ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

OFFICE OF GRADUATE STUDIES

Distribution of ABO and Rh (D) Blood Groups among Students Attending Secondary and Preparatory Schools in 2017 Bote Town, Oromia Regional State, Ethiopia

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

In Partial Fulfillment of the Requirement for the Degree of Master of Science in Applied Biology

By

Amsalu Wakgari Fufa

Adama, Ethiopia

September, 2017

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Advisor: Dr. Daniel Getahun

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APPROVAL SHEET OF BOARD OF EXAMINERS

We, the undersigned, members of the board of examiners of the final open defense by Amsalu Wakgari Fufa have read and evaluated his thesis entitled "*Distribution of ABO and Rh (D) Blood Groups among Students Attending Secondary and Preparatory Schools in 2017 Bote Town, Oromia Regional State, Ethiopia*." and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master in Applied Biology.

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Subject: <u>Thesis Submission</u>

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Signature _____

Date_____

DEDICATION

This piece of work is dedicated to my beloved family: my mother Likinesh Bekele, my father Wakgari Fufa, my wife Almaz Adamu and my son Immanuel Amsalu

.

BIOGRAPHICAL SKETCH

The author was born in Bonga town, Southern Nation Nationalities and People's Regional State of Ethiopia, on 24thAugust, 1988. He attended his Primary School at Bote Primary School from 1991 to 1998 and Secondary School at Nekempte Comprehensive High School 1999 to 2003. Then he joined Mekelle University in 2004 and graduated with B.Ed. degree in Biology in July 2006. Soon after graduation, he was employed by Oromia Regional Education Bureau, as Biology teacher at Bote Preparatory School. He joined Adama Science and Technology University in 2014 to 2017 pursues his postgraduate study in Applied Biology.

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LIST OF ABBREVIATIONS AND ACRONYMS

AHTR	Acute Hemolytic Transfusion Reaction
CSA	Central Statistical Authority
DIC	Disseminated Intravascular Coagulation
ELISA	Enzyme Linked Immuno Sorbent Assay
FMC	Flinders Medical Center
FFP	Fresh Frozen Plasma
HDN	Hemolytic Disease of the Newborn
HTR	Hemolytic Transfusion Reaction
IgG	Immunoglobulin Gama
IgM	Immunoglobulin Mue
ISBT	International Society Of Blood Transfusion
MN	M and N Blood Groups
RBCs	Red Blood Cells
Rh	Rhesus
vWF	Von Willebrad Factor

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ABSTRACT

The major concern of this study was to determine the distribution of ABO and Rh (D) blood group alleles, genotypes and phenotypes among students attending at secondary and Preparatory schools in 2017 in Bote town Oromia Regional State, Ethiopia. The Study of blood grouping is not only generating a simple database but also create a great social awareness about self-blood grouping, safe blood transfusion and have educational value among the population of a country. A total of 392 sample students gave bloods voluntarily for ABO and Rh blood grouping. Finger prick blood samples from both genders were tested for ABO and Rh blood groups determination by the open slide test method. A drop of each of the antisera, anti-A, anti-B and anti-D were placed and mixed with each blood sample with the help of sterilized applicator sticks and rocked gently to observe agglutination. The results revealed that phenotype O was the most frequent while blood group AB was the least frequent in the whole sample .The O, A, B and AB blood groups percentage, were as follows: 41% , 32%, 21% and 6%, respectively. Rhesus factor records: 93% for Rh+ and 7% for Rh-. The study reports the pattern of blood groups among Secondary and Preparatory Students in Bote town and would help the blood bank to prepare database and increase awareness which type of blood group should be stored more. So, the study has a significant implication regarding the inventory management of blood bank and transfusion services for those who need blood transfusion. The allele frequencies of ABO and Rh blood groups were calculated by Hardy-Weinberg equation and found to be: 0.65, 0.14 and 0.21 for I^O, I^B and I^A, respectively. The Rh allelic frequencies were 0.73 for D and 0.27 for d, respectively. In case of both ABO/Rh blood groups frequencies in the studied population blood group O+ was found to be the most common (38%), followed by groups A+ (30%), B+ (20%) and AB+ (5%), whereas among the Rh negative subjects, blood group O- was the most frequent (3%), followed by groups A− (2%), B− (0.1%) and AB− (1%). The distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy-Weinberg equilibrium (Goodness of fit X^2= , 0.4729, df=3, P>95%)

Keywords: *ABO Blood groups, Rh-antigens, Alleles, Students, Bote town*

1. INTRODUCTION

Blood is a specialized connective tissue in which cells are suspended in fluid extracellular material called plasma. About 5 L of blood in an average adult moves unidirectionally within the closed circulatory system. The so-called formed elements circulating in the plasma are erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (Anthony, 2013). More than 99 percent of blood cells are erythrocytes, which carry oxygen. The leukocytes protect against infection and cancer, and the platelets function in blood clotting. In the cardiovascular system, the constant motion of the blood keeps all the cells well dispersed throughout the plasma (Vander, 2001).

Anthony in 2013 explained the nature of Erythrocytes (red blood cells) is terminally differentiated structures lacking nuclei and completely filled with the O_2-carrying protein hemoglobin. Red Blood Cells (RBCs) are the only blood cells whose function does not require them to leave the vasculature and they are normally quite flexible, which permits them to bend and adapt to the irregular turns and small diameters of capillaries. Observations in vivo show that at the angles of capillary bifurcations, erythrocytes with normal adult hemoglobin frequently assume a cuplike shape. In larger blood vessels RBCs often adhere to one another loosely in stacks called rouleaux.

Tekade *et al.* (2011) suggested that the discovery of the ABO blood group over 100 years ago caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO blood group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different population. The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules (Daniel, and Clark, 2007).

Some of the red blood cell antigens are also present on the surface of other types of cells and body secretion like saliva, sweat, tear, urine, semen and serum which are used in forensic investigations. Several of these RBC surface antigens that stem from very closely linked genes collectively form blood group system. Blood groups are genetically determined and exhibit polymorphism in different populations. A total of 30 human blood group systems are now recognized by the international society of blood transfusion. In clinical practice ABO and Rhesus (Rh) blood groups are the most important among 30 blood groups (Jaff, 2010).

The ABO and Rh are recognized as the major clinically significant blood group antigens. The ABO system derives its importance from the fact that A and B are antigens and anti A and anti B occur naturally in the serum of persons lacking the corresponding antigens, these antibodies being capable of producing hemolysis in vivo. Rhesus blood group system was the fourth system next to ABO, MNS, and P1PK to be discovered and yet it is second most important blood group from the point of view of blood transfusion (Adeyemo *et al*., 2006). Furthermore the discovery of ABO and Rh blood group has contributed immensely to blood banking services and transfusion medicine in order to avoid morbidity and mortality in both adults and children. 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, and ABO and Rh are the most studied blood system among the human population due to their clinical, genetic and anthropological importance (Bakare *et al.*, 2006).

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

The distribution of the ABO and Rh blood group alleles vary worldwide and are not found in equal numbers. For example, Among African-Americans the distribution of ABO blood group, type O, 46%; type A, 27%; type B, 20%; and type AB, 7%. Among Caucasians in the United States, the distribution of type O is 47%; type A, 41%; type B, 9%; and type AB 3%;

Among Western Europeans type O, 46%; type A, 42%; type B, 9%; and type AB, 3% (Pramanik, 2000). Moreover Rh positive is documented as in United States of America (USA) 85%, Red Indians in USA 100% and 95% in Germany (khattak *et al*., 2008) and Siefu and Kinfe (1983) also reported distribution of Rh positive in Ethiopia is 95%.

The Rh blood group is named after the rhesus monkey, this group is determined by gene called D which has two alleles: D, d whatever other alleles a person may have, any one with genotype DD or Dd, has D antigens on his or her RBCs and is classified as Rh-positive (Rh+). In Rh-negative (Rh-) people, the D antigen is lacking. Anti-D antibodies are not normally present in the blood. If an Rh- person receives an Rh+ transfusion, the recipient produces anti-D. A related condition sometimes occurs when an Rh- woman carries an Rh+ fetus. The first pregnancy is likely to be uneventful because the placenta normally prevents maternal and fetal blood from mixing. However, at the time of birth, or if a miscarriage occurs, placental tearing exposes the mother to Rh+ fetal blood. She then begins to produce anti-D antibodies. If she becomes pregnant again with an Rh+ fetus, her anti-D antibodies may pass through the placenta and agglutinate the fetal erythrocytes. Agglutinated RBCs hemolyze, and the baby is born with a severe anemia called hemolytic disease of the new born (HDN), or Erythrblastosis fetalis (Saladin, 2003).

1.1 Significance of the Study

No research has been done on the distribution of ABO and Rh type of blood group in the study area like other countries in the world. It is hard to know which type of blood group could be found in large and/or less amount. The outcome of this research can also be seen as good input for the blood bank services for effective management of safe blood transfusion services. This study aimed to provide essential information about the frequency of 'ABO' and 'Rh-D' blood groups in Bote town blood donors to help in setting of blood bank policy for providing blood products for patients who require blood transfusion. World Health Organization (2007) reported that in countries where diagnostic facilities and treatment options are more limited, the majority of transfusions are prescribed for the treatment of complications during pregnancy and childbirth, severe childhood anemia, trauma and the management of congenital blood disorders. Hemorrhage, for example, accounts for over 25% of the 530 000 maternal deaths each year; 99% of these are in the developing world. Access

to safe blood could help to prevent up to one quarter of maternal deaths each year and blood transfusion has been identified as one of the eight life-saving functions that should be available in a first-referral level healthcare facility providing comprehensive emergency obstetric and newborn care. Apart from this by studying the distribution of the Rh (D) antigen it is possible to reduce the probability of maternal and infant death in the study area because it is possible to keep the health of both mothers and children by knowing about Rh antigen priory and giving genetic counseling and by making a healthy child to be born. From this study it is also possible to know the probability of hemolytic disease in the new born (HDN).

1.2 Objectives of the Study

General objective

- The general objective of this study is to assess distribution of ABO and Rh (D) blood groups among students attending Secondary and Preparatory Schools in 2017 Bote town, Oromia Regional State, Ethiopia.

Specific objectives

- To determine the frequency of ABO and Rh blood group phenotypes among Secondary and Preparatory Students in Bote Town
- To determine the allelic frequency of ABO and Rh blood groups among Secondary and Preparatory Students in Bote Town
- To determine genotypic frequency of the ABO and Rh blood groups among Secondary and Preparatory Students in Bote Town

2. LITERATURE REVIEW

2.1 A Brief History of the ABO and Rh (D) Blood Groups System

The ABO and Rhesus (Rh) both remain the most important and famous blood group systems clinically. The ABO blood group system was first discovered in 1901 by, an Australian Scientist Karl Landsteiner. The Rh system was later described by both Landsteiner and Weiner in 1940 by their joint work. Both are equally important in clinical and forensic medicine. Since the discovery, attention of scientists has been greatly focused on these two blood group systems because of the fact that they are highly polymorphic and immunogenic especially in human (Avent and Reid, 2000).

