex blood groups known in humans. It is the one of thirty (30) current human blood group systems. Clinically it is the most important blood group system after ABO at present. The commonly used terms Rhesus (Rh) factor, Rh -positive and Rh- negative refer to the D antigen only. Besides its role in blood transfusion, the Rh blood group system specially, the D antigen is used to determine the risk of hemolytic disease of the newborn (HDN) (Reid and Lomas, 2004).

People are Rh positive if they have Rh-D antigens on the surface of their red blood cells and are Rh- negative if they do not have this antigen. An individual either has or does not have the Rhesus factor on the surface of their red blood cells, this term strictly refers only to the most immunogenic D antigen of the Rh^+ blood group systems, or the Rh^-blood group system. The status is usually indicated by Rh positive (Rh^+ does have the D antigen) or Rh negative (Rh^-does not have the D antigen) suffix to the ABO blood type. However other antigens of this blood group, system are also clinically relevant. In construct to the ABO blood group, immunization against Rh can generally only occur through blood transfusion or placental exposure during pregnancy in women (Daniels, 2005). Rh-D antigen allelic and genotypic frequencies varies from one population to the other. Rh-D negative blood group is documented at 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria and 7.3% in Lahore (Greer *et al.,* 2010).

Table 2: Global allelic and genotypic frequencies of ABO and Rh-D blood groups studied in different population.

Study Area	A%	B%	O%	AB%	Rh+ve	Rh-ve	Reference
Pakistan	22.00	36.90	31.30	9.90	91.40	8.60	Anees and Jwad, 2011
W.India	22.20	36.40	9.40	31.70	91.75	8.25	Rajshpre *et al.,2013*
Bangladeshi	23.50	39.80	27.60	9.20	97.40	2.60	Sultan *et al.*,2013
Saudi Arabia	24.00	17.00	4.00	52.00	93.00	7.00	Sarhan *et al*.,2009
USA	42.00	10.00	44.00	4.00	85.00	15.00	Stanford Med. Blood Center, 2013
African American	27.00	20.00	46.00	7.00	95.00	5.00	Adeyemo and Soboyejo, 2006
NorthCameroon	25.07	21.86	48.62	4.45	96.32	3.68	Ndoula *et al*.,2014
Lagos Nigeria	23.10	21.30	52.90	2.70	97.00	3.00	Iyiola *et al* .,2012
NigerDelta	23.80	20.70	2.80	52.70	93.90	6.10	Enosolase *et al*.,2008
Sudan	23.30	13.20	52.70	10.80	98.00	2.00	Hanssan *et al*.,2010
Kenya	26.20	22.00	4.40	47.48	80.30	19.70	Lyko *et al*.,1992
Egypt	36.00	24.00	33.00	8.00	92.00	8.00	Tagny *et al* .,2010
Mekele (Ethiopia)	28.00	25.00	41.50	5.50	91.16	8.84	Megbaru Alemu *et al.,* 2014
Dilla (Ethiopia)	27.00	25.00	43.00	5.00	90.78	9.21	Fekadu Alemu Atire., 2015
Silte(Ethiopia)	28.11	23.35	43.08	5.44	92.06	7.94	Tesfaye K. *et al* ., 2014
	31.25	23.43	5.21	40.10	92.45	7.55	Present study

2.4 Rh –Antigens

Rh-D is the most important antigen after A and B antigens unlike the anti-A and anti B antibodies, anti D antibodies are only seen if a patient lacking D antigens is exposed to D^+ cells. The exposure of D^+ cells usually occur through pregnancy or transfusion. There are five principal antigens that may be found in most individuals, they are D, C, E, c and e (Daniels, 2002). The presence or absence of Rh antigens on red blood cells is determined by two alleles at another locus, Rh on chromosome 1. There are 6 types of Rh antigens in 3

groups Cc, Dd and Ee, every person acquire one from each group. Antigen D is the strongest and a person with this antigen was Rh positive while a person with no D antigen was Rh negative. Antibodies to D can cause transfusion reactions. Unlike ABO blood group spontaneous antibodies are never formed against Rh antigens and repeated exposure is needed to make significant amount of antibodies to cause a transfusion (Daniels, 2002).

2.5. Blood Typing System

A blood type system may be defined as a genetically discrete type of antigens controlled by a single gene or by a cluster of two or more closely linked homologous genes with virtually no recombination occurring between them (Yazdanbakhsh *et al.*, 2001).

Genetically Blood typing involves identifying antigen present on red blood cells (RBCs) membranes. Many different antibodies exist on human RBC and Rh group. A person normally produces against those antibodies not present on his /her RBCs but does not produce antibodies against those that are present. Thus a person with A blood group were have A antigen and anti B antibodies while a person with B blood group were have B antigen and anti A antibodies. Similarly a person with AB blood group were have both A and B antigens and none of both anti A or anti B antibodies contrary to a person with blood group O who were have none of A or B antigens but were have both anti A and anti B antibodies. Antibodies are of IgM type and are absent at birth. They presents in the blood after exposure in environmental antigens similar in structure to blood group antigens. Blood type is determined by multiple alleles I^AI^B and I^AI^O and I^BI^O are co-dominant and both are dominant over O. Individuals with genotype I^AI^A and I^AI^O have type A blood. Genotype I^BI^B and I^BI^O result in blood type B. When both I^A and I^B (I^AI^B) are present the blood type is AB, because A and B express a special dominance relationship. Co-dominoes, which means that A and B parents can have an AB child. When both alleles are O (I^O I^O) have type O blood. A type A and a type B couple can also have a type O blood. A type A and a type B couple can also have a type O child, If they are both heterozygous (I^AI^O, I^BI^O) (Aventy, 2009). The cis-AB phenotype has a single enzyme that creates both A and B antigens. The resulting red-blood cells do not usually express A or B antigen at the same level that would be expected on common group A and B red blood cells, which can help solve the problem of an apparently genetically impossible blood group (yazer, 2006). The ABO blood types 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 type substances A and B represent ABO blood type antigens are attached to oligosaccharide chains that project above the RBC surface two modified forms of a "stem" carbohydrate present on red blood cells and other tissues. These chains are attached to proteins and lipids that lie in the RBC membrane. ABO antigens are glycolipids in nature, meaning they are oligosaccharides attached directly to lipids on red cell membrane (Laura, 2005).

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

Table 3: Agglutination reaction of ABO blood typing sera (as cited in Avent, 2009; Laura, 2005)

Reaction		Blood type (Phenotype)	Genotype
A-antibodies (anti A serum)	B- antibodies (anti B serum)		
Clumping	No clumping	Type A	I^AI^A OR I^AI^O
No clumping	Clumping	Type B	I^BI^B OR I^BI^O
Clumping	Clumping	Type AB	I^AI^B
No clumping	No clumping	Type O	I^OI^O

2.6. Clinical Significance of Blood type

2.6.1. Blood Transfusion

Transfusion is a specialized branch of hematology that is concerned with the study of blood type, along with the work of a blood bank to provide transfusion services for blood and other blood products in transfusion medicine in order to avoid morbidity and mortality in both adults and children. Across the world, blood products must be prescribed by a medical doctor in a similar way as medicines. Much of the routine work of the blood bank involves testing blood from both doctors 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 hemolytic (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 (Nickel *et al*; 1999).

Patient should ideally receive their own blood specific blood products to minimize the chance of transfusion reaction. Risks can be further reduced by a cross-matching blood, but this may be skipped when blood is required for an emergency cross matching involves mixing a sample of the recipient's serum with a sample of the red blood cells and checking if the mixture agglutinates, or forms clumps. If agglutination is not obvious by direct vision, blood bank technicians usually check for agglutination with a microscope. If agglutination occurs that donor's blood cannot be transfused to that particular recipient (Bruce, 2002).

