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Assessment of Abo and Rh Blood Group Frequencies among Students of Tagel Secondary and Preparatory School, South Gondarzone, Ethiopia

Birtukan Shumet

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

ASSESSMENT OF ABO AND RH BLOOD GROUP FREQUENCIES AMONG STUDENTS OF TAGEL SECONDARY AND PREPARATORY SCHOOL, SOUTH GONDARZONE, ETHIOPIA

MSC THESIS

BY

BIRTUKAN SHUMET

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BAHIR DAR, ETHIOPIA

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ASSESSMENT OF ABO AND RH BLOOD GROUP FREQUENCIES AMONG STUDENTS OF TAGEL SECONDARY AND PREPARATORY SCHOOL, SOUTH GONDAR ZONE, ETHIOPIA

A THESIS SUBMITTED TO COLLEGE OF SCIENCE, BAHIR DAR UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

BY:

BIRTUKAN SHUMET

ADVISOR: ZIYIN MIHRETIE (PhD)

OCTOBER, 2020
BAHIR DAR, ETHIOPIA

DECLARATION

I declare that this thesis is my original work in partial fulfillment for the requirements for the degree of Master of Science in Biology. All the sources of the materials used for this thesis and all people and institutions who gave support for thesis work are fully acknowledged.

Name: Birtukan Shumet

Signature: _____

Date: _____

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Approval Sheet for Defense

As a thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my supervision, by Birtukan Shume ttitled as **"Assessment of ABO and Rh Blood Group Frequencies among Students of Tagel Secondary and Preparatory School, South Gondar Zone, Ethiopia"** I recommended the paper to be submitted as fulfilling the requirement for the Degree of Master of Science in Biology.

Advisor's name	Signature	Date
Department head name	Signature	Date

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Approval Sheet for Defense Result

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Birtukan Shumet and examined the candidate. We recommended the thesis to be accepted as fulfillment for the requirements of the Degree of Master of Science in Biology.

Board of Examiners

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DEDICATION

This paper is dedicate to my beloved family for their endless advise, support and encouragement for my success.

ABSTRACT

The distribution of blood groups was different from place to place and may not be found in similar numbers even among ethnic groups. Frequency distribution of blood groups is important for modern medicine, genetic research, anthropology, ancestral relations of humans and forensic science. The aim of this study was to determine the allelic, genotypic and phenotypic frequency distribution of ABO and Rh blood groups among students attending in Tagel preparatory and high school in Simada woreda, Ethiopia from September to August 2020. Blood samples were taken from 384 volunteer students by finger-pricks and blood groups were determined by haemagglutination method using commercially available anti-sera A, B, and Rh (D). Descriptive statistics was used to calculate percentage frequencies of blood phenotype. The genotypic and allelic frequencies of the blood groups were calculated from the observed phenotypes under the assumption of Hardy–Weinberg equilibrium. In the ABO system, type O was the most prevalent (34.63%) followed by A (32.03%), B (23.95%) and AB the least (9.37%). In case of Rh system, most of the students were Rh+ (96.1%) and the rest were Rh-(3.9 %). The allelic frequencies of O (I^o), B (I^B) and A (I^A) were 0.58, 0.22 and 0.19, respectively. While allelic frequencies of the Rh blood group of D and d were 0.803 and 0.197, respectively. Genotypic frequencies of OO, AA, AO, BB, BO, and AB were 0.336, 0.048, 0.255, 0.036, 0.220 and 0.084 respectively. While the genotypic frequency of DD, Dd and ddwere0.64, 0.31, and 0.039 respectively. The present study gives baseline information regarding the management of blood bank and transfusion services in the community. Large sample size with different areas is recommended to make strong generalizations.

Key words: Blood group, Tagel preparatory and high school, prevalence, Rh, ABO

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

Rh……Rhesus

CSA…….Central statistical agency

RBCs…..…….Red blood cells

Ig……....Immunoglobulin

SWARDO…….……. Simada woreda agriculture and rural development office

SPSS…….…..Statistical package for social science

1. INTRODUCTION

1.1. Background

Blood is the red liquid that circulates through the blood vessels of humans and other vertebrate animals, carrying substances such as nutrients and oxygen to the cells and metabolic waste products like carbon di oxide away from the cells (Okoduwa, 2013).

Blood group is the classification of blood depending on the presence or absence of antigen on the surface of red blood Cells (RBCs). Red blood cell antigens may be proteins, glycoproteins, or glycolipids. Most red blood cell antigens are synthesized by the cells; however, some antigens like the Lewis and Chido/Rogers systems are synthesized during entering to the red blood cell membrane from the plasma. Antigens such as ABO and Rh-D are specific to red blood cells and others are found on other cells throughout the body (John *et al.*, 2003).

Human red blood cell contains a variety of blood group antigens. The most important and best known are A and B antigen, which are actually complex oligosaccharide that differ in their terminal sugar on RBC. These are mostly glycolsphingo lipids. The antibodies against red blood cell antigens are called agglutinins. Individual are divided into four major blood groups A, B, AB and O, based on the presence of these antigens and agglutinins (Ganong, 2003). Different types of polysaccharide antigens, called agglutinogen (Ganong, 1995). The antigenic substances are capable of inducing a specific immune response (Novak, 1995) and that specific response results in the production of cells termed as antibodies (Hoffbrand, 1981). Antigens may either be erythrocytes, leukocytes or platelet related (Talib, 1991).

In human there are a total of 36 human blood group systems which recognized by the International society of blood transfusion (ISBT) Storry *et. al*., 2016.Butthe major blood group system is ABO blood type based on A and B antigen, and the second one is Rh blood type depends on the presence or absence of Rh antigens, (Alhowaish, 2013).

The ABO blood group system is discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900 (Garratty *et al*., 2000). He described A, B and O blood

groups for which he was awarded the Nobel Prize in 1930. After two years Alfred Von Decastello and Adriano Sturli additionally discovered the fourth type AB in 1902

(Vondecastella *et al.*, 1902). While Landsteiner and Weiner in 1940 discovered the Rhesus (Rh) blood group (Iyiola *et al.*, 2011).

The gene which controls the development of ABO blood group in human has three alleles: A, B, and O; it is said to be multiple allelism. Sometimes these A, B, and O notations may be written in the form of I^A, I^B, and I^O. The genotype of this blood type is: AA, AO, BB, BO, AB, and OO; and in case of phenotype there are four blood groups: A, B, AB, and O. The term genotype is used for the sum of the inherited alleles of particular gene (e.g. AA, AO), and genes controlling on the red blood cells of ABO group system expressed as either codominant (A and B) or complete dominant (A and B over O). The phenotype refers to the observable product of the alleles on the red blood cells surface structure as antigen (Lewis *et al.*, 2006).

The Rh blood group is named for the rhesus monkey, in which the Rh antigens were discovered in 1940. It is the most complex and immunogenic of the human blood-group systems with 52 well-defined antigens. The Rh blood group is found next to ABO groups in clinical importance because of their relation to haemolytic disease of the newborn (HDN) and their importance in blood transfusion (Adeyemo and Soboyejo, 2006; Bakare *et al.*, 2006).it is genetically complex but simply described in terms of a single pair of alleles, D and d. The genotype of this blood group is DD, Dd and dd (Daniels, 2002; Saladin, 2003).

The frequency of the ABO and Rh blood groups vary in the world and may not be found in the same numbers in various populations. The highest frequency of blood group in the world population had group O and Rh positive, whereas the least frequency blood group is AB and Rh negative (Boskabady *et al*., 2005; Nwaopoara *et al*., 2008; Pandey *et al*., 2013;Getaneh Alemu and Mohammedaman Mama, 2015; Kooffreh *et al*., 2015).

Different studies shows about ABO blood group distributions in Ethiopia there are variation in different ethnic groups (Falusi *et al*., 2000). In Silte zone, Kassahun *et al*., (2015) reported that frequency of O (41.0%), A (24.5%), B (21.3%) and AB (5.2%) and 92.06% Rh+ and 7.94% Rh-. Among Sidama ethnic group the phenotypic frequency of ABO blood group type O (51.3%), type A (23.5%), type B (21.9%) and type AB (3.3%) (Tewodros *et al.*, 2011).

According to Habtamu Tadesse (2019).the genotypic frequency of ABO blood group were OO (0.4) ,AO(0.24), BO(0.2),AB(0.064),AA(0.03) and BB (0.02) in west shewa zone oromia region. Previous studies conducted in Oromia National Regional State (Amsalu Waqgarie and Daniel Gietahun, 2017) the allelic frequencies of O (0.64), A (0.21) and B (0.15); In the similar way (Amsalu Wakgarie and Daniel Gietahun, 2017) reported that the frequency of Rh factor were DD (0.5329),Dd (0.3942), dd(0.0729).In Oromia National Regional State the allelic frequencies of D (0.73), and d (0.27) (Amsalu Waqgarie and Daniel Gietahun, 2017),

This is important test performed in blood banking services to avoid morbidity and mortality, which means patients with any blood type must rely on donors with matching blood types and apply successive blood transfusion (Nwaopara *et al*., 2008). Also currently used in forensic science that used to demonstrate crime sense like impossibility of paternity (Okoduwa, 2013).

