DISTRIBUTION OF ABO AND RH-D BLOOD GROUPS AND THEIR RELATIONSHIPS WITH ANTHROPOMETRIC MEASUREMENTS AMONG STUDENTS OF ADWA COLLEGE OF TEACHERS EDUCATION, NORTHERN ETHIOPIA

M.Sc. THESIS

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Distribution of ABO and Rh-D Blood Groups and Their Relationships with Anthropometric Measurements among Students of Adwa College of Teachers Education, Northern Ethiopia

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By

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APPROVAL SHEET

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As Thesis Research advisor, I hereby certify that I have read and evaluated this Thesis prepared, under my guidance, by **Haftom Gebremariam** entitled **"Distribution of ABO and Rh-D Blood Groups and their Relationships with Anthropometric Measurements among Students of Adwa College of Teachers Education, Northern Ethiopia".** I recommend that it be submitted as fulfillment of the Thesis requirement.

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As a member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, I certify that I have read, evaluated the Thesis prepared by Haftom Gebremariam Gerezihier and examined the candidate. I recommend that the Thesis be accepted as fulfilling the requirements for the Degree of Master of Science in Genetics.

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DEDICATION

This piece of work is dedicated to my beloved family: my father Gebremariam Gerezihier, my mother Abeba Kahsay, my sisters and brothers.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my work. I have followed all ethical and technical principles of scholarships in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that included in the Thesis has been given recognition through citation. This Thesis has been submitted in partial fulfillment of the requirements for MSc degree at the Haramaya University and is deposited at the University Library to be available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born on May 15, 1994 in Hawzen Wereda, Eastern Zone of Tigray Regional State from his father Gebremariam Gerezihier and his mother Abeba Kahsay. He attended his elementary school education at Adi Beles Elementary School, and secondary school education at Masho Secondary and Preparatory School. After completing his Preparatory school, he joined Adigrat University in October, 2013 and graduated with Bachelor of Science degree in Biology in July, 2016.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTE	Adwa College of Teachers Education
BMI	Body Mass Index
HDN	Haemolytic Disease of the New Born
HTR	Haemolytic Transfusion Reaction
HWE	Hardy – Weinberg Equilibrium
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
RBCs	Red Blood Cells
Rh	Rhesus Factor
SD	Standard Deviation
SPSS	Statistical Package for Social Science
WBCs	White Blood Cells
WHO	World Health Organization
PCR	Polymerase Chain Reaction
ELISA	Enzyme Linked Immunosorbent Assay

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES IN THE APPENDIX

Distribution of ABO and Rh-D Blood Groups and Their Relationships with Anthropometric Measurements among Students of Adwa College of Teachers Education, Northern Ethiopia

ABSTRACT

The ABO and Rh blood groups are the most important blood groups despite the long list of several other blood groups discovered so far. The distribution of ABO and Rh blood groups vary worldwide and are not found in equal numbers even among ethnic groups. They are also associated with some important human traits. Therefore, this study seeks to assess the distribution of ABO and Rh-D blood groups and to determine if there is any relationship between these blood groups and anthropometric measurements (height, weight and body mass index) among the students of Adwa College of Teachers Education. A cross sectional study was conducted from December 2017 to January 2018 in the college. A total of 614 students (331 males and 283 females) were took part in the study. Height and weight of the subjects were measured using tape measure and weighing machine respectively and blood types were determined using antisera. Blood type O has a highest frequency (44.8%) while blood type AB was found to be the least (5.4%). The allele frequencies of I^O, I^A, and I^B were found to be 0.67, 0.18, and 0.15 respectively. With regard to Rh-D blood group system, blood type Rh-D positive was found to be the most prevalent (94.6%) while Rh-D negative was the least (5.4%). In both Rh-D positive and Rh-D negative blood types, blood type O has a highest frequency and blood type AB has the least. The allele frequencies of D and d were 0.77 and 0.23 respectively. Comparison of overall mean of height and weight values among different blood types of ABO blood group system showed significant difference (p-value<0.05). Subjects with blood type O (1.64 ± 0.09 m) were relatively taller than the other blood types and the least mean height was recorded in blood type AB subjects (1.58 ± 0.08 m). With regard to weight, subjects with blood type A (51.4 ± 5.62 kg) were associated with high mean weight and blood type AB (47 ± 5.32 kg) subjects were found to have the least mean weight. However, mean BMI did not show such difference (p-value>0.05). Regarding Rh-D blood group system, comparison of mean height, weight and BMI among Rh-D positive and Rh-D negative blood types did not show significant difference (p-value>0.05).

Key words: Allele frequency, body mass index, genotype frequency, height, weight

1. INTRODUCTION

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 (Saladin, 2003). In many diseases, emergencies and accidents qualitative and quantitative deficiency of this precious fluid warrants replacement. Whole blood transfusion however remained unsafe and risky till the end of the 19th century. In early 20th century the mystery was ultimately solved with the discovery of ABO and Rh blood group antigens, in 1901 Karl Landsteiner, an Austrian discovered 'A', 'B' and 'O' blood group antigens for which he earned the Nobel prize in 1930 while group 'AB' was discovered in 1902 by Landsteiner's pupils von Decastello and Sturli (Dorers and Schwarz, 2003). Later in 1940 Rh blood group was discovered by Karl Landsteiner and Weiner (Garratty *et al,*. 2000).

The classification of blood groups into type A, B, AB and O in ABO system, Rh-positive and Rh-negative in Rh system is based on the presence or absence of inherited antigenic substances on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoproteins, and glycolipids depending on the blood group system (Alimba *et al.,* 2010). These antigens are genetically controlled (Talukder and Das, 2010).

The ABO blood group is controlled by a single gene (the *ABO* gene) with three alleles: I^O, I^A, and I^B. The I^A allele gives type A blood, I^B gives type B, and I^O gives type O blood. 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 co-dominance, in which both alleles are expressed in the heterozygous state (Yazer *et al.,* 2006). The ABO blood group is the most significant blood factor in clinical applications encompassing blood transfusions. The significance of the ABO blood group is not restricted to clinical applications; however with present capabilities to rapidly sequence genes, the ABO blood group is also proving to be a valuable asset for determining human migration patterns and origins (Criswell, 2008).

Rh-D blood group system is another important system. The significance of the Rh-D blood group is related to the fact that the Rh antigens are highly immunogenic. Rh-D blood group has 50 defined antigens. Among these antigens, five antigens namely C, D, E, c, and e are more important. The terms commonly used in Rh system like Rh factor, Rh positive and Rh negative refer to D antigen and the presence or absence of this antigen. The D antigen, besides its role in transfusion, is also used in the determination of haemolytic disease of the new born (or erythroblastosis fetalis) for Rh disease management. Individuals who do not form D antigens in their body will produce anti-D antibodies when they are exposed to D antigen present on RBCs in transfused blood (causing a haemolytic transfusion reaction, HTR) or on fetal RBCs (causing haemolytic disease of the new born, HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be. In order to prevent erythroblastosis fetalis, which arises due to the presence of Rh-D positive fetus in Rh-D negative mother, identification of Rh system becomes very important (Hemalatha, 2017).

The need for blood group prevalence studies is multipurpose, as besides their importance in blood transfusion and organ transplantation, they have application in genetic research, forensic pathology, anthropology and tracing ancestral relation of human, blood bank, paternity test, and some groups may have association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh incompatibility and ABO incompatibility of new born (Shakir *et al.*, 2012; Rehman *et al*., 2005).

All human populations share the same blood group systems; although they differ in the frequencies of specific types. The frequencies of ABO and Rh-D blood groups vary from one population to another and time to time in the same region (Srikant *et al.*, 2013). Among African-Americans, ABO blood group, the frequency of type O, is 46%; type A, 27%; type B, 20%; and type AB; 7%. In Caucasians of the United State, the frequency is type O, 47%; type A, 41%; type B, 9%; type AB, 3%. Among Western Europeans, type O, is 46%; type A, 42%; type B, 9%; and type AB, 3%. Moreover, Rh-D positive is documented as 95% among African-Americans. Rh-D negative is 5.5% in South India, 5% in Nairobi, 7.3% in Lahore, 4.8% in Nigeria (Iyiola *et al*., 2011). Among Ethiopians, the distribution is type O, 43%; type A, 27%; type B, 25%; and type AB, 5% (ISBT, 2006) and the distribution of Rh-D positive and negative blood types is 94.6% and 5.4% respectively (ISBT, 2008).

The ABO blood group appears to be a marker for various human diseases including: cardiovascular, neoplastic and infectious conditions (Liumbruno and Franchini, 2013). It has also been suggested that these blood groups are associated with certain personality characteristics (Atoom, 2014) and anthropometric characteristics. Height and weight are important human characteristics influenced by multiple pairs of genes and multiple environmental factors, rather than by a single pair of gene (Carol and Elizabeth, 2017) and Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m2) (WHO, 2006). Body mass index (BMI) usually defines body fatness as an index of weight relative to height, and is generally considered as a valid index of adiposity (Gundogdu, 2008). BMI more than or equal to 25 kg/m^2 is overweight and obesity is a BMI greater than or equal to 30 kg/m^2 (WHO, 2016).

Some studies have been conducted to find out associations between anthropometric measures and blood groups (ABO and Rh-D). In one study conducted among 898 young men, blood type B (B, AB) subjects were taller than non-B (A, O) subjects (Borecki *et al.,* 1985). Another study conducted in Jerusalem (Israel) found that those with blood types B or AB tended to be slightly shorter than blood types O and A (p = 0.011) (Kark *et al.,* 1986). Regarding weight, some studies reported, weights of individuals with blood type A were significantly more than other blood types (Kelso *et al.,* 1992; Jafari *et al.,* 2012). However, Kelso *et al.* (1994) showed no relationship between the ABO blood types and body weight. Qunq and Hamid (2012) from Malaysia found a high incidence of obesity (mild and moderate) in type "B" compared to other ABO types. Another study from India showed a correlation between the O blood type in children and high BMI (Siva *et al.,* 2012). Jafari *et al.* (2012) also revealed that mean weight and BMI are significantly higher in blood group A, females and those of Turkman ethnicity. Some other studies reported no relationship between anthropometric measurements and blood groups (Aboel-Fetoh *et al.,* 2016; Alwasaidi *et al.,* 2017). These findings have somewhat contradicting results for the different populations.