2.6.2. Blood Group Genotyping

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

2.6.3. Hemolytic Disease of the New born (HDN) (Erythroblastosis Fetalis)

Haemolysis is the break down or rupture of the red cell membrane by specific antibody (haemolysin) through the activation of complement with the release of hemoglobin, and the librated hemoglobin can easily be observed staining the supernatant fluid (Louise, 1995).

Levine and Stetson in 1939, was first described hemolytic disease of the new born (HDN). The cause of hemolytic disease was not well determined but maternal antibody was suspected. 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 (Avent and Reid, 2000). A pregnant woman can make IgG blood group antibodies if her fetus has a blood group antigen that does not have, this can happen if some of the fetus' blood cells pass into the mother's blood circulation (example, a small fetus maternal hemorrhage at the time of child birth or obstetric intervention) or sometimes after a therapeutic blood transfusion. Rh disease was the incompatibility and complication during and after pregnancy. This results Rh disease or other forms of hemolytic disease of the new born (HDN) in the current pregnancy and later pregnancies. If a pregnant woman is known to have anti-D anti bodies, the Rh blood type of a fetus can be tested by analysis of fetal DNA in maternal plasma to assess the risk to the fetus of Rh disease. Antibodies associated with some blood groups can cause severe HDN. Others can only cause mild HDN and others are not known to cause HDN (Letsky *et al*; 2000). A number of studies were conducted to investigate that there is association between ABO blood group systems and some disease conditions (Nakao *et al.*, 2011).

The importance of Rh factor in pregnancy can be seen in babies of mothers who are Rh and receive an Rh+ gene from their fathers who are Rh+, and the pregnancy is characterized by maternal-fetal Rh incompatibility. If an Rh- mother has already had a earlier pregnancy with an Rh+ fetus, and has another Rh+ baby, the baby is easily susceptible to Erythroblastosis fetalis if the first baby's blood mixed with the mother's blood and the mother secretes antibodies against Rh+ blood cells of her baby, which the mother's immune system recognizes as "foreign". Secretion of these antibodies is called sensitization or isoimmunization (Murray and Roberts, 2007).

Because sensitization usually occurs at delivery, the first Rh+ child of an Rh- mother rarely suffers from hemolytic disease. Ones the mother has developed the anti-Rh antibodies the problems occur with each successive Rh+ fetus. This triggers a secondary Ab response (activates memory B cells) so IgG is produced this time. IgG can cross the placenta and attack the RBC of the fetus and cause jaundice due to excessive bilirubin in their blood. Hemolytic disease can now be prevented by giving Rh negative mothers injections of Rhogam (anti-Rh Abs) within 72 hours of delivery. The Abs binds to circulating Rh+ fetal RBC and destroys them before they can act to sensitize her specific immune system. (Letsky *et al*., 2000).

2.6.4. Rh Blood Group System and Its Inheritance

It is common for D-negative individuals not to have any anti-D or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following sensitizing events: possibly a fetomaternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with D-positive RBCs. Rh disease develops in these cases (Moise, 2008). Rh-negative blood types are much less in proportion of Asian populations than they are in white hio, the presence or absence of the Rh antigens is signified by the + or – sign, so that for example the A- groups does not have any of the Rh antigens (Cummings, 2008).

A person is grouped as Rhesus (Rh) positive or negative based on the presence or absence of antigen D. Rh positive (+) is a person who inherits gene D and the red cell express antigen D. Whereas Rh negative (-) is a person who does not inherit gene D and the red cells do not express antigen D. For transfusion purpose, Rh positive blood can be given to Rh positive individuals and Rh negative blood can be given to both Rh+ and Rh- individuals. Rh+ blood should never be given to Rh- individuals especially to women of child bearing age. Rh antibodies are generally developed from two to six months after the initial immunization by red cells. Their production is consistent with the classical immune response in that the earliest antibody to appear is IgM, followed by IgG, some IgA have also been identified. The predominant Rh antibodies however, are immunoglobulin class IgG (Moise, 2008).

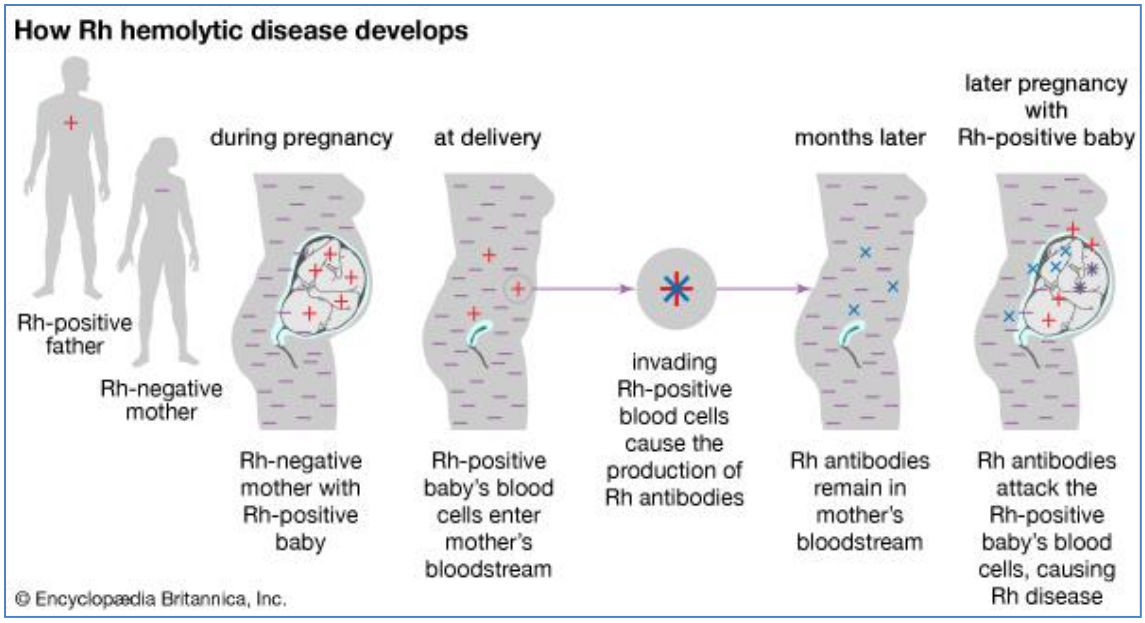

Figure 1: Hemolytic disease of the new born (HDN) in the current pregnancy.

