ALLELIC AND GENOTYPIC FREQUENCIES OF ABO AND Rh BLOOD GROUP AMONG STUDENTS ATTENDING TULU CHUKALA SECONDARY SCHOOL, ADULALA TOWN, EAST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

M.SC. THESIS

KENENI ABERA

DECEMBER, 2022

HARAMAYA UNIVERSITY, ETHIOPIA

Allelic And Genotypic Frequencies Of ABO And Rh Blood Group Among Students Attending Tulu Chukala Secondary School, Adulala Town, East Shoa Zone, Oromia Regional State, Ethiopia

A Thesis Submitted to the School of Biological science and Biotechnology
Post Graduate directorate
HARAMAYA UNIVERSITY

A Partial fulfillment of the Requirements for the Degree of Master of sciences in Biology

Keneni Abera

December, 2022
Haramaya University, Ethiopia

HARAMAYA UNIVERSIT

POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this thesis entitled **Allelic and Genotypic frequencies of ABO and Rh blood groups among students attending Tulu Chukala secondary school, Adulala town, East Shawa zone, Oromia Regional state, Ethiopia** prepared under my guidance by Keneni Abera. I recommend that it be submitted as fulfilling the thesis requirement.

Major advisor	signature	date
Co-advisor	signature	date

As a member of the Board of Examiners of MSc Thesis Open Defense Examination, I certify that I have read and evaluated the Thesis prepared by Keneni Abera and examined the candidate. I recommend that Thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Biology.

3. Chairman person	signature	date
4. Internal examiner	signature	date
5. External examiner	signature	date

DEDICATION

I dedicate this Thesis manuscript to my family for their support during my M.Sc. study.

STATEMENT OF AUTHOR

First, I explain that this thesis is the result of my own work and that all sources used for it have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at Haramaya University.

I confidently declare that this thesis has not been submitted to any other institutions anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made. In all other instances, however, permission must be obtained from the author.

Name: _____

Signature: _____

Place: Haramaya University

Date of submission: _____

BIOGRAPHY

The author was born in Bila town, in Western zone of Oromia region on august 30, in 1984 E.C. He attended his elementary and junior school in Boji Dirmeji elementary schools. At the completion of his elementary school, he attended his high school education (from grade 9th to 12th) in Boji Dirmeji secondary and preparatory school. After completion of preparatory education, he joined Dire Dawa University to attend a Degree program in Biology (B.Sc. in Biology) and graduated in June 2006 E.C. After graduation he was attended PGDT program at Wolaita Sodo University in 2007 and employed in teaching program in East Shawa zone, Liban Chukala Woreda and served for 3 years. The author is married and has one child. After this much time service he adjusted himself in July 2010 E.C to joined Haramaya University, college of Natural and computational sciences, School of Biological science and Biotechnology to follow his M.Sc. in Biology.

ACKNOWLEDGEMENTS

Firstly, I would like to thanks to Almighty God, for supporting me the opportunity, ordering of all aspects regarding my study and being with me in all aspects throughout my life. “Nothing is impossible with God”.

I would like to thanks my major adviser Dr. Yohannes Petros for his advice and guidance starting from proposal development to the completion of the research work and his provision of reference material. He worked hard to keep me in normal pathway and accomplishment of the study.

I am again need to forward my special thanks to laboratory technicians Ato Lenco Lemi and Ato Degu Lemma for their support throughout my laboratory sessions by preparing the whole materials

I would also like to express my sincere appreciation to Adulala health center for their hospitality and kind cooperation in all aspects on my research. I would like also to thank all management and staff members of Adulala health center.

ACRONYMS AND ABBREVIATIONS

FUT	Fucosyltransferases
Gal	Galactose
GT	Glycosyltransferase
HTR	High transfusion reactions
HDN	Hemolytic disease of the new born
HDFN	Hemolytic disease of the fetus and the new born
IgG	Immunoglobulin G
NV	Norwalk virus
Nac	N-acetylgalactosamine
RBC	Red blood cells
Rh	Rhesus
UDP	Uridinediphosphate

TABLE OF CONTENT

STATEMENT OF AUTHOR iv

BIOGRAPHY v

ACKNOWLEDGEMENTS vi

ACRONYMS AND ABBREVIATIONS vii

TABLE OF CONTENT viii

LIST OF TABLES x

LIST OF FIGURES xi

ABSTRACT xii

1. INTRODUCTION 1

2. LITERATURE REVIEW 4

2.1. Discovery of ABO and Rh Blood Group System 4

2.2. ABO Gene Evolution 5

2.3. Biosynthesis of ABO Antigens and ABO Molecular Genetics 5

2.3.1. ABO antigens 6

2.3.2. Rh-D antigen 7

2.4. Biochemical Composition of Rh Antigens 7

2.5. ABO and Rh Antibodies 8

2.5.1. ABO antibodies 8

2.5.2. Rh antibodies 9

2.6. Association of ABO Blood Group and Lifestyle 10

2.7. Clinical Significance of ABO and Rh Blood Group 10

2.7.1. Clinical Significance of ABO Antibodies 10

2.7.2. Clinical Significance of Anti-D 11

2.8. Population Genetics and the Hardy-Weinberg Principle 12

2.8.1. Estimation of Genotypic and Allelic Frequency Distribution 12

2.8.2. Extension of the Hardy-Weinberg Law to Loci with More Than Two Allele 13

3. MATERIALS AND METHODS 14

3.1. Description of the Study Area 14

3.2. Research Design 14

3.3. Study Population 14

TABLE OF CONTENTS (Continued)

3.4. Sample Size Determination 15
3.5. Blood Sample Collection and Technical procedures 15
3.6. Detection of ABO and Rh Blood Groups 16
3.7. Laboratory Analysis Techniques 16
3.8. Method of Data Analysis 16
3.8.1. Statistical Methods to Test the Goodness of Fit for Phenotypic frequencies 18
4. RESULTS AND DISCUSSION 19
4.1. Distribution of ABO Blood Group among students attending Tulu Chukala secondary school. 19
4.2. Distribution of Rh blood group among students attending Tulu Chukala secondary school 20
4.3. Combined phenotypic frequency of ABO and Rh blood groups 20
4.4. Allelic, Genotypic and Phenotypic frequency of ABO and Rh blood group among students attending Tulu Chukala secondary school 21
4.5. Chi-square for examining difference between the observed and expected number of ABO and Rh blood group among Tulu Chukala Secondary school students. 23
5. SUMMARY, CONCLUSIONS AND RECOMMENDATION. 25
5.1. Summary 25
5.2. Conclusions 25
5.3. Recommendations 26
6. REFERENCES 27
7. APPENDIX 32

LIST OF TABLES

Tables **Pages**

1:The punnet square is showing Hardy-Weinberg frequencies for three autosomal alleles. 13
2: Phenotypic frequency of ABO blood groups among students. 19
3: Phenotypic frequency of Rh blood groups among studens. 20
4: Combined ABO and RH blood group frequencies among students. 20
5: Allelic, Genotypic and Phenotypic frequencies of ABO and Rh bloof groups. 22
6: Observed and Expected frequency of ABO and Rh blood groups. 24

LIST OF FIGURES

Figures **Pages**

1: The three different antiserum (ant-A, Anti-B and anti-D) for identifying blood groups. 32

2: during blood sample taking by laboratory technicians for ABO blood typing 32

3: During adding antiserum on sample blood type identification 33

4: During the blood typing showing the difference of coagulation results (Blood type A^+ and B^+) 34

Allelic and Genotypic frequencies of ABO and Rh Blood Groups among Students Attending Tulu Chukala Secondary school, Adulala Town, east Shoa Zone, oromia Regional State, Ethiopia.