In Ethiopia, no study on this association has been conducted before. On the basis of these controversial results and lack of such studies in Ethiopia, this study was carried out to determine if there is any such association between blood groups (ABO and Rh) and each of the anthropometric measure (height, weight and BMI) in a population. In addition to this, as it is described above, different countries of the world have had well organized documents on the frequency of alleles, phenotypes and genotypes of ABO and Rh-D blood groups among their different ethnic groups and socio economic groups and made the information available for the purpose of blood transfusion and other blood related activities, and hence reduced problems with respect to blood transfusion and HDN. In most parts of Ethiopia, including the present research site, there was no prior study of this type and literature that provides information and creates awareness to reduce complication in relation to blood transfusion and HDN. So, this study is significant in coming up with document that shows the phenotypic, genotypic and allelic frequencies of ABO and Rh D blood groups of the students of Adwa College of Education that serves as a base line information in creating awareness to reduce complication occurred during blood transfusion activities and HDN. It also 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 researches of different version of this topic carried in the study site or other places of our country.

General Objective

The general objective of the study was to assess the distribution of ABO and Rh-D blood group systems and their relationships with human height, weight and body mass index (BMI) in students of Adwa College of Teachers Education, Tigray Regional State, northern Ethiopia.

Specific objectives

- ✓ To assess the frequency of ABO and Rh-D blood groups among students of Adwa College of Teachers Education.
- ✓ To determine the allelic and genotypic frequencies of ABO and Rh-D blood groups among students from the phenotypic frequencies.
- ✓ To analyze the relationships between the blood group systems (ABO and Rh-D) and height, weight, and BMI.

2. LITERATURE REVIEW

2.1. Antigens on Red Blood Cells and Blood Groups

Humans contain a series of glycoproteins and glycolipids on the surface of RBCs which constitute the blood group antigens. According to the presence or absence of antigens human blood can be classified into different blood group systems, example ABO blood group, MN blood group, Rh blood group systems, etc. All blood groups in human are under genetic control, each series of blood groups being under the control of genes at a single locus or of genes that are closely linked and behave in heredity as though they were at a single locus (Jaff, 2010). The human blood groups have been studied extensively for their involvement in incompatibility reactions. There are many blood group systems on the basis of different blood group antigens. ABO and Rh systems are important in clinical practice (Mandal, 2002).

2.2. ABO Blood Group System

2.2.1. History of ABO blood grouping

In the 1901, an Austria Scientist Karl Landsteiner established the existence of the first known blood group system. Landsteiner named the first two blood groups antigens A and B, using the first two letters of the alphabet while RBCs not reacting with anti- A and anti- B were called type C. Classification of the blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies present in the serum of individuals directed against these antigens. Where no agglutination had occurred, either the antigen or the antibody was missing from the mixture. In 1902, Von Decastello and Sturli described RBCs reacting with both anti-A and anti-B, but did not give these type a name, but continued calling RBCs that did not react with anti-A and ant-B type C (Garratty *et al.,* 2000), and this group was later called "O" after the German word "Ohne", which means "without". The following year the fourth blood type, AB, was added to the ABO blood group system. These RBCs expressed both A and B antigens. In 1910, scientists proved that the RBCs antigens were inherited, and that the A and B antigens were inherited co-dominantly over O. There was initially some confusion over how a person's blood type was determined, but the puzzle was solved in 1924 by Bernstein's "three allele model". The ABO blood group antigens are encoded by one genetic locus, the ABO locus, which has three alternative (allelic) forms I^A, I^B, and I^O (Avent and Reid, 2000). Development of the Coombs test in 1945, the advent of transfusion medicine, and the understanding of ABO hemolytic disease of the newborn led to the discovery of more blood groups (Anstee, 2009). As recognized by the HUGO gene nomenclature committee, currently there are about 35 human blood group systems (HUGO, 2014), and across the 35 blood groups, over 700 different blood group antigens have been found. Many of these are very rare or are mainly found only in certain ethnic groups (Anstee, 2009).

The 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, legal medicine, anthropology and the discovery of other blood group systems (Hillier *et al.,* 2008).

2.2.2. Genetics of ABO blood group system

The H antigen is an essential precursor to the ABO blood group antigens. The H locus is located on chromosome 19. It contains 3 exons that span more than 5 kb of genomic DNA, and it encodes a fucosyltransferase that produces the H antigen on RBCs. The H antigen is a carbohydrate sequence with carbohydrates linked mainly to protein (with a minor fraction attached to ceramide moiety). It consists of a chain of β-D-galactose, β-D-N-acetylglucosamine, β-D-galactose, and 2-linked, α-L-fucose, the chain being attached to the protein or ceramide (Yazer *et al.,* 2006). The O blood type has only this H antigen. In people of blood type A, N-acetyl-D-galactosamine (A determining) is added to the terminal D-galactose of the H antigen with the help of N-acetylgalactosaminyl transferase enzyme which is encoded by the I^A allele of the ABO gene located on chromosome 9 (9q34.2). A variant of this enzyme known as the D-galactosyl transferase, is encoded by the I^B allele of the ABO locus adds D-galactose to the terminal galactose of the H antigen, to form the B antigen. Both the A and the B antigens are found on the surfaces of the RBCs of individuals with AB blood type since they are heterozygotes for the two alleles. The I^O allele does not produce a functional enzyme at this locus and there is no any addition to the H antigen. The I^A and I^B alleles are co-dominant while the I^O allele is recessive to both of them (Ahmed *et al.,* 2007).

The ABO locus is located on chromosome 9. It contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. The ABO locus has three main allelic forms: I^A, I^B, and I^O. The I^A allele encodes a glycosyltransferase that bonds α-N-acetylgalactosamine to D-galactose end of H antigen, producing the A antigen. The I^B allele encodes a glycosyltransferase that joins α-D-galactose bonded to D-galactose end of H antigen, creating the B antigen. In case of I^O allele, the exon 6 contains a deletion that results in a loss of enzymatic activity. The I^O allele differs from the I^A allele by deletion of only one nucleotide – guanine at position 261. The deletion causes a frame shift, and results in premature termination of translation, and thus, degradation of the mRNA. This results in H antigen remaining unchanged in case of O blood type (Yazer *et al.*, 2006).

2.2.3. Antigens and Antibodies of ABO blood group system

An antigen can be defined as any substance which, when introduced into an individual who himself lacks the substance, stimulates the production of an antibody, and which, when mixed with the antibody, reacts with it in some observable way. Foreign substances, such as erythrocytes, can be immunogenic or antigenic if their membrane contains a number of areas recognized as foreign. These are called antigenic determinants or epitopes. The immunogenicity of a substance is influenced by a number of characteristics: such as foreignness, molecular weight, structural stability, structural complexity and route of administration (Garratty *et al.*, 2000).

The ABO blood groups are defined by the presence of two alternative antigens called A and B on red blood cells, determined by three alternative alleles at a single genetic locus. RBCs of type A have the A antigen on their surface, those of type B have antigen B, type AB red cells bear both antigens, while type O cells bear neither antigen (Yazer *et al.*, 2006).

ABO antibodies are naturally occurring antibodies that occur without exposure to RBCs containing the antigen. There is some evidence that similar antigens found in certain bacteria, like *Escherichia coli*, stimulate antibody production in individuals who lack the specific A and B antigens. They are absent at birth and start to appear around 3-6 months as result of stimulus by bacterial polysaccharides (Avent and Raid, 2000).

Normal healthy individuals produce antibodies against A or B antigens that are not expressed in their own cells. These naturally occurring antibodies are mainly immunoglobulin M (IgM). They attack and rapidly destroy red cells carrying the corresponding antigen. For example, anti-A attacks red blood cells of type A or AB. Anti-B attacks red blood cells of type B or AB (ISBT, 2008).

2.2.4. Frequencies ABO phenotypes in different populations

The ABO blood group phenotypes are not found in equal numbers in different populations. For example, in Brazilians, the distribution is blood type O, 47%; blood type A, 41%; blood type B, 9%; and blood type AB, 3%. Among Chinese-Canton, the distribution is blood type O, 46%; blood type A, 23%; blood type B, 25%; and blood type AB, 6%. Among Chinese-Peking, 27% have blood type A, 32% blood type B, 13% blood type AB and the remaining 29% blood type O. Among Indians, the distribution is blood type O, 37%; blood type A, 22%; blood type B, 33%; and blood type AB, 7% (Table 1).

Among Ethiopian blood donors, the frequency of blood type O is 40%; blood type A, 31%; blood type B, 23%; and blood type AB, 6% (Tibebu, 1998). In population of southwest Ethiopia, at Gilgel Gibe Field Research Center, the frequency of O, A, B and AB blood types are 42%, 31%, 21% and 6% respectively among a total of 1965 study participants (Abraham *et al.*, 2012). The phenotypic frequency of O,A, AB, and AB blood types of Sidama ethnic group was found to be 51.3%, 23.5%, 21.9%, and 3.3% respectively (Tewodros *et al.*, 2011).

Table 1: Distribution of ABO blood types studied in different population across the world

Population	O(%)	A(%)	B(%)	AB(%)
Arab	34	31	29	6
Armenian	31	50	13	6
Belgian	47	42	8	3
Brazilian	47	41	9	3
Chinese-Canton	46	23	25	6
Chinese-Peking	29	27	32	13

Population	O(%)	A(%)	B(%)	AB(%)
Egyptian	33	36	24	8
English	47	42	9	3
German	41	43	11	5
Greek	40	42	14	5
Indian	37	22	33	7
Japanese	30	38	22	10
Kikuyu (Kenya)	60	19	20	1
Russian	33	36	23	8
South African	45	40	11	4
Sudanese	62	16	21	0
USA (US black)	49	27	20	4
USA (US white)	45	40	11	4
Vietnamese	42	22	30	5

Source: - (ISBT, 2006).