Landsteiner named the first two blood group antigens A and antigen B, using the first two letters of alphabet while RBCs not reacting with anti A and anti B were called type C. In 1902 Von Decastello and Sturi described RBCs reacting to both anti A and anti B but did not give these type a name, continued calling RBCs that did not react with anti-A and anti-B type C (Garrattey *et al.*,2000). In 1911, Von Dungern and Herzfeld were the first to use the term O to describe RBCs not reacting with anti A and anti B and the term AB for RBCs reacting with both anti-A and anti-B (Mollison, 1994).

Hillier (2008) suggested that Landsteiner's discovery opened the door to the birth of a wide spectrum of discoveries in the field of immunohematology, blood transfusion among humans irrespective of their natives, unmatched-pregnancy, legal medicine, anthropology and the discovery of other blood group systems, all are deemed as an applications or as a result of Karl's discovery. The discovery of the ABO blood groups by Karl Landsteiner was an important achievement in the history of blood group determination. The vast majorities is inherited in a simple Mendelian fashion and are stable characteristics which are useful in paternity testing.

Since the early discovery of the D antigen in 1939, fifty related antigens have been assigned to the Rh system by the International Society of Blood Transfusion (ISBT). The major and the most important antigens are D, C, c, E, and e are which account for most clinical transfusion issues (Blaney and Howard, 2013).

2.2 Blood Group Systems

A complete blood type would describe a full set of 30 substances on the surfaces of RBCs, and an individual`s blood types in one of many possible combinations of blood group antigens. Across the 30 blood group, over 600 different blood groups antigens have been found, but many of these are very rare (ISBT, 2008). Clinically important and different types of blood group antigens including both ABO and Rh labeled in Figure 1.

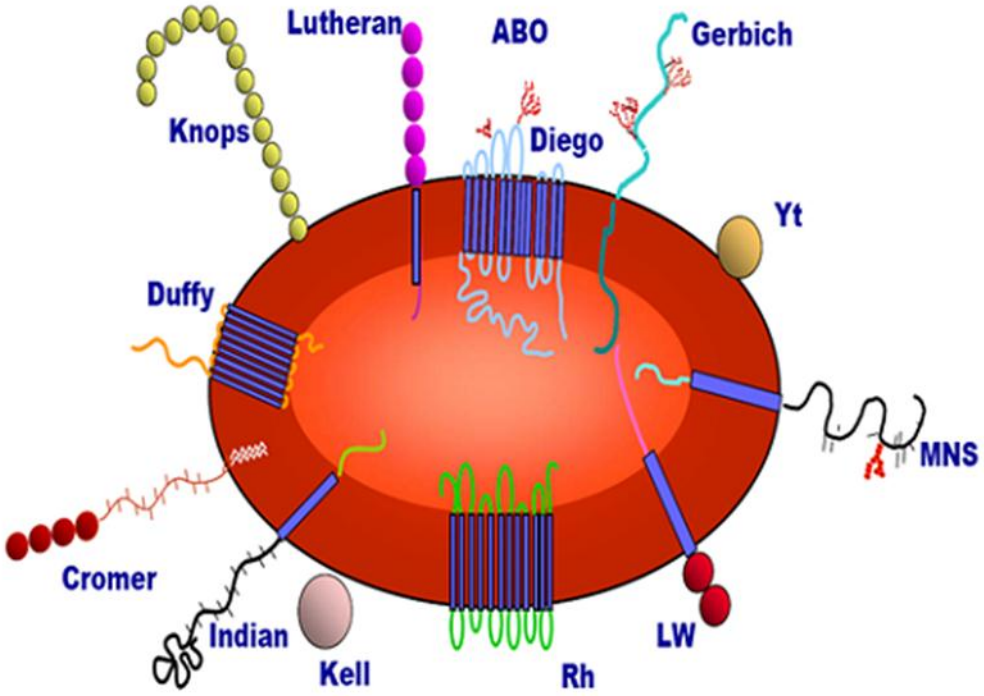


Figure 1: Schematic representing clinically significant blood group antigen

Source: (Wester *et al*., 2011).

Mader (2004) suggested that ABO blood typing is based on the presence or absence of two possible antigens, called type A antigen and type B antigen, on the surface of red blood cells. Whether these antigens are present or not depends on the particular inheritance of the individual. In the ABO system blood groups are classified as A, B, AB, and O. Classification of blood as, Rh-positive and Rh-negative in Rh system is based on presence or absence of inherited antigenic substance on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoproteins or glycolipids depending on the blood group system (Hasna *et al.,* 2010). Some of Human blood group systems recognized by the international

society of blood transfusion (ISBT) were listed in Table 1 and the first three digits represent the blood group systems (001-025)

Table 1: Currently recognized antigens within blood group systems recognized by the ISBT working party

System number	System name conventional	System symbol ISBT	Gene(s)
001	ABO	ABO	ABO
002	MNS	MNS	GYPA GYPB
003	P	PI	P
004	Rh	RH	RHD RHCE
005	Lutheran	LU	LU
006	Kell	KELL	KEL
007	Lewis	LE	FUT3
008	Duffy	FY	FY
009	Kidd	JK	HUT11
010	Diego	DI	SLC4A1
011	Yt	YT	ACHE
012	Xg	XG	XG
013	Scianna	SC	SC
014	Dombrock	DO	DO
015	Colton	CO	AQP1
016	LW	LW	LW
017	Chido/Rogers	CH/RG	C4A,C4B
018	H	H	FUT1
019	Kx	XK	XK
020	Gerbich	GE	GYPC
021	Cromer	CROM	DAF
022	Knops	KN	CR1
023	Indian	IN	CD44
024	Ok	OK	OK
025	MER2	RAPH	MER2

Source: (Lewis *et al*., 2006).

Weiner (2010) reported that almost an individual has the same blood group for life, but very rarely individual`s blood type change by additional or suppression of an infection, malignancy, or autoimmune disease Another more common in blood type change is bone marrow transplant. Bone marrow transplants are performed for many leukemia`s and lymphomas, among other disease. If a person blood type-A bone marrow receives blood from patients of blood type-O, the patient`s blood type will eventually convert to the donor`s type.

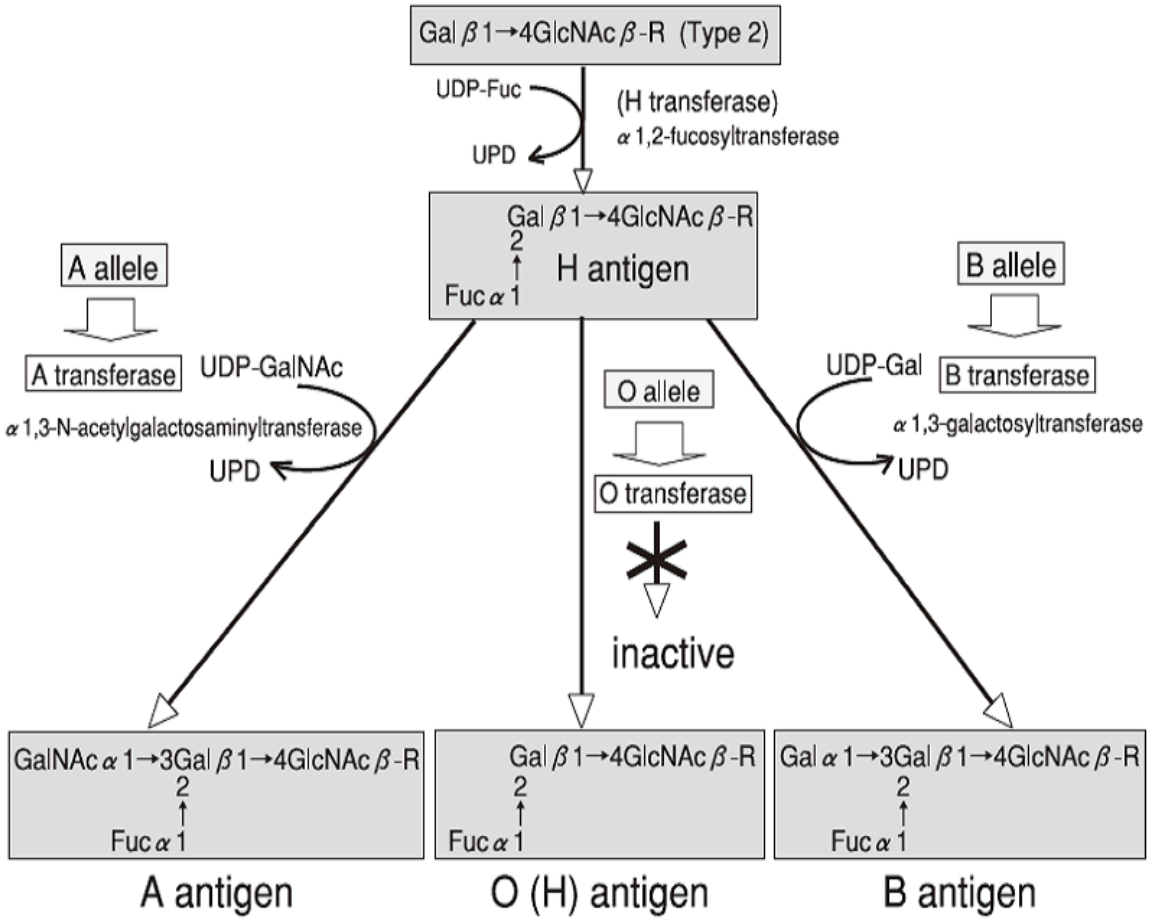
2.3 ABO Blood Group System

As Mader (2004) explained in the ABO system, blood type depends on the presence or absence of antigens A and B on the surface of red blood cells. ABO antigens exist on glycoproteins and glycolipids in red cell membranes and also on most cells and tissues in humans, and in animal tissues. The antigens are also present in the secretory fluids in the majority of humans. Thus the term "Histo-blood group system" is a more accurate description than "Blood group system". The antigens are unequally expressed among and within the different cells and tissues and in different species. Except for humans, only anthropoid apes, the orangutan and the gorilla have ABO antigens on their red cells, which suggest that the red blood cells are the last cells during evolution to obtain the ABO antigens.

2.3.1 Biosynthesis of ABO antigens

Antigens of the ABO system are oligosaccharides that are attached to the proteins and lipids on the surface of RBCs. These antigens are synthesized stepwise by the action of glycosyltransferase enzymes encoded for by the ABO gene locus located on chromosome 9 at 9q34.1-q34.2 (Oriol *et al*., 1986). In Figure 2 the ABO gene has three alleles: A, B and O. For the synthesis of the A and B antigens to occur, a precursor O antigen (also referred to as the H antigen) must be present. The O antigen, which is synthesized by an enzyme encoded for the by the H (FUT1) locus in RBCs is in the forms of —Lipid—Glucose—Galactose—N-acetylglucosamine— Galactose—Fucose. The A allele of the ABO locus codes for N-acetylgalactosamine (GalNAc) transferase which catalyzes the formation of an α-1, 3 glycosidic bond between the outermost galactose component of the O antigen and GalNAc. Meanwhile the B allele encoded the synthesis of Gal transferase which glycosidically adds an extra galactose to the O antigen at the α-1.3 position. The O allele however encodes an

enzyme with no function, and therefore neither A or B antigen is produced, leaving the underlying precursor (the H antigen) unchanged.