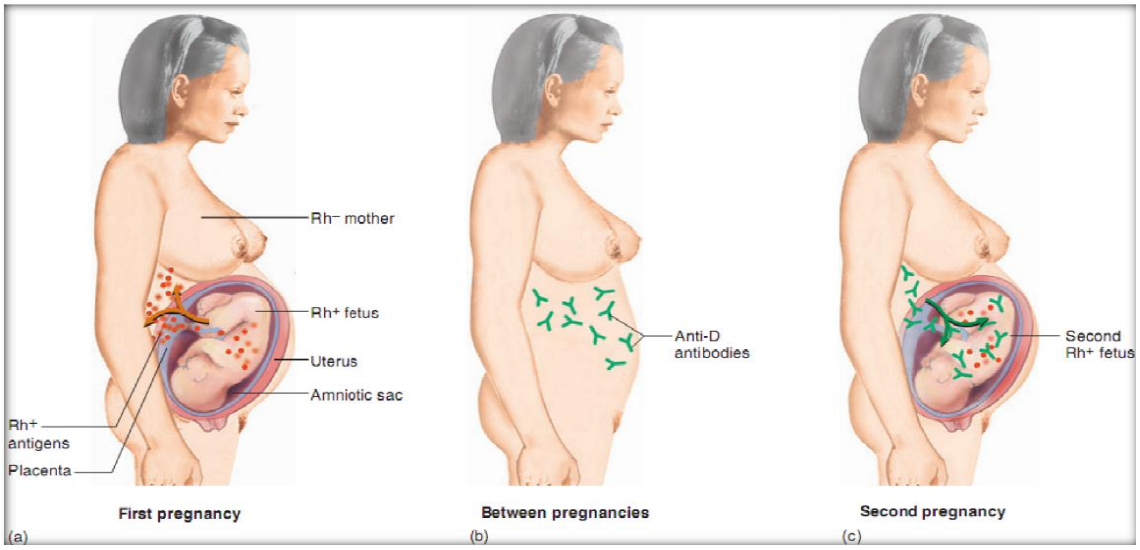

Figure 2: Hemolytic Disease of the Newborn (HDN):

(a) When an Rh- woman is pregnant with an Rh+fetus; she is exposed to D (Rh) antigens, especially during childbirth. (b) Following that pregnancy, her immune system produces anti-D antibodies. (c) If she later becomes pregnant with another rh +fetus, her anti-D antibodies can cross the placenta and agglutinate the blood of that fetus, causing that child to be born with HDN. (Source: Saladin, 2003).

2.4.1 **Blood Products**

Blood products may also be called blood based products to differ from blood substitutes. A blood product is any component of the blood which is collected from a donor for use in blood transfusion. In most cases whole blood is uncommonly used in transfusion medicine at present ,most blood products consists of specific processed components such as red blood cells, blood plasma or platelets Whole blood may be classified as a blood products or as a separate entity. Also, although many blood products have the effect of volume expansion, the group is usually distinguished from volume expanders, which generally refer to artificially produced substances and are there by within the scope of blood substitutes (Henrik *et al*; 2012).

2.6.5. Red Blood Cells Compatibility

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 a group A individual can receive blood only from individuals of groups A or O (with A being preferable). And can donate blood to individuals with type A or type AB (Simpkins and Williams, 1997).

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

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

Blood group O individuals do not have either A and B antigen on the surface of their RBCs, but their blood serum contains IgM anti A antibodies and anti B antibodies against the A and B blood group antigens. Therefore a group O individual can receive blood only from a group O individual, but can donate blood to individuals of any ABO blood group (A, B, AB and O). If anyone needs a blood transfusion in a dire emergency and if the time taken to process recipient's blood would cause a detrimental delay O negative blood can be issued (Griffith *et al.*, 2008).

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

2.7. Extension of the Hardy-Weinberg Law

The Hardy-Weinberg principle describes a mathematical relationship that allows the estimation of the frequency of offspring genotypes based on parental allele frequencies. It also estimates that allele frequencies was stable from generation to the next, indicative of non-evolution (Klug and Cummings, 2002, Mayo, 2008). For a population to be in Hardy-Weinberg equilibrium, the following assumptions are required to hold: random mating, no mutation, no migration, no stochastic effects or genetic drift due to small population size and equal fertility for all genotype groups so that no selection is occurring (Mollisi.,1994). Violation of any of these assumptions can result in evolutionary change in terms of allelic frequency distribution (Mayo, 2008).

When a single locus with two alleles p and q, the Hardy-Weinberg law tells us that at equilibrium the frequency of the genotype is $p^2+ 2pq+q^2 =1$,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 in to a diploid zygote (Daniel and Clark, 2007).

For this study three alleles are computed (I^A, I^B and I^O) with frequencies equal to p, q and r respectively. To find a single allele frequency of ABO and Rh-D blood groups the following formula was used:

For blood group A:

$$p' = 1 - \frac{\sqrt{f(B)+f(O)}}{n}$$

Where f= is frequency, B = is no of B blood group observed, O= is no of O blood group observed and n= is the total no of sample.

For blood group B:

$$q' = 1 - \frac{\sqrt{f(A)+f(O)}}{n}$$

For blood group O:

$$r' = \frac{\sqrt{f(O)}}{n}.$$

And for that of single Rh-D blood allele frequency:

$$d' = \frac{\sqrt{f(d)}}{n} \text{ and } D' = 1 - \frac{\sqrt{f(d)}}{n}$$ (Daniel and Clark, 2007).

The frequencies of the genotype at equilibrium will be computed by the square of the allelic frequencies.

$(p + q + r)^2 = p^2 (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO)$.

Where:

P^2	$= I^A I^A$	homozygous genotype for blood type A.
$2 p q$	$= 2 I^A I^B$	heterozygous genotype for blood type AB.
$q2$	$= I^B I^B$	homozygous genotype for blood type B.
$2 p r$	$= 2 I^A I^O$	heterozygous genotype for blood type A.
$2q r$	$= 2 I^B I^O$	heterozygous genotype for blood type B.
r^2	$= I^O I^O$	homozygous genotype for blood type O.

3. MATERIALS AND METHODS

3.1. Description of the Study Site

The study was conducted in Ethiopia; Oromia regional state, Arsi District, Arsi University. Arsi University is one of the youngest higher academic institutions in Ethiopia and established at Arsi Zone , Asella town in 2014 by Council of Ministers. Arsi University is located, 175 Km from Addis Ababa. This University has with an elevation of about 2210m-2700 meters above sea level. Additionally, Arsi University (AU) has three colleges. Namely: College of Agriculture, College of Health Science (Asella Referral Specialized Hospital and Teaching College), and College of social sciences and humanity. Currently, the University has a total of 6369 students of these 3784 are male while 2585 are female students who are attending the first degree program in different Schools but this study focus only in College of Agriculture with total population 1782 students 1101were male and 681 female 384 sample size selected which is equals to 27.97 % . (Arsi University Alumni Directorate, Registrar Office).

3.2. Research Design

A retrospective based quantitative cross-sectional study design farther explanation with qualitative description was conducted using data to determine the general allelic, Phenotypic and genotypic frequencies of ABO and Rh (D) blood groups in Arsi University, Ethiopia.

3.3. Study Procedure

Communicate with Arsi University research directorate and other concerned body then, to get permission to conduct research, the researcher started to communicate with the students Council and give awareness about blood group test and signing the consent forms that assure their willingness. The result of each student was secured and keeps confidentially. The blood samples were collected by qualified laboratory technician using standard clinical procedure by using sterile materials. Blood samples were collected after washing with 70% ethyl alcohol to obtain a drop of blood from left ring finger tip with the help of a glove, sterilized needle, cotton and lancet for each sample population. Then, it was placed on a clean dry glass each slide containing in three separated placed in a row and a drop of one of the anti- sera that is antibody coated, anti A, anti B and anti D was added to each of an

individual's drop of blood samples and mixed well with the aid of plastics to stirrer . After that, the result of each student's blood group was registered on the provided form.

3.4.Sampling Technique and Sample size

3.4.1. Sampling Technique

A random sampling techniques including both sexes were employed to select the voluntarily participating students in the present study process sample population until they reach estimated sample size from three selected predominantly populated ethnic group in the University. The blood sample was collected from each sample population recorded after agglutination and informed well about their ABO and Rhesus blood group . Blood group test and data collection was conducted from 16- 30 June, 2018.