2.3. The Rh Blood Group System

The Rh blood group system is the most polymorphic of the human blood groups. Next to ABO, is the most clinically significant in transfusion medicine (Avent and Reid, 2000). The Rh blood group system divides people into Rh positive and Rh negative groups depending on whether or not their erythrocytes carry the Rh antigen. Landsteiner and Wiener discovered this system in 1940. They showed that antisera raised in guinea pigs against erythrocytes from rhesus monkeys reacted with 85% of Caucasian blood donors in New York. The Rh system of blood group antigens is often described as if it is a single antigen. However, it consists of a complex series of antigens, which are specified by two genes: *RHD* and *RHCE*. The former encodes the Rh-D protein which expresses the D antigen while the latter encodes the RhCcEe protein which carries either the C or c antigen together with the E or e antigen. At one time it was thought that another antigen, termed the ‘d’ antigen, was present when the D antigen was absent. It is now recognized that the d antigen does not exist. However the term is still used to indicate the D negative phenotype (Garratty *et al.*, 2000). Antigen D, having antigen site between 110,000 and 202,000 per erythrocyte, is the most important of the rhesus antigens medically, because it is highly antigenic than the other Rhesus antigens and most likely to provoke an immune system response of the five main antigens (Flegel *et al.*, 1997).

It is common for D-negative individuals not to have any anti-D or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following sensitizing events: possibly a feto-maternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with D-positive RBCs. Rh disease develops in these cases (Moise, 2008).

2.3.1. Inheritance of Rh blood group system

An individual inherits one haplotype from each parent so that a large number of different genotypes are possible. Certain of these are more common than others, but there are differences between racial groups. However, it is the presence or absence of the D gene that is the most important. The D gene is sometimes called Rh1 and anti-D is called antiRh1 (WHO, 2002).

Table 2: Rh haplotypes and shorthand Rh notation ("d" is used to show the absence of the D gene)

Rh haplotypes	D positive	D negative (d)
ce	cDe Ro	cde r
Ce	CDe R1	Cde r'
cE	cDE R2	cdE r''
CE	CDE R3	CdE ry

Source: WHO, 2002

Where a person inherits a D gene, their red cells are positive when tested with anti-D and that person is said to be Rh-D positive. Where a person does not inherit a D gene, their red cells are negative when tested with anti-D and that person is referred to as Rh-D negative. It is not possible to determine whether a person reacting with anti-D is homozygous for D (i.e. has inherited a D gene from each parent: D/D) or heterozygous (i.e. has inherited a D gene from only one parent: D/d). A person who does not inherit a D gene is D negative (Table 2).

2.3.2. Antigens and antibodies in Rh-D blood group system

The Rh blood group is one of the most complex blood groups known in humans. Clinically, it is the most important blood group system after ABO. At present, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e which are only expressed on red cells and are encoded by two adjacent gene loci, the *RHD* which encodes the RhD protein with the D antigen and the *RHCE* which encodes the RhCE protein with the C, E, c and e antigens. They are not found in body fluids like saliva, amniotic fluid and not detected on leucocytes or platelets. The '*d*' gene is not expressed and there is no 'd' antigen, it only implies the absence of '*D*'. Individuals who lack any of these antigens may be stimulated to produce the corresponding antibodies (anti-D, anti- C, anti-c, anti-E, and anti-e) by transfusion or pregnancy. Antigen D, having antigen site between 110,000 and 202,000 per erythrocyte, is the most important of the rhesus antigens medically, because it is highly antigenic than the other Rhesus antigens and most likely to provoke an immune system response of the five main antigens (Flegel *et al.*, 1997).

Natural antibodies to Rh do not exist in humans, as they do for the AB antigens. Unlike the anti-A and anti-B antibodies, anti-D antibodies are only seen if a patient lacking D antigen is exposed to D+ cells. The exposure of D+ cells usually occurs through pregnancy or transfusion. Rh+ cells infused into an Rh negative recipient can give rise to a strong antibody response, mainly of the IgG class, which can result in dangerous reactions to subsequent transfusions. Blood typing and cross-matching are therefore important to ensure compatibility for the Rh factor as well as ABO. However, unlike the A and B antigens, the Rh antigens are present only on red blood cells. Therefore, while they are important for blood transfusion, they do not normally play a role in organ transplantation, and Rh typing of organ donors and recipients therefore is not a significant consideration (Laura, 2005).

2.3.3. The clinical importance of Rh-D blood group system

The clinical importance of the Rh blood group system was clearly demonstrated by Levine and Stetson in 1939 when, following the delivery of a stillborn baby, a patient urgently required a blood transfusion. ABO compatible blood was transfused, following which the patient had a near fatal transfusion reaction. Further laboratory studies showed that the mother's serum contained an irregular antibody that reacted strongly with the ABO-compatible donor red cells and also with the red cells of her fetus. Like the antibody reported by Landsteiner and Wiener, the mother's antibody reacted with approximately 85% of the random Caucasian population. When this antibody was compared with the one earlier discovered by Landsteiner and Wiener, it was shown that the two had similar specificity. As a result of these findings, not only had a new blood group system been discovered, but a scientific explanation had been provided to help understand the cause of unexplained transfusion reactions and why babies were occasionally born suffering from an anaemia caused by maternal–fetal blood group incompatibility (WHO, 2002).

2.3.4. Distribution of Rh-D blood group phenotypes in different populations

Rh blood group distribution varies worldwide. The allele D which gives rhesus positive status is at its lowest in Europe. 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 % (Neil, 2006).

Rh negative blood group is documented as 5.5% in south India (Chavhan *et al.*, 2010), 0% in red Indians (USA), 19.7% in Kenya, 5.7% in Nigeria, 5% in Germany, 7% in Saudi Arabia and 15% in USA (Table 3). About 95% of African - Americans are Rh-positive, (Chavhan *et al.,* 2010).

Table 3: Allele Frequency of Rh-D blood groups studied in different populations across the World.

Population	Rh (D)	Rh (d)
Ethiopia	0.94644	0.05356
Germany	0.9500	0.0500
Kenya	0.8030	0.1970
Lagos (Nigeria)	0.9400	0.0600
Mandi Bahauddin (Pakistan)	0.9140	0.0860

Population	Rh (D)	Rh (d)
Nigeria	0.9430	0.0570
Ogbomoso (Nigeria)	0.9670	0.0330
Port Harcourt (Nigeria)	0.9677	0.0323
Red Indians(USA)	100	0
Saudi Arabia	0.9300	0.0700
U.S.A	0.8500	0.1500

Source: ISBT, 2008

2.4. Clinical Significance of Blood Typing

2.4.1. Blood Transfusion

Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups, along with the work of a blood bank to provide a transfusion service for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor in a similar way as medicines. Much of the routine work of a blood bank involves testing blood from both donors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between a donor and recipient, a severe acute hemolytic reaction with 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).

2.4.2. Universal donors and universal recipients

With regard to transfusions of packed red blood cells, individuals with type O Rh-D negative blood are often called universal donors, and those with type AB Rh-D positive 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. One exception is individuals with hh antigen system (also known as the Bombay phenotype) who can only receive blood safely from other hh donors, because they form antibodies against the H antigen present on all red blood cells. Blood donors with particularly strong anti-A, anti-B or any typical blood group antibody are excluded from blood donation. The possible reactions of anti-A and anti-B antibodies present in the transfused blood to the recipients RBCs need not be considered, because a relatively small volume of plasma containing antibodies is transfused (Letsky *et al.*, 2000).

By way of example, considering the transfusion of O Rh D negative blood (Universal donor blood) into a recipient of blood group A Rh D positive, an immune reaction between the recipient's anti-B antibodies and the transfused RBCs is not anticipated. However; relatively small amount of plasma in the transfused blood contains anti-A antibodies, which could react with the A antigens on the surface of the recipient RBCs, but a significant reaction is unlikely because of the dilution factors. Rh-D sensitization is not anticipated. Additionally, red blood cell surface antigens or 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. Transfusion are further complicated because platelets and white blood cells (WBCs) have their own systems of surface antigens, and sensitization to platelets or WBC antigens can occur as a result of transfusions (Avent, 2009).

2.4.3. Hemolytic diseases of the new born (HDN)

2.4.3.1. ABO incompatibility

If maternal anti-Rh antibodies to fetal red blood cells can damage the RBC of the developing fetus, why is incompatibility for ABO blood groups not as dangerous as Rh-incompatibility, particularly since ABO isoagglutinins normally exist in mothers which could potentially damage the infant even during a first pregnancy? The answer lies in the isotype of antibody produced in the two cases. Anti-Rh-antibodies are mainly IgG which are capable of crossing the placenta and entering the fetal circulation. The natural antibodies (isoagglutinins) to A and B blood group substances, however, are mostly of the IgM class (typical of anti-carbohydrate responses) and therefore do not cross the placenta. IgG antibodies against the A and B blood group antigens may develop in some individuals, and the resulting ABO incompatibility actually accounts for about two thirds of all discernable cases of HDN. Such cases, however, are generally very mild and require little or no treatment. Thus, while ABO incompatibility is actually much more common than Rh incompatibility, it is much less likely to cause significant disease (Daniel and Elizabeth, 2009).

2.4.3.2. Rh incompatibility

HDN due to anti-Rh D occurs when mother and infant are always incompatible with respect to the Rh factor: The mother Rh-D negative, and the infant Rh-D positive (inherited the D factor from the father). ABO incompatibility between the mother and fetus reduces the chance of maternal immunization to the D antigen. This is probably because the fetal cells, which are incompatible with the maternal ABO antibodies, are destroyed by existing ABO antibodies before they have a chance to act as an antigenic stimulus. The first Rh-D incompatible infant is usually unaffected because the number of fetal cells that cross the placenta during pregnancy (after 24 weeks gestation) is small and insufficient to cause IgG anti-D production, unless a prior transfusion of D positive blood has been given. During transplacental hemorrhage, the amount of fetal blood that enters the maternal circulation increases and in six months' time after delivery only 10% of these Rh-D negative women could produce detectable antibodies. The actual production of anti-D antibodies depends on the dosage and antigenecity of the D antigen, and the mother's ability to respond to these foreign antigens. During a second pregnancy with Rh-D positive fetus, small number of fetal cells cross the placenta stimulating the antibody to high concentration, mainly IgG anti-D that passes into the fetal circulation is destroying fetal red cells. The severity of the disease increases with each Rh-D positive pregnancy (Wagner *et al.*, 2002).