1.2. Statement of the problem

Many scholars carried out research in different countries like Nigeria, India, America and others on the frequency distribution of ABO and Rh blood groups, until this time, no research was conducted on simada woreda related with this study. So not known ancestral relationship between their parent, no documented data used for genetic research and for clinical aspects, maternal mortality, hemolytic disease for infants, and lack of awareness about blood transfusion. Due to this reason the study was designed to determine the phenotypes and genotypes, and the allele frequencies distribution of ABO and Rh-D blood group among the students.

1.3. Significance of the study

The knowledge of frequency distribution of ABO and Rh blood group at local and regional level is helpful in the effective management of blood banks and safe transfusion services. ABO and Rhesus (Rh) blood group are useful in population genetic studies, researching population migration patterns. The need for blood group prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease and environment. And also prevent

hemolytic disease of new born infant and reducing many preventable diseases. Currently used in forensic science that used to demonstrate crime sense like impossibility of paternity.

1.4. Objective of the study

1.4.1. General objective

- ✓ To determine the ABO and Rh blood group frequencies among students of Tagel preparatory and secondary school.

1.4.2. Specific objective

- ✓ To determine the phenotypic frequency of ABO blood types among students at Tagel preparatory and secondary school.
- ✓ To determine the phenotypic frequency of Rh blood types among students at Tagel preparatory and secondary school.
- ✓ To determine the allelic frequency of ABO blood group among students at Tagel preparatory and secondary school.
- ✓ To determine the allelic frequency of Rh blood group among students at Tagel preparatory and secondary school.
- ✓ To determine the genotypic frequency of ABO blood group among students at Tagel preparatory and secondary school.
- ✓ To determine the genotypic frequency of Rh blood group among students at Tagel preparatory and secondary school.

1.5. Limitation of the study

The extent of the study was limited from wogeda town especially. tagel preparatory and secondary school of Simada wereda. Moreover, the study was limited in content focusing only ABO and RH blood group not relate with other traits. The other limitation was smaller sample size .The reasons for such limitation were finance and time

2. LITERATURE REVIEW

2.1. Human blood group system

People have different blood groups, based on the presence or absence of antigen on the red blood cell (Dean, 2005). There are a total of 36 human blood group systems recognized by the International society of blood transfusion (ISBT) Storry *et al.*, 2016.The two main blood groups are called ABO and Rh type. Both are useful in blood transfusion and organ transplantation (Khan *et al.*, 2009; Eweidah *et al.*, 2011).

2.1.1. ABO blood group

The ABO blood group system was first discovered by Karl Landsteiner in 1901 and consists of three main alleles: these are two co dominant A and B and the other recessive allele is called O (Owen, 2000; Loscertales *et al.*, 2007). There are four major phenotypes, namely A, B, AB, and O (Ghasemi *et al.*, 2010; Yamamoto *et al.*, 2012) depend on the presence or absence of A and B antigens and antibodies at the surface of red blood cells (Calafell, 2008; Criswell, 2008).

Red blood cell antigens will be proteins, glycoproteins, or glycolipids. Most Red blood cell antigens are synthesized by the cells; however, some antigens, such as those of the Lewis and Chido/Rogers systems, are adsorbed onto the red blood cell membrane from the plasma. Some antigens such as ABO and Rh-D are specific to red blood cells, and others are found on other cells throughout the body (John *et al.*, 2003).

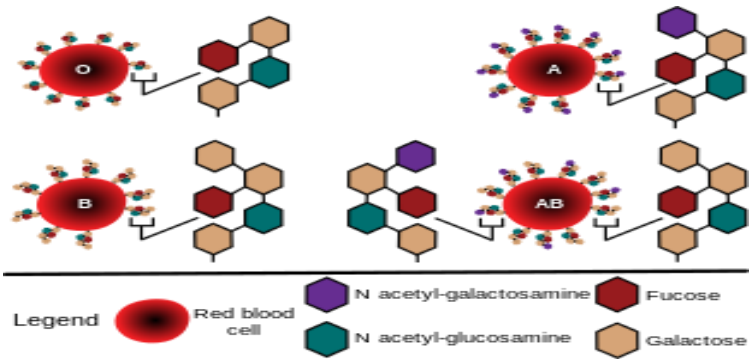

Figure 1: A diagram showing the structural differences on the surface of red blood cells used to classify them into ABO blood groups. All the four blood groups contain fructose, galactose and N acetylglocusamine in common. (Wikimedia Commons, 2019)

The ABO blood type alleles A and B thus codes for glycosyltransferase which transfer GalNac and galactose respectively (Kitano *et al*., 2009), while O is a null allele incapable of coding for a functional glycosyltransferase (Daniels, 1997). In addition to expression on red blood cells, ABO antigens are also highly expressed on the surface of epithelial cells (Xie, 2010; Than *et al.*, 2011). On RBCs, they are mostly glycosphingolipids. The antibodies against red cell antigens are called agglutinins and individuals are divided into four major blood groups A, B, AB and O according to the presence of these antigens and agglutinins (Jeremiah, 2006) .

In humans, AB antigens are widely expressed on a variety of cell surface molecules in many tissues and hormones, depending on the ABO genotypes of the individuals. These include glycoproteins and glycolipids on mucous cells, nerve cells, red cells, epidermis, and vascular endothelium (Suzuki, 2005; Than *et al*., 2011; Yamamoto *et al*., 2001). ABO blood types are also present in some other animals, like apes such as chimpanzees, bonobos, and gorillas (Dean, 2005; Yamamoto *et al*., 2001).

2.1.1.1. Phenotypic Frequency Distribution of ABO blood group

The frequency distribution of ABO blood group was different in the world. From these population blood type O is higher frequency and blood type AB is the least while A and B is vary or interchangeable in different studies. For example, 55.2% of Calabar in Nigeria, 63.7% of Ekpoma in nigeria, 38% of Bihar in India are shows type O (Nwaopoara *et al*., 2008; Pandey *et al*., 2013; kooffreh *et al*., 2015).

The population of Ekpoma in Nigeria showed that a higher percentage of the population (63.73%) were blood group O, while those with blood group A, B and AB are (17.62%), (16.58%) and (2.07%), respectively (Nwaopara *et al*., 2008). In the same way (Pandey *et al*., 2013) in India Bihar population showed that the frequency of blood group O (38%) was highest followed by B (33.4%), A (21.8) and AB (6.8),but the difference is the frequency of blood group A and B.

According to (kooffreh *et al.,* 2015) in Calabar, Nigeria, observed that blood group O had the highest frequency (55.2%), followed by B (21.6%), A (18.8%) and AB had the least (4.4%)

and Boskabady *et al*., (2005) showed that the percentage of blood groups A, B, AB and O among the city of Mashhad population was 23.1%, 23.3%, 8.9% and 34.7% respectively.

Table 1: World distribution of ABO blood type by groups of people from different Nations based on Beckman (2008) report.

People	O	A	B	AB	People	O	A	B	AB
Aborigines	61	39	0	0	Chinese-Canton	46	23	25	6
Abyssinians/Ethiopia	43	27	25	5	Chinese-Peking	29	27	32	13
Albanians	38	43	13	6	Chuvash	30	29	33	7
Ainu (Japan)	17	32	32	18	Czechs	30	44	18	9
Americans	31	49	14	6	Danes	41	44	11	4
Arabs	34	31	29	6	Dutch	45	43	9	3
Asian (in USA - General)	40	28	27	5	Egyptians	33	36	24	8
Austrians	36	44	13	6	British	47	42	9	3
Bantus	46	30	19	5	Eskimos-Alaska	38	44	13	5
Basques	51	44	4	1	Eskimos-Green land	54	39	5	2
Blackfoot (N. Am. Indian)	17	82	0	1	Estonians	34	36	23	8
Belgians	47	42	8	3	Fijians	44	34	17	6
Bororo (Brazil)	100	0	0	0	Finns	34	41	18	7
Brazilians	47	41	9	3	French	43	47	7	3
Bulgarians	32	44	15	8	Georgians	46	37	12	4
Burmese	36	24	33	7	Germans	41	43	11	5
Buryats (Siberia)	33	21	38	8	Grand Andamanese	9	60	23	9
Bushmen	56	34	9	2	Latvians	32	37	24	7

As it can be seen from Table 1 , Aborigines have 61% “O” type but 0% for “B” and “AB” type; Bororo/Brazilians have 100% “O” type; and Ethiopians have 45% “O” type and 5% “AB” type.