Fuc: L-fucose, Gal: D-galactose,

GalNAC: N-acetylgalactosamine, GlcNAc: N-acetylglucosamine

Figure 2: Biosynthesis of ABO antigen

Source: (Oriol *et al*., 1986).

2.3.2 ABO antibodies

The ABO blood group system is unusual in that antibodies are almost always present in individuals who lack the specific ABO antigens. The plasma of blood group A, B, AB and O will contain anti-B, anti-A, neither anti-A nor anti-B, and anti-A, B antibodies, respectively. ABO antibodies can be detected by the age of three month and reach the maximum level at the age of 5 to 10 years (Bruce, 2002).

The antibodies (also called natural antibodies) are mainly of IgM class, complement activating and hemolytic at 37˚C, but antibodies of IgG and IgA classes may also be present. After immunization IgG ABO antibodies will increase and IgA will also become present. A characteristic of weak-subgroups and weak-phenotypes is often an unusual alloantibody against ABO antigens (e.g. anti-A1 is often seen in some weak A subgroups). Because of the lack of variability, and unreliability of antibody detection, these allo-antibodies should not be used to define a weak-subgroup or phenotype, but should instead only be used as an indicator of something unusual (Greenwalt, 1997).

2.3.3 ABO blood group typing

Blood typing is performed with anti-sera blood serum that contains specific antibodies. For ABO blood typing antibodies against A and B antigen (these antibodies are also called anti-A and anti-B antibodies) are used.

Table 2: Agglutination reaction of ABO blood group system

Blood type	Reaction	
	A antibodies (anti-A serum)	B antibodies (anti-B serum)
A	Clumping	No clumping
B	No clumping	Clumping
AB	Clumping	Clumping
O	No clumping	No clumping

Source: (Avent and Reid, 2000)**.**

Agglutination of RBCs by an Antibody, Anti-A and anti-B have 10 binding sites, located at the 2 tips of each of the 5 Ys, and can therefore bind multiple RBCs to each other (Figure 3)

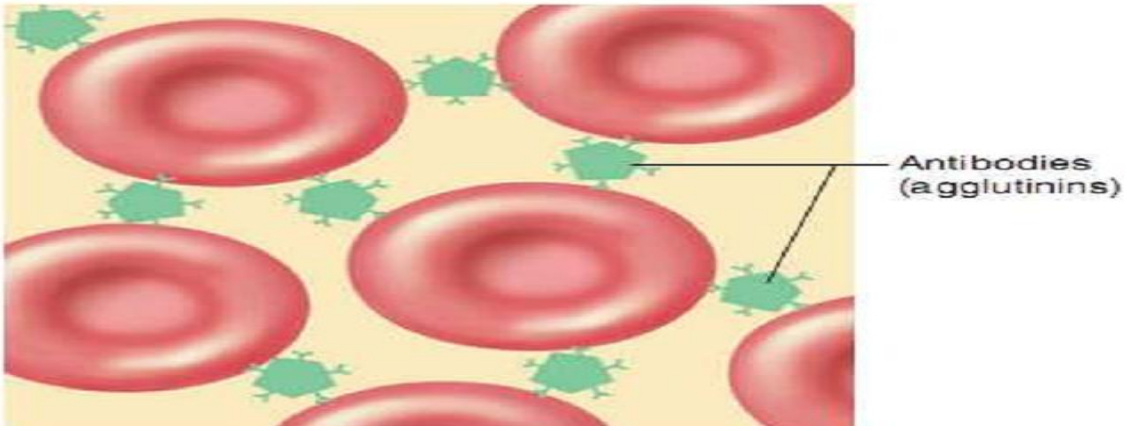


Figure 3: Agglutination of RBCs by an antibody

Source: (Saladin, 2003).

2.3.4 ABO blood group genotyping

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three alleles: I^0, I^A, and I^B. The I^A allele gives type A, I^B gives type B, and I^O gives type O. As both I^A and I^B are dominant over I^O, only I^OI^O people have type O blood. Individuals with I^AI^A or I^AI^O have type A blood, and individuals with I^BI^B or I^BI^O have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship; co dominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^BI^O, I^AI^O). Four blood group phenotypes and six genotypes were listed in Table 3. In addition to the current practice of serologic testing of blood types, the progress in molecular diagnostics allows the increasing use of blood group genotyping. In contrast to serologic tests reporting a direct blood type phenotype, genotyping allows the prediction of a phenotype based on the knowledge of the molecular basis of the currently known antigens (Anstee, 2009).

Table 3: Blood phenotype and genotype

Phenotype	Genotype
A	$I^A I^A$ and $I^A I^O$
B	$I^B I^B$ and $I^B I^O$
AB	$I^A I^B$
O	$I^O I^O$

Source: (Anstee, 2009).

2.4 Rh Blood Group System

Since the early discovery of the D antigen in 1939, fifty related antigens have been assigned to the Rh system by the International Society of Blood Transfusion (ISBT). The major and the most important antigens are D, C, c, E, and e are which account for most clinical transfusion issues (Blaney and Howard, 2013). The second major blood grouping system is the Rhesus (Rh) system. Like the ABO blood types, the Rh factor is an inherited blood protein, or antigen, on red blood cells. People who have it are "Rh positive"; those who don't are "Rh negative". The Rh system is more complex than the ABO system in that there are 35 different possibilities that can be inherited from each parent. These, however, are roughly grouped into positive and negative types. Being Rh positive is more common than being Rh negative. In Rh blood group system specifically, the D antigen is used to determine the risk of hemolytic disease of the new born (HDN) (Reid and Lomas, 2004).

A mother who is Rh-negative may develop antibodies to an Rh-positive baby. If a small amount of the baby's blood mixes with the mother's blood, which often happens in such situations, the mother's body may respond as if it were allergic to the baby. The mother's body may make antibodies to the Rh antigens in the baby's blood. This means the mother has become sensitized and her antibodies may cross the placenta and attack the baby's blood. Such an attack breaks down the fetus's red blood cells, creating anemia (Mais, 2009).

2.5 Hemolytic Disease of the Newborn (HDN) or Erythroblastosis Fetalis

Letsky *et al.* (2000) suggested that Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh negative can bear an Rh positive child if the father is Rh positive (either homozygous or heterozygous). Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy. At the time of birth, however, tissue damage resulting from the separation of the placenta from the uterine wall can result in a significant amount of fetal blood entering the maternal circulation; which may stimulate a strong IgG anti-Rh response in the mother. If the same mother then bears a second Rh+ child, the existing anti-Rh antibodies can cross the placenta during the pregnancy and destroy fetal red blood cells. The ensuing damage to various organs results in the potentially dangerous condition Erythroblastosis Fetalis also known as Hemolytic Disease of the Newborn or HDN. This can be diagnosed prenatally by carrying out amniocentesis, and examining the amniotic fluid for the presence of free hemoglobin and its degradation products. The progress of hemolytic disease of the new born (HDN) (Figure 4). (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 Hemolytic Disease of the Newborn (HDN), 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.

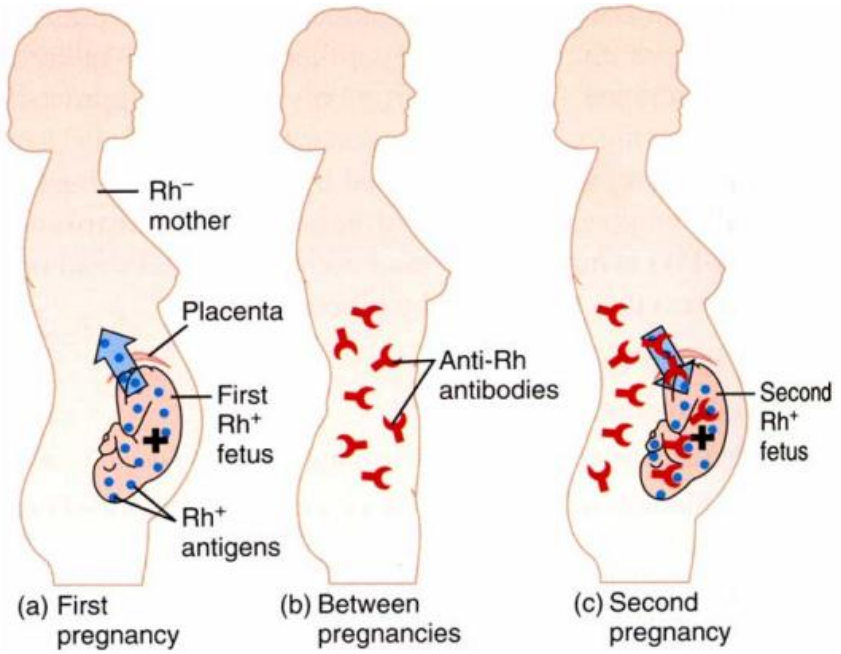


Figure 4: Hemolytic disease of the new born (HDN)

Source: (Saladin, 2003).

A mother who is Rh negative can bear an Rh positive child if the father is Rh positive (either homozygous or heterozygous) (Table 7).

Table 4: Illustration of Rhesus compatibility match

Father	Mother	Compatibility
Rh-positive	Rh-positive	Compatible
Rh-negative	Rh-negative	Compatible
Rh-positive	Rh-negative	Incompatible
Rh-negative	Rh-positive	Compatible

Source: (Okoduwa, 2013).

Various approaches can be used during and after birth to rescue the infant, including exchange transfusion, complete replacement of the infant's blood to remove the anti-Rh antibodies and provide undamaged red blood cells. However, the production of anti-Rh antibodies in an Rh- mother can often be prevented by administering anti-Rh immune globulin (e.g. RhoGAM) into the mother, typically at around 28 weeks of gestation and again within 72 hours of the birth of her Rh+ baby. By mechanisms which are still not fully

understood, these antibodies greatly reduce the likelihood of sensitization of the mother's immune system by the Rh+ erythrocytes. If this procedure, developed in the 1960's, is successfully carried out during each Rh+ pregnancy, anti-Rh antibodies are not produced by the mother, and subsequent pregnancies will not be at risk (Okoduwa, 2013).

2.6 Worldwide Distribution of ABO Blood Group and Rh factor

Dean (2005) suggested that the ABO blood group distribution varies among the different people all over the world. For instance, Blood group B has its highest frequency in Northern India and neighboring Central Asia, and its incidence diminishes both towards the west and the east, falling to single digit percentages in Spain. It is believed to have been entirely absent from Native American and Australian Aborigines populations prior to the arrival of Europeans in those areas. Blood group A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigines populations and the Blackfoot Indians of Montana. O blood type is very common around the world. Type O is particularly high in frequency among the indigenous populations of central and South America where it approaches 100%. The lowest frequency of (O) is found in Eastern Europe and central Asia, where B is common (Table 4).