3.4.2 Sample size

The sample size of the study was estimated by taking the prevalence of 50% as there was no reported study in the area. As stated by (Daniel, 1999) cited in Naming *et al*., 2006. By using the formula:

$n = z^n (p) (1-p) /d^2$ Where:

n= no of the study responds selected for work.

z = 95% confidence interval = 1.96

P= prevalence (frequencies of ABO and Rh-D blood groups) = 0.5

d = marginal error between the samples and the population = 0.05

$n = (1.96)^2 (0.5) (1-0.5) / (0.05)^2$

n = 384

To minimize an error from the likelihood non-voluntaries and non-responsive participants, 9% of the sample size would be added to the normal sample three hundred eighty four(384). n = 384+ (384x 0.09) n=418, but all the sample population were volunteer students which actively participating in the study process and no need of using error minimizing formula So, the study were conducted on 384 volunteer students 219 were males and 165 were females.

3.5. Determination of ABO and Rh -D Blood Groups

The ABO and Rh-D blood grouping was done using monoclonal ABO anti-sera and RhD Anti-D. The Anti-sera used are manufactured by Tulip Diagnostics LTD India, and were bought from Asella Medical Drugs Store and Distributer. A drop of blood from each sample student was placed on a clean dry glass slide in three orderly separate places in a raw. Three types of sera were employed, anti-A serum for determining blood group A, anti B serum for blood group B and anti D serum for Rh factor determination. Agglutination or clumping of blood cells in anti -A serum indicates blood group A, and clumping of cells in anti B serum indicates blood group B. No clumping or agglutination of cells in both anti A and anti B sera indicate blood group O. Agglutination of cells in both anti- A and anti -B sera indicates blood group AB. Agglutination of blood cells of in any blood type in anti D serum indicates Rh positive and no agglutination indicates Rh negative. Results for agglutination were recorded immediately for ABO blood groups and after two minutes in Rh -D blood group. Blood groups were determined on the basis of agglutination reaction and recorded blood samples were collected by qualified laboratory technicians using the standard clinical procedure with sterilized lancet blade, as blood group A^+, B^+, AB^+, O^+ OR A^-, B^-, AB^-, and O^-(Alimba *et al*., 2010).

3.6.Method of Data Analysis

This research use qualitative method approach to come up with validated findings that were stored, edited and analyzed both qualitatively and quantitatively. The blood sample collected from students ' were tabulated and analyzed using descriptive statistics. Data was entered into and then analyzed performed by using Microsoft Excel SPSS version 20 for allelic and genotypic frequencies of ABO and Rh-D blood grouping would be used for data analysis. After collecting qualitative data, the results were coded on designed excel sheet. The results of each item were tabulated, summed, arranged and treated using statistical analysis such as observed, expected and percentage, independent chi-square to test if there are statistically significant variations among the volunteer students/ respondents in terms of responding to the given item. The analyzed data was presented in the form of Table and Figures where necessary .

Phenotypic frequencies were determined as percentages of observed numbers. Allelic, phenotypic and genotypic frequencies were calculated by using blood group frequencies

and the result expressed as percentages under the assumption of Hardy-Weinberg Law (HWL) (Griffith *et al*., 2008). For this study three alleles was computed (I^A I^B and I^O), with frequencies equal to p, q and r respectively. The frequencies of the genotypes at equilibrium was computed by $(p + q + r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO). Chi-square test was used to compare observed and expected allelic, phenotype and genotypic frequencies of both ABO and Rh -D blood groups to that expected under the HWL. Pearson chi-square goodness of fit test statistic is: . $\chi^2 = \sum \frac{(O-E)^2}{E}$. Where: O is observed count and E is corresponding expected counts. And a value of $p < 0.05$ considered as statistically significant. (Griffith *et al*., 2008).

3.6.1. Phenotypic Frequency Determination of ABO and RhD Blood Groups

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

frequency of observed value = $\frac{\text{Observed number}}{\text{Total Number}}$

3.6.2. Estimation of Allele Frequency

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

$r = \sqrt{O}$

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

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

Where r =O, p=A and q=B (Chavhan, 2010)

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

(d) was calculated according as

$d = 1 – p – q – r.$

The final allele frequencies were then calculated as follows:

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

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

r1 = (r +d / 2) (1 + d/ 2) Where : d= Rh- valeu and D= Rh+ value (Chavhan, 2010)

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

The genotypic frequencies of ABO blood groups was calculated as follows

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

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

Genotype $I^{AO} = 2pr$

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

Genotype $I^{BO} = 2qr$

Genotype $I^{AB} = 2pq$

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

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

Genotype $DD = p^2$

Genotype $Dd = 2pq$

Genotype $dd = q^2$

3.6.4. Chi-square Test

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

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

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

Expected phenotypic frequencies were calculated as:

E_f = Genotypic frequency X number of total sample

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

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

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

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

4. RESULTS AND DSICUSSION

4.1.ABO and Rh (D) Phenotypic Frequency

The scope of this study was restricted to the students of Arsi University college of Agriculture. The Allelic and genotypic frequencies of ABO and Rh (D) blood groups among the three ethnic groups were conducted on three hundred eighty four (384) volunteer students blood samples obtained from randomly selected individuals belonging to the three ethnic group Amhara , Oromo and Wolayita . (Table 4)

Table.4: Frequency of ABO blood group and Rh factor among students of Amhara, Oromo and Wolayita ethnic groups in AU.

Ethnic group	Rh Blood group	Blood Group				Total
		O (%)	A (%)	B (%)	AB (%)	
Amhara n= 129	Rh+ve	45(34.88)	37(28.68)	32(24.8)	7(5.43)	121(93.80)
	Rh-ve	3(2.32)	2(1.55)	2 (1.55)	1 (0.77)	8 (6.20)
	Total	**48(37.21)**	**39(30.23)**	**34(26.35)**	**8(6.20)**	**129 (100)**
Oromo n= 128	Rh+ve	50(39.06)	38 (29.69)	27(21.09)	6(4.68)	121(94.53)
	Rh-ve	1(0.78)	3 (2.34)	2 (1.56)	1 (0.78)	7(5.47)
	Total	**51(39.84)**	**41(32.03)**	**29(22.6)**	**7(5.46)**	**128 (100)**
Wolaita n= 127	Rh+ve	47(37.01)	35(27.56)	26(20.47)	5(3.93)	113(88.98)
	Rh-ve	8 (6.29)	5 (3.93)	1 (0.78)	0 (0)	14 (11.02)
	Total	**55(43.30)**	**40(31.49)**	**27(21.25)**	**5(5)**	**127 (100)**
Total	**Rh+ve**	**142 (30.73)**	**110 (23.43)**	**85 (19.54)**	**18 (4.68)**	**355 (92.45)**
	Rh-ve	**12 (9.37)**	**10 (7.82)**	**5 (3.89)**	**2 (0.52)**	**29 (7.55)**

Where :- Number of Amhara Ethnic Group = 129
Number of Oromo Ethnic Group = 128
Number of Wolayita Ethnic Group = 127 and
The overall population sample size were 384 .

There are large variation among phenotypic percentage frequency in ABO blood group among different ethnic groups in various studies. Many studies have shown that blood

group O is the most common blood group and blood group AB is the least common blood group in different ethnic group (Nwauche and Ejele, 2004).

In all of the three ethnic groups as well as in the total sample, blood group O Rh^+ has the highest percentage (30.73%) while blood group AB Rh^- has the least percentage (0.52%) in the population. The percentage of blood group O Rh^+ was 39.06, 37.01 and 34.88 % in Oromo, Wolayita and Amhara ethnic group respectively, the percentage of blood group A Rh^+ was 29.69, 28.68 and 27.56% Oromo, Amhara and Wolayita ethnic group respectively, the percentage of blood B Rh^+ was 24.8, 21.09 and 20.47% in Amhara, Oromo and Wolayita ethnic group respectively similarly the percentage of blood AB Rh^+ was 5.43, 4.68 and 3.93 % in Amhara, Oromo and Wolayita ethnic group in descending order respectively (Table 4).