2.5. Blood Based Products

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

2.6. Blood Typing

Blood typing involves identifying substances called antigens present on RBCs membranes. Many different antibodies exist on human RBCs but those of clinical importance include only the ABO and Rh-D groups. Blood typing is performed with antiserum, blood serum that contains specific antibodies. For ABO blood typing, antibodies against A and B antigen (these antibodies are also called anti-A and anti-B antibodies) are used. If clumping or clotting occurs in the test blood upon exposure to the A antibody, the blood contains the A antigen. If clumping occurs in the test blood upon exposure to the B antibody (anti-B serum), the blood contains the B antigen. If clotting occurs with both A and B antibodies (anti-A and anti-B sera), the blood type is AB, and if no clumping occurs with either serum type, the blood type is O (Rai and Kumar, 2010).

2.7. Anthropometric Measurements

2.7.1. Height and weight

Height and weight are complex traits which are determined by more than one factor; these can be several genes and/or the influence of environmental factors. Traits primarily controlled by two or more genes are called polygenic traits: those controlled by two or more genes and significant environmental interactions are called multifactorial traits. Although each gene controlling complex traits is inherited in Mendelian fashion, the interaction of genes with each other and with environment produces variable phenotypes that often do not show clear-cut Mendelian ratios. Human height and weight for examples, are multifactorial traits; they are controlled by several genes, and environmental factors make significant contributions to variations in their expressions (Carol and Elzabeth, 2017; Michael, 2015).

2.7.2. Body Mass Index (BMI)

Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2) (WHO, 2006). BMI is a simple, inexpensive, and noninvasive surrogate measure of body fat. In contrast to other methods, BMI relies solely on height and weight and with access to the proper equipment, individuals can have their BMI routinely measured and calculated with reasonable accuracy (CDC, 2010). For adults, WHO defines overweight and obesity as follows: overweight is a BMI greater than or equal to 25 and obesity is a BMI greater than or equal to 30 (WHO, 2016).

BMI values are age-independent and the same for both sexes. However, BMI may not correspond to the same degree of fatness in different populations due, in part, to different body proportions. The health risks associated with increasing BMI are continuous and the interpretation of BMI grading's in relation to risk may differ for different populations (WHO, 2006). The common interpretation is that it represents an index of an individual's fatness. It is widely used as a risk factor for the development of or the prevalence of several health issues. It also is widely used in determining public health policies. The BMI has been useful in population-based studies by virtue of its wide acceptance in defining specific categories of body mass as a health issue (Nuttall, 2015).

2.8. 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 allele frequencies and genotype frequencies. Significant deviations from Hardy-

Weinberg expectations in a sample of subjects would lead to the rejection of the hypothesis of genetic equilibrium in the population (Kalmes and Huret, 2001).

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

$$p^2 \text{ (AA)}: 2pq\text{(A a)}: q^2 \text{ (aa)}$$

ABO blood type is a trait determined by three alleles at a single locus. The alleles are commonly denoted A, B, O. These alleles combine to give the following phenotypic blood types: AA and AO (type A), BB and BO (type B), AB (type AB), and OO (type O). Denote the frequencies of alleles A, B, O as p, q, r respectively. Under the assumptions of Hardy-Weinberg, we would expect the genotypic frequencies: AA with frequency p^2, AB with frequency 2pq, AO with frequency 2pr, BB with frequency q^2, BO with frequency 2qr, and OO with frequency r^2. Notice that, again, these frequencies can be calculated by squaring the appropriate multinomial. This time it is $(p + q + r)^2 = p^2+2pq +2pr + q^2+2qr + r^2$ (Kalmes and Huret., 2001).

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

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

2pq is frequency of genotype I^AI^B

2pr is frequency of genotype I^AI^O

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

r^2 is the frequency of genotype I^OI^O

ABO allele frequencies are estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates are calculated as: p = 1 - √B+O, q = 1 - √A+O, r = √O (p, q, and r denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O). A correction factor (θ) is calculated according to θ = 1 – p – q – r. The final allele frequencies are then calculated as follows: p1 = p (1 + θ/2); q_1 = q (1 + θ/2); r_1 = (r + θ/2) (1 + θ/2) [where p_1, q_1, and r_1 denote corrected allele frequencies. Rh-D allele frequencies are calculated according to the Hardy-Weinberg equation (Al-Arrayed *et al.*, 2001). The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium are tested using chi-square test to check whether population is at Hardy-Weinberg genetic equilibrium or not (Chakraborty, 2010).

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

2.9. Relationships between the Blood Groups and Anthropometric Measurements

Various studies have been conducted to establish an association between blood groups (ABO and Rh-D) and anthropometric measurements (Height, Weight and BMI). However, these studies have been unable to establish an equal link between these blood groups (ABO and Rh-D) and the anthropometric measurements. Borecki *et al.* revealed that blood type B (B, AB) subjects are taller than non-B (A, O) subjects (Borecki *et al.*, 1985). In other study conducted in a sample of 4,472 boys, aged 17–18 years, resident in Jerusalem (Israel), those with blood types B or AB tended to be slightly shorter than blood types O and A (p = 0.011) (Kark *et al.*, 1986). However, a study conducted in Iran showed no significant difference in mean height among the blood types (Jafari *et al.*, 2012).

A study conducted on Brazilian infants reported that weights of females with blood type A were significantly more than other blood types. This difference was not found among male infants (Kelso *et al.,* 1992). Similarly, a study conducted in different ethnicities of the Golestan Cohort population, Iran, revealed that the mean weight was significantly higher in blood type A compared with other blood type (68.25 kg; p= 0.002) (Jafari *et al.,* 2012). However, Kelso *et al.* (1994) showed no relationship between the ABO blood types and body weight among four samples from different culturally distinct populations.

A study from India showed a correlation between B blood type and high BMI (Tulika and Ashish, 2012). Similarly, Sukalingam and Ganesan (2015) from Malaysia revealed that blood type B and Rh-D positive were more susceptible to get obesity ($p<0.05$) as compared to blood type O and A; whereas AB blood type had a lesser chance of getting obesity. However, another study from India found a correlation between the O blood type in children and high BMI (Siva *et al.*, 2012). In contrast, a study conducted in Pakistan found a highest BMI in subjects with blood type A (24.3 ± 5.04 kg/m^2) and lowest in blood type AB (23.0 ± 2.91 kg/m^2). The Rhesus-D positive and male students had greater BMI (23.6 ± 3.56) than females (23.2 ± 3.44). Comparison of overall mean BMI values among different blood types showed significant difference with p-value < 0.001 (Parveen *et al.,* 2016). Similarly, Jafari *et al.* (2012) from Iran showed that, BMI is significantly higher in blood type A compared with other blood types (26.75kg/m^2; p=0.001). Some other studies showed no association between blood groups (ABO and Rh-D) and BMI (Aboel-Fetoh *et al.*, 2016; Alwasaidi *et al.*, 2017).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Adwa College of teacher education, Adwa town, Central Tigray Zone, Tigray Regional State. Adwa is located at 977 km north of Addis Ababa, the capital of Ethiopia, and 190 km northwest of Mekele, the capital of the regional state. The average altitude of the district of Adwa is 1882 meter above sea level. Its astronomical location is 14.163647° North Latitude and 38.893718° East Longitude.

3.2. Study Design

A cross-sectional study was conducted to assess the distribution of ABO and Rh blood groups and their correlation and/or association with anthropometric measurements (height, weight, and BMI) in students of Adwa College of Teachers Education during the period of November 2017 to January 2018.

3.3. Study Population

Adwa College of Teacher Education has a total of 2964 regular students (1449 males and 1515 females) during the 2017/2018 academic year. The students came from different parts of Tigray Regional State (about 98%) (ACTE registrar office's personal communication).

3.4. Sample Size Determination and Sampling Technique

The study was conducted on 614 sample students. Volunteer sampling technique was used to select samples from the regular students of the college after discussing with them about the purpose and objective of the research. Sample size was calculated using Yamane (1967) formula.

$$n = \frac{N}{1 + Ne^2}$$

Where n= sample size, N= total population= 2964, and e= margin of error= 0.04

Therefore, n= 2964 ÷ 1 + 2964$(0.04)^2$= 516 + 98 (19%) for contingency= 614

3.5. Data Collection Methods

Data such as blood type for ABO and Rh-D systems, height (in centimeter), weight (in kilogram) and some socio-demographic information including age, gender, place of birth, place of residence (district) before joining the college were collected from each participant student following getting written informed consent (agreement) of participation in the research process.

3.5.1. Measurement of Height and Weight and calculation of BMI

Height was measured using a measuring tape with the individual standing bare-footed against a wall and by making a mark on the wall. Subjects were asked to stand upright, barefoot on the ground with heels, buttocks, upper back, and back of head making firm contact with the wall. Chin was tucked in slightly and the head held erect. Cardboard was pressed firmly onto the subject's head to form a right angle to the wall and the subject was asked to bend his/her knees slightly when he/she steps away to avoid the bending or dislocation of the cardboard before the height is recorded (Nanaware *et al.,* 2011).

Participants' weight was measured with the participant standing on a balance scale without shoe and any external materials that may increase weight. The participant was asked to place in the anatomical position; head, eyes facing forward and upright position, foot slightly apart and palms of hand facing forward. Weight was recorded by the researcher squatting in front of the scale so as to avoid error due to parallax (Aliyu *et al.,* 2014). Then, BMI was calculated for each subject as:

$$\text{BMI} = \text{weight in kilogram}/(\text{height in meter})^2$$

3.5.2. Blood sample collection and typing

3.5.2.1. Blood sample collection

Blood sample was collected from each participating student after he/she agrees to be involved in the research process, and written informed consent was obtained from all study participants to assure their willingness. Blood samples were taken from finger pricks by qualified laboratory technicians, using the standard clinical procedure, with disposable lancet.