Other unique distributions in Grand Adamanese scored 60%for "A" type blood group. Like Nwauche and Ejele (2004) and many other researchers have reported that blood group O was the most common blood group and blood group AB was the least common blood group in some ethnic groups.

In African-Americans blood type O, A, B, and AB have 46%, 27%, 20% and 7% distributions, respectively. Among the Caucasians in the United State, the distribution was O = 47%, A = 41%, B = 9% and AB = 3%. This distribution, with little difference, was reported as the distribution among Western Europeans: O = 46%, A = 42%, B = 9% and AB = 3% (Adeyemo and Soboyejo, 2006).

The O blood type is very common around the world; about 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of central and South America where it approaches 100%. The lowest frequency of O type is found in Eastern Europe and central Asia, where B is common (Khan *et al.*, 2009). From the Bangladish society the ABO blood group type distribution showed that type "B" covered the highest frequency (39.8) whereas type "O" covered 27.6% which is the second highest frequency (Sultana *et al.*, 2013).

Different studies shows about ABO blood group distributions in Ethiopia there are variation in different ethnic groups (Falusi *et al*., 2000). In Silte zone, Kassahun *et al*., (2015) reported that frequency of O (41.0%), A (24.5%), B (21.3%) and AB (5.2%) and 92.06% Rh+ and 7.94% Rh-. Among Sidama ethnic group the phenotypic frequency of ABO blood group type O (51.3%), type A (23.5%), type B (21.9%) and type AB (3.3%) (Tewodros *et al.*, 2011). Similarly, (Teklu and Shiferaw, 2016) reported that blood type O (43.0%), A (32.0%), B (21.5%) and AB (3.5%). Fekadu (2015) reported the general Ethiopians ABO blood group distribution of type O, 40%, type A (31%), type B (23%) and type AB (6%).

2.1.1.2. Allelic frequencies of ABO blood groups

Previous studies conducted in Oromia National Regional State (Amsalu Waqgarie and Daniel Gietahun, 2017) the allelic frequencies of O (0.64), A (0.21) and B (0.15); Kassahun Tesfaye *et al*., (2015) in a similar study conducted in Silte zone, Ethiopia, the allelic frequencies of O (r), A (p) and B (q), were 0.65, 0.19 and 0.15 respectively. The pattern of allelic frequencies were

$I^{o}>I^{A}>I^{B}$ (Kassahun Tesfaye *et al*., 2015). In the general Indian subcontinent, (Yassin, 2013) reported that allelic frequencies of p (0.154), q (0.249), r (0.591),

2.1.1.3. Genotypic frequency of ABO blood group

According to Habtamu Tadesse (2019).the genotypic frequency of previous study OO (0.4) ,AO(0.24), BO(0.2),AB(0.064),AA(0.03) and BB (0.02) in west shewa oromia region. In the similar way (Amsalu Wakgarie and Daniel Gietahun, 2017) reported that OO (0.413) AA (0.043) AO (0.268) BB (0.021) BO (0.186) AB (0.06) in oromia region.

2.1.1.4. Genetics of ABO Blood Group

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene with three types of alleles (multiple alleles) explains by classical genetics: I^O, I^A, and I^B. I designation stands for isoagglutinogen or antigen. The gene encodes a glycosyltransferase that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome. (Criswell, 2008)

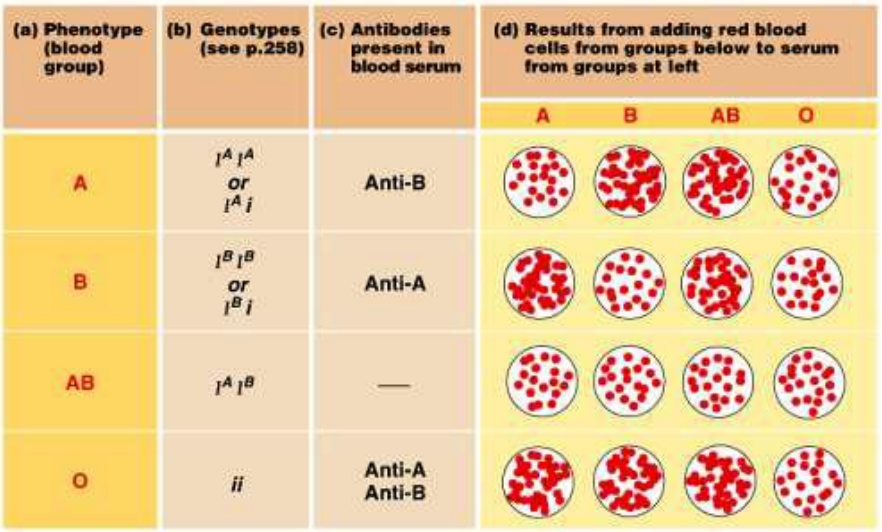

Figure 2: A diagram showing the possible types of interactions, blood coagulation, made when bloods from different individuals are mixed up in the form of blood donor and recipient. [The picture from Wikimedia Commons freely licensed media file repository, 2008]

The I^A allele gives type A, I^B gives type B, and *i* gives type O. As both I^A and I^B are dominant over *i*, only *ii* people have type O blood. A person with I^AI^A or I^Ai have type A blood, and with I^BI^B or I^Bi have type B. I^AI^B people have AB blood type , because A and B express a special dominance relationship: codominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi,I^Ai). The *cis-AB* phenotype has a single enzyme to creates both A and B antigens (Yazar *et al.*, 2006).

2.1.1.5. Characteristic feature and life style of ABO blood group

Blood group A; A person of this blood type was Intelligent, Not suitable for Leadership positions by instinct. Needs much relaxation and focus on doing exercises. It is favorable to eat Beans, fruits like pineapples, and sea food for his/her digestive and immune system is sensitive. Who will be susceptible to some diseases like diabetes, anemia, and heart diseases (Atoom, 2014)

Blood Group B**:**in this blood type a person will be Flexible, creative, live and eat in a balance way, love to work exercises, strength full over difficulties in life. High intellectual activity, have harmony in addition to inner peace. Fewer tendencies to challenge and confront. Who eat all kinds of food but in a balanced way. He is Susceptible for different diseases but rare in viruses (Atoom, 2014).

.Blood Group (AB): the person of this blood type Called spirituals. Gently receive all life style and without any negative perception of the consequences. Mostly charming and exciting people which sometimes fall them in an Emotional problems. It advises to feed fish, vegetables, and milk products. (Atoom, 2014).

Blood Group (O): in this blood type there is physical and personal strength, stamina, self-reliance, courage, pursuit of success, leadership features, power, and optimism. It is favorable to eat meat, fish, vegetables, and fruit and they are advised to focus on proteins it is susceptible to Infectious diseases (Atoom, 2014).

2.1.2. Rh blood group system

According to, (Dubroff and Joseph, 2003; Adeyemo and Soboyejo, 2006) the Rh blood type is known as Rhesus factor. It is an antigen that exists on the surface of red blood cells in most people. People who have the Rh antigen to have Rh positive (+) blood type while others who don‘t are considered as Rh negative (-) blood type. In contrast to the situation encountered in antigen A and B, the plasma of Rh negative individual does not have ant-Rh (Martini, 2004).

The Rh-D gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have not a gene it does not produce D antigen, and therefore the Rh-D protein is absent from their red blood cells (Saladin, 2003). But this is important in blood transfusion because the Rh-D immune response in Rh D negative women is primary expose for hemolytic disease of the newborn (Apoil and Blancher, 2000;Okeke *et al.,* 2012).

The Rh antibody unlike the IgM antibodies found against the ABO antigens, IgG antibodies do cross the placenta. This can lead to a very harmful condition during pregnancy (even several years after immunization) called severe hemolytic disease of the newborn (HDN) (Gurevitz, 2010; Iyiola *et al.,* 2011).

Unlike the ABO antibodies, Rh antibodies do not occur naturally, but are formed only when blood from Rh-positive donors is transfused into Rh-negative recipients. In such a case the recipients become sensitized to the Rh antigen, that is, they form antibodies against Rh-positive erythrocytes. Subsequently, during a second transfusion, large numbers of antibodies are formed rapidly, and these promptly agglutinate the erythrocytes of the Rh-positive donor (Bakare *et al.,* 2006).

2.1.2.1. Phenotypic Frequency distribution of Rh blood group

The frequency of Rh positive gene is higher in most populations in the world as compared to Rh negative gene. According to Nwaopara *et al*., (2008) in the population of Ekpoma in Nigeria observed that a higher percentage of the population (97.41%) were Rh positive while a lower percentage of the population (2.59%) were Rh negative.