ABSTRACT

The four blood types are known as A, B, AB, and O. Blood type A contains A antigen on the surface of red blood cells and B antibody on blood plasma. Type B blood contains the reverse combination. Serum of blood type AB contains neither antibody, but red cells in this type of blood contain both A and B substances. In type O blood, neither substance is present on the red cells, but the individual is capable of forming antibodies directed against red cells containing substance A nor B. Frequency distribution of ABO and Rh blood groups is helpful for effective management of blood banks and safe blood transfusion services. The prevalence of these blood groups varies worldwide and may not be found in equal numbers even among the same ethnic groups. The study aimed to determine ABO and Rh-D blood groups distribution frequencies among 422 students at Liban Chukala Secondary School from March to May 2022.Blood samples were taken from volunteer students by using commercially available anti-sera A, B, and Rh (D) from finger-pricks and blood groups were determined on open slide and blood types were determined by agglutination reaction test. The genotypic and allelic frequencies of the blood groups were calculated from the observed phenotypes under the assumption of Hardy–Weinberg equilibrium. In the ABO system, type O was the most prevalent (45%) followed by A (30.8%), B (19%) and AB the least (5.2%), in the pattern O > A > B > AB. Most of the students were found to be Rh+ (96.21%) and some students were found to be Rh- (3.79). The allelic frequencies of O (r), A (p) and B (q), D and d were 0.6707, 0.1999, 0.1294, 0.0.8053 and 0.1947, respectively. Genotypic frequency of I^OI^O was the most (0.45) frequent while that of I^BI^B was the least (0.0167) while DD and dd were 0.6485 and 0.038, respectively. The observed and expected frequencies of individuals having ABO and Rh blood were is significance between in both blood systems (goodness-of-fit χ^2 for ABO = 0.0025, df = 3 and χ^2 for Rh = 0.0001, df = 1; P < 0.05). In general this study provides information for Tulu Chukala Secondary School students to know their own blood type, because of the knowledge of blood group distribution is very important for blood transfusion.

***Keywords**: Allele, Antibody, antigen, Genotype, phenotype, Rh-factor*

1. INTRODUCTION

Blood is a fluid connective tissue and the most crucial component of the circulatory system. The blood plays more roles than one might expect, it is involved in respiration, nutrition, waste elimination, thermoregulation, immune defense, water and acid base balance and internal communication (Raja *et al.*, 2016).

Most adults have 4 to 6 liter of blood containing Erythrocytes (Red blood cells), leukocytes (White blood cells) and platelets. The classification of blood into groups is based on the presence or absence of inherited antigenic substances on the surface of red blood cells, RBCs (Swelem *et al.,* 2018). Some of these antigens are also present on the surface of other types of cells and body secretions like saliva, sweat, tear, urine, semen, serum etc., which are used in forensic investigations (Lekha *et al.*, 2016). Several of these RBC surface antigens that stem from one allele (Or very closely linked genes) collectively form blood group system (Ohiengbomwan *et al.,* 2018*)*.

According to the ABO blood group system, there are four different kinds of blood groups: A, B, AB and O. Blood group A, has A antigens on the surface of RBCs and B antibodies in blood plasma; blood group B, has B antigens on the surface of RBCs and A antibodies in blood plasma; blood group AB, has both A and B antigens on the surface of RBCs and no A or B antibodies at all in blood plasma and blood group O, has neither A nor B antigens on the surface of RBCs but it has both A and B antibodies in blood plasma (Fekadu, 2015). The ABO blood group system is 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 alleles I^O in intra allelic interaction (Fareed *et al.,* 2014).

The Rh system is one of the most polymorphic of the human blood groups. Many people also have a so called Rh factor on the red blood cells surface. This is also an antigen and those who have it are called Rh+. Those who haven't are called Rh-. A person with Rh- blood does not have Rh antibodies naturally in the blood plasma; as one can have A or B antibodies, for instance. But

a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh+ blood can receive blood from a person with Rh- blood without any problems (Enosolease and Bazuaye, 2008).

More than 400 antigens have been genetically identified; five are the most common known as D, C, c, E and e. The Rh is genetically complex but it is simply described in terms of a single pair of alleles, D and d. An Rh positive (Rh+) person has DD and Dd and Rh negative (Rh-) have dd. The Rh blood groups rank with ABO blood groups in clinical importance because of their relation to hemolytic disease of the new born (HDN) and their importance in blood transfusion (Wester *et al.,* 2011). The ABO and Rh blood group alleles vary worldwide and are not found in equal frequencies even among the same ethnic groups. For example, among African-Americans, the distribution of ABO blood group is type O is 46%; 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%. Also, among Western Europeans, type O is 46%; type A, 42%; type B, 9% andtype AB, 3% (Mollison, 1994).

The study of blood grouping is very important as it plays an important role in blood transfusion, forensic study, genetics, blood bank, organ transplantation, paternity test, and some groups may have association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh incompatibility and ABO incompatibility of newborn (Rehman *et al.,* 2005). ABO blood group system is shared by all human populations but frequencies of distribution differ. The frequency of the ABO and Rh blood groups vary worldwide and may not be found in equal numbers in various parts of Ethiopia. There have been no known data of the distribution pattern and frequency of ABO and Rh blood groups of Tulu Chukala secondary school. So, this study is significant because it will come up with documents that show the allelic, phenotypic and genotypic frequencies of ABO and Rh blood groups of Tulu Chukala secondary school, that serves as a base line information in creating awareness to reduce complications occurring during blood transfusion activities and Hemolytic disease of newborn infants (HDN). It will also be used in adding knowledge to the already existing body of

knowledge and serves as a reference material for another research of the same type or different version of this idea carried in another area.

The aim of this study is to provide information on the distribution pattern of the alleles, phenotype and genotype of the human ABO and Rh blood group systems in Tulu Chukala secondary school.

General objectives

The general objective of this research is to investigate the allelic and genotypic frequencies of ABO and Rh blood groups among the students of Tulu Chukala Secondary school in Liban Chukala Wereda, Oromia Regional state, Ethiopia.

Specific Objectives

- To determine the frequencies of the ABO and Rh blood group phenotypes among students of Tulu Chukala secondary school
- To determine the frequencies of the ABO and Rh blood group alleles among students of Tulu Chukala secondary school students.
- To estimate the frequencies of the ABO and Rh blood group genotype using the Hardy-Weinberg genetic equilibrium equation.

2. LITERATURE REVIEW

2.1. Discovery of ABO and Rh Blood Group System

Until the discovery of the ABO blood group over 100 years ago, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood (Tekade *et al*., 2011). A number of blood group systems have so far been discovered in man of which ABO blood type polymorphism and Rhesus (Rh) blood grouping received highest clinical, anthropological, immunological and biological attention (Iyiola *et al.,* 2012). Karl Landsteiner (1900) and his pupil Von Decastello and Sturli (1902) in Austria demonstrated that four blood group phenotype (A, B, AB and O) exist in humans, being formed out of three polymorphic alleles (A,B and O). This happens according to the presence or absence of red blood cell (RBC) surface antigen viz. A, B and O.The discoverer showed that an individual possessed antibodies against those antigens which lacked on his/her red cells (Eweidah *et al*., 2011).

Landsteiner in 1901 discovered the first human blood group system that is the ABO blood group system. Later, Landsteiner and Wiener discovered the Rh blood group in 1940 (Chakraborty, 2010). The ABO and Rh D blood group vary worldwide and are not found in equal numbers even among members of the same ethnic group. The ABO blood group system is of four major types, based on the presence or absence of A and B surface antigens. The ABO and Rhesus (Rh D) blood groups are the most important blood groups among the long list of several other blood groups discovered so far. A total of 30 human blood group systems are now recognized by the international society of blood transfusion of which the ABO is the most important in clinical practice (Tekade *et al*., 2011).

Rhesus blood group system (Rh) is the second most important blood group system due to hemolytic disease of the newborn. It is determined by a gene located on the short arm of chromosome 1 with two alleles D and d (Murphy *et al*., 2003). Individuals who have the D antigen on their red cells are known as Rhesus-positive (Rh+) (DD or Dd

genotype) while those without antigen D (dd genotype) in their red blood cells are Rh- (Knowles and Poole, 2002).

2.2. ABO Gene Evolution

The gene of ABO blood group is located in chromosome 9 in human (autosomes). Human ABO blood type is determined by genes located on the long arm of the chromosome 9 (9q34.1-q34.2) which encode glycosyltransferase. While the Rh (D) gene encoding the Rh protein located on chromosome 1p34-p36 (Carpeggiani *et al.,* 2010).

The human A and B alleles of the ABO blood group gene code for glycosyltransferase, which transfer N-acetylgalactosamin+e and galactose, respectively, to a common precursor (Yamamoto *et al.,* 1990. 1995). Based on the nucleotide and deduced amino acid sequences of the primate. ABO genes critical substitution differentiating the A and B gene occurred before the divergence of the lineage leading to humans, chimpanzees, gorillas, and orangutans (Yamamoto *et al., 2001*). They suggested that some kind of balancing selection might have been operating at the ABO locus (Storryand Olsson, 2004). The view of convergent evolution over trans-species inheritance of ancestor alleles was supported by the study of primates O alleles (Auton *et al.,* 2012). A and B antigens are not restricted to humans but are widely present in nature (Yamamota *et al.,* 2001). The kind of ABO type varies depending on species. For examples, only A and B groups are known to exist in chimpanzees, whereas only the B group is found in gorilla (Socha *et al.,* 1995)

2.3. Biosynthesis of ABO Antigens and ABO Molecular Genetics

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 (RBC) and the antibodies are in the blood plasma (Daniel and Clark, 2007). The ABO blood group system is governed by a single gene located on chromosome 9 with three alleles (I^A, I^B and I^O) (Zahid *et al*., 2016). The I^A and I^B alleles are co-dominant but both of them are dominant over the recessive allele I^O in intra-alelic interaction in diploid condition (Murphy *et al*., 2003).