3.5.2.2. Blood group determination

The ABO and Rh-D blood groups were typed using commercial kits based on serological test of agglutination. The ABO and Rh blood groups of each subject was determined using cell grouping anti-A, anti-B and anti-D. Three drops of blood from each subject was placed on a clean slide in three places i.e. one drop at each place on the slide. A drop of one of the antisera, anti A, anti B and anti D was added and mixed with the aid of glass rods. Blood was mixed thoroughly with the antisera and rocked gently for 60 seconds to observe agglutination.

Blood group was determined on the basis of agglutination and recorded as blood type as compound blood types for ABO and Rh-D: A^+, B^+, AB^+, O^+ or A^-, B^-, AB^- and O^-. The blood sample was collected and typed by qualified laboratory technician following the standard clinical procedure with sterilized lancet, slides, and chemicals like Anti-A, Anti-B and Anti–D.

3.6. Methods of Data Analysis

In this study, the phenotypic distribution of blood groups was presented as simple percentages and frequencies. Allele and genotype frequencies were calculated from phenotypic frequencies by considering two alleles at the same locus for Rh-D system and three alleles at the same locus for ABO system using Hardy-Weinberg equilibrium equations.

3.6.1. Calculation of Blood Group Phenotype Frequencies

$$\text{Observed percentage} = \frac{\text{Observed number}}{\text{Total number}} \times 100$$

$$\text{Observed frequency} = \frac{\text{Observed number}}{\text{Total number}}$$

3.6.2. Allelic frequency determination of ABO and Rh-D blood groups

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

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

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

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

A correction factor (d) was calculated according to $d = 1 – p – q – r$. Then the final corrected allele frequencies would be:

$p_1 = p\ (1+d/2)$

$q_1 = q\ (1+d/2)$

$r_1 = (r+d/2)\ (1+d/2)$

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

$q = \sqrt{Rh-}$

$p = 1 - q$

3.6.3. Genotypic frequency determination of ABO and Rh-D blood groups

The genotypic frequencies of ABO blood group was calculated as follows:

$I^AI^A = p^2$ for homozygote AA

$I^AI^O = 2pr$ for heterozygote AO

$I^BI^B = q^2$ for homozygote BB

$I^B I^O$= 2qr for heterozygote BO

$I^A I^B$ =2pq for heterozygote AB

$I^O I^O$ = r^2 for homozygote OO

The genotypic frequencies of Rh-D blood group was calculated as follows

Genotype DD = p^2

Genotype Dd = 2pq

Genotype dd = q^2

3.6.4. Goodness-of-fit test between observed and expected phenotype frequencies

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test.

$$\text{Chi} - \text{square} = \frac{\sum(\text{Oi} - \text{Ei})^2}{\text{Ei}}$$

Where Oi and Ei are observed and expected frequencies respectively.

Expected phenotypic frequencies ABO and Rh-D blood groups 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 A blood group Ef = frequency of OO X number of total sample

For Rh+ = frequency of (DD + Dd) X number of total sample

For Rh- = frequency of dd X number of total sample

The number of degrees of freedom is simply the number of classes minus one; for intrinsic hypotheses it is usually determined as the number of classes minus one minus the number of independent parameters estimated from the sample. Hardy Weinberg equilibrium is a useful indicator of genotype frequencies within a population and whether they are based on a valid definition of alleles and a randomly mating sample. HWE assumes a stable population of adequate size without selective pressures and is used in human genetic studies as a guide to data quality by comparing observed genotype frequencies to those expected within a population (Griffith *et al.,* 2008).

3.6.5. Relationship between blood group systems and anthropometric measures

Mean and standard deviation were used to summarize the height, weight, and BMI of the participants. In addition, the association between blood groups (ABO and Rh-D) and height, weight and BMI of the participants was done using ANOVA using SPSS. The level of significance was set at ($P < 0.05$).

3.7. Ethical Consideration

Ethical clearance was obtained from Tigray Regional State Health Bureau Ethical Committee.

4. RESULTS AND DISCUSSION

4.1. Phenotypic Distribution of ABO and Rh-D Blood Groups

For this study, six hundred fourteen (614) individuals were selected randomly which consist of 331(53.9%) males and 283(46.1%) females. Mean age of the subjects was 19.04 ± 1.334 (male = 19.51 ± 1.367 and female = 18.48 ±1.049). Phenotypic distributions of ABO and Rh-D blood groups of the participants are presented in simple frequencies and percentages.

Table 4: Numbers and percentages of ABO blood types

ABO Blood types	Number	Percentage (%)
A	168	27.4
B	138	22.5
O	275	44.8
AB	33	5.4
Total	614	100

As shown in table 4, blood type O has the highest frequency while blood type AB has the least frequency. The percentage frequency of blood type O is 44.8% followed by blood type A, 27.4% and blood type B, 22.5% and the least percentage frequency is that of blood type AB which is 5.4%. The overall series of phenotypic frequencies of ABO blood group is phenotype O>A>B>AB. It shows phenotype O is the commonest blood type in the study area.

The distribution of ABO blood groups varies markedly in different ethnic groups and socioeconomic groups in different part of the world. It also varies from time to time in the same region (Srikant *et al.,* 2013). Many studies have shown that blood type O was the most common blood type and blood type AB was the least common blood group in different populations and ethnic groups (Nwauche and Ejele, 2004). The present data is in agreement with previous findings from Ethiopia and other parts of the world. For example, the study conducted in population of south west Ethiopia (at Gilgel Gibe Field Research Center) showed that the distribution of type O, is 42%; type A, is 31%; type B, is 21%; and type is AB, 6%

(Abraham *et al.,* 2012). The present study is also in agreement with the distribution of ABO blood group in Oromo, Amhara and Wolayita ethnic groups where blood type O is 42 %, 43% and 44.5% followed by blood type A, 28%, 29% and 27% and blood type B, 25%, 23% and 24% in Oromo, Amhara and Wolayita respectively and the least percentage frequency is that of blood group AB in the three ethnic groups which is 5%, 5% and 4.5% in Oromo, Amhara and Wolayita respectively (Nigusu, 2013). Kassahun and colleagues also found that blood type O was the most common blood type and blood type AB was the least common blood type in Silte Zone (Kassahun *et al.*, 2014).

When compared with other reports from similar studies, the results of this study are also consistent with previous findings from different parts of the world which found ABO blood type frequencies in order of O > A > B > AB (Saleh and Abood, 2016; Akwanjoh, 2014; Bakare *et al.,* 2006; Adeyemo and Soboyejo, 2006; and Iyiola *et al.*, 2011).

However; the present findings does not agree with the relative frequency of blood types determined to some population. For example, frequency of ABO blood group among the Banjara Population of Akola District, Maharashtra, India where ABO blood group frequency occurred in the order of B> O>A>AB (37.45%, 27.64%, 22.91%, and 12% respectively) (Chavhan *et al.*, 2012). 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). It is also not consistent with ABO phenotypic frequency of Armenian population in which A blood type has highest frequency (50%) followed by O blood type (31%), B blood type (13%) and AB blood type (6%) (ISBT, 2006).

Table 5: Percentage frequency of Rh-D blood types (n= 614)

Rh-D blood types	Number	Percentage (%)
Rh-D Positive	581	94.6
Rh-D Negative	33	5.4
Total	614	100

This study has shown that Rh-D positive (94.6%) has the higher percentage frequency while Rh-D negative (5.4%) has the least percentage frequency. These results are consistent with previous findings of Ethiopian populations (Abraham *et al.,* 2012; Tewodros *et al.,* 2011; and Nigusu, 2013). Again, the findings of this study are in agreement with reports from previous similar studies in different parts of the world where the Rh-D positive was found to be higher in the population sampled than the Rh-D negative (Ahmed *et al.,* 2007; Bakare *et al.,* 2006; Adeyemo and Soboyejo, 2006; Iyiola *et al.,* 2011). Rh-D negative blood group was documented as 5.5% in south India, 5% in Nairobi, 4.8% in Nigeria, 7.3% in Lahore, 7.7% in Rawalpindi. About 95% of African-Americans are Rh-positive (Chavhan *et al.,* 2010; Abraham *et al.,* 2012).

The results, however; differ from the work reported by Yousaf and colleagues where the population sampled among Bahawalpur division of Pakistan population were all Rh D positive (Yousaf *et al.,* 1988). It also disagrees with that of Njoku *et al.* (1996) who reported rhesus positive values of 100% in Nigeria. In addition, it is dissimilar to that in Indians with a preponderance of the Rh-D negative (89.7%) over the Rh-D positive (10.3%) (Thangaraj *et al.,* 1992).

Table 6: Distributions of the combined ABO and Rh-D blood group phenotypes among the students (n= 614)

ABO blood group Phenotypes	Rh-D blood group phenotypes		Total
	Rh-D Positive (+) frequency (%)	Rh-D Negative (-) frequency (%)	
O	262 (42.67%)	13 (2.12%)	275 (44.8%)
A	156 (25.4%)	12 (1.95%)	168 (27.4%)
B	130 (21.17%)	8 (1.3%)	138 (22.5%)
AB	33 (5.4%)	0 (0%)	33 (5.4%)
Total	581 (94.6%)	33 (5.4%)	614 (100%)

Table 6 presents the combined frequency distributions of ABO and Rh blood group phenotypes. The prevalence of the ABO phenotypes linked with Rh positive phenotypes were O+ (42.67%), followed by A+ (25.4%), B+ (21.17%), and AB+ (5.4%). In Rh negative phenotypes the frequencies were O^- (2.11%), followed by A^- (1.95%), B^- (1.3%), and there were no individuals with blood type AB who were Rh-D negative among the students of ACTE. In both Rh-D positive and Rh-D negative phenotypes O blood type has the highest percentage frequency while AB blood type has the least. This illustrates that Rh-D Positive and Rh-D negative incidences were recorded highest in O blood group, followed by A, B and AB. These results are consistent with previous findings of Ethiopian populations. For example, the study conducted in Silte zone shown that the frequency of ABO blood groups in both Rh positive and negative subjects among the three ethnic groups (Sodo, Silte and Meskan) was O> A >B > AB, except in the Sodo ethnic group where the blood group A was the commonest among Rh negative subjects (Kassahun *et al.,* 2015). The present study is also in agreement with the study conducted in south west Ethiopia (Jimma town blood bank) where blood type O has highest frequency in both Rh-D positive and Rh-D negative individuals followed by blood type A, B and AB blood type has the least frequency in both the Rh-D positive and Rh-D negative subjects (Teklu and Shiferaw, 2016). Similar pattern of frequency was also observed in other studies (Khattak *et al.,* 2008 and Anees *et al.,* 2007).