Getaneh Alemu and Mohammedaman Mama (2015) in Arba Minch, Ethiopia also observed that most of the populations were Rh+ (92.8%) and kooffreh *et al*., (2015) among the families

in Calabar, Nigeria showed that Rh positive had the highest frequency (91.6%) and Rh negative had the least frequency (8.4%) in the population. In the similar way in Tanzania population 98% are shown Rh positive (had D antigen in the blood) and 2% hadn't D antigen (Rh negative) (Jahanpour *et al*., 2017) and in the city of Mashhad88.71% of the studied population was Rhesus positive and 11.3% rhesus negative (Boskabady *et al*., 2005). Further study conducted in western nation (Britain) by Garratty *et al*., (2004), they reported that Rh factor frequency showed that 83% of the population had Rh (D) and 17% hadn't Rh (D) antigen.

2.1.2.2. Allelic frequencies of Rh (D) blood groups

Previous studies conducted in Oromia National Regional State the allelic frequencies of D (0.73), and d (0.27) (Amsalu Waqgarie and Daniel Gietahun, 2017), and Rh factor were very close to the findings of Kassahun Tesfaye *et al*., (2015) in a similar study conducted in Silte zone, Ethiopia.They also found that 92.06% were Rh+ while 7.94% were Rh-.(Kassahun Tesfaye *et al*., 2015).In the general Indian subcontinent, (Yassin, 2013) reported allelic frequencies of D (0.676) and d (0.324).

2.1.2.3. Genotypic frequency of Rh (D) blood group

According to HabtamuTadesse, 2019 the genotypc frequency of Rh factor was DD (0.86), Dd (0.07) and dd (0.064) in west shewa zone, oromia region. In the similar way (Amsalu Wakgarie and Daniel Gietahun, 2017) reported that the frequency of Rh factor were DD (0.5329), Dd (0.3942), dd (0.0729).

2.1.2.4. Genetics of the Rh Blood Group System

Rh blood group is the second most common and has 50 defined antigens. Among these antigens, six antigens namely C, D, E, c,d and e are more important. The genes for the Rh system were located on Chromosome 1. The genetic composition of the Rh blood type includes two genes R*hD* and *RhCE* located in close proximity. These genes encode for the proteins RhD and RhCE. The RhD protein carries the D antigen while the RhCE latter carries C and E antigens. C and E can present in various combinations (e.g. CE, ce, Ce, cE). There is no antithetical component for

the RhD antigen. Therefore, a “d” does not exist. If the D antigen is not present, it is totally absente or deletes this location. This corresponds to the Rh negative phenotype (Whitlock, 2010).

Lack of any antigenic material is the result of absence of the RhD gene. The RhD and RhCE genes each have ten exons, are 97% identical, and most likely arose from gene duplication. RhD and RhCE differ by 32 to 35 of their 416 amino acid composition. The difference of antithetical antigens (e.g. C and c are antithetical) comes from a difference of fewer amino acids than the comparison of antigens from alternative blood group. This fact also explains the large degree of foreignness when the RhD antigen is introduced into an RhD negative individual. The highly antigenic nature of the RhD antigen is in contrast to other antigen systems (Whitlock, 2010).

2.2. Hardy-Weinberg Equilibrium

Hardy-Weinberg Equilibrium, also known as the Hardy-Weinberg principle, is used to compare allele frequencies in a given population over a period of time. A population of alleles must be similar when there is No gene mutation, migration, genetic drift and natural selection but Random mating must occur, meaning that individuals mate by chance .Hardy-Weinberg Equilibrium never occurs in nature because there is always at least one rule being violated. Hardy-Weinberg Equilibrium is an ideal state that provides a baseline against which scientists measure gene evolution in a given population. The Hardy-Weinberg equations can be used for any population; the population does not need to be in equilibrium (Johnson *et al.*, 2008*).*

Hardy-Weinberg (H-W) principle is very important used to determine the phenotypic, genotypic and allelic frequencies of the ABO and Rh-D blood group systems. The points of the principle are there is no selection, mutation and migration (genetic drift), but there is random mating and equal fitness between the two sexes. As far as these conditions are fulfilled the frequency of each allele can be maintained stable from generation to generation and the situation is referred as Hardy-Weinberg "equilibrium". Based on the H-W principle the genotypic frequencies can be determined from the frequencies of its alleles and vice-versa.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study area (Simada) is part of South Gondar Zone found in Amhara region, which is situated 774 km north of the capital city of Addis Ababa and 209 km southeast of Bahir Dar (Fig.3). The topographic elevation ranges from 1196 meter above sea level to 3525m. It is bordered on the southeast by the Sedieamuja, on the southwest by the Abay (Nile) which separates it from the East Gojjam, on the northwest by Este, and on the east by Tach Gayint. Part of this district's boundary with Este is defined by the Wanka, a tributary of the Nile. The major town of Simada is called wogeda.

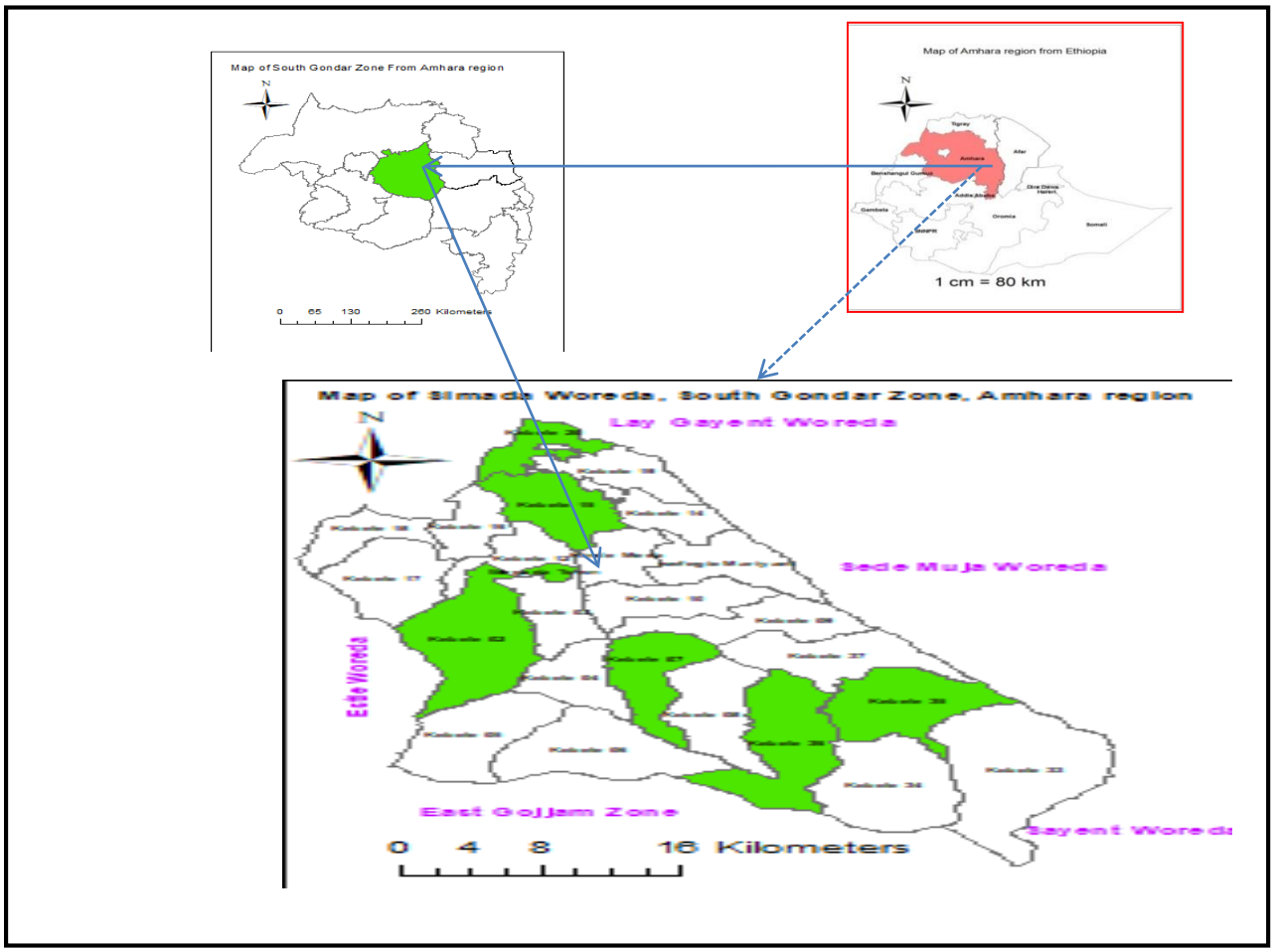

Figure 3: Location Map of the study area

The district has three climatic zones: 40%Woynadega (intermediate elevation), 10% dega (high land) and 50% kola (low lsand). The urban center where 5% of the population lives in the district

is located in the Woynadega zone. The climate is monsoonal and varies with elevation. The primary wet season extends from April through October; among these, July and August are the wettest months. The mean annual rainfall is 900-1100mm and the mean annual temperature is 23^{o}C (SWOARD, 2011).