Neil (2006) also reported that of the Rhesus blood group system, the allele D which gives rhesus positive status is at its lowest in Europe and it increases in frequency east ward and south ward 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%. Population genetics has made extensive use of these genetic markers. It seems that adjacent populations differ only slightly in the frequencies of particular alleles and that these differences tend to increase with the distances separating populations (Rubbia, 1975).

Table 5: ABO blood group and Rh factor distribution in the world populations

Population	Abo blood group (%)				Rh factor (%)		References
	O	A	B	AB	Rh+	Rh–	
All donors in US	46.5	37	12.4	4.10	85.4	14.6	Garratty *et al*., 2004
Asian in US	40	28	25	7	98.3	1.7	Garratty *et al*., 2004
Bangladesh	40.5	26.5	23.5	9.5	96.8	3.2	Chandra and Gupta, 2012
Black non-Hispanic in US	50	26	20	4	93	7	Garratty *et al*., 2004
Britain	47	42	8	3	83	17	Chaurasia *et al*., 2015
Bulgaria	36	40	17	7	ND	ND	Salduz *et al*., 2015
Greece	34	48	12.	6	ND	ND	Salduz *et al*., 2015
Hispanic in US	56.5	31	10	2.5	92.7	7.3	Garratty *et al*., 2004
Iran	40	28.5	25	6.5	92.4	7.6	Maatoghi *et al.*, 2016
Nepal	32.5	34	29.5	4	96.7	3.3	Chaurasia *et al.*,2015
North American Indian in US	54.5	35	8	2.5	90.3	9.7	Garratty *et al*., 2004
Northern India	29	22	40	9	95.7	4.3	Chandra and Gupta, 2012
Pakistan	30.5	28.7	32.4	8.4	93	7	Chaurasia *et al.*, 2015
Saudi Arabia	52	25	19	4	93	7	Chandra and Gupta, 2012
Turkey	29.7	38.2	14.4	6.8	89.6	10.4	Kayiran *et al.*,2012

2.6.1 ABO blood group and Rh factor distribution in Africa

The ABO blood group distribution varies among the different people all over Africa. O blood type is very common in most countries in Africa. The pattern of distribution of ABO in Africa is $O > A > B > AB$ but in Egypt $A > O > B > AB$ (Table 5)

Table 6: ABO blood group and Rh factor distribution in Africa

Country	Blood group (%)				Rh factor (%)		References
	O	A	B	AB	Rh+	Rh-	
Cameroun	49	25	22	4	96	4	Ndoula *et al* ., 2014
Egypt	31	37	23.	9	92	8	Homouda, 2012
Ethiopia (silte)	43	28	24	5	92	8	Kassahun *et al*., 2012
Guinea	49	22	24	5	96	4	Ndoula *et al* ., 2014
Kenya	69	16	13	2	ND	ND	Iyiola *et al*., 2011
Madagascar	41	23	30	6	99	1	Randriamanantany *et al*.,2012
Mauritania	49	28	18	4	94	6	Ndoula *et al* ., 2014
Morocco	47	33	16	4	91	9	Benahadi *et al*., 2013
Niger	53	24	20	3	94	6	Enosolease and, Bazuaye , 2008
Nigeria (Lagos)	55	25	17	3	94	6	Iyiola *et al*., 2011
Sudan	58	24	14	4	96	4	Nahid, 2007

2.6.2 ABO blood group and Rh factor distribution in Ethiopia

Misganaw (2004) reported that the distribution of ABO blood groups among Ethiopians blood type O, 40%; type A, 31%; type B, 23%; and type AB; 6%. Many other studies have shown that blood group O was the most common blood group and blood group AB was the least common blood group in different ethnic groups (Table 6). For example, The distribution of type O is 40%; type A is 31%; type B is 23%; and type AB is 6% in Ethiopian blood donors, In population of south west Ethiopia (at Gilgel Gibe Field Research Center), the distribution of type O, is 42%; type A, is 31%; type B, is 21%; and type is AB, 6%. Among

Sidama ethnic group, the distribution is type O, 51.3%; type A, 23.5%; type B, 21.9%; and type AB, 3.3% and the distribution pattern were ($O > A > B > AB$)(Table 6).

Table 7: ABO blood group distribution in Ethiopia

Ethinic group (Town)	Abo Blood group (%)				Rh factor (%)		References
	O	A	B	AB	Rh+	Rh -	
Sodo (Ethiopia)	37	32	26	5	91	9	Kassahun *et al.*,2012
Meskan (Ethiopia)	50	24	21	5	92	8	Kassahun *et al.*,2012
Silte (Ethiopia)	43	29	23	5	93	7	Kassahun *et al.*,2012
Ethiopians (blood donors)	40	31	23	6	ND	ND	Tibebu , 1998
Population of South West Ethiopia- (Gilgel Gibe Field Research)	42	31	21	6	97	3	Abraham *et al.*, 2012
Sidama (Ethiopia)	51	24	22	3	ND	ND	Tewodros, *et al.*,2011
Jima (Ethiopia)	43	32	21.5	3.5	93	7	Teklu and Shiferaw, 2016

ND = Not determined

2.7 Clinical and Biological Significance of ABO Blood group

2.7.1. Blood transfusion

A blood transfusion is the transfer of blood from one individual into the blood of another. In order for transfusions to be safely done, it is necessary for blood to be typed so that agglutination (clumping of red blood cells) does not occur. Blood typing usually involves determining the ABO blood group and whether the individual is Rh (negative) or Rh (positive) (Mader, 2004).

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

Laura and Dean (2005) supposed that the success of blood transfusions depends on ensuring the compatibility of the blood types between donor and recipient. If the recipient has antibodies to the transfused red cells, these red cells will be rapidly destroyed, resulting in a potentially lethal transfusion reaction. Type A blood given to a type B recipient, for instance, can result in such a reaction, since the recipient's serum contains anti-A antibodies.

2.7.2 Universal donors and universal recipients

With regard to transfusions of whole blood or packed red blood cells, individuals with type O Rh (D) negative (O^-) blood are often called universal donors, and those with type AB Rh (D) positive (AB^+) blood are called universal recipients; however, these terms are only generally true with respect to possible reactions of the recipient's anti-A and anti-B antibodies to transfused red blood cells, and also possible sensitization to Rh (D) antigens (Fauci *et al.*, 2008). Additionally, red blood cell surface antigens other than A, B and Rh D, might cause adverse reactions and sensitization, if they can bind to the corresponding antibodies to generate an immune response. Transfusions are further complicated because platelets and white blood cells (WBCs) have their own systems of surface antigens, and sensitization to platelet or WBC antigens can occur as a result of transfusion (Baloch and Ali, 2004). With

regard to transfusions of plasma, this situation is reversed. Type O plasma, containing both anti-A and anti-B antibodies, can only be given to O recipients. The antibodies will attack the antigens on any other blood type. Conversely, AB plasma can be given to patients of any ABO blood group due to not containing any anti-A or anti-B antibodies (Anees, 2007)

2.7.3. Red blood cell 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 AB (Greenwalt, 1997).

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, a group B individual can receive blood only from individuals of groups 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 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 (with AB being preferable), but cannot donate blood to any group other than AB. They are known as universal recipients (Griffiths *et al.*, 2008).

Blood group O individuals do not have either A or B antigens on the surface of their RBCs, and their blood serum contains IgM anti-A 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 (i.e., A, B, O or AB). If a patient in a hospital situation needs 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 (Griffiths *et al.*, 2008).

Table 8: ABO blood phenotypes and transfusion

Blood type	Antigens on erythrocytes	Antibodies in plasma	Can received blood from groups	Can give blood for 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

Source: (Mader, 2004).

2.7.4. Blood group and nutrition

D'Adamo a naturopathic physician, in his book "Eat Right For Your Type" published in 1996 postulates that the ABO blood group reveals the dietary habits of our ancestors and people with different blood groups process food differently; thus, adherence to a diet specific to one's blood group could improve health and decrease risk of chronic diseases such as cardiovascular disease. Based on the "Blood-Type" diet theory, group O is considered the ancestral blood group in humans, so their optimal diet should resemble the high animal protein diets typical of the hunter gatherer era. In contrast, those with group A should thrive on a vegetarian diet as this blood group was believed to have evolved when humans settled down into agrarian societies. Following the same rationale, individuals with blood group B are considered to benefit from consumption of dairy products because this blood group was believed to originate in nomadic tribes.

Blood group A is primarily associated with vegetarian food sources and individuals in this group secrete smaller amounts of stomach acid and have lesser chances for gastric ulcers, heart diseases, cancer and diabetes (Viola and Carolyn**,** 1991) There has been extensive scientific research over the past 30 years that shows evidence that your individual blood type

determines your predisposition toward getting certain diseases, such as cancer, heart disease, diabetes, lupus, muscular sclerosis, allergies, etc. Sokolov (1993) also suggested that. Our blood type determines what type of biochemistry our digestive systems are made of.

2.8 Comparison of ABO and Rh (D) Groups

Along with the ABO blood type system, the Rh blood type system is also important when transfusing blood. The major difference between the ABO system and the Rh system is the following: In the ABO system, the plasma agglutinins responsible for causing transfusion reactions develop spontaneously, whereas in the Rh system, spontaneous agglutinins almost never occur. Instead, the person must first be massively exposed to an Rh antigen, such as by transfusion of blood containing the Rh antigen, before enough agglutinins to cause a significant transfusion reaction will develop. The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens. Anyone who has this type of antigen is said to be Rh positive, whereas a person who does not have type D antigen is said to be Rh negative (Blaney and Howard, 2013). The biochemistry and nature of ABO and Rh blood group similarity and differences compared in (Table 9)

Table 9: Comparison of ABO and Rh (D) groups

Parameter	ABO group	Rh(D) group
Location of gene	Chromosome 9	Chromosome 1
Antigens	A, B, AB	D
Distribution of antigens	Red cell, platelets, many tissues, body fluids	Red cell only
Development of antigens	Weak expression at birth	Fully developed at birth
Dosage effect	No	Present
Nature of antibodies	Naturally occurring	Immune
Antibody class	IgM	IgG
Whether antibodies fix complement	Yes	No
Optimal reaction temperature of Antibody	$4^{o}C$	$37^{o}C$
Optimal reaction medium	Saline	Anti-human globulin

Source: (Kwathalkar, 2013).

2.9. Blood Products

Benjamin *et al.,* (1996) suggested that to provide maximum benefit from each blood donation and to extend shelf-life, blood banks fractionate some whole blood into several products. The most common of these products are packed RBCs, plasma, platelets, cryoprecipitate, and fresh frozen plasma (FFP). FFP is quick frozen to retain the labile clotting factors V and VIII, which are usually administered to patients who have a potentially fatal clotting problem caused by a condition such as advanced liver disease, overdose of anticoagulant, or disseminated intravascular coagulation (DIC). Units of packed red cells are made by removing as much of the plasma as possible from whole blood units. Clotting synthesized by

modern recombinant methods are now in routine clinical use for hemophilia, as the risks of infection transmission that occur with pooled blood products are avoided.