4.2. Genotypic and Allelic Frequencies of ABO and Rh Blood Groups

By using the extension of the Hardy-Weinberg law employed by Griffith *et al.* (2008) the genotypic and the allelic frequency distribution for the sample in the present study is calculated and listed in Table 7.

Allelic frequencies show a high frequency of allele I^O over I^A and I^B alleles in the order of $I^O > I^A > I^B$. The allelic frequencies were obtained as $I^O = 0.67$, $I^A = 0.18$ and $I^B = 0.15$ (Table 7). It shows similar patterns of allelic frequencies with those documented from earlier studies among various segments of the world population including Ethiopia. For instance in Ethiopia, study by Nigusu (2013) found allelic frequencies of $I^O = 0.6540 > I^A = 0.1821 > I^B = 0.1639$ in Oromo ethnic group; $I^O = 0.6600 > I^A = 0.1881 > I^B = 0.1519$ in Amhara ethnic group; and a frequency of $I^O = 0.6772 > I^A = 0.1729 > I^B = 0.1549$ for Wolayita ethnic groups. Similarly, previous studies among various segments of the world population have documented similar pattern of allelic frequencies. For instance, studies by Saleh *et al.* (2016) in north Baghdad population (Iraq), Chavhan *et al*. (2012) in Banjara Population of Akola District, Maharashtra, India and, Iyiola *et al.*, (2011) in Ilorin, Kwara State of Nigeria all found the allelic frequencies to occur in $I^O > I^A > I^B$ order.

Table 7: Allelic and Genotypic frequencies of ABO and Rh blood groups

Allele	Allelic frequency	Genotype	Genotypic frequency
I^O	0.67	I^OI^O	0.4489
I^A	0.18	I^AI^A	0.0323
		I^AI^O	0.2408
I^B	0.15	I^BI^B	0.0226
		I^BI^O	0.2015
		I^AI^B	0.0541
D	0.77	*DD*	0.5892
		Dd	0.3568
d	0.23	*Dd*	0.054

The genotypic frequency of the O blood type (I^OI^O) is obtained as 0.4489; whereas genotype I^AI^A is 0.0323; as well genotype I^AI^O is 0.2408. Genotype I^BI^B is obtained as 0.0226; whereas genotype I^BI^O is 0.2015. The genotypic frequency of I^AI^B for this study is 0.0541. Genotype I^OI^O has the highest frequency while genotype I^BI^B has the least frequency (Table 7). Similar results were reported by Iyiola *et al.* (2011), Saleh *et al.* (2016), Bakare *et al.*, (2006).

With respect to Rhesus blood group system, allelic frequencies show a high frequency of allele *D* over the *d* allele with a frequency of 0.77 and the *d* allele is obtained a frequency of 0.23. Genotypic frequency of Rh-D blood group was also calculated using the Hardy-Weinberg law. Accordingly, the genotypic frequency of homozygous Rhesus positive (*DD*) obtained for this study is 0.5901; whereas the heterozygous Rhesus positive (*Dd*) and homozygous Rhesus negative (*dd*) are obtained a frequency of 0.35615 and 0.05374 respectively (Table 7).

On the predominance of blood allele I^O over other blood alleles in the population sampled, the researcher agreed with the suggestion of Bakare *et al.* (2006), that predominance of I^O allele may be as a result of heterozygous advantage that many A and B blood types have been heterozygous carrying I^O allele silently thereby maintaining I^O allele in the heterozygous population. For example, this finding shows that, the frequency of I^AI^A genotype was 0.03236 while I^AI^O genotype was 0.24088. Thus, among those who are blood type A, 11.85 % were homozygous I^AI^A, while about 88.15% were heterozygous I^AI^O.

4.3. Chi-square Test for the Goodness of Fit of ABO and Rh Blood Group Distribution

This section presents the observed versus expected frequencies of ABO and Rh blood groups of the students treated in the research.

Table 8: Observed versus expected proportions of ABO and Rh-D blood groups frequency

	ABO blood group system					Rh-D blood group		
Blood type	A	B	AB	O	Total	Rh-D+	Rh-D-	Total
Observed	168	138	33	275	614	581	33	614
Expected	167.68	138.21	33.22	275.62	614.74	580.84	33.16	614
X^2= 0.00383 and, P value < 0.05						X^2= 0.00082, P value < 0.05		

Table 8 presents the observed proportions of ABO and Rh individuals in the studied population when compared with expected proportions. It also shows the chi-square ($\chi2$) and probability (P) value for the student population sampled in the study.

As it is shown in Table 8, the application of extended Hardy-Weinberg principle for three or more alleles yields little variation in the observed and expected genotypic frequencies which serves as a base in determining the chi-square ($\chi2$) values that further used in determining the goodness-of- fit. 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 $\chi 2 = 0.00383$, df=3, P-value <0.05) (Table 8). This shows that the population is at genetic equilibrium.

Similarly, Table 8 shows observed versus the expected values of Rh-D blood group phenotypes in the total sample. The variation of distribution of the overall observed frequencies of Rh-D blood group phenotypes from those expected under Hardy-Weinberg equilibrium were also insignificant (Goodness of-fit $\chi 2 = 8.2X10^{-4}$, df=1, P-value <0.05) (Table 8).

4.4. Relationship between Blood Groups (ABO and Rh-D) and Anthropometric Measurements

Table 9: Mean height, weight and BMI in relation to ABO blood group system

ABO blood types	Mean height (m) ± SD	Mean weight (Kg) ± SD	Mean BMI (kg/m^2) ± SD
A	1.63 ± 0.09	51.36 ± 5.62	19.29 ± 2.08
AB	1.58 ± 0.08	47 ± 5.32	18.77 ± 1.90
B	1.63 ± 0.09	50.80 ± 6.72	19.12 ± 2.09
O	1.64 ± 0.09	50.69 ± 6.30	18.93 ± 1.95

Mean height of subjects with blood type A, B, AB and O were found to be 1.63 ± 0.09 m, 1.63 ± 0.09 m, 1.58 ± 0.08 m and 1.64 ± 0.09 m respectively. Highest mean height was found in the subjects with blood type O (1.64 ± .09 m) where the lowest value was found in subjects with blood type AB (1.58 ± .08 m). This is not consistent with the result reported by Jafari *et al.* (2012) where individuals with blood type O had lowest value of mean height as compared to the other blood types. It also differs from the work reported by Borecki *et al.* (1985) where individuals with blood type B (B and AB) were taller than non-B (A and O) subjects.

As indicated in Table 9, mean weight of subjects with blood type A, B, AB and O were found to be 51.36 ± 5.62 kg, 50.80 ± 6.72 kg, 47 ± 5.32 kg and 50.69 ± 6.30 kg respectively. The highest mean weight was recorded in the subjects with blood type A i.e. 51.36 ± 5.62 kg and the lowest value was found in the subjects with blood type AB i.e. 47 ± 5.32 kg. These results are in agreement with the result reported by Jafari and colleagues where individuals with blood type A had highest mean weight as compared to the other blood types, however unlike to the result of this study individuals with blood type O had lowest mean weight (Jafari *et al.*, 2012).

Table 9 also presents the comparison of overall mean BMI values among different blood types of ABO blood group system. Mean BMI of subjects with blood type A, B, AB and O were found to be 19.29 ± 2.08 kg/m^2, 19.12 ± 2.09 kg/m^2, 18.77 ± 1.90 kg/m^2 and 18.93 ± 1.95 kg/m^2 respectively. The highest BMI was found in subjects with blood type A i.e. 19.29 ± 2.08 kg/m^2 where the lowest value was found in blood type AB 18.77 ± 1.90 kg/m^2. These results are in accordance with previous studies in Golestan province (Iran) which stated that mean of BMI were higher in blood type A compared to other blood types (Jafari *et al.*, 2012). Similarly, Parveen and colleagues found highest BMI in subjects with blood type A and lowest in blood type AB (Parveen *et al.*, 2016).

The present finding seems to deviate from the results obtained by Tulika and Ashish (2012) from India which found a correlation between blood type B and prevalence of obesity (high BMI) in blood donors. It also seems not to agree with another study from India where subjects with blood type O were found to have higher BMI in children as compared to other blood types (Siva *et al.*, 2012). It is also not consistent with the study conducted by Hercegovac and colleagues which found a highest average value of BMI in subjects with the blood type AB where the lowest value was found in subjects with blood type B (Hercegovac *et al.*, 2017).

Table 10: Mean height, weight and BMI of individuals in relation to Rh-D blood group system

Rh-D blood types	Mean height (m) ± SD	Mean weight (Kg) ± SD	Mean BMI (kg/m^2) ± SD
Rh-D Positive	1.63 ± 0.09	50.64 ± 6.27	19.06 ± 2.04
Rh-D Negative	1.65 ± 0.09	51.73 ± 5.40	19.10 ± 1.75

As shown in Table 10, subjects with blood type Rh-D negative were found to have higher Mean height (1.65 ± 0.09 m) as compared to the subjects with blood type Rh-D positive (1.63 ± 0.09 m). Similarly, individuals with blood type Rh-D negative were found to have higher mean weight (51.73 ± 5.40 kg) as compared to the individuals with blood type Rh-D positive (50.64 ± 6.27 kg).