Addition of monosaccharide's are built oligosaccharides. The addition of each monosaccharide requires a specific transferase, an enzyme that catalysis the transfer of the monosaccharide from its donor substrate, a nucleotide molecule carrying the relevant monosaccharide, to its acceptor substrate, the non-reducing end of the growing oligosaccharide chain. A-transferase, the product of the *A* allele, is a GalNActransferase, which catalyzes the transfer of GalNAc from UDP-GalNAc (donor) to the fucosylated Gal residue (acceptor). B-transferase, the product of the *B* allele, is a Gal-transferase, which catalyzes the transfer of Gal from UDP-Gal to the fucosylated Gal residue of the acceptor .The *O* allele produces no active enzyme, and so the fucosylated Gal residue remains not substituted (and expresses H antigen). The genetic basis for oligosaccharide blood groups is fundamentally different from that of the protein blood groups. Protein antigens are encoded directly by the blood group genes, but the genes governing carbohydrate polymorphism encode the transferase enzymes that catalyze the bio-synthesis of the blood group antigens. *A* and *B* alleles of the *ABO* gene (Daniels and Bromilow, 2007).

2.3.1. ABO antigens

The ABO system is determined by the presence of red blood cell antigens, an antigen A (group A), B antigen (group B), or both antigens (group AB), or neither of the antigens (group O), which classifies all human blood in one of four groups (phenotype) A, B, AB, O (Zahid *et al*., 2016) with six genotype, that is OO (type O), OA (type A), OB (type B), AA (type A), BB (type B), AB (type AB (Khalid *et al*., 2013).

Agglutination tests are used to detect A and B antigens on red cells. Reagent antibodies frequently produce weaker reactions with red cells from newborns than with red cells from adults. Although A and B antigens can be detected on the red cells of 5- to 6-week-old embryos, A and B antigens are not fully developed at birth, presumably because the branching carbohydrate structures develop gradually. By 2 to 4 years of age, A and B antigen expression is fully developed and remains fairly constant throughout life (Brecher, 2005).

2.3.2. Rh-D antigen

Rh antigens are highly immunogenic and till now 49 Rh antigens are identified. It is also a major player of hemolytic disease of the newborns (HDN) often called Rh disease (Dean L., 2005). There are five Rh antigens that may be found in most individuals. These are D, C, E, c and e. Rh-D is the most important antigen after A and B antigens. The D antigen is the primary antigen in the Rh system. When present on red cells, the individual is designated as "Rh positive." An individual may inherit one D gene from each parent. The inheritance of either one or two D genes will designate that person as "Rh positive." The incidence of Rh positive individuals is 85% in the Caucasian population and 92% in the African-American population (Whitlock, 2010).

Conversely when no d gene is inherited from both parents, the individual is designated as "Rh negative." Rh negative individuals comprise about 15% in the Caucasian population and 8% in the African-American population. The D antigen is very immunogenic. More than 80% of Rh negative (D negative) individuals transfused with Rh positive blood will develop an anti-D on initial exposure. Rh positive individuals may be transfused with either Rh positive or Rh negative blood. Rh negative individuals, however, should always be transfused with Rh negative blood unless the situation is life threatening and only Rh positive blood is available. Exclusive administration of Rh negative blood is crucial for women of childbearing age. Rh negative women who developed anti-D are likely to develop Hemolytic Disease of the Fetus and Newborn (HDFN) if an Rh positive infant is born to an Rh negative mother. The amount of D antigen present on the red cells varies with an individual's genotype (Whitlock, 2010).

2.4. Biochemical Composition of Rh Antigens

Rh antigens are located on the surface of red blood cells, as with the ABO system. In contrast to the ABO system, the major Rh antigens are found exclusively on red cells and not on tissue cells or in body fluids in soluble form. The biochemical nature of Rh D and Rh CE antigens is protein. Protein relies on lipids in the red cell membrane for physical support. Each of the antigens is constructed of 416 amino acids. The string of amino acids loops through the red

cell membrane and displays short loops on the exterior .The active amino acids vary with an individual's genetic coding. Rh antigens are integral to the red cell membrane. This theory is supported by the fact that cells without any Rh antigens, Rh null, present an altered physical appearance and decreased red cell survival. Glycoproteins that are associated with the biochemical structure of the Rh system have been identified. These glycoproteins are not related to antigenic properties of any blood group system but rather are associated with the red cell membrane. These glycoproteins play a role in association of the Rh D and Rh CE with the red cell membrane. The glycoprotein associated with the red cell membrane is Rh Ag. Mutation or absence of these glycoproteins results in lack of expression of any Rh antigens (Rh null). There have been comparable glycoproteins identified in the brain, the liver, the kidney, and the skin. These glycoproteins have been labeled RhBG and RhCG. They have not been associated with any specific blood group antigens but research indicates involvement with ammonia transport (Whitlock, 2010).

2.5. ABO and Rh Antibodies

2.5.1. ABO antibodies

Antibodies directed against ABO antigens are the most important antibodies in transfusion medicine. The ABO blood group presents a unique situation in Immunohematology. It is the only example of a blood group where each individual produces antibodies to antigens not present on the red cells. These ABO antibodies were originally thought to be natural antibodies formed with no apparent antigenic stimulus. Since the antibodies are not stimulated by exposure to red cells, they may also be considered non-red cell stimulated antibodies. However, some form of an antigenic stimulus must exist. The proposed mechanism is environmental. These "naturally occurring" substances resemble A and B antigens and stimulate the production of complementary antibodies to the antigens that are not present on the red cell surface (Whitlock, 2010).

Newborns have no ABO antibodies. When newborns are tested, only a forward group is performed. Newborns may exhibit passive ABO antibodies that have crossed the placental

barrier. Reverse grouping of a newborn or umbilical cord serum indicates the blood group of the mother. The child will begin antibody production, and have a detectable titer, at three to six months of age. ABO antibody production peaks at age five to ten years of age and continues in immune-competent individuals throughout life (Whitlock, 2010).

2.5.2. Rh antibodies

ABO and Rh blood group antigens are hereditary characters and are useful in population genetic studies, researching population migration patterns, as well as resolving certain medico legal issues, particularly of disputed paternity and are of great importance in blood transfusion and organ transplantation in that the donor blood type should match that of the recipient (Tesfaye *et al*., 2015).

Most Rh antibodies result from exposure to human red cells through pregnancy or transfusion. Occasionally, Rh antibodies (eg, anti-E, anti-Cw) are naturally occurring. D is the most potent immunogen, followed by c and E. Although a few examples of Rh antibodies behave as saline agglutinins, most react best in high protein, anti-globulin, or enzyme test systems. Even sera containing potent salinere active anti-D are usually reactive at higher dilutions in anti-globulin testing. Some workers find enzyme techniques especially useful for detecting weak or developing Rh antibodies (Brecher, 2005).

Antibody usually persists for many years. If serum antibody levels fall below detectable thresholds, subsequent exposure to the antigen characteristically produces a rapid secondary immune response. With exceedingly rare exceptions, Rh antibodies do not bind complement, at least to the extent recognizable by techniques currently used. As a result, primarily extravascular hemolysis, instead of intravascular hemolysis, occurs in transfusion reactions involving Rh antibodies (Brecher, 2005).

2.6. Association of ABO Blood Group and Lifestyle

The presence of A and B blood group antigens expressed on red blood cells and other cells and molecules within the body has been associated with susceptibility to disease like cancer, leukemia, cardiovascular disease and risk of both arterial and venous thrombosis. Most studies indicated an increased risk of thrombosis associated with the non-O blood group (Tregou *et al.,* 2009).

ABO blood groups have shown to have some association with various non-infectious and infectious diseases (Jefferys *et al.,* 2005). Host genetic and environmental factors may be important in the genesis of diseases. The probable association between infectious agents and ABO blood antigens is dependent on its carbohydrate moieties on red blood cell (RBC) surface. This structure may act as a receptor for some viruses, bacteria, and parasites and mediate their entrance (Chandra and Gupta, 2012). Some parasites cannot bind to RBCs that lack other blood group antigens, thus, these are important structure for adherence (Cartron and Colin, 2001).