2.10 The Hardy-Weinberg Genetic Equilibrium

The 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).

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) = p^2 for the AA homozygote in the population, frequency of q^2 for the aa homozygote, and frequency 2*pq* for the Aa heterozygote (Russell, 2005).

p^2 (AA): 2pq (Aa): q^2 (aa)

2.10.1. Estimation of genotypic and allelic frequencies

An important application of the Hardy-Weinberg law is estimating the genotype 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 with different genotypes we usually begin by counting the number of homozygous recessive individuals; these homozygous individuals can be distinguished from the rest of the population by the phenotype. By using the Hardy-Weinberg law we can calculate the frequency of the heterozygous and homozygous conditions (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 (Griffith *et al.,* 2008). In other words, when you add up the frequency of the I^A, I^B and I^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 allelic frequencies are:

p = I^A, q = I^B and r =I^O.

$(p + q + r)^2 = p^2 (I^A I^A) + 2pq (I^A I^B) + q^2 (I^B I^B) + 2pr (I^A I^O) + 2qr (I^B I^O) + r^2 (I^O I^O)$ (Griffith *et al.*, 2008). Three alleles are computed (I^A, I^B and I^O), with frequencies equal to p, q and r respectively. The frequencies of the genotype at equilibrium will be computed by the square of the allelic frequencies. This system has six possible genotypic combinations but only four phenotypic blood groups. Because the alleles I^A and I^B are co-dominant and both are dominant to I^O Homozygous $I^A I^A$ individuals and heterozygous $I^A I^O$ individuals are phenotypically identical, as are $I^B I^B$ and $I^B I^O$ individuals. This results in four phenotypic combinations, known as blood types A, B, AB, and O (Table 10).

Table 10: Punnet square showing frequencies for the alleles of the ABO blood groups

Alleles	Alleles		
Female gamete	Male gamete		
	I^A	**I^B**	**I^O**
I^A	$I^A I^A$ (p^2)	$I^A I^B$ (pq)	$I^A I^O$ (pr)
I^B	$I^A I^B$(pq)	$I^B I^B$ (r^2)	$I^B I^O$ (qr)
I^O	$I^A I^O$ (pr)	$I^B I^O$ (qr)	$I^O I^O$ (r^2)

Source: (Griffith *et al.*, 2008).

Frequency of homozygote $I^A I^A$ = the square of allelic frequency e.g. (p^2)

Frequency of heterozygote $I^A I^B$ = 2×product of allelic frequency e.g. (2×pq)

Frequency of homozygote $I^B I^B$= the square of allelic frequency e.g. (q^2)

Frequency of heterozygote $I^A I^O$ = 2×product of allelic frequency e.g. (2pr)

Frequency of heterozygote $I^B I^O$ = 2×product of allelic frequency e.g. (2qr)

Frequency of homozygote $I^O I^O$ = the square of allelic frequency e.g. (r^2)

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was carried out at Bote town in Bora district which is found in East Shoa Zone Oromia Regional State. Bote town is located at 110 km South of Addis Ababa. It is found at $8^0$8'N, $38^0$57'E and an altitude of 1,611 meters above sea level. The district has a total population of 28,469 of whom 14,594 are males and 13,875 are females; The ethnic composition of the population indicates more than half of the population in this town is Oromo which is 55.1%, 24.2% Amhara, 18.3% Gurague, 1.1% Tigrie, and both Hadiya and Walayta account 1.3% (CSA, 2007).

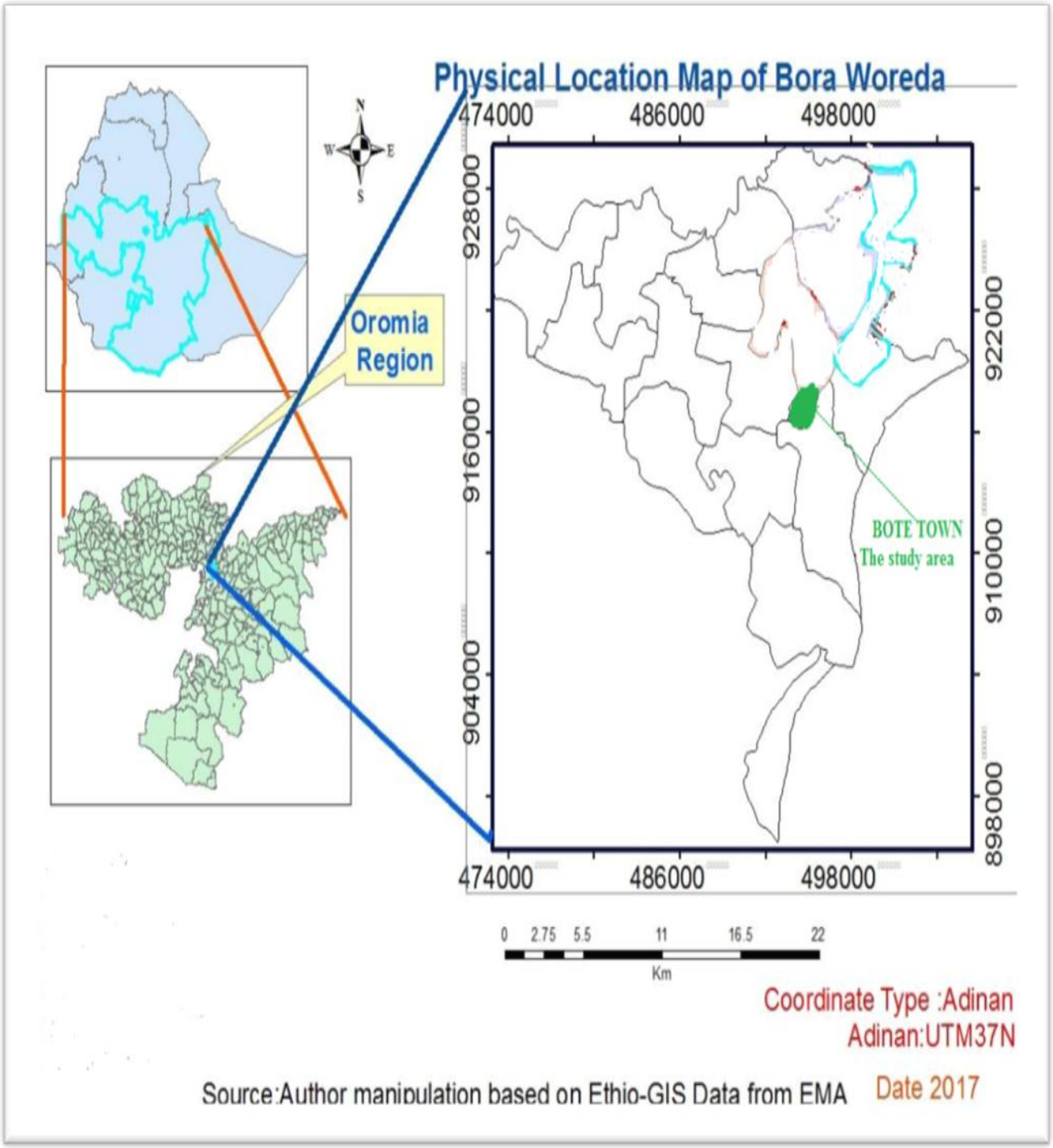


Figure 5: Map of the study area

Source: (Author manipulation based on Ethio-GIS Data from EMA).

3.2. Study Population and Sampling Techniques

The study populations were all students of Secondary and Preparatory schools in Bote town. There are three schools namely Bora Secondary School with 540 student, Bote Preparatory School with 165 and Professor Badeg Academy (Secondary School) with 70 students and the total population size in these schools was 775 students. Discussion was made with all the students in Secondary and Preparatory schools in Bote town concerning the purpose of the study and the importance of knowing own blood group in the aspects of nutrition, health, blood transfusion and blood donation. Moreover they were asked to give their blood voluntarily. Accordingly 392 students or more than 50% students from each school gave their blood. This study was carried out at Secondary and Preparatory Schools biology laboratory in Bote town, Ethiopia. The blood sample collection and identifying ABO and Rh blood group carried out during March 6, 2017 – April 14, 2017.

3.3. Blood Sample Collection Method

Blood samples were taken from finger pricks, and the usual open slide method were used for testing ABO blood groups and Rh (D) factors, following Bhasin and Chahal (1996). All the necessary precautions were taken for blood sampling. The blood samples were collected under aseptic precautions by three professional laboratory technicians from the Woreda Health Center.

3.4. Determination of ABO and Rh Blood Groups

The samples were tested for blood groups by using Anti-A, Anti- B, Anti-D. Two types of blood group system were tested; ABO blood group and Rh (D) blood group system. The ABO blood grouping and Rhesus factors (Rh) typing determined by glass slide method, which is based on antigen antibody agglutination. Commercially available standard anti-sera A, anti-sera B and anti-sera D were used for the study. Blood grouping was done by commercial reagent kit supplied by SPINREACT, S.A.U.-Ctra. Santa Coloma, 7E-17176 SANT ESTEVE DE BAS-(Gerona) Spain and obtained from Adama blood bank service. Blood was treated with anti-A, anti-B and anti-D anti-sera on separate glass slides, marked as A, B and D and were mixed with separate sterilized applicator sticks. The mixture was observed for agglutination. The blood group was determined based on agglutination with the

corresponding anti-sera. If agglutination was present in the blood drop A, then it belongs to A blood group, agglutination in blood drop B, B group, agglutination in both A and B blood drops, AB group and if no agglutination in both A and B drops, then O group. Similarly, agglutination in blood drop D was considered as Rh Positive and no agglutination Rh negative (Avent, 2009). Therefore, the results were recorded as A+, B+, AB+, O+ and A-, B-, AB-, and O- and blood groups Rh factor were recorded in tabulated form in register book.

Figure 6 shows the A positive blood group results on adding Antigen-D, Antigen-B and Antigen-A respectively. The blood sample on drop A and D was form aggregates (agglutinate) but the blood sample found on drop B remain fluid (No aggregates).

