JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

Frequency Distribution of ABO and Rh Blood Groups Among Students of Saja Secondary and Preparatory School, Yem Special Woreda, Southern Ethiopia

MSc. Thesis

By:

Habte Garkebo

August, 2012 E.C

Jimma, Ethiopia

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A Thesis Submitted to Jimma University, College of Natural Sciences, Department of Biology in Partial Fulfillment of the Requirements for Master of Science degree in Biology

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A Thesis presented to School of Graduate Studies, Jimma University, in partial fulfillment of the requirements for the Degree of Master of Science in General biology

Approved by Examining board

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DECLARATION

I declare that this thesis of a title Frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, Yem Special Woreda, Southern Ethiopia is my original work and all sources of materials used for this thesis work have been duly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for the Master of Science degree in general biology at Jimma University and is reserved at the university library to be made available to users.

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LIST OF ACRONYMS AND ABBREVIATIONS

SNNPR	Southern Nations, Nationalities, and People Region
Rh	Rhesus
EDTA	Ethylene Diamine Tetra acetic Acid
USA	United States of America
RBC	Red Blood Cell
HWE	Hardy–Weinberg Equilibrium
SPSS	Statistical Package for the Social Sciences
CI	Confidence Interval

ABSTRACT

Different types of blood groups are hereditary and determined based on the presence or absence of surface antigens on the RBCs. The ABO and Rh blood groups are the most important blood groups in humans due to their vital role in blood transfusion and used in the blood typing. The frequency distribution of ABO and Rh blood groups vary in different races and ethnic groups in different parts of the world. Information on the frequency distribution of ABO and Rh blood groups is vital to the effective management of blood banks and safe blood transfusion service. The aim of this research is to determine the frequency distribution of ABO and Rh blood groups among students of Saja secondary and preparatory school. The sample included a total of 232 blood donor students during the period of study (2012 E.C). Selection of students were obtained on the basis of their willingness to participate by filling all their profile and signed on the consent agreement format. Blood samples were collected from each student by finger-prick method and blood groups were examined by slide-test method using antisera. Blood groups were determined on the basis of agglutination. Differences in allelic, phenotypic and genotypic frequencies of the ABO and Rh blood groups among each ethnic group (Yem and Oromo) of the students were observed. The most frequent ABO blood group in the present study was found to be 'O' (34.9 %) followed by 'A' (30.2%), 'B' (26.3%) and 'AB' (8.6%). The frequency of Rh positive group (94.4%) was more than Rh negative group (5.6%). The allelic frequencies of each ethnic group were calculated under Hardy-Weinberg equation. There was no positive relation pattern between ABO and Rh blood groups with sexes and ethnic groups. It is important to conduct similar well designed studies in different parts of Ethiopian in order to determine frequency distribution of national ABO and Rh blood groups for policy making and implementation to face the future National health challenges.

Keywords: ABO, Agglutination, allele, Blood group, Genotype, Phenotype and Rh

1. INTRODUCTION

1.1. Background of the study

The membrane of human red blood cell is complex and contains a number of blood group antigens, the most clinically significant being the ABO and Rh antigens. The ABO blood group system is based on the presence of 'A' and 'B' antigens. Rh blood group is mainly determined by the 'D' antigen (Knowles, Poole, 2002).

All human populations share the same blood group systems, although they differ only in the frequencies of specific types, which mean the incidence of ABO and Rh blood groups varies in different races, ethnic groups and socio-economic groups in different parts of the world (Khattak *et al*., 2008). The frequency distribution of ABO and Rh blood groups varies worldwide and is not found in equal numbers in different races and even among ethnic groups. Apart from the spatial and ethnic variations, the ABO and Rh blood group frequencies may change temporally in a single population (Dhot *et al.,* 2003; Pourfath *et al.,* 2004).

Having knowledge on the frequency distribution of ABO and Rh blood groups at local and regional levels are helpful in the effective management of blood banks and safe blood transfusion services (Patel *et al*., 2012). Hence, studies on the frequency distribution of ABO and Rh blood groups are precondition not only for formulation of appropriate control strategies but also to predict risk for communities under consideration.

In Ethiopia, the frequency distribution of the ABO and Rh blood group system is little explored. Some studies have been conducted on the frequency distribution of ABO and Rh blood groups in students of secondary and preparatory school in Ethiopia. There was no information on the frequency distribution of blood groups ABO and Rh phenotypes in the study area. Therefore, the present study is intended to determine and provide information on the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, Yem special woreda to give an insight in to the current status of blood group distribution and the corresponding genetic composition of blood of the participants and compare with other data from similar studies of other country.