With regard to BMI, subjects with blood type Rh-D negative were found to have higher mean BMI (19.10 ± 1.75 kg/m^2) as compared to the subjects with blood type Rh-D positive (19.06 ± 2.04 kg/m^2). In contrast, a study conducted by Parveen and colleagues found a higher mean BMI in subjects with blood type Rh-D positive compared to blood type Rh-D negative (Parveen *et al.*, 2016).

Table 11: Mean height, weight and BMI in relation to blood groups (ABO and Rh-D) in males and females

Blood type	Gender	Anthropometric characteristics		
		Height (m) ± SD	Weight (kg) ± SD	BMI (kg/m^2) ± SD
A	Female	1.57 ± 0.08	48.91 ± 5.50	19.56 ± 2.57
	Male	1.68 ± 0.06	53.75 ± 4.65	19.03 ± 1.43
B	Female	1.55 ± 0.05	47.52 ± 6.60	19.66 ± 2.44
	Male	1.69 ± 0.07	53.40 ± 5.61	18.70 ± 1.66
AB	Female	1.53 ± 0.05	45.30 ± 5.63	19.30 ± 2.06
	Male	1.67 ± 0.04	50.00 ± 3.02	17.85 ± 1.15
O	Female	1.57 ± 0.06	47.92 ± 6.35	19.44 ± 2.45
	Male	1.69 ± 0.07	52.80 ± 5.39	18.54 ± 1.36
Rh-D positive	Female	1.56 ± 0.06	47.91 ± 6.24	19.54 ± 2.49
	Male	1.69 ± 0.07	53.01 ± 5.25	18.64 ± 1.41
Rh-D negative	Female	1.60 ± 0.09	48.23 ± 4.00	18.88 ± 1.09
	Male	1.68 ± 0.08	54.00 ± 5.01	19.24 ± 2.09
Total	Female	1.56 ± 0.07	47.93 ± 6.22	19.51 ± 2.45
	Male	1.69 ± 0.07	53.10 ± 5.23	18.68 ± 1.46

As indicated in Table 11, Males were found to have higher mean height and weight as compared to females in all phenotypes of ABO blood group system. Highest mean height was found in male subjects with blood types B and O (1.69 ± 0.07 m and 1.69 ± 0.07 m respectively) whereas the lowest value was found in female subjects with blood type AB i.e. 1.53 ± 0.05 m. This is in contrast with the result obtained by Borecki and colleagues where blood type B (B, AB) subjects were taller than non-B (A, O) subjects (Borecki *et al.,* 1985). With regard to weight, highest mean value was found in male subjects with blood type A i.e. 53.75 ± 4.65 kg whereas the lowest value in female subjects with blood type AB i.e. 45.30 ± 5.63 kg. This is not in agreement with the study by Kelso and colleagues where weights of females with blood type A were more than other blood types (Kelso *et al.,* 1992).

With regard to BMI, highest mean BMI was found in females with blood type B i.e. 19.66 ± 2.44 kg/m^2 and the lowest mean BMI was found in males with blood type AB i.e. 17.85 ± 1.15 kg/m^2. Females were found to have higher mean BMI than males in all blood types of ABO blood group system. The result of this study is in agreement with the result obtained by Jafari and colleagues where mean BMI of females were higher as compared to males in all blood types (Jafari *et al.,* 2012). In contrast, a research conducted by Parveen and colleagues showed that males had greater mean BMI than females (Parveen *et al.,* 2016).

The result of the present study showed that males were found to have higher mean height and weight in both Rh-D positive and negative blood types of Rh-D blood group system. Highest mean height and weight were found in males with blood type Rh-D positive (1.69 ± 0.07 m) and Rh-D negative (54.00 ± 5.01 kg) respectively whereas the lowest value of mean height and weight were found in female subjects with blood type Rh-D positive (height: 1.56 ± 0.06 m; weight: 47.91 ± 6.24 kg). With regard to BMI, highest mean BMI was found in female subjects with blood type Rh-D positive i.e. 19.54 ± 2.49 kg/m^2 whereas the lowest mean BMI was found in males with blood type Rh-D positive i.e. 18.64 ± 1.41 kg/m^2. The result of the present finding is not in agreement with result obtained by Parveen and colleagues, where highest mean BMI was found in male subjects with blood type Rh-D positive (Parveen *et al.,* 2016).

Table 12: Comparison of mean height, weight and BMI among ABO, Rh-D blood groups and gender, by using ANOVA.

Variables	Anthropometric characteristic	P value
ABO blood types	Height	0.014
	Weight	0.003
	BMI	0.234
Rh-D blood types	Height	0.262
	Weight	0.329
	BMI	0.908
Gender	Height	< 0.001
	Weight	< 0.001
	BMI	< 0.001

The results of this study showed statistically significant differences among the different blood types of ABO blood group system regarding height and weight (P value < 0.05). Subjects with blood type O were found to have highest mean height (1.64 ± 0.09 m) and the lowest mean height was recorded in subjects with blood type AB (1.58 ± 0.08 m). With regard to weight, subjects with blood type A were found to have highest mean weight (51.4 ± 5.62 kg) and the lowest mean weight was found in subjects with blood type AB (47 ± 5.32 kg) (Table 9). This is not in accordance with the study conducted by Jafari and colleagues where height and weight were not associated with ABO blood group (Jafari *et al.*, 2012).

In the present study, the comparison of BMI among different blood types of ABO blood group systems did not show significant difference (P value > 0.05). This is in agreement with the study conducted by Ainee and colleagues in which comparison of BMI among different blood groups did not show significant difference (P >0.05) (Ainee *et al.,* 2014). Aboel-Fetoh and colleagues also found no statistically significant differences between different ABO blood types as regards BMI in Arar, Northern Saudi Arabia (Aboel-Fetoh *et al.,* 2016). Another study from Saudi Arabia also found no statistically significant difference between the prevalence of obesity or high BMI and ABO blood types (Alwasaidi *et al.*, 2017). In contrast, the study conducted by Parveen and colleagues found significant difference with P-value < 0.001 from the comparison of overall mean BMI values among different ABO blood types (Parveen *et al.*, 2016).

With regard to Rh-D blood group system, comparison of anthropometric characteristics (height, weight and BMI) among the two blood types of Rh-D blood group system (Rh-D positive and negative) did not show significant difference (P value >0.05). This is not in accordance with the study done by Parveen and colleagues where the comparison of overall mean BMI values among different blood types of Rh-D blood group system showed significant difference with *p*-value < 0.001 (Parveen *et al.*, 2016).

The overall comparison of mean height, weight and BMI among male and female subjects showed statistically significant difference with P value < 0.001. Males were found to have higher mean height and weight (height: 1.69 ± 0.07 m; weight: 53.10 ± 5.23 kg) compared with females (height: 1.56 ± 0.07 m; weight: 47.93 ± 6.22 kg). Gender differences of mean BMI showed that females had greater mean BMI (19.51 ± 2.45 kg/m^2) than males (18.68 ± 1.46 kg/m^2) (Table 11). This is in agreement with the result obtained by Jafari *et al.* (2012) where mean BMI was significantly higher among females than males. The result, however, differ from the work reported by Parveen *et al.* (2016) where males were found to have significantly higher mean BMI compared to females (P value < 0.05).

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

The research was conducted in Adwa College of Teachers Education (ACTE), Central Zone of Tigray regional state, Northern Ethiopia. The aim of the research was to provide information on the distribution pattern of phenotypes, genotypes and the allelic frequencies of ABO and Rh-D blood groups and to analyze the relationship between blood groups (ABO and Rh-D) and anthropometric characteristics (height, weight and BMI) among the students in ACTE.

A total of 614 individuals were selected randomly from the students of ACTE. Blood sample was taken from finger pricks of students by qualified medical laboratory technicians, using the standard clinical procedures, with disposable lancet. The ABO and Rh-D blood groups of each subject was determined using cell grouping anti-A, anti-B and anti-D. A drop of each of the antisera, anti A, anti B and anti D was added and mixed by shaker for 60 minutes with each blood sample and rocked gently to observe agglutination.

The frequency of blood type O was the highest with percentage frequency of 44.8% followed by blood group A which is 27.4% and blood group B is 22.5% and the least percentage frequency is that of blood group AB with percentage frequency of 5.4%. With regard to Rh-D blood group system, 94.6% of the total samples were Rh-D positive and 5.4% were Rh-D negative. The frequencies of I^O, I^A and I^B alleles were 0.67, 0.18 and 0.15 respectively. The frequency of allele *D* and *d* of Rh-D blood group were 0.77 and 0.23 respectively. Regarding the genotypic frequencies of ABO blood group, genotype I^OI^O has the highest frequency with 0.4489 followed by I^AI^O, I^BI^O, I^AI^B and I^AI^A with a frequency of 0.2408, 0.2015, 0.0541 and 0.0323 respectively and genotype I^BI^B has the least frequency with 0.0226. In the Rh-D blood group system, frequency of genotype *DD*, *Dd* and *dd* was 0.5892, 0.3568 and 0.054 respectively. The distribution of ABO and Rh-D blood group systems in the students did not differ significantly from those expected under the hardy Weinberg law (P-value < 0.05).

With regard to the relationships between blood groups (ABO and Rh-D) and anthropometric characteristics (height, weight and BMI), the present finding found a significant difference among blood types of ABO blood group system regarding height and weight (P value < 0.05)

but no significant difference was shown regarding BMI (P value > 0.05). Similarly, Rh-D blood types showed no relationship with anthropometric measurements (P-value > 0.05).

5.2. Conclusion

The distribution of ABO and Rh-D blood groups of this study has similar trends with the data from previous studies in Ethiopian populations and with most populations of the world. In ABO blood group system, blood type O has the highest frequency and blood type AB has the lowest frequency. In the Rh-D blood group system, blood type Rh-D positive has the highest frequency while Rh-D negative blood type has the lowest frequency. The order of the frequencies of ABO blood group alleles is $I^O > I^A > I^B$. In Rh-D system, frequency of allele *D* is higher than frequency of *d* allele. The chi-square test shows that the population is at the Genetic equilibrium.