This study is in similar fact with ethnic group ABO highest percentage frequency in Amhara, Oromo and Afar people where blood group O is 43.3 %, 51.7 % and 50.6 % followed by blood group A 27.8 %, 21.1 % and 23.3 % and blood group B 25 %, 19.4% and 22.8% in Amahra, 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 Amahra, Oromo and Afar respectively (Mohammed Saied,2013).

Many other studies has indicated that blood group O was the most common blood group and blood group AB was the least common blood group in different populations and ethnic groups(Nwauche *et al*., 2004). For example, among Ethiopians, the distribution is type O (42%), type A (30%), type B (22%) and type AB (6 %) (www.rhesusnegative.net, 2012). Thus, in different ethnic groups with exceptional cases the allele pathway for ABO always followed a particular pattern for its frequencies (Nwauche and Ejele, 2004 and Falusi *et al*., 2000). In the previous study reports, concerning the frequency of blood groups among Ethiopians, blood type O was 40%, type A 31%, type B 23%, and type AB, 6% (Misganaw, 2004). The frequencies of ABO blood group in this studies samples in agreement with studies in different parts of Ethiopia A^+, B^+, AB^+ and O^+ blood group in the three ethnic group (overall) were 23.43%, 19.54%, 4.68% and 30.73% respectively similarly A^-, B^-, AB^- and O^- blood group were 7.82, 3.89 , 0.52 and 9.37 respectively (Table 4).

As it is shown in (Table 4)blood group O were the highest frequency while blood group AB were the least frequency in the total observed study population. The frequency of blood group O+ and AB+ were 30.73 % and 4.68% respectively. This study is in agreement 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*)*. Among Sidama ethnic group (Ethiopia), the distribution is type O 51.3%; type A, 23.5%; type B, 21.9%; and type AB, 3.3% (Tewodros *et al*., 2011). 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). Therefore the results of this study are in agreement with the data from previous studies in Ethiopia populations.

As observed from other reports of similar studies, the results of this study are also consistent with previous findings from other parts of the world. For example in Britain (Anees, 2007) the frequencies of the ABO blood group for A, B , AB and O were 41.7% , 8.6% , 3% and 46.7% 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).

The comparisons among the three ethnic group frequency of ABO blood groups in this studies , blood types A^+ has the highest frequency in Oromo ethnic group than in amhara and Wolayita ethnic group were 29.69%, 28.68% and 30.23% respectively . Blood group B^+ has the highest frequency in Amhara ethnic group than in Oromo and Wolayita ethnic groups 24.8% , 21.09% and 20.47% respectively and blood group AB^+ has the highest frequency in Amhara ethnic group than in Oromo and Wolayita ethnic group as follow 5.43%, 4.68% and 3.93% respectively (Table 4). But these differences are not significant, ($\chi^2 = 0.02088$ df= 2 $p > 0.05$).This implies that the population is homogenous which might be due to intermixing or random intermarriage higher genetically similarity of their parents among the three ethnic group and small sample sizes used.

In Ilorin, Kwara state of Nigeria, the frequencies of O, A, B and AB blood group were 58.1, 18.7, 17.6, and 5.6% respectively (Iyiola *et al*., 2011). In Ogbomoso, South-west Nigeria, phenotypic frequencies of 50% for O; 22.9% for A; 21.3% for B and 5.9% for AB has been reported among 7653 individuals sampled (Bakare *et al.*, 2006). However, the results of this study do not agree with the results from some Asian countries where blood

group B has the highest frequency in some and blood group A in the others. For example Rai and Kumar (2010); Chavhan, (2010); Kumar *et al*., (2009); Rai, (2011); Giri *et al*., (2011); Warghat *et al*., (2011), and Rai *et al*., (2009) reported high incidence of B blood group in Indian population. Khan *et a*l. (2009); Rahman and Lodhi (2004); Khan *et al*., 2004 and 2006) and Mahamood *et al*., 2005 also reported high incidence of B blood group in Pakistan populations. The highest frequency of A blood group was documented in Jordan populations (Hanania *et al*., 2007); and Saudi Arabian populations (Khan *et al.,* 2006). Among African Americans, the frequency 46% (O); 27% (A); 20% (B); and 7% (AB). Among Western Europeans, 42%(A); 9%(B); , 3% (AB) and the remaining 46%(O). However, the findings of this study significant with the results of some Asian countries where blood type-B has the most common frequency in some and blood type-A in the others. Highest frequency of type-B blood was also reported in Pakistani populations (Khan *et al*., 2009; Rahman and Lodhi, 2004; Khan *et al*., 2004 and 2006) and Mahamood *et al*., 2005) and in Indian population (Giri *et al*., 2011; Warghat *et al*., 2011, and Rai *et al*., 2009).

Table 5: Phenotype of the ABO blood groups and their observed frequencies among the students of Arsi University

Blood Group	Observed number	Phenotype %	frequencies
A	120	31.250	0.312
B	90	23.437	0.234
AB	20	5.210	0.052
O	154	40.100	0.401
Total	384	100	1

The phenotype percentage and observed frequencies of ABO blood groups similarly shows true about dominancy of blood group O as presented in (Table 5). There are differences in observed frequency of the ABO blood group phenotypes percentage O has the highest percentage and AB has the lowest among the three ethnic groups of the study population . In the observed frequencies ABO blood group O (0.401%) has the highest frequently followed by frequencies of A (0.312) as the frequencies of B (0.234) and blood group AB (0.052) has the least frequency count phenotype in the population under the total study sample (Table 5).

Table 6: Percentage frequencies of Rh (D) blood group phenotypes among students of Amhara, Oromo and Wolayita ethnic group in AU

Ethnic Group	Rh positive (D) (%)	Rh negative (d) (%)	Total
Amhara	121(93.79%)	8(6.20%)	129
Oromo	121(94.53%)	7(5.46%)	128
Wolayita	113(88.97%)	14(11.02%)	127
Total	355 (92.45%)	29 (7.55%)	384 (100%)

This study has shown that Rh- positive has the higher percentage frequency while Rh negative has the lower percentage frequency in the total sample as well as in each of the three ethnic groups. The frequency of Rh-D positive blood group was 94.53 % in Oromo 93.79%, in Amhara and 88.97 % in Wolayita ethnic group, while the frequency of Rh negative blood was 11.02 %, 6.20 % and 5.46 % and in Wolayita, Amhara and Oromo ethnic group respectively. The frequencies of Rh blood group in the overall sample were 92.45% Rh positive and 7.55% Rh negative. The phenotypic percentage frequencies in study population and the overall sample students Rh blood group, Rh+ve has higher percentage frequency than Rh-ve which has lower percentage, Rh+ 92.45% and Rh- 7.55% respectively (Table 6). These results are consistent with previous findings of Ethiopian populations (www.rhesusnegative.net, 2012) Again, the findings of this study are in agreement with most of 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 a*l., 2006, Akhigbe *et al*., 2009, Adeyemo and Soboyejo, 2006, Iyiola *et al*., 2011). Rh-D negative blood group was 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 and Abraham *et al.*, 2012). The difference in the frequencies of Rh-D phenotypes among the three ethnic group is not significant, ($\chi 2$ =3.20074 df=2, $p< 0.05$) (table 8 .)

4.2. Estimated allele and genotype frequencies for ABO and Rh-D Blood Groups

Table 7. Frequencies of ABO and Rh blood groups among the three studied ethnic groups.