This was approved in Norwalk virus (NV) infection which is more common in blood type O but individuals with blood type B are resistance to NV infection. This ability may occur due to the expression of ABH carbohydrate antigens. The existence terminal α-galactose can modify the NV ligand and make it hidden for NV binding and block the binding site. The lack of ABH antigen expression in O lead to susceptibility of individuals to infections after exposure to NV (Hutson *et al.,* 2002).

2.7. Clinical Significance of ABO and Rh Blood Group

2.7.1. Clinical Significance of ABO Antibodies

ABO antibodies are capable of causing both Hemolytic Disease of the Fetus and Newborn (HDFN) and Hemolytic Transfusion Reactions (HTR). These issues explain the clinical significance of “naturally occurring” antibodies. HDFN usually presents itself with a maternal

antibody of an IgG isotype that corresponds to an antigen on the surface of the baby's red cells. The most common scenario is a group O mother and a group A baby. ABO hemolytic disease may affect a woman's first pregnancy. This is in contrast to Rh HDFN where the antigenic stimulation usually occurs in the first pregnancy and subsequent antigen-positive newborns are affected. Hemolytic transfusion reaction occurs when a recipient is transfused with red cells that are an ABO group incompatible with the antibodies in his or her serum. Because of the complement-binding ability of the ABO antibodies, this is always a life-threatening situation. As the recipient antibodies react with the incompatible red cells, complement is activated and in vivo hemolysis, agglutination, and red blood cell destruction occurs. ABO compatibility is also significant in solid organ transplantation. For most organs, an ideal scenario for transplant is an ABO compatible solid organ. Post-transfusion antibody titer, and apheresis to reduce the titer of the incompatible antibody, will assist in achieving a positive outcome when an ABO incompatible organ is transplanted. (Whitlock, 2010).

2.7.2. Clinical Significance of Anti-D

Anti-D is clinically the most important red cell antibody in transfusion medicine after anti-A and -B. It has the potential to cause severe HTRs and D+ blood must never be given to a patient with anti-D. As at least 30% of D-recipients of transfused D +red cells make anti-D, D-positive red cells are not routinely transfused to D-patients (Daniels and Bromilow, 2007).

Anti-D can cause severe HDFN. This occurs when IgG anti-D in an immunized mother crosses the placenta and facilitates destruction of D+ fetal red cells.
The effects of HDFN caused by anti-D at its most severe are fetal death at about the 17th week of pregnancy. If the infant is born alive, the disease can result in hydrops and jaundice. If the jaundice leads to kernicterus, this usually results in infant death or permanent cerebral damage. In most cases of HDFN, the mother was immunized to produce anti-D by fetal D+ red cells during a previous pregnancy. These D+ red cells leak into the maternal circulation via a trans-placental hemorrhage, which generally occurs during delivery, but sometimes happens earlier in the pregnancy. Anti-D immunization can be prevented, in most cases, by administration of a dose of anti-D immunoglobulin to the D -mother immediately after delivery of a D+ baby. It is

still not absolutely clear how the anti-D immunoglobulin prevents immunization, but it is probably due to rapid removal of the D+ fetal cells from the maternal circulation (Daniels and Bromilow, 2007).

2.8. Population Genetics and the Hardy-Weinberg Principle

The Hardy-Weinberg law states that in a large, random mating population with no mutation, no migration, and no selection affecting the gene, there is a simple mathematical relationship between the genotype frequencies and allele frequencies. If no other force is applied, the population will remain at this equilibrium (Dar *et al.,* 2010*)*. Significant deviations from Hardy-Weinberg expectation is in a sample of subjects would lead to the rejection of the hypothesis of genetic equilibrium in the population. Hardy-Weinberg principle at equilibrium for binomial (Rh), the frequencies of the genotypes become $(p+q)^2 = p^2 + 2pq + q^2 = 1$. While the genotypic frequencies for trinomial (ABO) expansions were represented by: $(p+q+r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) = 1

2.8.1. Estimation of Genotypic and Allelic Frequency Distribution

The Hardy-Weinberg law is an important principles to in estimating the heterozygous frequencies in a population. The majority of deleterious recessive genes in human population are carried in heterozygous condition. To calculate the frequency of individuals who have heterozygous recessive traits, it usually began by counting the number of homozygous recessive individuals. These homozygous individuals can be distinguished from the rest of the population by clinical symptoms that indicate the defects. By using the Hardy-Weinberg law we can calculate the frequency of the heterozygous condition (Cummings, 2000). For this study, the genotypic and allelic frequencies of ABO blood group were estimated using the extension of the Hardy-Weinberg law as employed by (Griffith *et al.,* 2008). The genotypic frequencies are given by the following equation, when the frequencies are p, q and r for the alleles I^A, I^B and I^O, respectively. Thus, $(p+q+r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) (Griffith *et al.,* 2008).

The genotypic frequencies at equilibrium will be computed by the square of the allelic frequencies. In this systems, the alleles I^A and I^B are co-dominant and both are dominant to O. It has six possible genotypic combination but only four phenotypic blood groups. Homozygous I^AI^A individuals and heterozygous I^AI^O individuals are phenotypically identical, as also I^BI^B and I^BI^O individuals. This result in four phenotypic combinations known as blood types A, B. AB and O.

2.8.2. Extension of the Hardy-Weinberg Law to Loci with More Than Two Allele

Extension of the Hardy-Weinberg Law to multiple alleles of a single autosomal gene can be illustrated by a three allele case. The result of random mating in which three alleles are considered is shown in table 1.

The allele are designated by p, q and r, where the uppercase latter represents the gene and the subscript designates the particular allele. The allele frequencies are p, q and r, respectively (Daniels and Clark, 2007).

Table 1.The punnet square is showing Hardy-Weinberg frequencies for three autosomal alleles.

		Male gametes		
Allele		I^A	I^B	I^O
Frequency		p	q	R
Female gametes		I^AI^A	I^AI^B	I^AI^O
Allele	Frequency	p^2	pq	Pr
I^A	P	I^AI^B	I^BI^B	I^BI^O
I^B	Q	pq	q^2	Qr
I^O	R	I^AI^O	I^BI^O	I^OI^O
		pr	qr	r^2

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Tulu Chukala Secondary school. Tulu Chukala Secondary School is found in Oromia regional state of East Shewa zone Liban Chukala woreda Adulala town. The town is located 80km from Addis Ababa/Finfine/ city to the South East. Liban Chukala is one of the woreda in the Oromia Regional state part of the (East Shewa) located in the Great Rift Valley. The woreda town of Liban Chukala is called Adulala. The coordinates of Adulala town are 8° 32' 54" N and 38° 5' 18" E. According to the 2007 national census report (CSA, 2007) a total population for this woreda as being 76, 351, of whom 39,754 were men and 36,597 were women; 2,930 or 3.84% of its population were urban dwellers.

3.2. Research Design

The design of the study is laboratory based cross sectional survey at Liban Chukala Woreda. Allelic and genotypic frequencies of the ABO and Rh blood groups among schools' students was determined. The study was carried out from March 2022 to May 2022 academic year regardless of sex and age.

3.3. Study Population

Currently Tulu Chukala secondary school has a total of about 1452 students who attending school in 2022. A total of 422 samples of both sexes from Tulu Chukala secondary school were randomly selected from the total students and included in this study. From this 745 male students and 707 female students. All students are between 17 and 22 years of age. Therefore, the study populations for the present research were the secondary school students who are enrolled in the school in 2021/2022 academic year regardless of sex and age.

3.4. Sample Size Determination

The sample size for this study was determined by using the formula as described for calculating sample size.

$$n = \frac{z^2(p)(1-p)}{d^2}$$

Where: n_o = required sample size

z – Statistic for a level of confidence, usually set at 1.96 (≈ 2.0)

p – Expected prevalence or proportion (proportion in the population) having the particular trait (when no estimate, p = 0.5, and

q = 1.0 - p

e/d – is the desired level of precision (in proportion of one; if 5%, e = 0.05).

Therefore, $n = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2} = 384$

To minimize errors arising from the likelihood of non-compliance, 10% of the sample size were added to the normal sample. Therefore, four hundred twenty two (422) students was selected to participate in this study. For this study systematic random sampling were used. The first subject were selected by lottery method and then every thirty eight voluntarily participants was selected.