According to the Central Statistical Agency (CSA) (2007), the district has an estimated total population of 228,271, an increase of 22% from the 1994 Census. This is an average of 4.2 persons per household. The population density of 102 persons per square kilometer is less than the zonal average of 145 people per km^2.

3.2. Sample size determination

The representative samples of this study was 384 individuals will selected using random sampling technique from 3754 individuals to give each blood samples for the diagnosis of blood types in the school. The sample population will stratified into four depends on grade level (9, 10, 11 and 12). For estimating the sample size (N) the researcher used statistical formula at 5% level of significance and considered ABO/ Rh blood group. The formula is found below;

$N=Z^2P\ (1-P)/d^2$ (Niang *et al.*, 2006)

$1-P=q$

N=the minimum required sample size

Z=1.96 at 95% confidence interval

d= margin of sampling error 5%

p=prevalence of ABO/Rh blood group

Since the distribution of blood group was not known in the study area P was taken to be 50% for the calculation.

N= (1.96)0.5 (1- 0.5) / (0.05) =384

Therefore the total sample size becomes 384.

3.3. Data collection

The study was conducted in Tagel preparatory and secondary school from September to August in2020. The data was collected from a total of 384 individuals from this 204 female and while 180 male. The age of the student was from 17-22 attained in the school for learning. The sample

of the blood collected by laboratory physician in wogeda health center. To collect the sample of blood the following steps necessary.

1. Wiped their finger vigorously with alcohol immersed cotton wool.
2. Pricked their finger with fresh sterile blood lancet and press gently.
3. Taken blood immediately and put three area of a slide
4. Dropped different anti-sera (monoclonal antigen A, B & D) on each prepared blood sample accordingly.
5. mixed with fresh sterile wooden stick and wait a minutes.
6. Observed the reaction and recorded.

3.4. Data analysis

All statistical analyses was done by SPSS software package version 20 and Microsoft excel. Frequency table were used for summarizing key findings. Allele and genotypic frequencies calculated under the assumption of Hardy–Weinberg equilibrium .Chi-square test will be used to compare observed and expected value of phenotypic, allelic and genotypic frequency distributions of the ABO blood group and Rh antigens under the Hardy–Weinberg. Descriptive statistics was used to calculate frequencies of the phenotype of the blood ABO and Rh blood groups and results were reported as frequencies and percentages. The genotypic and allelic frequencies of the ABO and Rh blood groups were calculated from the observed phenotypes of ABO and Rh under the assumption of Hardy–Weinberg equilibrium.

Under H-W equilibrium, the following equations and representations are commonly used:

1. “A” stands for dominant allele and represented by “p”;
2. “a” stands for recessive allele and represented by “q”;
3. $p + q = 1$ (1)
4. $(p + q)^2$ =which implies $p^2 + 2pq + q^2 = 1$. (2)

Again it implies that $AA + 2Aa + aa = 1$

Following the same principle and notion one can determine the frequencies of genotypes, phenotypes and alleles for multiple allelic traits. For the ABO blood group types the alleles can also be represented by p, q, and r.

5. $p + q + r = 1$. (3)

6. $(p + q + r)^2 = 12$. This gives $p^2 + 2pq + q^2 + 2qr + 2pr + r^2 = 1$. (4)

$P^2 + 2pq + q^2 + 2qr + 2pr + r^2$ represent "AA", "AO", "BB", "BO", "AB", "OO" genotypes, respectively

The alleles of ABO blood groups, i.e., I^A, I^B, and I^O, and their frequencies were represented by p, q, and r, respectively. The frequencies were calculated as follow

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

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

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

Therefore, the genotypic frequencies are represented as

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

Where, p^2 is the genotypic frequency of I^AI^A, q^2 is the genotypic frequency of I^BI^B, 2pq is the genotypic frequency of I^A I^B, 2pr is the genotypic frequency of I^AI^O, 2qr is the genotypic frequency of I^BI^O and r^2 is the genotypic frequency of I^OI^O as cited in Hanania *et al,*.(2007).

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

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

P = 1 – q = Allele D

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

DD + 2Dd + dd = 1

Genotype DD = p^2

Genotype Dd = 2pq

Genotype dd = q^2

3.5. Ethical clearance

Ethical clearance was obtained from Bahir Dar University, College of Science Research and Community Service, Ethical Clearance Committee. Informed consent was obtained from the study subjects.

4. RESULT

4.1. Phenotypic Frequency Distribution of ABO and Rh blood groups

As the result in figure 2 illustrates that the distribution pattern of A, B, AB and O blood groups among the total of 384 studied subjects were123 (32.03 %),92 (23.95%),36 (9.37%) and133 (34.63%) respectively. In this distribution the male participant of blood were 50 (13%), 39 (10.15%), 21 (5.46%) and 70 (18.22%), for A,B, AB, and O blood group respectively, whereas, in females, were 73 (19%), 53 (13.8%), 15 (3.9%) and 63 (16.4%) for A, B, AB and O groups respectively. The result of statistical data indicates that blood group O was the first most common prevalent blood group followed by A and then B, while the least prevalent blood group in the studied subject was AB. The distribution pattern of ABO blood groups in both sexes were the same trend (O>A>B>AB).

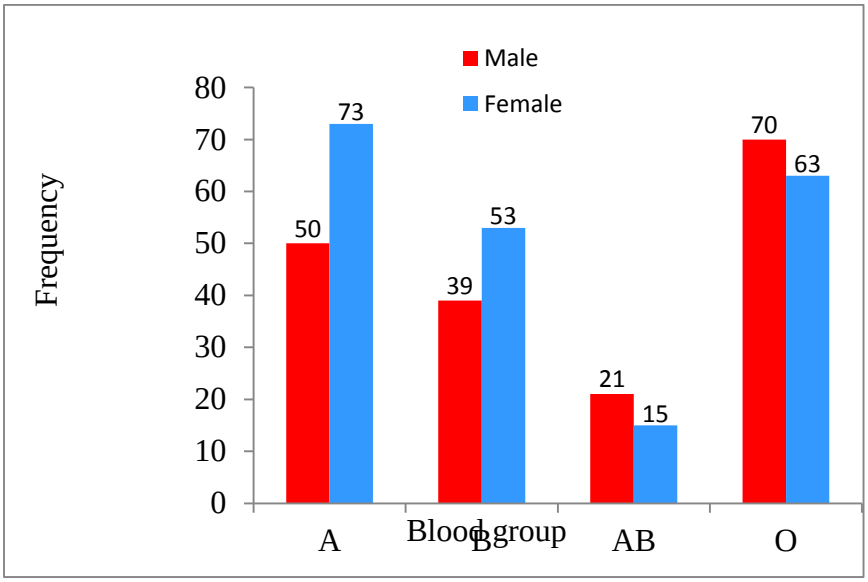

Figure 4: Phenotypic Distribution of ABO blood group

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Table 2: Frequency distribution of Rhesus factor in the studied subject

	Rhesus factor (Rh-D)	
Sex	Rh positive	Rh-negative
Male	175	5
Female	194	10
Total	369	15

From all sampled population 369 (96.1%) of them were Rh positive. The remaining 15 (3.9%) of them were Rh negative. As distribution pattern of Rh blood group showed that among 180 male individuals 175 (97.2%) and 5 (2.78%) were found for Rh-positive and Rh-negative respectively, while in 204 female individuals 194 (50.5) and 10 (2.6) were Rh-positive and Rh negative respectively. that is given in (Table 2).

4.2. Allelic and genotypic frequencies of ABO blood group and Rh-factor for the studied groups

The genotypic and allelic frequencies of the ABO and Rh blood groups were calculated from the observed phenotypes of ABO and Rh under the assumption of Hardy–Weinberg equilibrium.

Table 3: The phenotypic allelic and genotypic frequency distribution of the studied group

blood group type	Observed number and phenotypic frequency	Genotype	Allelic frequency	Genotypic frequency
A	A=123 (0.32)	AA	p=0.22	p^2 (p×p)=0.22x×0.22=0.05
		AO		2pr=2×0.22×0.58=0.26
B	B=92 (0.24)	BB	q=0.19	q^2= (q×q)=0.19×0.19=0.04
		BO		2qr=2×0.19×0.58=0.22
AB	AB=36 (0.09)	AB		2pq=2×0.22×0.19=0.09
O	O=133 (0.35)	OO	r=0.59	r^2 (r×r)=0.58×0.58=0.34
RH-	RH-=15 (0.039)	Dd	d=0.197	d^2 (d×d)=0.197×0.197=0.0388
RH+	RH+=369 (0.96)	DD	D=0.803	D^2 (D×D)=0.803×0.803=0.6448
		Dd		2Dd=2×0.803×0.197=0.3168

Results of allelic and genotypic frequencies of ABO and Rh blood groups are listed in Table 3. The allelic frequencies of the ABO blood group of r (I^o), p (I^B) and q (I^A) were 0.58 , 0.22 and 0.19, respectively ($I^O > I^A > I^B$) while allelic frequencies of the Rh blood group of D and d were 0.803 and 0.197,respectively. Genotypic frequency of OO, AA, AO, BB, BO, AB was 0.336, 0.0484, 0.255, 0.036, 0.22 and 0.0836 respectively. In case of Rh factor DD, Dd and dd was 0.6448, 0.368 and 0.0388 respectively.