Blood group phenotypes	Observed No of students	Frequencies
A+	110	0.2864
B+	85	0.2213
AB+	18	0.0468
O+	142	0.3697
A-	10	0.0260
B-	5	0.0130
AB-	2	0.0052
O-	12	0.03125
Total	384	1

The combined frequency of the overall study sample ABO and Rh(D) blood group among each ethnic groups are shown in (Table 7). This study has shown that O+ has the predominant(0.3697) while AB- has the small frequencies(0.0052) in the total sample as well as in overall of the three ethnic groups. The frequency of Rh-positive (+) was 0.3697 , 0.2864, 0.2213 and 0.0468 for O+, A+, B+, AB+ respectively, Among Rh -negative (-) Rh-D frequencies compared to their total sample of their corresponding ethnic group and the Rh-negative(-) was high frequent in O-(0.03125) than A- (0.0260) B- (0.0130) and AB-(0.0052) (Table 7).

Table 8: Allelic, phenotypic and Genotypic frequencies of the ABO and Rh-D blood groups in the three ethnic groups

Ethnic Group	Allele	Allelic frequency	Genotype	Genotype frequency	Phenotype (Blood Group)	Phenotype frequency
Amhara	$I^{O}(r)$	0.6099	$I^{O}I^{O}$	0.3721	O	0.3721
	$I^{A}(P)$	0.2028	$I^{A}I^{A}$	0.0411	A	0.0411
			$I^{A}I^{O}$	0.2472	A	0.2472
	$I^{B}(q)$	0.1872	$I^{B}I^{B}$	0.0350	B	0.0350
			$I^{B}I^{O}$	0.2283	B	0.2283
			$I^{A}I^{B}$	0.0759	AB	0.0759
	I^{D}	0.7808	$I^{D}I^{D}$	0.6096	Rh-D	0.6096
			$I^{D}I^{d}$	0.3423	Rh-D	0.3423
	I^{d}	0.2192	$I^{d}I^{d}$	0.0480	Rh-d	0.0480
Oromo	$I^{O}(r)$	0.6311	$I^{O}I^{O}$	0.3984	O	0.3984
	$I^{A}(P)$	0.1522	$I^{A}I^{A}$	0.0231	A	0.0231
			$I^{A}I^{O}$	0.1921	A	0.1921
	$I^{B}(q)$	0.2165	$I^{B}I^{B}$	0.0469	B	0.0469
			$I^{B}I^{O}$	0.2733	B	0.2733
			$I^{A}I^{B}$	0.0659	AB	0.0659
	I^{D}	0.7827	$I^{D}I^{D}$	0.6126	Rh-D	0.6126
			$I^{D}I^{d}$	0.3402	Rh-D	0.3402
	I^{d}	0.2172	$I^{d}I^{d}$	0.0472	Rh-d	0.0472
Wolayita	$I^{O}(r)$	0.6580	$I^{O}I^{O}$	0.4329	O	0.4329
	$I^{A}(P)$	0.1352	$I^{A}I^{A}$	0.0182	A	0.0182
			$I^{A}I^{O}$	0.1779	A	0.1779
	$I^{B}(q)$	0.2334	$I^{B}I^{B}$	0.1089	B	0.1089
			$I^{B}I^{O}$	0.3071	B	0.3071
			$I^{A}I^{B}$	0.0631	AB	0.0631
	I^{D}	0.9132	$I^{D}I^{D}$	0.8339	Rh-D	0.8339
			$I^{D}I^{d}$	0.1585	Rh-D	0.1585
	I^{d}	0.0868	$I^{d}I^{d}$	0.0075	Rh-d	0.0075
Total	$I^{O}(r)$	0.633	$I^{O}I^{O}$	0.4011	O	0.4011
	$I^{A}(P)$	0.1634	$I^{A}I^{A}$	0.0275	A	0.0275
			$I^{A}I^{O}$	0.2057	A	0.2057
	$I^{B}(q)$	0.2124	$I^{B}I^{B}$	0.3816	B	0.3816
			$I^{B}I^{O}$	0.2695	B	0.2695
			$I^{A}I^{B}$	0.4098	AB	0.4098
	I^{D}	0.8256	$I^{D}I^{D}$	0.6816	Rh-D	0.6816
			$I^{D}I^{d}$	0.0452	Rh-D	0.0452
	I^{d}	0.1744	$I^{d}I^{d}$	0.2732	Rh-d	0.2732

Allelic frequency of I^A, I^B, *and* I^O were calculated by the modified Hardy- Weinberg law of equilibrium as indicated in (Table 8).The three alleles are computed (I^A, I^B *and* I^O) with frequencies equal to the derived p, q and r respectively by using the formula allele frequency of ABO and Rh-D blood groups. The overall allelic frequencies of three ethnic groups of the populations as it was calculated using the extension of the Hardy–Weinberg law were 0.6330, 0.2124 and 0.1634 for I^O, I^B and I^A alleles respectively ($I^O > I^B > I^A$**).**

As indicated in (Table 8). The frequency of homozygous genotype allele in the three ethnic group $I^O I^O$ make up 0.3721, 0.3984 and 0.4329 in Amhara, Oromo and Wolayita respectively is higher in Wolayita than in Oromo and Amhara, heterozygus genotype $I^A I^O$ were 0.2472, 0.1921 and 0.1779 in Amhara, Oromo, and Wolayita respectivel more common in Amhara than in Oromo and Wolayita. While $I^B I^O$ makes up 0.2283, 0.2733 and 0.3071in Amhara, Oromo, and Wolayita, respectively predominant in Wolayita than in Oromo and Amhara. $I^A I^B$ 0.0759, 0.0659 and 0.0631 Amhara, Oromo, and Wolayita respectively more common in Amhara than in Oromo and Wolayita. The results of this study in agreement with the finding of (Bakare *et al.*, 2006) that the O allele was common may also be as a result of the fact that many A and B may have been heterozygous carrying O allele as recessive therefore holding O allele in the heterozygous population. Which agrees with result reported by Iyiola *et al*., (2011), Hanania *et al*., (2007), and Bakare *et al*., (2006).

The ABO blood group alleles of the three ethnic groups were 0.6099 I^O, 0.2028 I^A and 0.1872 I^B in Amhara ethnic group, 0.6311 I^O, 0.1522 I^A and 0.2165 I^B in Oromo ethnic group and 0.6580 I^O, 0.1352 I^A, and 0.2334 I^B in Wolayita ethnic group. The overall order of allele frequencies of ABO blood group in three ethnic groups in Amhara were $I^O > I^A > I^B$ which in agreement with these results when compared similar with the other previous studies in the southern and central parts of Iraq which stated that the gene frequencies were (I^A = 0.19 , I^B = 0.20 and I^O = 0.61) in Basrah (Ferhan, 1970) and the northern part of Iraq which stated that the allele frequencies were in the order of ($I^O > I^A > I^B$), were (I^O = 0.647 , I^B =0.172, I^A = 0.194), but in both Oromo and Wolayita were $I^O > I^B > I^A$ *(*0.633 I^O , 0.1634 I^B and 0.2124 I^A*)* *(*Table 4.7) similarly (I^A = 0.187, I^B = 0.22 and I^O = 0.59) in Baghdad (AlRubeai, 1975) and also with the results of (Sadik, 1989) in Nianwa. Therefore , the result of this finding was not significant variation with the other studies in different parts of the world .

The allelic, phenotypic and genotypes frequencies various in the ABO and Rh-D blood groups system in different population of the world. The overall homozygous blood group genotype I^AI^A, I^BI^B and I^OI^O were calculated 0.0275, 0.3816 and 0.4011 respectively. Were as heterozygous blood group of genotype I^AI^O, I^BI^O and I^AI^B were 0.2057, 0.2695and 0.4098 respectively(Table 8). In the case of Rhesus or Rh-D blood group system of the sampled population 92.45% of the population sampled were Rh +ve and 7.55% were Rh-ve as explained in(Table 6). The genotypic Rh-D blood group system among the homozygous DD, the heterozygous Dd and homozygous recessive dd were 0.6816, 0.0452 and 0.2732 respectively calculated by using Hardy-Weinberg Law. So the homozygous DD were more common than the heterozygous Dd genotype and dd homozygous recessive genotype .