3.5. Blood Sample Collection and Technical procedures

The blood samples was collected from finger pricks by professional laboratory technicians from the District Health Center. The technical procedure of blood sample collection is simple than other branches of pathology. Blood collected were fresh without any anti-coagulant by finger picks with sterile lancet using an open slide method of testing for the ABO blood types and Rh (D) factor.

The thumb were sterilized with alcohol swab before pricking, compressed and pricked slightly with the help of a lancet, the oozed blood is placed on a clean slide at three places on a glass slide. All the necessary precautions was taken for blood sampling.

3.6. Detection of ABO and Rh Blood Groups

The samples was then tested for ABO and Rhesus factor blood groups by using Anti-A, Anti-B, Anti-D on open glass slide. Commercially available standard anti-sera A, anti-sera B and anti-sera D was used for the study. Blood were treated with anti-sera on separate glass slides, marked as A, B and D and was 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. The agglutination with anti-A is considered as group A, agglutination with anti-B group B, the agglutination with both anti-A and anti-B considered as group AB and if blood is not agglutinated with both anti-A and ant-B, it considered as group O. The agglutination in Rh blood drop with anti-D is considered as Rh^+ and non-agglutination with anti-D is considered as Rh^-. The grouping is done by antigen-antibody agglutination test. Therefore, the results was recorded as A^+, B^+, AB^+, O^+ and A^-, B^-, AB^- and O^- (Alimba, 2010).

3.7. Laboratory Analysis Techniques

This analysis were done with the assistance of two qualified laboratory technicians. The participants are first observed physically to ensure personal safety before taking blood sample from them. To ensure quality and safety of all the laboratory procedures including collection and handling of specimen were carried out.

3.8. Method of Data Analysis

The quantitative data generated from the clinical diagnosis were analyzed using SPSS window version 20 software. Chi-Square (x^2) test were conducted with SPSS statistical package (Wayne, 2010) to compare observed and expected phenotype frequencies of ABO and Rh blood groups among the students.

The three alleles of ABO blood groups; I^A, I^B, and I^O, and their frequencies were represented by p, q, and r, respectively.

The frequencies were calculated as follows:

$(p + q + r)^2 = p^2$ (AA) + 2pq (AB) + q^2 (BB) + 2pr (AO) + 2qr (BO) + r^2 (OO) =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 and r^2 is the genotypic frequency of I^OI^O. (Hanania et al., 2007). The frequencies of the blood group phenotypes were calculated as the number of individuals belonging to the phenotypic class divided by the total number of students.

Formula for the calculation of allelic frequency:

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

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

$r = \sqrt{O}$

p, q and r denote allele frequencies and A, B, O blood groups.

A correction factor or precision (d) is used and calculated according to d = 1 – p – q – r. Then the final corrected allele frequencies were:

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

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

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

The frequencies of the Rh blood group allele D (dominant allele) and d (recessive allele) were determined as:

$q = \sqrt{Rh-}$ = Allele d

p = 1 – q = Allele D

Using Hardy-Weinberg equation, at equilibrium the frequencies of the genotype where represented as:

$(p + q)^2 = p^2 + 2pq + q^2$

The Rh blood (D) group genotypic frequency were calculated from the allelic frequency under the assumption of Hardy-Weinberg equilibrium as follows:

DD + 2Dd + dd = 1

Genotype DD = p^2

Genotype Dd = 2pq

Genotype dd = q^2

3.8.1. Statistical Methods to Test the Goodness of Fit for Phenotypic frequencies

Observed and expected phenotype frequencies were compared on the basis of the Hardy-Weinberg equation using the Chi-square test (Chakraborty, 2010).

Chi-square test were used to compare the observed phenotypic frequencies of the A, B, O and Rh blood groups to that expected under Hardy-Weinberg law (Griffith *et al.,* 2012).

The Chi-square test statistic is, then

$$x^2 = \sum_j^c = 1 \left(\frac{{}^{O_j - E_j)^2}}{E_j}\right) \qquad \text{OR} \qquad x^2 = \frac{(O-E)2}{E}$$

Where O_j is observed count; E_j is corresponding expected count; x^2 is Chi-square test and the number of classes for which counts per frequencies were being analyzed and added together.

Expected phenotypic frequencies were calculated as:

Expected number of A = $(p_c^2 + 2p_cr_c)$ times N (number of total samples);

Expected number of AB = $(2p_cq_c)$ times N (number of total samples);

Expected number of B = $(q_c^2 + 2q_cr_c)$ times N (number of total samples);

Expected number of O = (r_c^2) times N (number of total samples);

3.9. Ethical Consideration

An authorization to carry out the study was obtained from Adulala Health center bureau. All the information that was obtained about the subjects will be kept confidential.

4. RESULTS AND DISCUSSION

4.1. Distribution of ABO Blood Group among students attending Tulu Chukala secondary school.

For this study, were four hundred twenty two individuals are selected randomly. The phenotypic frequency of ABO and Rh Blood group is calculated in Table 2 and Table 3 respectively.

Table 2: Phenotypic frequency of ABO blood groups among students.

Blood type	Observed number of students	Frequency	Percent
A	130	0.308	30.8%
B	80	0.19	19%
AB	22	0.052	5.2%
O	190	0.45	45%
Total	422	1	100%

There are large differences in frequency distribution of the ABO blood group phenotypes among individual of students at Tulu Chukula Secondary School. The Blood type ‘AB’ has the lowest frequency (0.052) while Blood type ‘O’ has the highest frequency (0.45).Blood type ‘B’ has the frequency of (0.19) whereas, blood type ‘A’ has the frequency of (0.308).The percentages of all ABO blood group phenotype among the students of Tulu Chukala secondary school where 30.8%, 19%, 5.2% and 45%for the A, B, AB and O blood type respectively.

The result of this study shows that the frequencies of the blood group distribution was in the order ‘AB < B < A < O’

Many other studies have shown that blood type ‘AB’ is the least common blood group and blood type ‘O’ is the most common blood group in different ethnic group (Nwauche and Ejele, 2004).

4.2. Distribution of Rh blood group among students attending Tulu Chukala secondary school

Table 3: Phenotypic frequency of Rh blood groups among students.

Rh Phenotype	Observed number of individual	Frequency	Percent
Rh –ve	16	0.0379	3.79%
Rh +ve	406	0.9621	96.21%
Total	422	1	100%

The frequency distribution of Rh positive (Rh+) and Rh negative (Rh-) blood type among the students of Tulu Chukala Secondary school was obtained as 0.96 and 0.04 respectively. Thus, the frequency of Rh positive blood type was widely distributed and the frequency of Rh negative blood type was less distributed among the students.

4.3. Combined frequencies of ABO and Rh blood groups

Table 4: Combined ABO and Rh blood group frequencies among students.

Blood type and phenotypic frequency					
Rh blood	A frequency	B frequency	AB frequency	O frequency	Total frequency
Rh –ve	5 (0.012)	6(0.0142)	2(0.0047)	3(0.007)	16(0.0379)
Rh +ve	125(0.2962)	74(0.1754)	20(0.0474)	187(0.4431)	406(0.9621)
Total	130 (0.308)	80(0.19)	22(0.052)	190(0.45)	422(1.00

Blood group AB negative was not frequent among the students than the other blood group. Blood group 'O' positive was widely distributed among the students. The frequency of Rh – negative was rare among the students of Tulu Chukala secondary school.

Frequency of Rh positive blood group A, B, AB and O was 0.2962, 0.1754, 0.0474 and 0.4431 respectively. The frequency of O Rh positive was relatively higher among the students. The frequency of Rh negative blood group A, B, AB and O was 0.012, 0.0142, 0.0047 and 0.007 respectively (Table 4).

4.4. Allelic and Genotypic frequencies of ABO and Rh blood group among students attending Tulu Chukala secondary school.

Formula for the calculation of allelic frequency:

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

$p = 1 - \sqrt{0.19 + 0.45} = 1 - 0.8 = 0.2$

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

$q = 1 - \sqrt{0.308 + 0.45} = 1 - 0.8706 = 0.1294$

$r = \sqrt{O}$

$r = \sqrt{0.45} = 0.6708$

p, q and r denote allele frequencies and A, B, O blood groups.

A correction factor or precision (d) is used and calculated according to $d = 1 - p - q - r$.