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5. DISCUSSION

5.1. Phenotypic Distribution of Blood Group ABO and Rh factor

5.1.1. Phenotypic Distribution of ABO Blood Groups

In this study the sampled population distribution regard to ABO blood phenotype, blood group O was the first most common prevalent 133 (34.63%), and type A 123 (32.03%) was the second most prevalent followed by B 92 (23.95%), while the least blood type in the studied subject was AB 36 (9.37%). The result on the distribution of the population based on ABO blood phenotype was given by O>A>B>AB.

The result of this study on the ABO blood group phenotype frequency distribution was similar with the pattern seen in the previous studies that reported high frequencies of group O and low frequency of AB (Boskabady *et al*., 2005; Nwaopara *et al*., 2008; Pandey *et al*., 2013; Getaneh Alemu and Muhammadaman Mama, 2015; kooffreh *et al.,* 2015, Habitie Worede, 2018). .

The population of Ekpoma in Nigeria showed that a higher percentage of the population (63.73%) were blood group O, while those with blood group A, B and AB are (17.62%), (16.58%) and (2.07%), respectively (Nwaopara *et al*., 2008). In the same case Pandey *et al*., (2013) in India Bihar population showed that the frequency of blood group O (38%) was found highest followed by B (33.4%), A (21.8) and AB (6.8).

According to Kooffreh *et al*., (2015) in Calabar, Nigeria observed that blood group O had the highest frequency (55.2%), followed by B (21.6%), A (18.8%) and AB had the least (4.4%) and Boskabady *et al*., (2005) showed that the percentage of blood groups A, B, AB and O among the city of Mashhad population was 23.1%, 23.3%, 8.9% and 34.7% respectively.

All the above previous studies are supported the present investigation; the only difference is the distribution of blood A and B, in some reports. In this the result of Boskabady *et al*.(2005), Pandey *et al*., (2013), and kooffreh *et al., (*2015) are different from the present study. But the result of Nwaopara *et al*., (2008) Getaneh Alemu and Mohammadaman Mama

(2015) and Habitie Worede (2018) showed similar result in terms of the distribution of blood A and B. In the present study the frequency distribution of ABO blood groups in both sexes was the same trend (i.e. O>A>B>AB).

Previous studies stated that the distribution of blood groups difference and similarities occurred in the population may due to the cause of geographical area, natural selection (infectious disease), genetic drift, gene flow between populations (marriage distance such as consanguineous and short marriage distance can cause the homogeneity in genetic of the population, increasing the chance of recessive allele expression) (Baskabady *et al*., 2005; Pandey *et al*., 2013).

5.1.2. Phenotypic frequency Distribution of Rh (D) factor

In this study the distribution of Rh positive was higher prevalent than Rh negative blood. From all sampled populations 369 (96.1%) of them were Rh positive and the remaining 15 (3.9%) of them were Rh negative. Related studies reported that the frequency of Rh positive gene is higher in most populations in the world than Rh negative gene. This result is similar with the study conducted in western nation (Britain) by Garratty *et al*., (2004), they reported that Rh factor frequency showed that 83% of the population had Rh (D) and 17% had Rh (d) antigen.

In the population of Ekpoma in Nigeria observed that a higher percentage of the population (97.41%) were rhesus positive while a lower percentage of the population (2.59%) were rhesus negative Nwaopara *et al*., (2008). GetanehAlemu and Muhammadaman Mama (2015) in Arba Minch, Ethiopia observed that most of the populations were Rh+ (92.8%) and kooffreh *et al*.,(2015) among the families in Calabar, Nigeria showed that Rh positive had the highest frequency (91.6%) and Rh negative had the least frequency (8.4%) in the population.

In Tanzania population 98% are shown Rh positive (had D antigen in the blood) and 2% had not D antigen (Rh negative) (Jahanpour *et al., 2017*). The result in Ekpoma, Arba Minch, Calabar, and Tanzania was far from the result of present study by percentage. In the city of Mashhad 88.71% of the studied population was rhesus positive and 11.3% rhesus negative (Boskabady *et al*., 2005).

5.1.3 Allelic frequencies of ABO and Rh (D) blood groups

In this study the allelic frequencies of O (0.58), A (0.22) and B (0.19); D (0.8), and d(0.2).So the pattern of allelic frequencies were I^{o}> I^{A}> I^{B}.in the same way the results of previous studies conducted in Oromia National Regional State (Amsalu Waqgarie and Daniel Gietahun, 2017) the allelic frequencies of O (0.64), A (0.21) and B (0.15); D (0.73), and d (0.27), and Rh factor were very close to the findings of Kassahun Tesfaye *et al*., (2015) in a similar study conducted in Silte zone, Ethiopia. In their study, the allelic frequencies of O (r), A (p) and B (q), D, and d were 0.65, 0.19 and 0.15, 0.72 and 0.28, respectively. They also found that 92.06% were Rh+ while 7.94% were Rh-.The pattern of allelic frequencies were I^{o}>I^{A}>I^{B} (Kassahun Tesfaye *et al*., 2015). In the general Indian subcontinent, (Yassin, 2013) reported allelic frequencies of p (0.154), q (0.249), r (0.591), D (0.676) and d (0.324).

5.1.4. Genotypic frequency of ABO and Rh (D) blood group

The result of the genotypic frequency in this study were OO, AA, AO, BB, BO, AB was 0.336, 0.0484, 0.255, 0.036, 0.22 and 0.0836 respectively. In case of Rh factor DD,Dd and dd was 0.644,0.368and0.0388respectively.Thepatternofgenotypic frequency OO>AO>BO>AB>AA>BB this result was similar with the previous study OO(0.4),AO(0.24),BO(0.2),AB(0.064),AA(0.03) and BB (0.02) while Rh factor was DD (0.86), Dd (0.07) and dd (0.064)in west shewa oromia region (HabtamuTadesse,2019). In the similar way (Amsalu Wakgarie and Daniel Gietahun, 2017) reported that OO (0.413) AA (0.043) AO (0.268) BB (0.021) BO (0.186) AB (0.06) in case of Rh factor DD (0.5329),Dd (0.3942), dd(0.0729).

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

It was observed that in the population of Tagel secondary and preparatory school students in the ABO blood group distribution showed that blood group O the first most frequent and blood group A as the second most frequent blood followed by blood B while blood AB were the less frequent blood in the total studied subjects. In case of Rh factor most of the studied population had high frequency of Rh positive (having antigen) and low frequency of Rh negative. This study provides information on the phenotypic, allelic and genotypic frequencies of ABO and Rh blood groups. Blood group O in the studied population is most predominant. The researcher believes that the result would serve as a reference for other studies and future utilities in health planning and other needs.

6.2. Recommendation

Further studies with larger sample size, at different area are needed to investigate the frequency distribution of ABO blood groups to make strong generalizations. .

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APPENDICES

Appendix 1: Consent form

My name is Birtukan Shumet I am a post graduate student in Bahir Dar University and I will conducted research for the fulfillment of a master degree on the assessment of ABO and Rh blood group type frequencies among students of tagel secondary and preparatory school. Therefore conducting purposive cross-sectional study on this issue is very important. Knowing the blood group of each individual essential to effective blood banking and safe transfusion during any emergency, also it is important to avoid the problem related to blood group by adjusting life style and medical care.

If you are volunteer to participate and will agreed with the written informed consent in this study0.05ml blood will give. The venous blood will be used to identify the blood group type. During this time the participants not gain any incentive or injury, of course during blood drawn from the vein you may feel some minor pain and discomfort. Though this pain will lose after few minute. But the participants who will involve in this study will have a chance to know their blood type. Knowing blood group is important for blood transfusion during emergency. The participants' have full right to refuse or withdraw taking part in the study even they didn't want at the beginning or any time.

I have been wall informed that Bahir Dar University would like to have permission to the post graduate students to carry out a study on assessing the ABO and Rh blood group type frequencies among students of tagel secondary and preparatory school in simada woreda, south Gondar zone, Ethiopia. The investigator verified me that there are no risks on blood sampling procedure. As a result I am aware that the study requires about 0.05 ml of blood for blood group type identification and done by careful procedure. My right to refuse or withdraw from the study any time therefore I would like to approve that I have willingness to include this part by reading and sign the form at the end.

1. I understand that the investigator has legal ethical clearance from Bahir Dar University.
2. I understand that the aim of the study.