Regarding the relationship between blood groups (ABO and Rh-D) and anthropometric measurements (human height, weight and BMI), height and weight showed significant difference among different blood types of ABO blood group system with P-value < 0.05. Subjects with blood type O were relatively taller than the other blood types and the least mean height was recorded in blood type AB subjects. With regard to weight, subjects with blood type A were associated with high mean weight and blood type AB subjects were found to have low mean weight. However, BMI did not show significant difference among different blood types of ABO blood group system (P-value > 0.05). In Rh-D blood group system, height, weight and BMI were not associated with Rh-D blood types (p-value > 0.05).

To sum up, this study provides information on the phenotypic, genotypic and allelic frequencies of ABO and Rh-D blood group systems of the students in Adwa College of Teachers Education. In addition to this, the present finding provided data on the relationships between blood groups (ABO and Rh-D) and anthropometric characteristics (height, weight and BMI). The knowledge of frequencies and distribution of the different blood groups is very important for blood banks and transfusion services so that they could contribute significantly to the National health system to formulate the policy. Having knowledge of own blood group is important for everyone. It saves lives when transfusion is needed and neonates. It is also important for geographical information, genetic studies and for forensic studies in the population. Furthermore, the data generated in this study would serve as a reference for other similar studies and future utilities in health planning.

5.3. Recommendations

- ✓ The data generated in this study would be helpful as a base for researchers who are interested to conduct similar type of study.
- ✓ The sample size used to conduct this study was small. Therefore, it is advisable to use larger sample size to obtain more accurate data regarding the pattern of distribution on these blood groups, and the association between blood groups (ABO and Rh-D) and anthropometric characteristics (height, weight and BMI).
- ✓ There are techniques like PCR, ELISA that allows a more detailed determination of blood group typing and therefore a better match for transfusion. So, the researcher recommend to all concerned governmental and non-governmental organizations to facilitate the use of such blood group typing mechanisms in hospitals where blood transfusion services are highly prevalent.

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7. APPENDICES

Appendix I: Consent Form

I the undersigned have been informed and understand the purpose and objectives of the study that plans to assess the distribution of ABO and RhD blood group systems and their correlation with anthropometric measurements (height, weight and BMI). I have been informed that qualified and experienced laboratory technician would do the blood collection according to the established aseptic procedures by using sterile disposable lancet. I have been also informed that all laboratory results would be kept confidential.

I have been given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I have gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name of the student ___________________

Age ___________ Sex _______ year ___________

Signature ___________________

Date: ___________________

Appendix II: Data Collection Format

Appendix Table 1

Sample code	Age	sex	Place of birth	Place of residence before joining the college	Blood group		Height (m)	Weight (Kg)	BMI
					ABO	Rh			
AD001									
AD002									
AD003									
AD004									
AD005									
AD006									
AD007									
AD008									
AD009									
AD010									
AD011									
AD012									
AD013									
AD015									
AD016									
AD017									
AD018									
AD019									
AD020									

Appendix III: Probability Values for Chi-square Analysis

Appendix Table 2

Degree of freedom	Probability										
	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.09	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59
	Non-significant								Significant		

Appendix IV: Calculation of Allele, Genotype and Expected Frequencies of ABO and Rh-D Blood Groups

Phenotypic frequencies: (blood type O= 44.8, A= 27.4, B= 22.5 and AB= 5.4; Rh-D^+= 94.6, Rh-D^-= 5.4)

$r = \sqrt{O} = \sqrt{0.448} = 0.6693$

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

$p = 1 - \sqrt{(0.225+0.448)}$

$p = 1 - 0.8204 = 0.1796$

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

$q = 1 - \sqrt{(0.274+0.448)}$

$q = 1 - 0.8497 = 0.1503$

A correction factor (d) was calculated according to $d = 1 – p – q – r$.

Therefore, $d = 1 - 0.1796 – 0.1503 – 0.6693 = 0.0008$

The final allele frequencies were then calculated as follows: $p1 = p (1 + d/2)$; $q1 = q (1 + d/2)$; r1

$= (r + d/2) (1 + d/2)$ where p1, q1, and r1 are corrected allele frequencies of allele I^A, I^B, I^O respectively.

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

$p1 = 0.1796 (1 + 0.0008/2)$

$p1 = 0.18$

q1 = q (1 + d/2)

q1 = 0.1503 (1 + 0.0004)

q1 = 0.15

r1 = (r + d/2) (1 + d/2)

r1= (0.6693 + 0.0004) (1 + 0.0004)

r1 = 0.67

So the allelic frequencies of I^A = 0.18, I^B = 0.15, I^O = 0.67

Calculating the Genotypic frequencies and Expected number

- Genotype I^{AA} = p^2 = $(0.18)^2$ = 0.0323, Exp. No. = 0.0323 x 614 = 19.83
- Genotype I^{AO} = 2pr = 2 (0.18 * 0.67) = 0.2408 Exp. No. 0.2408*614= 147.85

Therefore, the expected number of blood type A is 167.68

- Genotype I^{BB} = q^2 = $(0.15)^2$ = 0.0226 Exp. No. 0.0226*614= 14.49
- Genotype I^{BO} = 2qr= 2 (0.15*0.67)= 0.2015 Exp. No. 0.2015*614= 123.72

So, the expected number of blood type B is 138.21

- Genotype I^{OO} = r^2 = $(0.67)^2$ = 0.4489 Exp. No. 0.4489*614= 275.62
- Genotype I^{AB} = 2pr = 2 (0.18*0.15) = 0.0541 Exp. No. 0.0541*614= 33.22

Therefore, the expected number of blood type O and AB are 275.62 and 33.22 respectively.

The Chi-square (χ^2) test statistic is then

$$\chi 2 \text{ ABO } = \frac{\sum(\text{Oi} - \text{Ei})^2}{\text{Ei}}$$

$$\chi 2 \text{ ABO } = \frac{(275 - 275.62)^2}{275.62} + \frac{(168 - 167.68)^2}{167.68} + \frac{(138 - 137.21)^2}{137.21} + \frac{(33 - 33.22)^2}{33.22}$$

$\chi 2$ ABO= 0.00383

The degree of freedom is 4-1= 3, P-value < 0.05

Calculating the allelic frequencies of Rh-D positive and Rh-D negative blood types

p + q = 1 for allele frequencies and,

$p^2 + p^2 + 2pq = 1$ for genotype frequencies

q= √Rh-D negative = √0.054= 0.23

p= 1 - q = 1- 0.2324 = 0.77

So the allelic frequencies of D and d alleles are 0.77 and 0.23 respectively.

Calculating the Genotypic frequencies and Expected number

- Genotype DD = $p^2 = (0.77)^2$ = 0.5892 Exp. No. 0.5892*614 = 361.77
- Genotype Dd = 2pq = 2(0.77*0.23) = 0.3568 Exp. No. 0.3568*614 = 219.07

Therefore, the expected number of Rh-D positive is 580.84

- Genotype dd = $q^2 = (0.23)^2$ = 0.054 Exp. No. 0.0529*614 = 33.16

Therefore, the expected number of Rh-D negative is 33.16

The Chi-square (χ^2) test statistic is then

$$\chi 2 \text{ Rh} - \text{D} = \frac{(581 - 580.84)^2}{580.84} + \frac{(33 - 33.16)^2}{33.16}$$

χ^2 Rh-D = 0.00082

The degree of freedom is 1, P-value < 0.05

Appendix V: Ethical Clearance

ብሄራዊ ክልላዊ መንግስቲ ትግራይ
ቢሮ ሓለዋ ጥዕና

The Government of the National Regional
State of Tigray, Bureau of Health

ቁፅሪ ትፃ/1918/2010

ዕለት 27/3/2010

ናብ ዓድዋ መምህራን መሰልጠኒ ኮሌጅ
ዓድዋ

ዋኒኑ:- ትሕብብር ይምልከት

ኣብ ላዕሊ ክም ዝተገለፀ ኣብ ሃራማያ ዩኒቨርሲቲ ባዮሎጂካል ሳይንስን ባዮቴክኖሎጂ ትምህርቲ ክፍሊ ካልኣይ ድግሪ \ማስተርስ ተምሃራይ ዝኾነ ሃፍቶም ገብረማርያም "Distribution of ABO and Rh Blood Groups and Their Association with Anthropometric Measurements Among Students of Tigray Ethinic Group in Adwa College of Teachers Education, Tigray Region, Ethiopia, 2017/18" ብዝብል ኣርእስቲ በቲ መፅናዕታዊ ስነ ምግባር ብዝፈቐዶ መሰረት ገይሩ መፅናዕቲ ስለዘካይድ ኣብ ዘካይዶ መፅንዓቲ ዘድሊ ምትሕብባር ኹሉ ንኽገብረሉ ነተሓሳስብ::

"ሽቶ ካልኣይ ትልምን ዕብየትን ስግግርን ንምዕዋት ኩሉ ይረባረብ!!"

ኣብራሂም ሓሰን
መተሓባበሪ መፅናዕትታት፣ፕሮጀክትታትን ዓለም ለኸ ርክባትን

ቅዳሕ
- ተምሃራይ ሃፍቶም ገብረማርያም/ Principal Investigator/
ሃራማያ ዩኒቨርሲቲ

ብሄራዊ ክልላዊ መንግስቲ ትግራይ ቢሮ ሓለዋ ጥዕና
The Government of the National State of Tigray Bureau of Health

✉	☎	ፋክስ Fax	ኢ.መይል E-mail	መቐለ ትግራይ ኢትዮጵያ
07	251034-440-02-22 251-034-440-31-10	251-034-440-88-30	tigrayhealth@telecom.net.et	Mekelle, Tigray, Ethiopia

251-034-441-01-00 251-034-441-01-03 251-034-441-01-02