So, $d = 1 - 0.2 - 0.1294 - 0.6708 = -0.0002$

Then the final corrected allele frequencies were:

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

$p_c = 0.2(1 + (-0.0002/2))$

$p_c = 0.2(0.9999) = 0.1999$

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

$q_c = 0.1294(1 + (-0.0002/2))$

$q_c = 0.1294(0.9999) = 0.1294$

$r_c = (r + d/2)(1 + d/2)$ or $r_c = 1 - (p_c + q_c)$

$r_c = 1 - (0.1999 + 0.1294)$

$r_c = 0.6707$

So, the corrected allelic frequencies of A, B, and O blood group is 0.0199, 0.1294 and 0.6707 respectively

The frequencies of the Rh blood group allele D (dominant allele) and d (recessive allele) were determined as:

$q = \sqrt{Rh-}$

Allele d (q) = $\sqrt{0.0379} = 0.1947$

$p = 1 - q$

Allele D (p) = $1 - 0.1947 = 0.8053$

Table 5: Allelic and Genotypic frequencies of ABO and Rh blood groups.

Blood group	Allele	Allele frequency	Genotype	Genotype frequency	Phenotype
ABO	I^A	0.1999	I^AI^A	0.04	A
			I^AI^O	0.2681	A
	I^B	0.1294	I^BI^B	0.0167	B
			I^BI^O	0.1735	B
	I^O	0.6707	I^OI^O	0.45	O
			I^AI^B	0.0517	AB
Rhesus	D	0.8053	DD	0.6485	Rh +ve
			Dd	0.3135	Rh +ve
	D	0.1947	dd	0.038	Rh –ve

Table 5: indicates the Allelic and Genotypic frequencies of ABO and Rh blood groups of Tulu Chukala secondary school students. The allelic frequency of ABO blood group shows a high frequency of the allele in the order of $I^O > I^A > I^B$ in Students of Tulu Chukala secondary school, where in order of ($I^O = 0.6707$, $I^A = 0.1999$ and $I^B = 0.1294$).

With respect to Rh D blood grouping system of the total 422 sample population were Rh D positive and Rh (d) negative, with frequency of 0.8053 for allele (D) and 0.1999 for allele (d)

Table 5: also presents the frequency of the various Genotype in the ABO and Rh blood group. The genotypic frequency of ABO blood group in the students of Tulu Chukala secondary school shows that AA = 0.04, AO = 0.2681, BB = 0.0167, BO = 0.1735, OO = 0.45 and AB = 0.0517.

The genotypic frequency of Rh blood group among Tulu Chukala secondary school students were DD = 0.6485, Dd = 0.3135 and dd = 0.038

4.5. Chi-square for examining difference between the observed and expected number of ABO and Rh blood group among Tulu Chukala Secondary school students.

Calculating the genotypic frequencies and expected number of ABO and Rh blood group.

$AA = p_c^2 = (0.1999)^2 = 0.04$, Exp.No. = 0.04 x 422 = 16.88

$AO = 2p_cr_c = 2(0.1999 \times 0.6707) = 0.2681$, Exp. No. = 0.2681 x 422 = 113.138

The expected number of blood type A is 130.02

$BB = q_c^2 = (0.1294)^2 = 0.0167$, Exp. No. = 0.0167 x 422 = 7.0474

$BO = 2q_cr_c = 2(0.1294 \times 0.6707) = 0.1735$, Exp. No. = 0.1735 x 422 = 73.217

The expected number of blood type B is 80.27

$AB = 2p_cq_c = 2(0.1999 \times 0.1294) = 0.0517$, exp. No. = 0.0517 x 422 = 21.8174

The expected number of blood type AB is 21.82

$OO = r_c^2 = (0.6707)^2 = 0.45$, exp. No. = 0.45 x 422 = 189.9

The expected number of blood type OO is 189.9

$DD = p_c^2 = (0.8053)^2 = 0.645$, Exp. No. = 0.645 x 422= 273.667

$Dd = 2p_cq_c = 2(0.8053x\ 0.1947) = 0.3135$, Exp. No. = 0.3135 x 422 = 132.297

The expected number of Rh positive blood group is 405.96

$dd = q_c^2 = (0.1947)^2 = 0.038$, Exp. No. = 0.038 x 422 = 16.036

The expected number of Rh negative blood group is 16.036

Table 6: Observed and Expected frequency of ABO and Rh blood groups.

Blood group	Observed	Expected	(O – E)	$(O – E)^2$	$(O – E)^2/E$
A	130	130.018	-0.018	0.0003	0
B	80	80.2684	-0.2684	0.072	0.0009
AB	22	21.8174	0.1826	0.0333	0.0015
O	190	189.9	0.1	0.01	0.0001
Total	422	422.0038			x^2= 0.0025
Rh negative	16	16.036	-0.036	0.0013	0.0001
Rh positive	406	405.964	0.036	0.0013	0
Total	422	422			*x^2= 0.0001*

The calculated Chi-square for ABO blood group in table 6 was 0.0025, which has < 0.05 p value with 3 degree of freedom. This means that there is significance between the observed number of individuals in each of the four classes of the ABO blood group phenotype and those expected in each the phenotype classes. The result indicated that the study population is stable, because it can be affirm the next generation, the frequency of the ABO blood group will be the ones obtained in the present study.

And also, the calculated Chi-square for Rh blood group in table 6 were 0.0001, which has < 0.05 p value with 1 degree of freedom for Rh blood group phenotypes and ones expected assuming Hardy-weinberg genetic equilibrium.

This shows that the population was at genetic equilibrium. This might be due to no mobilization into or from the area and random intermarriage in the population. These result is were consistent with previous findings of Ethiopian populations (www.rhesusnegative.net, 2012).

5. SUMMARY, CONCLUSIONS AND RECOMMENDATION.

5.1. Summary

The research was conducted at Tulu Chukala Secondary School, in Liban Chukala Wereda Eastern Zone of Shoa. The distribution of allele and Genotype frequency of ABO and Rh D is unknown information among the students of Tulu Chukala Secondary school. So, the objective of this study was to provide information on the distribution patterns of ABO and Rh blood groups among the students.

A total of 422 individuals were selected randomly among the students of Tulu Chukala secondary school. The blood type were determined by open slide test method and the research work conducted in three months from May to July 2021. A drop of each of the anti-sera (anti A, anti B and anti D) was added and mixed by shake with each blood sample.

The frequency of blood group O was the highest percentage frequency of 45% followed by blood type A which is 30.8% and blood type B was 19% and the least percentage frequency was that of blood group AB (5.2%). And the genotype frequency of AA, AO, BB, BO, OO and AB was 0.04, 0.2681, 0.0167, 0.1735, 0.45 and 0.0517 respectively. About 96.21% were Rh+ and 3.79% were Rh negative. The frequency of Rh D allele DD, Dd and dd was 0.64, 0.32 and 0.04 respectively.

5.2. Conclusions

The implication of this finding the distribution of ABO and Rh blood group this study had similar trends with the data from the previous studies in Ethiopian population and with most population of the world. The study has a significant implication regarding the management of blood bank and transfusion services in the area. In general this study provides information for Tulu Chukala Secondary School students to know their own blood type, because of the knowledge of blood group distribution is very important for blood transfusion.

5.3. Recommendations

The following recommendations are drawn from the study.

- The Rhesus factor and ABO blood grouping are the two most important compatibility factors to be considered in blood transfusion. In transfusion work, it is important to ensure that Rh (D) negative patients receive Rh (D) negative blood. This is very important to reduce the disease of hemolytic death of the newborn (HDN).
- Further study at molecular level would definitely reveal the degree of genetic proximity among the population.

6. REFERENCES

Alimba, C.G., Adekoya K.O, Oboh BO. 2010. Prevalence and gene frequencies of phenylthiocarbamide (PCT) taste sensitivity, ABO and Rhesus factor (Rh) blood groups, and hemoglobin variants among a Nigerian population. *Egyptian Journal of Medical Human Genetics;* 11:153-158.

Auton A, Fledel-Alon A, Pfeifer S, Venn O. 2012. A fine scale chimpanzee genetic map from the population sequencing. Science; 336:193-8.

Brecher Mark E. 2005 .Technical Manual .15th edition. United State: AABB.

Cartron, J. P. and Colin, Y. 2001. Structural and functional diversity of blood group antigens. *Tarnsfusion Clinical Biology*, 8:163-99. Cell trait *Ind. Journal Physiol. Pharmacol*.; 40(2): 171-174.

Carpeggiani, C., Coceani, M., Landia, P., Michelassia, C., L. Abbatec, A. 2010. ABO blood group alleles; A risk factor for coronary artery disease, an angiographic study, *Atherosclerosis;* 211:461-466.