Allelic, phenotypic and genotypic frequencies of ABO and Rh blood group are holding varying in different populations throughout the human race. In this study, the researcher attempted to determine the allelic, phenotypic and genotypic frequencies of ABO and Rh blood group in three ethnic group (Amhara, Oromo and Wolayita) in Arsi University. The finding show that variations existed in the allelic, phenotypic and genotypic frequencies of ABO and Rh blood group in three ethnic groups. It implies that there was significant variation among the population.

In the present study, the gene frequencies of ABO and Rh-D blood groups of individuals typed in Arsi University students were considered. Though the allelic, phenotypic and genotypic frequencies of each blood group are similar, there was significant variation in the population of individuals belonging to the various ABO blood groups. This may be due to distinct variation in the number of observed and expected individuals or small numbers of sample (Table 3). And also data among the three ethnic group showed that there was dominance of allelic frequency of O blood group in this study 0.6099 I^O, 0.6311 I^O and 0.6580 I^O in Amhara ,Oromo and Wolayita respectively . This is due to the fact that many of the individuals who are of blood group A and B may have been heterozygous, carrying single O gene together with an A or B gene (Bemstein, 1924) reported that the genetics of the ABO blood group system were dependent on a set of three allelic genes I^A, I^B and I^O. I^A and I^B are co-dominant in blood group AB and both are dominant to I^O allele.

The observed values and frequency figures are similar to those previously reported by other works as indicated (Table 4) blood group O is more prevalent in Saudi Arabia, USA, American African, North-Cameron, Lagos-Nigeria, Sudan, and Kenya that is in the order O > A > B > AB. This shows these populations are characterized by high frequencies of the O allele and an average of about 14% each of the A and B genes (Odeigah, 1990). This is similar with the fact that in Arsi University Students of the Amhara, Oromo and Wolayita ethnic group are characterized by high frequencies of the O allele. While in Pakistan, Western-India and Bangladesh blood group B was more common that is B > O > A > AB and in Egyptian population the order of frequency ABO blood groups were A > O > B > AB and also in Madagascar population O > B > A > AB. These all listed that there is no agreement with the result obtained with this study. Because the suddenly of ABO and Rh-D blood groups emplies that variation occur enter changeably in population at different parts of the world and ethnic groups (Garraty *et al*., 2000).

The result of this study is relevant result with studies from other place of Ethiopia. In Ethiopia, the blood type O is most prevalent in Mekele (Megbaru *et al.,* 2014), Dilla (Fekadu , 2015) and Silte (Kassahun *et al*., 2014) as indicated in Table 4.That is: (O > A > B > AB and showing consistency with the results of the this study). On the Rhesus factor, the present study Rh-negative result (5.97%) was in contrasted to that of documented at 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria and 7.3% in Lahore (Greer *et al.,* 2010).

4.3. Goodness-of-Fit Tests of Hardy-Weinberg Frequencies

The chi-square tests for the study population were calculated based on the expected and observed results. It is very important to know whether the study population is in Hardy-Weinberg equilibrium or not. The chi-square tests in ABO and Rh (D) blood distribution in the populations were calculated from the expected and observed values. The ABO and Rh (D) blood groups distribution is compared with the calculated expected value by using the Chi-square test at P value <0.05, 95% confidence level .

In present study, there was no significant relationship between ethnic group and ABO and Rh-D blood groups, which was confirmed by study done in Pakistan (Khattak et al., 2008). The chi-square test value of ABO and Rh (D) blood groups for the three ethnic groups calculated above are insignificant, this shows that populations of the three ethnic groups

remains constant implies that they are at Hardy-Weinberg Law. It is predicted that there was random mating or intermarriage among the ethnic group, no migration and might be small sample size of the population, etc and thus the allelic frequency will not change from one generation to the next.

Table 9. Chi-square test in ABO blood groups among students of AU Oromo, Amhara and Wolayita ethnic groups

ABO blood group						
Ethnic group	Blood Groups	Observed No (O)	Expected No(E)	Differences D=(O-E)	D^2	D^2/E
Amhara	A	39	40.3	-1.3	1.69	0.41935
	B	34	30.2	3.8	14.44	0.47814
	AB	8	6.7	1.3	1.69	0.25223
	O	45	51.7	-6.7	44.89	0.86827
	$\chi 2 = \Sigma (Of - Ef)2/ Ef = 2.01799$ df=3, P< 95%)					
Oromo	A	41	40	1	1	0.02500
	B	29	30	-1	1	0.03333
	AB	7	6.7	0.3	0.09	0.01343
	O	51	51	0	0	0
	$\chi 2 = \Sigma (Of - Ef)2/ Ef = 0.07176$ df=3 , P< 95%)					
Wolaita	A	40	39.7	0.3	0.09	0.00226
	B	27	29.8	-2.8	7.84	0.26308
	AB	5	6.6	-1.6	2.56	0.38787
	O	55	50.9	4.1	16.81	0.33025
	$\chi 2 = \Sigma (Of - Ef)2/ Ef = 0.98346$ df=3, P< 95%)					

Where:- Chi-square (χ^2) =$\Sigma(Of-Ef)^2/Ef$

Goodness of fit Value of χ^2 0.02088, df=2, P<95%)

χ^2= chi-square

df= degree of freedom

Table 10: Observed versus expected frequency of Rh (D) blood groups of Amhara , Oromo and Wolayita ethnic group

Ethnic group	Rh-D blood group system					
	RhD	Observed No (O)	Expected No(E)	Differences D=(O-E)	D^2	D^2/E
Amhara	Rh-D+	121	119.3	1.7	2.89	0.02422
	RhD-	8	9.7	-1.7	2.89	0.29793
	χ^2 = 0.32215 df = 1, P > 0.05					
Oromo	RhD+	121	118.3	2.7	7.29	0.06162
	RhD-	7	9.7	-2.7	7.29	0.75154
	χ^2 =0.81316 df 1, P >0.05					
Wolaita	RhD+	113	117.4	-3.6	12.96	0.11039
	RhD-	14	9.6	4.4	19.36	2.01666
	χ^2 = 2.12705 df 1, P >0.05					

Where Chi-square (χ^2)= $\Sigma(Of-Ef)^2/Ef$

Goodness of fit Value of $\chi 2$ =3.20074 df=2, p> 0.05

χ^2 = chi-square

In Table 9. the calculated Chi-Square value for three ethnic group were 2.01799 degree of freedom 3 P value < 0.05, 0.07176 P value < 0.05 df 3 , 0.98346 which has the P value is < 95% with 3 degrees of freedom for Amhara ,Oromo and Wolayita respectively the total χ^2 value 0.02088 df= 2 p < 0.05. The chi square test shows that there is no significant difference between values expected for the frequency of ABO blood group and observed for total study sample populationemplies that the population was at Hardey Weinbegy Law (Table 9). The calculated Chi-Square value of Rh D blood group were Pearson chi-square 0.32215 df 1 with P value > 0.05, 0.81316 df 1 p value > 0.05 and 2.12705 df 3 with P value > 0.05 in Amhara , Oromo and Wolayita respectively and the overall chi-square (goodness-of-fit) is 3.20074 df=2 for study ethnic group (Table 4.9). so that, overall significance level of the research is acceptable at P value < 0.05% in the three ethnic group and there is no significant in Variation between values obtained for study ethnic group

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

- The data from this study has a significant implication regarding the blood transfusion services in the area.
- Knowledge of blood group distribution is also important for clinical studies, for reliable geographical information and for forensic studies in the population.
- Such studies need to be carried out at all University of Ethiopia.
- The chi-square test shows that the ethnic group is at the Hardy-Weinberg Genetic Law.