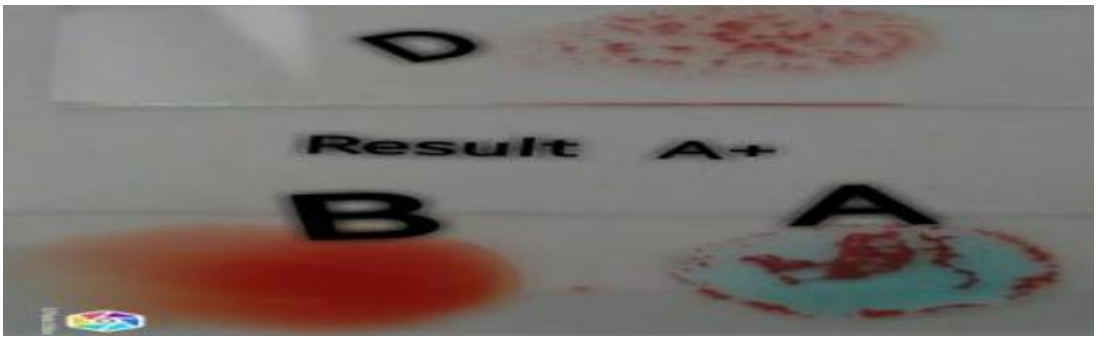


Figure 6: A positive blood group sample
Photo by Amsalu Wakgari, 2017

Figure 7 shows the A negative blood group results on adding Antigen-D, Antigen-B and Antigen-A respectively. The blood sample on drop A was form clump (agglutinates) but the two blood sample found on drop B and D remain fluid (No clump).

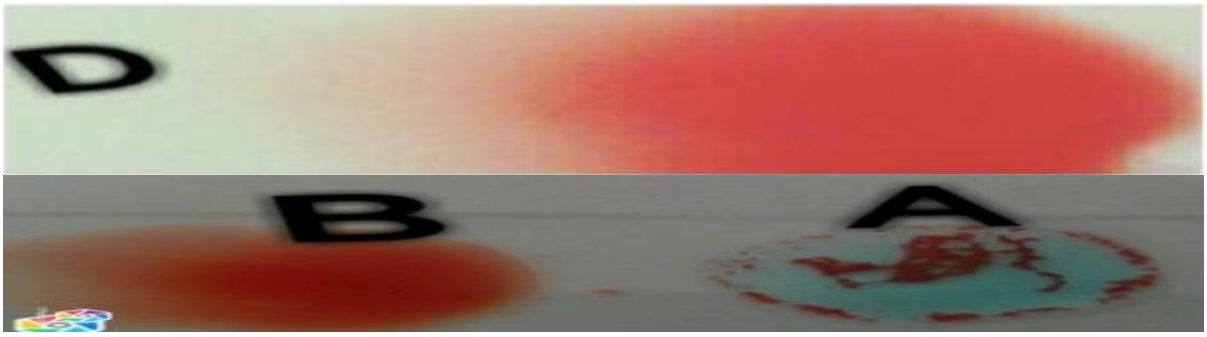


Figure 7: A negative blood group sample

Photo by Amsalu Wakgari, 2017

Figure 8 shows the B positive blood group result on adding Antigen-D, Antigen-B and Antigen-A respectively. The blood sample on drop B and D was form aggregates (agglutinates) but the blood sample found on drop A remain fluid (No aggregates).

3.5. Ethical Considerations

An authorization to carry out the study was obtained from Oromia Regional State Health Bureau ethical committee using an agreement letter prepared by Adama Science and Technology University. All the information that was obtained from the subjects was kept confidential. Before data collection, an informed consent was obtained from the students.

3.6. Statistical Analysis

All data were analyzed using the Statistical Package for Social Science (SPSS Inc., IBM software package 20. For the present study the frequency of the blood phenotypes were used to calculate the frequency of the ABO blood group alleles and the genotype.

3.6.1 Extension of the hardy-Weinberg equation with more than two alleles

Modified Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies (Strickberger, 1976). When two alleles, for example, P and q are present at a locus, based on the Hardy Weinberg principle at equilibrium the frequency of the genotypes become $p^2+2pq+q^2$, which is the square of allelic frequencies $(p+q)^2$. This is a simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are inherited two at a time in to a diploid zygote (Daniel and Clark, 2007).

The three alleles of ABO blood group which are I^A, I^B, and I^O are represented as p, q, r, respectively. In which p is the frequency of I^A, q, is the frequency of I^B, r, is the frequency of I^O. Therefore, the genotypic frequencies are represented as: $(p+q+r)^2=1$

$p^2+2pq+q^2+2pr+2qr+r^2=1$ and $p+q+r = 1$

Where: p^2 is the genotypic frequency of I^AI^A;

q^2 is the genotypic frequency of I^BI^B

2pq is the genotypic frequency of I^AI^B;

2pr is the genotypic frequency of I^AI^O

2qr is the genotypic frequency of I^BI^O;

r^2 is the genotypic frequency of I^OI^O (as cited in Hanania, *et al.*, 2007).

The genotypic frequencies of Rh (D) blood groups are calculated as follows:

$(D+d)^2 = 1$

DD+2Dd+dd = 1

Genotype DD = p2

Genotype Dd = 2pq

Genotype dd = q2

In this study, first, the distributions of blood groups among Secondary and Preparatory students of Bote town were expressed in percentage and frequencies. Gene frequency were calculated considering two alleles at the same locus for Rh system and three alleles at the same locus for ABO using Hardy-Weinberg equilibrium equation. ABO alleles were estimated according to a published method which yields results that are closer to maximum likelihood estimate.

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

$r = \sqrt{O}$

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

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

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

$q = \sqrt{Rh-}$

$P = 1 – q$ (Daniel and Clark, 2007).

Chakraborty (2010) suggested that Observed and expected genotype frequencies in Hardy-Weinberg were calculated on the basis of genes frequency and Chi-square tests was done to test the independence and the goodness of fit for frequencies.

Phenotypic frequency determination ABO and Rh (D) blood groups

Observed percentage = Observed number / Total number x 100%

Observed frequency = Observed number / Total number

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

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

Expected phenotypic frequencies were calculated as:

- Ef = Genotypic frequency X number of total sample
- For A blood group Ef = frequency of (AA + AO) X number of total sample
- For B blood group Ef = frequency of (BB + BO) X number of total sample
- For AB blood group Ef = frequency of AB X number of total sample
- For O blood group Ef = frequency of OO X number of total sample

A chi-square test was used to compare the allelic frequencies (ABO) students of each school with the allelic frequency of expected analyzed result.

4. RESULTS

4.1 Socio-Demographic Characteristics of the Sample Students

For this study, three hundred ninety two (392) sample students were gave blood voluntarily from Secondary and Preparatory schools of Bote town (Table 11).

Table 11: Socio-demographic characteristics of the Secondary and Preparatory students in Bote town in the year 2017

Gender				Total	
Males		Females			
Number	%	Number	%	Number	%
204	52	188	48	392	100

4.2 Frequency of ABO Blood Groups among Secondary and Preparatory Students in Bote Town

In this study there were differences in frequency distribution of the ABO blood group phenotypes in Secondary and Preparatory Students in Bote town.

Table 12: Distribution of the ABO blood groups among 392 sample students of Secondary and Preparatory Schools in Bote town in the year 2017.

ABO blood group	Sample students	%	Frequency
Type A	125	31.88	0.319
Type B	84	21.41	0.214
Type AB	21	5.4	0.054
Type O	162	41.32	0.413
Total	392	100%	1

.

4.3 The Distributions of Rh factor among Secondary and Preparatory Students in Bote town

The frequency distribution of Rh blood group among Secondary and Preparatory students in Bote town is shown by comparing male student with females (Figure.14).

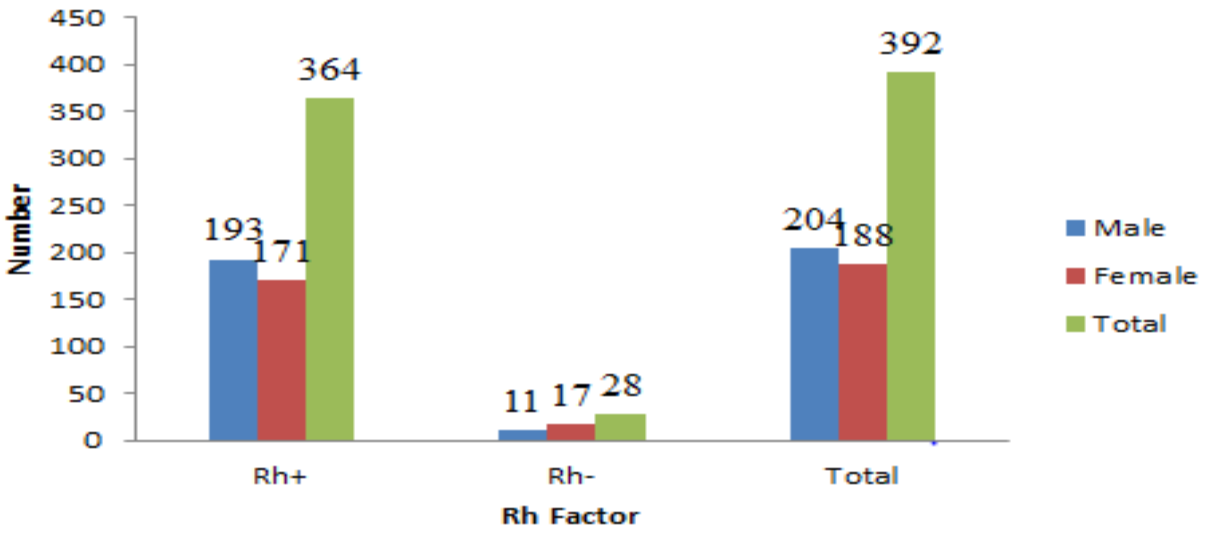


Figure 8: Rh factor frequency among Secondary and Preparatory students in Bote town in the year 2017.

4.4 ABO and Rh Blood Group Combined Frequency

Based on the present study ABO/Rh blood groups frequencies in the studied population blood group O Rh positive was found to be the most common (38%) and AB Rh negative were the least (0.8 %) (Table 13).

Table 13: Combined frequency of ABO and Rh blood groups among Secondary and Preparatory students in Bote town in the year 2017.

ABO Blood Group (Phenotype)	Rh factor		TOTAL
	Rh positive	Rh negative	
A	118(30%)	7(2%)	125(31.9%)
B	78(20%)	6(1.5%)	84(21.4%)
AB	18(4.5%)	3(0.8%)	21(5.4%)
O	150(38.3%)	12(3%)	162(41.3%)
Total	364 (92.86%)	28(7.14%)	392(100%)

4.5 Estimation of ABO and Rh (D) blood groupAlleles

By using the extension of the Hardy-Weinberg equation Allelic frequencies of the three ABO blood group alleles (p, q, and r) were calculated as

$r = \sqrt{O}$ = Allele I^o

$= \sqrt{0.41} =$ **0.64**

$p = 1 - \sqrt{B+O}$ =Allele I^A

$=1-\sqrt{0.21+0.41}$

$=1-0.79 =$ **0.21**

$q = 1 - \sqrt{A+O}$ =Allele I^B

$=1-\sqrt{0.32+0.41}$

$=$**0.15**

A high frequency of the allele I^O over I^A and I^B alleles in the order of ($I^O > I^A > I^B$) in Secondary and Preparatory students in Bote town shown in the pi chart (Figure 15).