Chakraborty S. 2010. Comparative study of various genetic distance measures between populations for the ABO gene. *Not Sci Biol*; 2(4):12-17. Www.notulaebiologicae.ro. Retrieved on 24.5.2014.

Chandra T and Gupta A. 2012. Frequency of ABO and Rhesus blood groups in blood donors. *Asian Journal Trans Sci*; 6(1):52-3.

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

Daniel HL and A.G Clark, 2007. *Principle of population genetics. 4th ed. Sinaur*. Associates. Sunderland, Massachusetts P 633.

Daniels Geoff and Bromilow Imelda, 2007. Essential Guide to Blood Groups, 1st edition. Oxford: Blackwell Publishing.

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

Dean L. 2005. Blood Groups and Red Cell Antigens; Bethesda (MD): *National center for Biotechnology Information(US)*, Chapter 2, Blood group antigens are surface markers on the red blood cell membrane.

Enosolease, M.E. and Bazuaye G.N., 2008. Distribution of ABO and Rh- D blood groups in the Benin area Of Nigeria-delta implication for regional blood transfusion. Asian J. Transfuse Sci., 2(1): 3-5.

Eweidah MH, Rahiman S, Ali MDH and Al-Shamary AMD. Distribution of ABO and Rhesus blood groups in Al-Jouf province of Saudi Arabia. Anthropologist 2011; 13(2):99-102.

Fareed, M., Hussain, Ruqaiya, Shah, Ahsana, Afzal, 2014. A1A2BO and Rh gene frequencies among six populations of Jammu and Kashmir,India. Transfusion and nApheresis Science, 50(2): 247.

Fekadu, A. A. 2015. Distribution of ABO and Rh blood groups among students of some Ethnic Groups at Dilla University, Ethiopia, *International Journal of Genetics*, 3(1): 8-19.

Guyton AC, Hall JE. 2006. Textbook of medical physiology. 11th. WB Sounders Company, Philadelphia.

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

Hutson AM, Atmar RL, Graham DY, Estes MK. 2002. Norwalk virus infection and disease is associated with ABO histo-blood group type. *Journal Infect Dis.; 185(9):1335-7 PMID:*

Iyiola OA, Igunnugbemi OO and Bello OG. 2012. Gene frequencies of ABO and Rh (D) blood group alleles in Lagos, South-West Nigeria. *The Egyptian J medical human genetics*, 13: 147-153.

Jefferys, S. D., Kenneth C. A. 2005. Transfusion biology and therapy. In Gerad L. Mandell, *Principles and practice of infectious diseases 6th ed.* Philadelphia: Churchill Livingston, 46: 708.

Khalid M, Aslam N, Aslam M, Ahmad R. 2013. Distribution of ABO and Rh (D) blood groups among blood donors in district Mardan, Pakistan. *Journal of Saidu Medical College* 3(2):318-322.

Knowles S and Poole G. 2002. Human blood group systems. In: Murphy MF, Pamphilon DH, editors. Practical Transfusion Medicine. 1st ed. London, UK: Blackwell Science pp. 24-31.

Lekha, M., Archana Shetty and Vijaya Chowdappa, 2016. Distribution of ABO, Rhesus Blood Groups,Transfusion Transmitted Diseases and AdverseDonor Reactions in Blood Bank of Sapthagiri Tertiary Care Hospital, India: A Five Year Study. *NationalJournal of Laboratory*

Mollison, P.L., 1994. The genetic basis of the Rh blood group system. Transfusion, 34: 539–41.

Murphy WJ, Fronicke L, O'Brien SJ, Stanyon R. 2003. The Origin of Human Chromosome 1 and Its Homologs in Placental Mammals". Genome Research 13(8):1880-1888.

Ohiengbomwan, O.T., N.L. Idemudia, O. Owoicho and 30. A.A. Adeyanju, 2018. Gene Frequencies of Haemoglobin Genotype, ABO and Rhesus Blood Groups among Students Population of a Private University in Nigeria-Implications for Blood Banking. Int. J. Life. Sci. Scienti. Res., 4(4): 1851-1857.

Raja, K.A., G.H. Dobariya, C.A. Unagar, A.N. Pandya, J.N. Patel and S.N. Wadhwani, 2016. Frequency and distribution of ABO and Rh blood groups among blood donors in tertiary care hospital of South Gujarat, India. Int. J. Res. Med. Sci., 4: 5377-5381.

Saladin K. 2003. *Anatomy and Physiology: The unity of Form and Function, 3rd edition*. The McGraw- Hill Companies, USA pp. 679698.

Socha, W. W., Blancher, A., and Moor-Jankowski, J. 1995. Red cell polymorphism in non-human primates: A review. *Journal of medical primatology;* 24:282-305.

Storry J. R. Olsson M. L. 2004. Genetic basis of blood group diversity, *British Journal of Haematology;* 126:759-771.

Swelem, O., F. Goubran, S. Younis and N. Kamel, 2018. ABO, Rh phenotypes and cell blood groups frequencies in an Egyptian population. Hematol Transfus Int. J., 6(2): 71-75.

Tekade R, Warghat N, Sharma N, Wankhade A, Baig M. 2011. Gene Diversity among some endogamous population of Amravati District. Journal of Biotechnology 2(5):558-567.

Kassahun Tesfaye, Yohannes Petros and Mebeaselassie Andargie, 2014. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. *The Egyptian Journal of Medical Human Genetics,* 9(2): 2-5.

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

Nwauche CA and Ejele OA, 2004. ABO and Rhesus antigen in a cosmopolitan Nigeria population. *Nigerian Journal of Medicine* 13(3):263 – 266.

Tregout DA, Heath S, Saut N, Andreani C, Schved JF, Perdon G, Galan P, Drouet L, Zelenika D, Vague I, Alessi MC, Tiret L, Lathrop M, Emmerich J, Morange PE, 2009. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. Blood; 113(21): 5298-5303.

Wester, E.S., J.R. Storry and M.L. Olsson, 2011. Characterization of JK (a+ (weak)): a new blood group phenotype associated with an altered JK*01 allele. *Transfusion*, 51(2): 380-92.

Whitlock Sheryl A, 2010. Immunohematology for Medical laboratory Technicians. United State of America: Delmar.

www.rhesusnegative.net, 2012

Yamamoto F, Clausen H, White T, Marken J, Hakomori S. 1990. Molecular genetics basis of the histo-blood group ABO system. *Nature;* 345:229-33.

Yamamoto F, Clausen H, White T, Marken J, Hakomori S. 2001. Molecular genetics basis of the histo-blood group ABO system. *Nature;* 345:229-33.

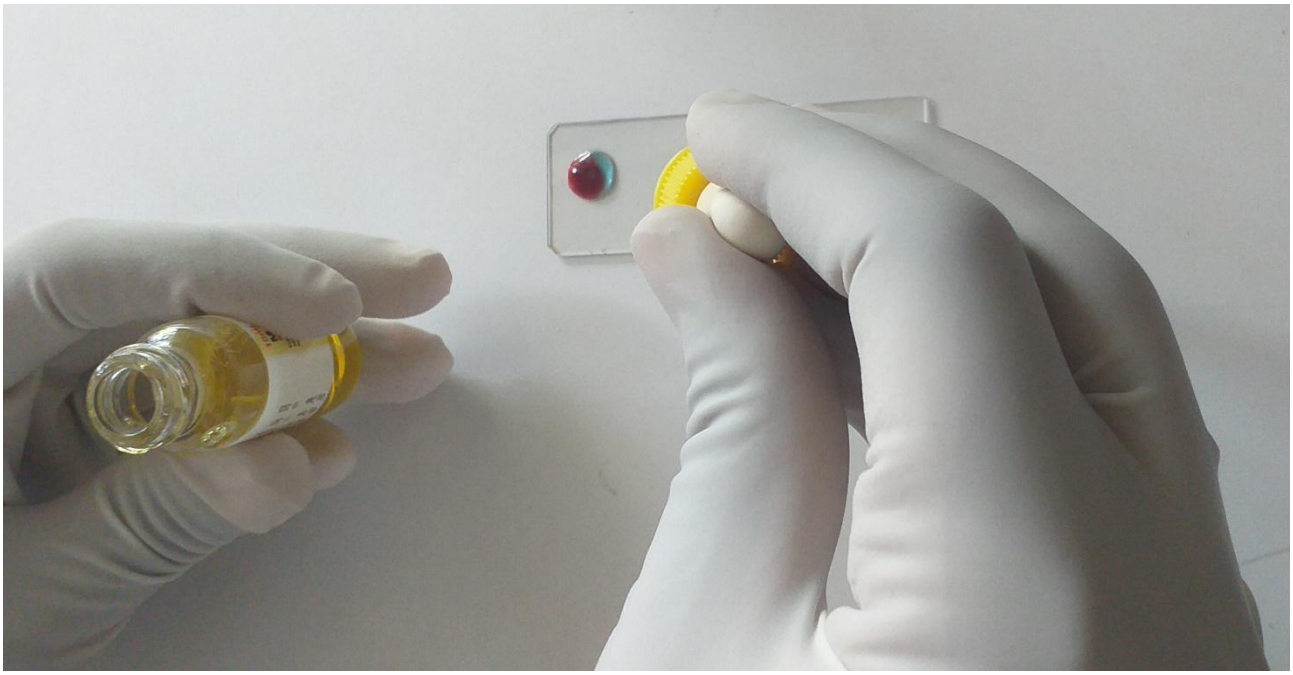
Zahid H, Yahyaoui A, Uwingabiye J, El Khazraji A, El Hadef R, Messaoudi N (2016). Phenotypic and allelic distribution of ABO and Rh-D blood Groups in Blood Transfusion Center of Avicenna Military Hospital, Marrakech, Morocco. *International Journal of Medicine and Health Research* 2(1):1-9.