3. I understand that blood that taken from participants' is without risk and only useful for the study.
4. I understand that I am free to withdraw from the study.
5. I am willing to fulfill any information for the successes of this study.
6. I understand that if I want to refuse from this study there is no any effect in schooling processes.
7. I understand that I will not get any financial benefits from this study

   I have been given enough time to think over before I signed this informed consent. Therefore with full understanding of the situation, I will give my informed consent to participate in this study.

Researcher signature_____ date_____

Participant signature _____ date _____

Thanks for your willing to participate!!

Appendix 2: Amharic version consent form

የስምምነት ቅፅ

መለያ----

ብርቱካን ሹመት እባላለሁ፡፡በባህርዳር ዩንቨርሲቲ በድህረ-ምረቃ ት/ቤት በስነ-ህይወት ት/ት ክፍል የሁለተኛ ድግሪ ተማሪ ነኝ፡፡ የሁለተኛ ድግሪ ትምህርቴን ለማተናቀቅ ጥናታዊ ፅሁፍ በመስራት ላይ እገኛለሁ፡፡ ጥናቱ የሰዎች የደም አይነት ስርጭት ምን እንደሚመስል በት/ቤታችሁ የዳሰሳ ጥናት ለማድረግ ዩንቨርሲቲው ፈቅዶልኛል፡፡ ይህ ጥናት የእያንዳንዱን ሰዉ ደም አይነት ማወቅ በደም ልገሳ ጊዜ ያገለግላል፣ በተጨማሪም ለደም አይነቱ ምቹ የሆኑትን ና የማይመቹትን ምግብ አይነቶች ለይቶ ጤናዉን ለመጠበቅና በተለይ ደግሞ ሴቶች በርግዝና ወቅት ቅድመ ክትትል ለማድረግ ይጠቅማል፡፡ ስለዚህ እርስዎ በፈቃደኝነት በዚህ ጥናት ቢሳተፉ ማንኛዉንም የአካል አውቃቀር ለምልከታ ና 0.05 ሚ.ሊ ደም በመስጠት እንዲተባበሩኝ ስል እጠይቃለሁ ፡፡ደሙ የሚወሰደዉ የደም አይነትን ለመለየት ነው፡፡ እርስዎ የሚሰ ጡት ማንኛውም አይነት መረጃ ለጥናታዊ ፅሁፉ ካልሆነ በስተቀር ለሌላ ጉዳይ ስለማይውል እርስዎ ከዚህ ስጋት ነፃ ሆነው በራስ መተማመን ሊሆርዎ ይገባል፡፡

ተሳታፊው በዚህ ጥናት ስለተሳተፈ ምንም አይነት ችግር የማይደርስበት መሆኑና ምንም አይነት የገንዘብም ሆነ የቁሳቁስ ድጋፍ የሌለው መሆኑን አረጋግጣለሁ፡፡ በእርግጥ ተሳታፊው ደም በሚሰጡበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል ፡፡ ይሁን እንጅ ይህ ህመም ከተወሰነ ደቂቃ በኋላ ይለቀዎታል፡፡ ምክንያቱም ሁሉን ነገር ጥንቃቄ በትሞላበት ና ከምንም ብክለት ንፁህ በሆነ ሁኔታ ይከናወናል፡፡እንዲሁም በዚህ ጥናት ተሳታፊወች የደም ዓይነታቸውን የማወቅ ዕድል ያገኛሉ፡፡የደም ዓይነታቸውን ማወቃቸው ደግሞ በደም ልገሳ ጊዜ ያገለግላቸዋል ና ለደም አይነቱ ተስማሚ የሆኑ የአመጋገብ ሁኔታዎችን ለማስተካከል ና ጤናውን ለመጠበቅ ይረዳዋል፡፡

በዚህ ጥናት ተሳታፊው ከመጀመሪያም ይሁን በማንኛውም ጊዜ ላይ ከዚህ ጥናት ራሱን የማግለል እና የመውጣት መብቱ የተጠበቀ ነው፡፡

እኔ የጥናቱ ተሳታፊ ግለሰብ በባህርዳር ዩንቨርሲቲ በድህረ-ምረቃ ት/ቤት በስነ-ህይወት ት/ት ክፍል በሰዎች የደም አይነት ስርጭት እንድታጠና ለአጥኝዋ የተፈቀደላት መሆኑን

ተረድቻለሁ፡፡ አጥኝዋ ደም በምሰጥበት ጊዜ ምንም ችግር እንደማያጋጥመኝ አረጋግጣልኛለች፡፡ ይህንም ተረድቸ አስፈላጊ መረጃዎችን እና 0.05 ሚ.ሊ ደም የደም አይነቴን ለመለየት ሰጥቻለሁ፡፡ከዚህ በተጨማሪ ውጤቱ እንደሚገለፅልኝ ተረጋግጦ በጥናቱ ያለመሳተፍም ሆነ በማነኛውም ጊዜ እራሴን የማግለል መብቱ የተጠበቀልኝ መሆኑ ተረጋግጦልኛል፡፡

ስለዚህ በዚህ ጥናት ለመካተት ፈቃደኛነቴን የሚከተለውን ቅፅ አንብቤ በፊርማየ አረጋግጣለሁ፡፡

1. አጥኝው ከባህር ዳር ዩንቨርሲቲ ህጋዊ ፈቃድ የተሰጠው መሆኑን ተረድቻሁ፡፡
2. የጥናቱን ዓላማ ተገንዝቤያለሁ፡፡
3. ያለምንም ችግር የሚወሰደው የደም ናሙና ለጥናቱ ያለውን አስፈላጊነት ተረድቻለሁ፡፡
4. ከዚህ ጥናት ከፈለኩ አቋርጬ መውጣት እንደምችል ተረድቻለሁ፡፡
5. ለጥናቱ ስኬት ከእኔ የሚጠበቀውን ለማድረግ ሙሉ ፈቃደኛ ነኝ፡፡
6. በጥናቱ ባለመሳተፌ ምክንያት በትምህርቴ ላይ ምንም ችግር እንደማይደርስብደኝ ተረድቻለሁ
7. ከዚህ ጥናት የገንዘብ ጥቅማጥቅም እንደማላገኝ አውቄያለሁ፡፡

በዚህ ጥናት ለመሳተፍ ከመፍቀዴ በፊት ስለጥናቱ እንዳስብበት በቂ ጊዜ ተሰጥቶኛል፡፡ ስለዚህ ሁሉን ነገር በመረዳት በጥናቱ ለመሳተፍ በሙሉ ልቤ መፍቀዴን በፊርማየ አረጋግጣለሁ፡፡