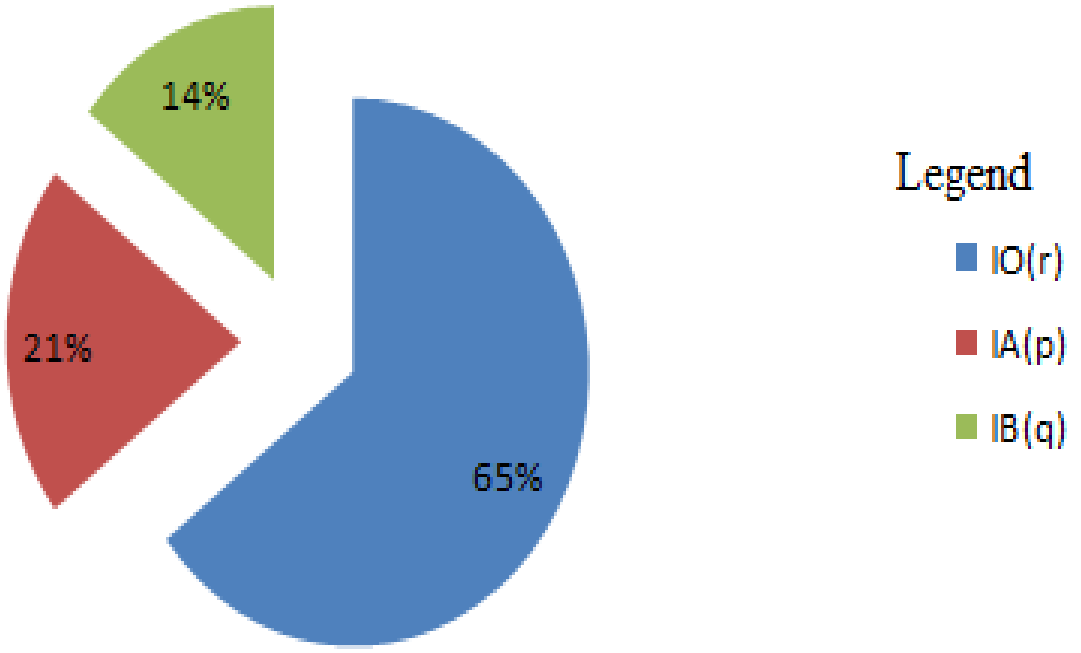


Figure 9: Calculated ABO alleles among Secondary and Preparatory students in Bote town in the year 2017

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

q = √Rh- =Allele d (Daniel and Clark, 2007).

= √0.07 = **0.27**

P = 1 – q = Allele D (Daniel and Clark, 2007).

1-0.27 =**0.73**

Rh blood group allele D (the dominant allele) and d (recessive allele) were calculated using the Hardy-Weinberg equation 73% and 27% respectively.

4.6 Estimation of ABO and Rh (D) Genotypes among Students in Bote Town

ABO blood group genotypic frequency in secondary and preparatory students in Bote town calculated from the allelic frequency by Hardy-Weinberg equation (Table 15). The three alleles of ABO blood group which are I^A, I^B, and I^O are represented as p, q, r, respectively. In

which p is the frequency of I^A, q, is the frequency of I^B, r, is the frequency of I^O. Therefore, the genotypic frequencies are represented as: $(p+q+r)^2=1$

$p^2+2pq+q^2+2pr+2qr+r^2=1$ and $p+q+r = 1$

Where: p^2 is the genotypic frequency of I^AI^A;

q^2 is the genotypic frequency of I^BI^B

2pq is the genotypic frequency of I^AI^B;

2pr is the genotypic frequency of I^AI^O

2qr is the genotypic frequency of I^BI^O;

r^2 is the genotypic frequency of I^OI^O (as cited in Hanania, *et al.,* 2007).

Table 14: Genotypic frequency of ABO blood groups among Secondary and Preparatory students in Bote town in the year 2017.

ABO blood group genotype	Frequency	(%)
I^OI^O	0.413	41.3
I^AI^A	0.043	4.3
I^AI^O	0.268	26.8
I^BI^B	0.021	2.1
I^BI^O	0.186	18.6
I^AI^B	0.060	6

Rh blood group genotypic frequency in secondary and preparatory students in Bote town calculated from the allelic frequency by Hardy-Weinberg equation (Figure 15).

The genotypic frequencies of Rh (D) blood groups are calculated as follows:

$(D+d)^2= 1$

DD+2Dd+dd = 1

Genotype DD = p2

Genotype Dd = 2pq

Genotype dd = q2

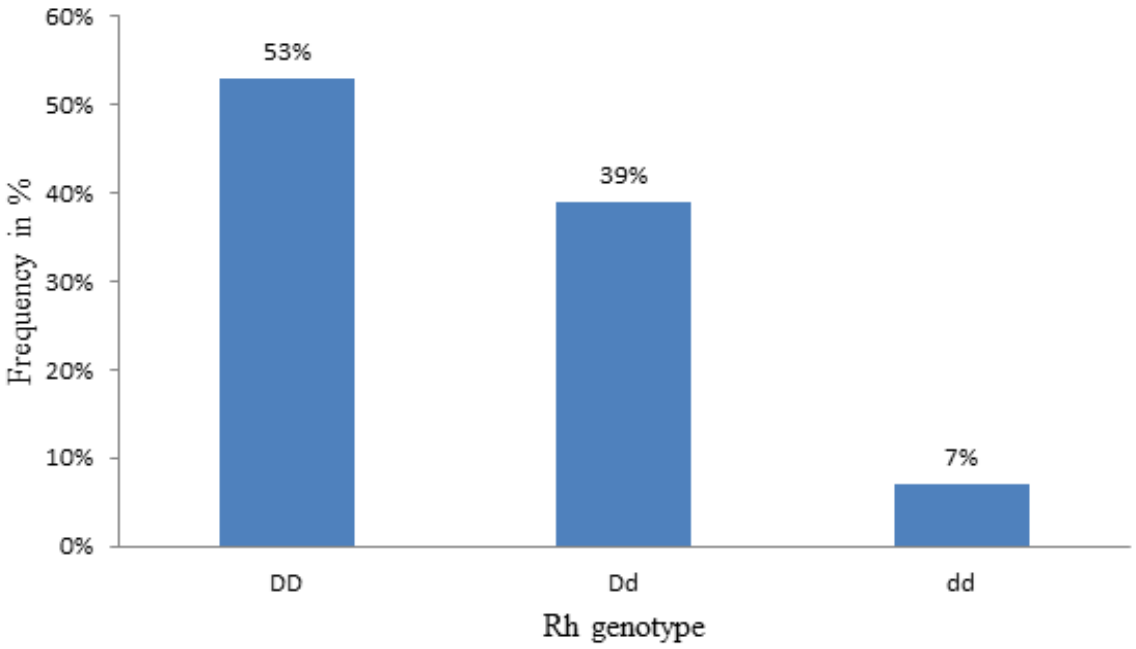


Figure 10 : Rh blood group genotypic frequency in Secondary and Preparatory students in Bote town in the year 2017

4.7 The Chi-Square for the Goodness of Fit of ABO and Rh Blood Group Distribution

The chi-square tests in ABO blood distribution were calculated from the expected and observed values. The ABO blood group distribution is compared with the calculated expected value by using the Chi-square test at P value < 0.05, 95% confidence level with 3 degrees of freedom (Table 16).

Table 15: Chi-square test for ABO blood group distribution among Secondary and Preparatory students in Bote town in the year 2017

ABO Blood group	Observed number (O)	Expected number (E)	Difference d (O –E)	d^2/ Expected
A	125	121.88	3.12	0.0799
B	84	80.95	3.05	0.115
AB	21	23.56	2.56	0.278
O	162	161.94	0.06	0.00002
Total				0.4729

The calculated Chi-Square value of Rh blood group is 0.0119 for Secondary and Preparatory Students in Bote town (Table 17).

Table 16: Observed versus Expected Rh blood groups and the chi square value of Secondary and Preparatory students in Bote town in the year 2017

Rh Blood group	Observed number (O)	Expected number (E)	Difference d (O –E)	d^2/Expected
Rh+	364	364.56	-0.56	0.0009
Rh-	28	27.44	0.56	0.011
Total				0.0119

5. DISCUSSION

The data collected revealed that the ABO blood group frequencies were found in the order O > A > B > AB. In phenotypic distribution of ABO blood group O has the highest frequency while blood group AB has the least 41% and 5.4% respectively. Blood group A and blood group B the 2nd and the 3rd with the frequency 32% and 21% respectively. Many other studies have shown that blood group O was the most common blood group and blood group AB was the least common blood group in different ethnic groups (Nwauche and Ejele, 2004).

This study is in agreement with ABO frequency in population of Southwestern Ethiopia (Gilgel Gibe field research), Sodo, Silte, and Meskan people where blood group O is 42%, 37%, 43% and 49.7% respectively. Blood group A, 31%, 32%, 24% and 29% in population of Southwestern Ethiopia (Gilgel Gibe field research), Sodo, Meskan and Silte, respectively and the least percentage frequency is that of blood group AB which is 6% in West in Ethiopia (Gilgel Gibe field research) and 5% in Sodo, Silte, and Meskan people (Abreham *et al*., 2012 and Kassahun *et al*., 2012). Among Sidama ethnic group (Ethiopia) the ABO blood group distribution is type O, 51.3%; type A, 23.5%; type B, 21.9%; and type AB, 3.3% (Tewodros *et al*., 2011).The results of this study are also consistent with previous findings in Jima town among blood donors the ABO blood group distribution of A, B and AB, O, reported as 32%, 21.5% and 3.5%, 43% respectively (Teklu and Shiferaw, 2016). Therefore the results of this study are in agreement with the data from previous studies among Ethiopian populations with the same pattern O >A>B>AB.

Among the Caucasians in the United States of America, the frequency of blood group O, A, B and AB are 47.0, 41.0, 9.0 and 3.0 %, respectively with the same pattern O >A>B>AB (Adeyemo and Soboyejo, 2006) which is also in agreement with this study. However, the present findings seem to deviate from the results obtained by Khan and his colleagues on the genotype frequencies of blood group antigens from Bannu region in Pakistan where ABO blood group frequency occurred in the order of B> A> O> AB (Khan *et al*., 2009). It also seems not to agree with the results obtained from Swat district in Pakistan where the percentage frequencies were A =27.92%, B= 32.40 %, O = 29.10% and AB=10.58% (Khattak *et al*., 2008).

Among ABO blood group, Students in the study area are more of "O" blood group has its advantages which may include emergency blood transfusion as blood group O is a universal donor (lack both A an B antigen) hence it is readily available. This can also be seen as good input for blood bank services for effective management of blood and safe blood transfusion services. As the student in these schools are also young and at conducive age in order to donate their blood for the patients who are being treated in hospitals, particularly for women who needs a great medical treatment during giving birth to a baby and for traffic accident emergency cases in hospitals. This study reports that pattern of blood groups among Secondary and Preparatory Students in Bote town would help the blood bank to prepare database and increase awareness which type of blood group should be stored more. As a result, the study might have a significant implication regarding the inventory management of blood bank and transfusion services for those who need blood transfusion.