7. APPENDIX

Figure 1: The three different antiserum (ant-A, Anti-B and anti-D) for identifying blood groups.

Figure 2: during blood sample taking by laboratory technicians for ABO blood typing

Figure 3: During adding antiserum on sample blood type identification



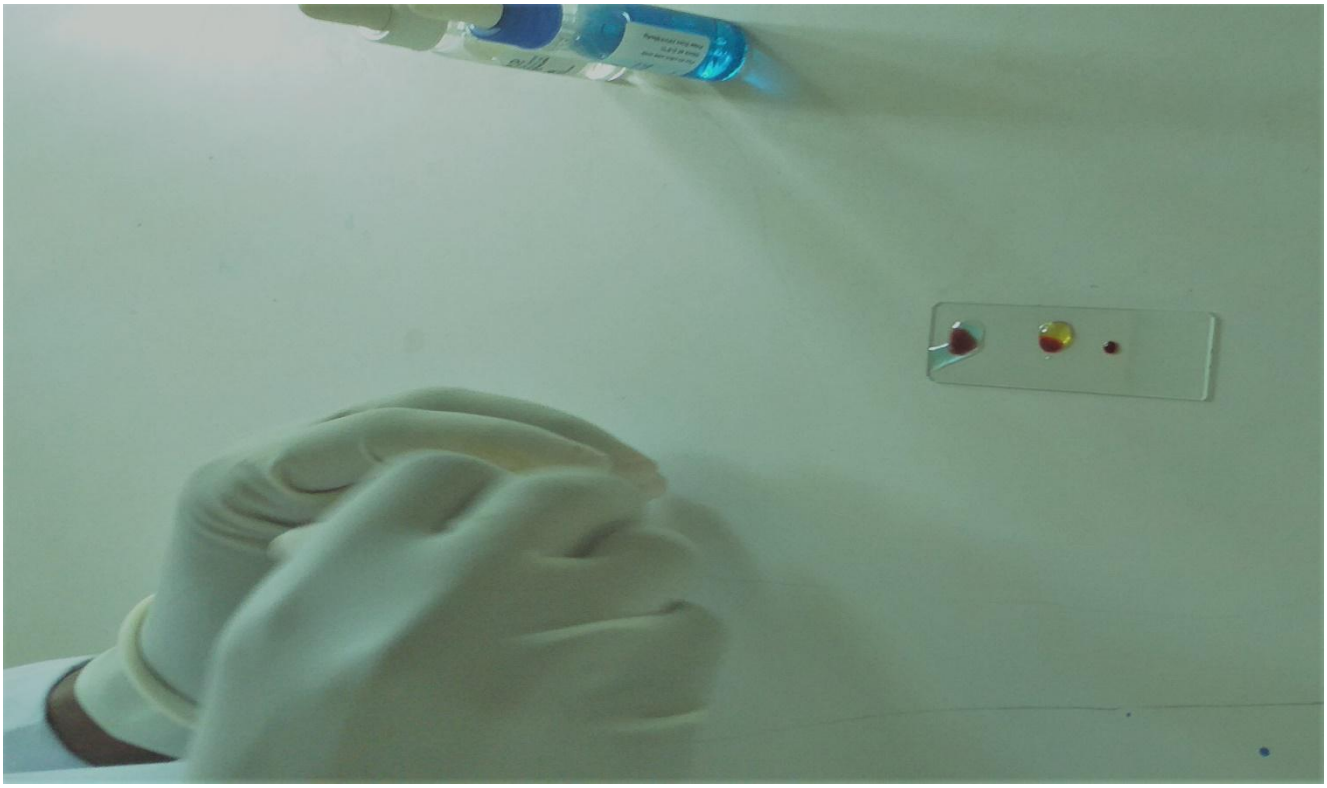
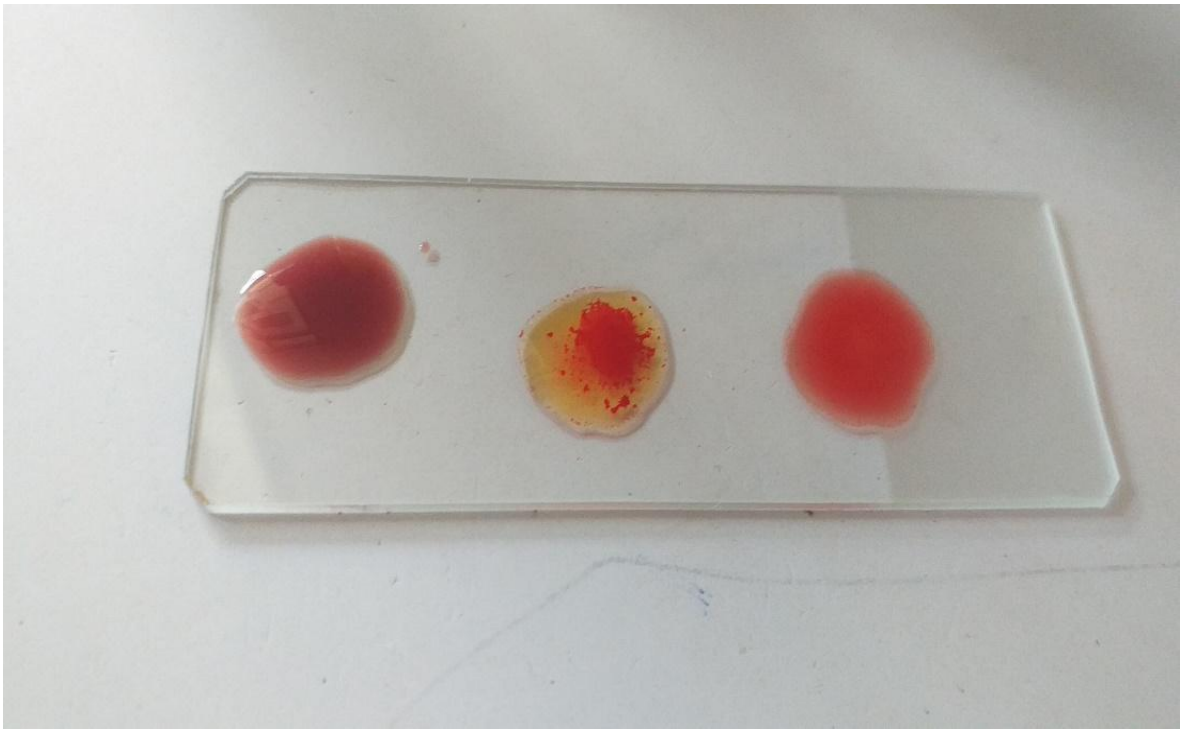


Figure 4: During the blood typing showing the difference of coagulation results (Blood type A^+ and B^+)



Ref No. BIHA 161/2015

Date 26/03/2015

Ethical letter

To: Haramaya University

We are writing this reference letter for **Mr. Keneni Abera** to witness that he has worked his laboratory work on ABO and Rh blood group test on Tulu Chukala Secondary School students to differentiate " **Allelic, Genotypic and phenotypic frequencies of ABO and Rh blood groups among students of Tulu Chukala secondary school**". Therefore we are hindly request that he completed his laboratory activities in our health centre with the help of our laboratory technicians.

With best regards

Yideneku Kebede

Oromiya Adeemsa noo Biuroo