5.2. Recommendation

- The data generated in this study would be helpful as an initial base for researchers that generates data on the allelic, phenotypic and genotypic frequencies of ABO and Rh-D blood group system related studies across the country or at the group level.
- The sample size used to conduct this study from each of the three ethnic group was small and may not represent the total number of population of the three ethnic group as well as the College. Therefore it is advisable to use larger sample size from each the three ethnic group to obtain more accurate data regarding the pattern of frequencies of these blood groups.

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7. APPENDIX

Appendix 7.1: **Students Agreement Consent Form**
Consent format

I the undersigned have been informed and understand that the purpose of this particular research project is to find out the allelic, phenotypic and genotypic frequencies of ABO and Rh-D blood group among Amhara, Oromo and Wolayita ethnic Students of Arsi University students ,Oromia Regin , Ethiopia. I have been requested to participate in the study and give a drop of blood from left ring 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. 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.

Signature_____

Appendix 7.2: Blood donors of ABO and Rh (D) Blood Group Phenotypes recording format

No	Full Name	sex	Ethnic group	ABO & Rh Blood Phenotype	Sign.	Remark
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Remark O = Oromo, A = Amhara, W= Wolayita

Appendix 7.3: Supporting letters and ethical clearance to conduct research

ቀን/06/10/2010 ዓ.ም

ማስታወቂያ

ለ አርሲ ዩኒቨርሲቲ ተማሪዎች በሙሉ::

ጉዳዩ፤- የደም አይነት (Blood group) ለማወቅ የምትፈልጉ ተማሪዎች በሙሉ ::

ከላይ ለመጥቀስ እንደተሞከረው የጊቢያችን ተማሪዎች የሆናችሁ በፍቃደኝነት ከ 7/10/2010 እስከ 15/10/2010 ዓ.ም ድረስ ብቻ በክሊኒካችን እየመጣችሁ ማወቅ እንደምትችሉ እናሳውቃለን::



አርሲ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ

ስልክ: 251-223-311-2 31
ፋክስ: +251-223-313-533
✉ 04 , 396 አሰላ, ኢትዮጵያ
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Hailu Fekadu (MPH, Research& community service coordinator)
Asella, Ethiopia

Ref No. A/ሠ/ዘ/ሰ/ር/ሲ/20/8866/10
Date: 10/08/2010

To- Asela Teaching and Retreral Hospital

Assela

Subject : **Request for support**

Suleman Deko is Msc student at College of Natural and compititional Science ,Medwelabu, University is going to undertake a research project **entitled " Allelic and genotypical frequencies of ABO and Rhesus RH(D) blood groups among students of Amhara, Oromo and Wolayita Ethinic group In Arsi University, Ethiopia"**

This research is seen by our college ERB and endorsed as ethicaly no problem to use in practical situation

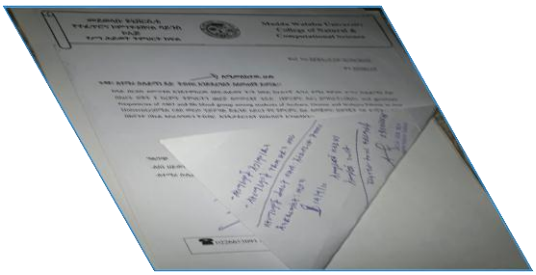
Hence, therefore: to request your good office to allow him to use our laboratory for this spesfic research. The University appreciates your usual cooperation in advance!

With regards!

ሀይሉ ፍቃዱ ደምሴ
Hailu Fekadu
Demssie (MPH)
ሥነ ማህበረሰብ አገልግሎት ተ/ዲን
Dean For Research and Community Service

CC//

V/D for research and community service



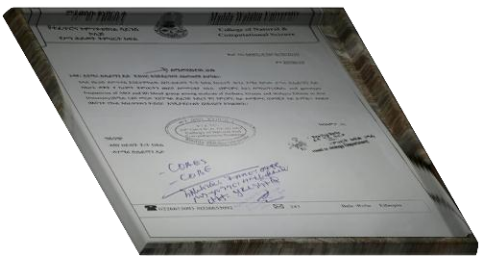


Figure 7.1: The three different ABO and RhD anti-serums (anti-A, anti-D and anti-B) in order



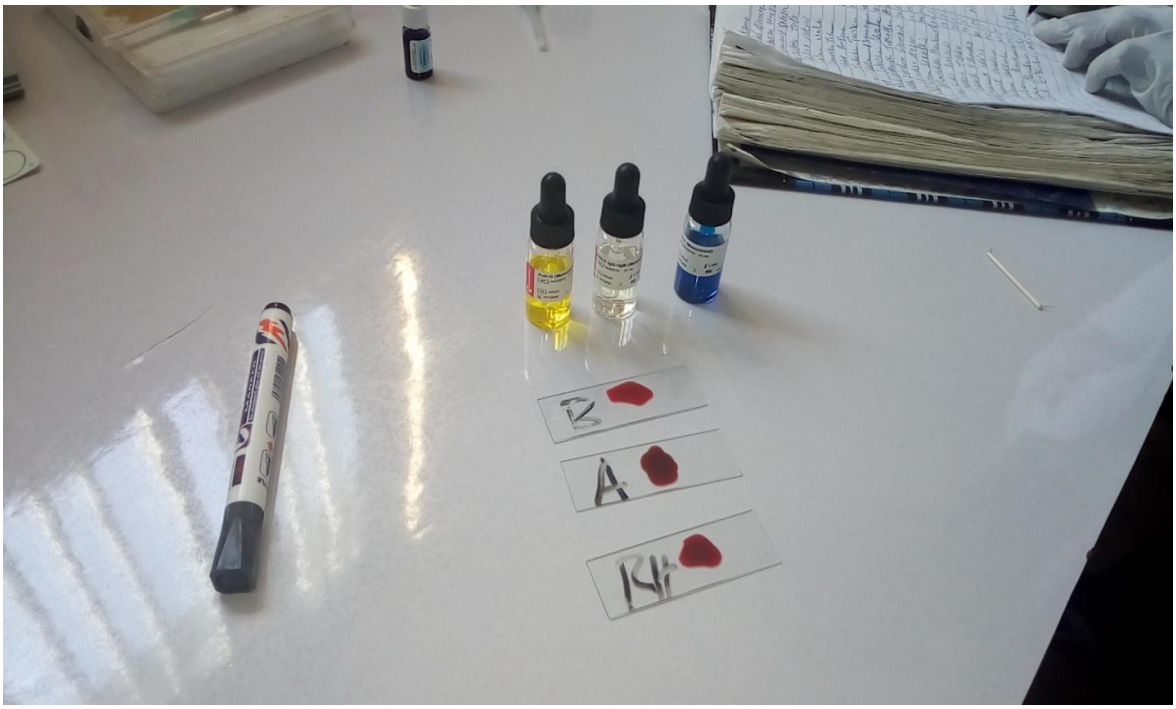


Figure7. 2: Pictures of determining and recording of ABO and Rh(D) blood types of the students









Figure 7.3: laboratory technicians and researcher during blood sample taking for ABO and Rh (D) blood typing test.



Figure 7. 4. After observation of agglutination of ABO and Rh (D) blood group system result register.