Due to the fact that ABO blood group play germane role in human diseases, it is thus beyond immunohaematology and the higher prevalence of O blood group observed in this study might have an evolutionary advantage in conferring resistance to some disease like malaria (Anifewoshe *et al*., 2017). Thus dominance of O group could be protective against malaria because studies have shown that the erythrocytes of an individual with blood type O might not be suitable for rosette formation by *Plasmodium falciparum*. Blood group O may have disadvantages including higher risk of cholera and plague by individuals, as well as developing duodenal and peptic ulcers than other blood groups .as well as cancer of the stomach has been significantly associated with blood group A individuals than other blood groups (Khan *et al*.,2004). In short, generation of a simple database of blood groups, not only provides data about the availability of human blood in case of regional calamities, but also serves to enables insight into possibilities of future burden of diseases.

This study has shown that Rh positive has the higher percentage frequency while Rh negative has the lower percentage frequency in Secondary and Preparatory students in Bote town. The frequency of Rh (D) positive blood group was 93% and Rh negative 7%. From the total Rh negative sample students 61% students were females. These results indicate high prevalence of hemolytic disease of the new born (erythroblastosis fetalis) which increases the probability of maternal and infant death.

This finding is consistent with Ethiopian population previous study. For example among blood donors in Jimma town 93% Rh positive and 7% Rh negative (Teklu and Shiferaw, 2016). In addition, the incidence of rhesus negativity in the Silti Secondary and Preparatory School was found to be 6.8, 8.16, and 8.84% among Silti, Meskan and Sodo, respectively (Kassahun *et al*., 2012).

Allelic frequencies of ABO blood groups among Secondary and Preparatory students in Bote town were I^A, I^B, and I^O calculated 21%, 15% and 64% respectively according to the modified Hardy -Weinberg Law of equilibrium. The order of allelic frequencies of ABO blood group among Secondary and Preparatory students in Bote town were $I^O > I^A > I^B$.

Previous studies among various part of Ethiopian population have documented similar pattern of allelic frequencies. For instance, Arsi clan 0.19 I^A, 0.16 I^B, and 0.65 I^O in Guji 0.21I^A, 0.16 I^B, and 0.63 I^O and 0.22 I^A, 0.15 I^B, and 0.63 I^O in Borena clan (Yassin Woliye, 2013). The ABO allele frequency shows the same pattern with $I^O > I^A > I^B$.

On the other hand Khan *et al*. (2009) studied the distribution in the Poonch district of Azad Jammu and Kashmir and Nagariya in Maharastra, India (Hindu and Muslim caste) demonstrated the order of human ABO allele frequencies as $I^O > I^B > I^A$ which deviate from the allele frequency in the study population.

The allele frequencies of Rh (D) blood group were calculated according to the Hardy-Weinberg equation. The frequency of allele D and d are found to be 73% and 27%, respectively in Secondary and Preparatory students in Bote town. This shows that allele D has higher frequency than allele d. This also agrees with many studies where Rh positive has higher incidence than Rh negative in different populations and ethnic groups (Nwauche and Ejele, 2004; Bakare *et al*., 2006).

In this study the genotype I^OI^O has the highest frequency while genotype I^BI^B has the least frequency. The frequencies of ABO genotypes in the overall sample were 4.3% I^AI^A, 26.8 I^AI^O, 2.1% I^BI^B, 18.6% I^BI^O, 6% I^AI^B and 41.3% I^OI^O. Among individuals with blood group A, homozygous A or I^AI^A calculated as 13% and hybrid A (heterozygous A) I^AI^O was 87%. Among those who are blood group B, 10% were homozygous I^BI^B while about 90% were heterozygous

I^BI^O. Most of the A and B blood types are heterozygous dominant (I^AI^O and I^BI^O respectively) in Secondary and Preparatory students in Bote town. The predominance of O allele may also be as a result of the fact that blood group A and B may have been heterozygous carrying O allele silently there by maintaining O allele in the heterozygous population.

With respect to Rhesus blood grouping system, the frequency of heterozygous Rh (Dd) obtained for this study is 39%, in Secondary and Preparatory students in Bote town and the homozygous Rh genotype (DD) were the highest frequency 53% and Rh (D) with homozygous recessive (dd) were with the frequency 7% which is equal to students with Rh factor. Similar results were reported by Kassahun *et al*., (2012) and Zelalem, (2014).

Comparison of observed and expected number of ABO blood group distribution in Secondary and Preparatory school in Bote town reported that blood group A and B shows only 3 students variation with expected result. The calculated Chi-Square value for ABO blood group in Bote town Secondary and Preparatory students were 0.4078 which has the P value is between 0.95 and 0.90 with 3 degrees of freedom. This means that there is no significant difference between values expected for the distribution of ABO blood group and observed for total sample students in Bote town. The calculated Chi-Square value of Rh blood group is 0.0119 with 1 degree of freedom at P value > 95% for Secondary and Preparatory Students in Bote town. Hence the distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy-Weinberg equilibrium (Goodness of fit X^2= , 0.4729, df=3, P>95%)

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The distributions of ABO and Rh blood groups of this study have similar trends with the data from the previous studies in Ethiopian population. In ABO blood group system in Secondary and Preparatory Students in Bote town. O blood group record the highest frequency 41.3% followed by blood groups A 32%, blood group B 21.41% and AB 5.4% with the order of O > A > B > AB while the Rh positive 93% and Rhesus negativity 7%.

The data from this study provides information on the genetic variability and polymorphism of the blood group and rhesus antigens among secondary and preparatory students in Bote town. This information would be useful to the population geneticists and to the clinicians, especially in the planning of blood transfusion program since they play integral role of the genetic profile of Secondary and Preparatory Schools in Bote town. The study has a significant implication regarding the management of blood bank and transfusion services in the study area. Knowledge of blood group distribution is also important for clinical studies, for reliable geographical information and for forensic studies in the population.

6.2. Recommendations:

- The sample size used to conduct this study from Bote town was small and may not represent the population of Bora wereda. Therefore it is advisable to use larger sample size from different areas including the rural areas to obtain more accurate data regarding the pattern of distribution of these blood groups.
- Special attention should be given to Rh negative women's to be included in maternal health programs by Ministry of health

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APPENDIX

Appendix I. Probability values for Chi-square analysis

Table 1. Probability Values for Chi-Square Analysis

	Probabilities								
df	0.95	0.90	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	.004	.016	.15	.46	1.07	1.64	2.71	3.84	6.64
2	.10	.21	.71	1.39	2.41	3.22	4.61	5.99	9.21
3	.35	.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35
4	.71	1.06	2.20	3.36	4.88	5.99	7.78	9.49	13.28
5	1.15	1.61	3.00	4.35	6.06	7.29	9.24	11.07	15.09
6	1.64	2.20	3.83	5.35	7.23	8.56	10.65	12.59	16.81
7	2.17	2.83	4.67	6.35	8.38	9.80	12.02	14.07	18.48
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.21
	← Acceptable →								Unacceptable

Note. From Statistical Tables for Biological and Medical Research (6th ed.), Table IV, by R.Fisher and F.Yates, Edinburgh: Longman Essex, 1963.

Appendix II. Consent Form

Name of the study participant _____ Age_____Sex_____

School _____ Grade and Section_____

I have been informed about the purpose and objectives of the study that plans to determine "*Distribution of ABO and Rh (D) Blood Groups among Students Attending at Secondary and Preparatory Schools in 2017 Bote Town, Oromia Regional State, Ethiopia*.". For the study I have been requested to participate in the study and give a drop of blood from finger. They told me that the qualified and experienced laboratory technician would do the blood collection according to the established aseptic procedures by using sterile disposable lancet. Based on this, I have agreed to participate in the study based on my interest. I have been given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I have gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant)_____Signiture_____Date_____

Name (witness) _____Signiture_____Date_____

Appendix III. Identification of ABO and Rh blood group and data collection in Secondary and Preparatory School Bote town in the year 2017.

Plate: Laboratory technicians, Data recorders and Students during ABO and Rh blood group identification and data collection

Photo by Amsalu Wakgari, 2017

Appendix IV. Students' ABO and RhD Blood Group Phenotypes recording sheet

Code	Name of student	Sex	Age	school	ABO Blood group				Rh factor		Remark
					A	B	AB	O	Rh +ve	Rh -ve	

Appendix V: Ethical clearance

BIIROO EEGUMSA FAYYAA OROMIYAA

OROMIA HEALTH BUREAU
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Lakk/Ref. No. AGBHEG/1016/09

Guyyaa /Date 07/10/2009

Waajjira Eeg/Fay/God/Shawaa Bahaa tiif

Adaamaa

Dhimmi: Xalayya Deggersa Ilaala.

Akkuma beekamu Biiroon Keenya Ogeyyii , dhabbile akkasumas namoota qorannoo geggessuuf propozaala dhiyeffatan propoozaala isaani madaaluun akkasumas iddo biratti ilaalchisani fudhatama argatan (approved) dhiyeffatan,propoozaala isaanii ilaaluudhaan waraqa deggersa ni kenna.

Haaluma kanaan mata dure **"Phenotypic distribution of ABO and RH(D) blood groups among high school students in Bote town, Easy Shoa zone"** jedhuun dhabbatni **"Obbo Amsaaluu Waaqgaarii"** Godina kessan kessatti qorannoo geggessuuf propoozaala isaani koree "Health Research Ethical Review Commite" Biiroo keenyatti dhiyeffatani jiru.

Haaluma kanatti hunda`uudhaan koreen "Health Research Ethical Review Committee"Biiroo keenya piropoozaal kana ilaaluun mirkanesse qorannoon kun akka hojii irra oolu murtesse jira.

Kanaafuu,hojji qorannoo kana irratti deggersa barbaachisa ta`e akka gootaniif ,xalayya deggersa Aanaalee fi Buufatalee fayyaa filatamaniif xalayya deggersa akka barresitanii fi akkasumas akka hordoftan jecha," **Obbo Amsaaluu Waaqgaarii"** qorannoon kun qacceffame xumurame firii isa koppi tokko BEFO tiif akka galii godhan galagalcha xalayya kanaan isaan beeksiifna.

Anis, **Obbo Amsaaluu Waaqgaarii"**wayitti qorannoon kun qaaceffame xumurame firii isa koppi tokko BEFO tiif galii gochuuf mallatoo kootiin mirkanessa.

Nagaa wajjin!

Mallattoo

Maqaa **Amsaaluu Waaqgaarii**

Bilbila 0912684198

G/G

Obbo Amsaaluu Waaqgaarii tiif

Bakka jiranutti

Biiroo Eegumsa Fayyaa Oromiyaatti Adeemsaa Hojii Qabnii Karooraa Misooma Fayyaa Baajata,Hordoffii fi Gamaaggama * Planning Budgeting Monitoring & Evaluation Process Oromia Regional Health Bureau

Darajjee Abdiinnaa (BSC,M
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