መረጃውን የገለፀው ግለሰብ (አጥኝዋ) ፊርማ _____ ቀን _____

የጥናቱ ተሳታፊ ግለሰብ ፊርማ _____ ቀን _____

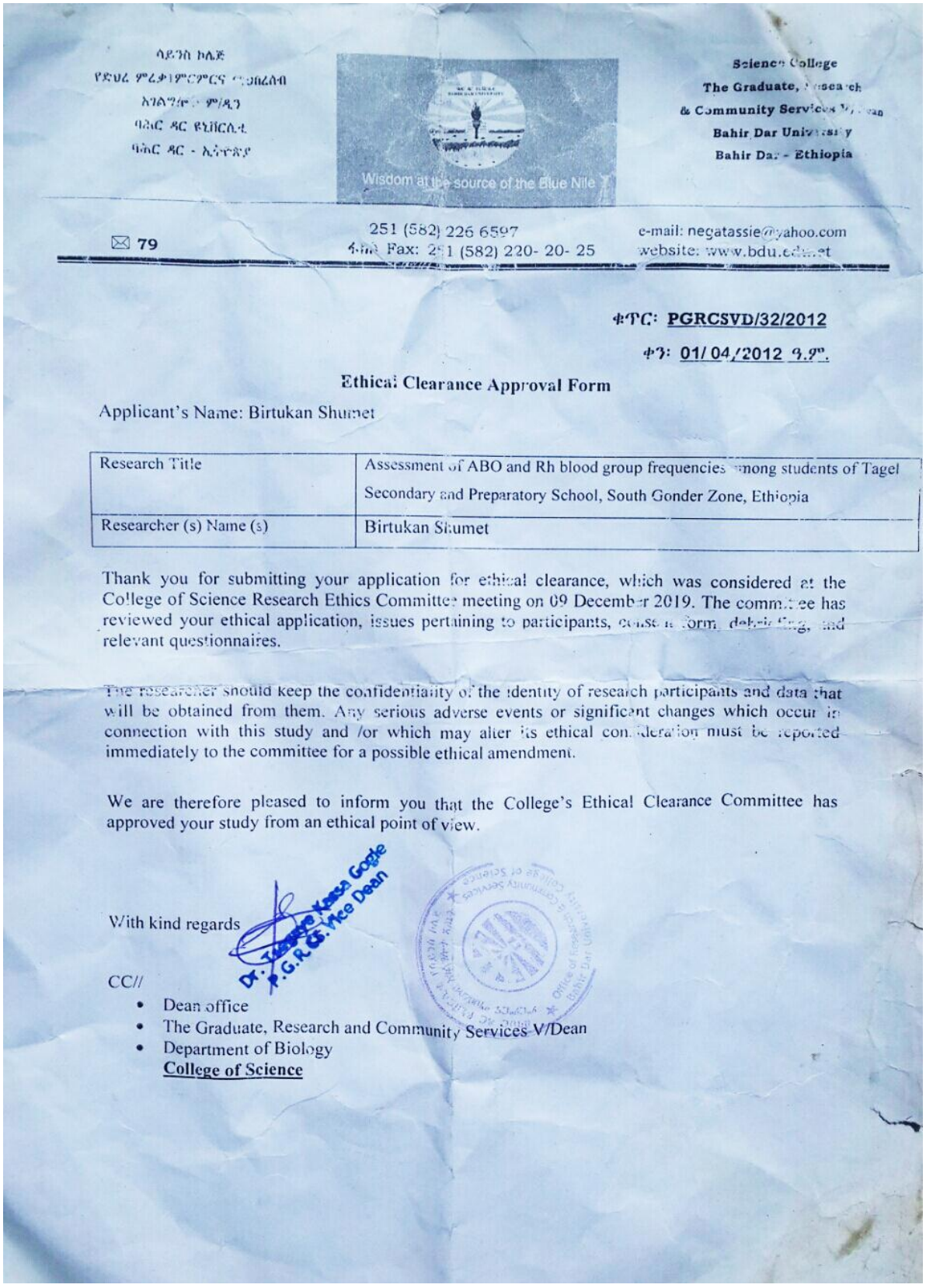
በጥናቱ ለመሳተፍ ሰለፈቀዱ ከልብ አመሰግናለሁ፡፡

Appendix 3: Figure showing participation of students during blood test in wogeda health center

Appendix 4: Figure showing chemicals and materials used for ABO and Rh blood group blood testing

Appendix 5: Figure shows blood sample of the student

Appendix 6: Documents of ethical clearance for BahirDar University



ሳይንስ ኮሌጅ
የድህረ ምረቃ፣ምርምርና ማህበረሰብ
አገልግሎት ም/ዲን
ባሕር ዳር ዩኒቨርሲቲ
ባሕር ዳር - ኢትዮጵያ

Wisdom at the source of the Blue Nile

Science College
The Graduate, Research
& Community Services V/Dean
Bahir Dar University
Bahir Dar - Ethiopia

✉ 79
251 (582) 226 6597
ፋክስ Fax: 251 (582) 220- 20- 25
e-mail: negatassie@yahoo.com
website: www.bdu.edu.et

ቁጥር: PGRCSVD/32/2012
ቀን: 01/04/2012 ዓ.ም.

Ethical Clearance Approval Form

Applicant's Name: Birtukan Shumet

Research Title	Assessment of ABO and Rh blood group frequencies among students of Tagel Secondary and Preparatory School, South Gonder Zone, Ethiopia
Researcher (s) Name (s)	Birtukan Shumet

Thank you for submitting your application for ethical clearance, which was considered at the College of Science Research Ethics Committee meeting on 09 December 2019. The committee has reviewed your ethical application, issues pertaining to participants, consent form, debriefing, and relevant questionnaires.

The researcher should keep the confidentiality of the identity of research participants and data that will be obtained from them. Any serious adverse events or significant changes which occur in connection with this study and /or which may alter its ethical consideration must be reported immediately to the committee for a possible ethical amendment.

We are therefore pleased to inform you that the College's Ethical Clearance Committee has approved your study from an ethical point of view.

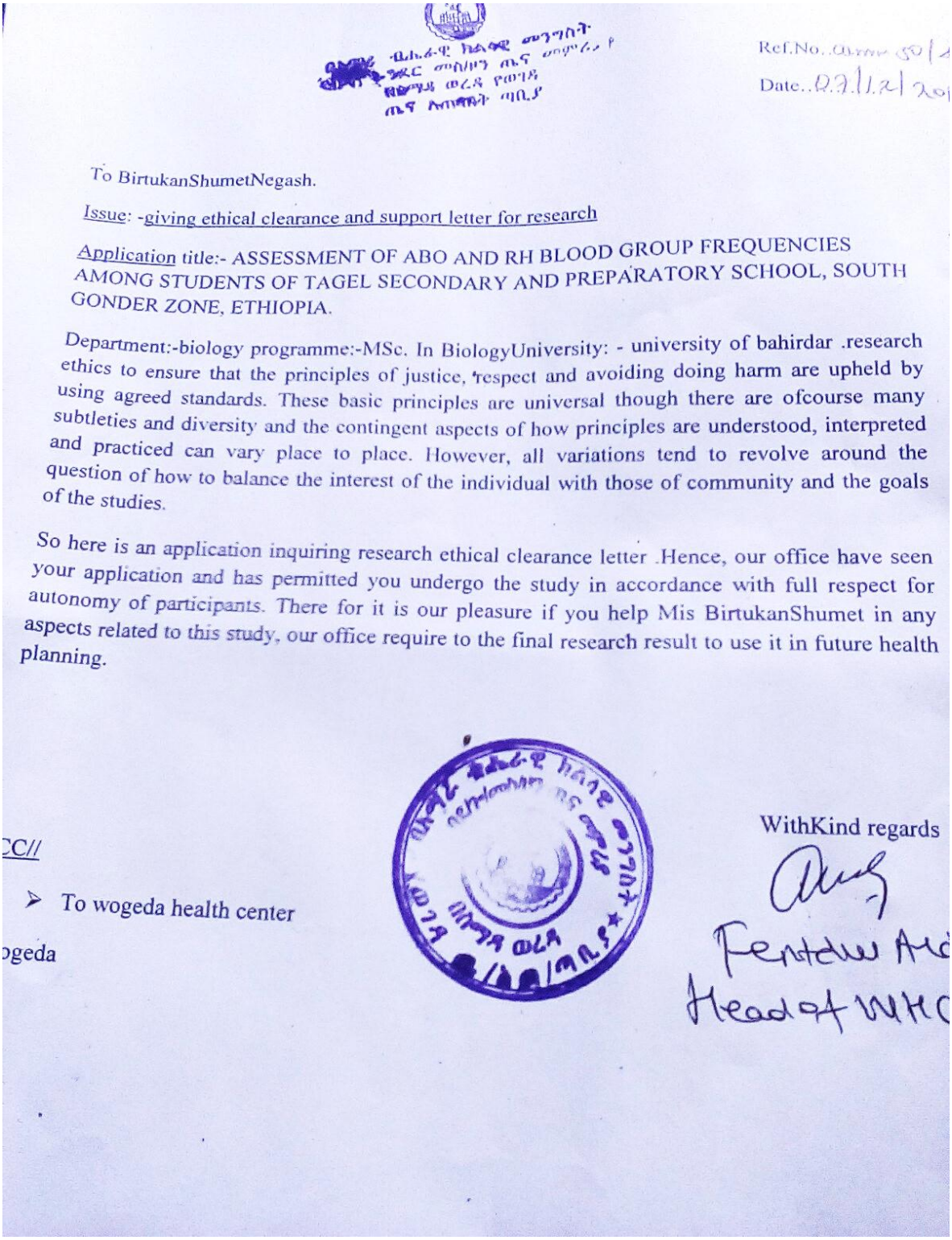
With kind regards

Dr. Tesfaye Yaregal Google
P.G.R.C. Vice Dean

CC//
- Dean office
- The Graduate, Research and Community Services V/Dean
- Department of Biology

College of Science

Appendix 7: Document for support scanned letter from south Gonder zone Simada Woreda to Wogeda Health Center



Ref.No.

Date..

To BirtukanShumetNegash.

Issue: -giving ethical clearance and support letter for research

Application title:- ASSESSMENT OF ABO AND RH BLOOD GROUP FREQUENCIES AMONG STUDENTS OF TAGEL SECONDARY AND PREPARATORY SCHOOL, SOUTH GONDER ZONE, ETHIOPIA.

Department:-biology programme:-MSc. In BiologyUniversity: - university of bahirdar .research ethics to ensure that the principles of justice, respect and avoiding doing harm are upheld by using agreed standards. These basic principles are universal though there are ofcourse many subtleties and diversity and the contingent aspects of how principles are understood, interpreted and practiced can vary place to place. However, all variations tend to revolve around the question of how to balance the interest of the individual with those of community and the goals of the studies.

So here is an application inquiring research ethical clearance letter .Hence, our office have seen your application and has permitted you undergo the study in accordance with full respect for autonomy of participants. There for it is our pleasure if you help Mis BirtukanShumet in any aspects related to this study, our office require to the final research result to use it in future health planning.

WithKind regards

Fentdw A

Head of WH

CC//

- To wogeda health center

ogeda