1.2. Statement of the problem

Study on the frequency distribution of ABO and Rh blood groups are helpful especially for health sector to manage blood banks and safe blood transfusion service effectively, but it was little studied in Ethiopia. There are many occasions on which knowing your ABO blood group or blood group check can come in handy such as an accident in which you or your family member requires blood transfusion due to heavy loss of blood during an accident. And also identification of the Rh system is important to avoid a potential risk of erythroblastosis fetalis; however, most people still now did not know their own ABO and Rh blood types.

For the safe blood transfusion from donor to recipient, it is necessary that donor's blood type (especially the ABO and Rh blood groups) be determined in order to avoid the negative immune response (antigens and antibodies reaction). There was no written document in the study area about the frequency distribution of ABO and Rh blood groups. The main objective of this paper was to determine the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, Yem special woreda, Southern Ethiopia.

1.3. Research questions

In order to achieve the objectives presented below, the study was attempted to answer the following research questions:

1. Which phenotypic and allelic frequency distribution of ABO and Rh blood groups are the most and the least prevalent among students in the study area?
2. Is there variation between the phenotypic and allelic frequency distribution of ABO and Rh blood groups among students of each ethnic group (Yem and Oromo) in the study area?
3. How to estimate the observed and the expected allelic frequency distribution of ABO and Rh blood groups among students of each ethnic group in the study area?
4. Is there relation between the frequency distribution of ABO and Rh blood groups with sexes and ethnic groups?

1.4. Objective of the study

1.4.1. General objective

- To determine the frequency distribution of ABO and Rh blood groups of Saja Secondary and Preparatory School Children, Yem Special Woreda, Southern Ethiopia.

1.4.2. Specific objectives

- To evaluate the phenotypic and allelic frequency distribution of ABO and Rh blood groups of school children,
- To compare the phenotypic and allelic frequency distribution of ABO and Rh blood groups among students of each ethnic group,
- To estimate the observed and expected allelic frequency distribution of ABO and Rh blood groups among students of each ethnic groups, and
- To determine the relation between ABO and Rh blood groups with respect to sexes and ethnic groups.

1.5. Significance of the study

This research includes the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School. Study on the frequency distribution of ABO and Rh blood groups plays an important role in safe blood transfusion practice, which will help in reducing morbidity and mortality rate. Therefore, the finding of this study will have significance mainly for health sector to manage blood transfusion service. And it also provides information on the phenotypic and allelic frequencies distribution ABO and Rh blood groups among students of each ethnic group in the study area. It also indicates information on the frequency distribution of ABO and Rh blood groups in relation with diseases association and it enhances the stakeholders to design and implement appropriate prevention and control measures. Moreover, it serves as a starting point by motivating other researches to conduct deeper study on the frequency distribution of ABO and Rh blood groups in the study area.

1.6. Scope and Limitations of the study

This study was focus on the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, Yem Special Woreda, Southern Ethiopia. Due to time, man power and financial problem, the study was only limited to three months of investigation based on volunteers' blood sample donor students for ABO and Rh blood groups test.

1.7. Definition of basic terms

The following are some of the key words that were used in the study.

Allele: — a version of a gene that determines a particular trait.

Allelic frequency: — the frequency of an allele, as a proportion of all alleles of the gene in the population.

Frequency distribution: — in statistics, it is described as a tabular representation that display the frequency of various outcomes in a sample.

Genotype: — pair of alleles for a particular gene in an organism.

Morbidity: — any departure from a state of physiological well-being or it is illness or sickness.

Phenotype: — the physical/ observable characteristics of an organism.

Prevalence: — the total number of individual who have an attribute or disease at a particular time.

2. LITERATURE REVIEW

2.1. The discovery of ABO and Rh blood groups

Blood group refers to the entire blood group system comprising red blood cell antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. A specific pattern of reaction to testing antisera within a given system is called blood types. Over a period of time, our understanding on blood groups has evolved to encompass not only transfusion-related problems but also specific disease association with red blood cell surface antigens (Owen, 2000).

In 1900, Karl Landsteiner discovered that some blood transfusions were successful while others could be deadly; and he identified that red blood cells contain antigens on their surfaces, and that blood plasma contains antibodies targeted to particular antigens. He also demonstrated that the serum of some people agglutinated the red cells of other (Hosei, 2008). His extensive research on serology based on simple but strong scientific reasoning led to identification of major blood groups such as O, A, and B types, compatibility testing, and subsequent transfusion practices. He was awarded Noble Prize in 1930 for this discovery. Later, Jan Jansky described classification of human blood groups of four types that is 'A', 'B', 'AB' and 'O' (Owen, 2000). The fourth less frequent blood group 'AB' was also discovered by Alfred Von Decastello and Adriano Sturli in 1902 (Dariush and Marjanzarif, 2013). After the discovery of the ABO blood groups, the Rh blood group was defined by Landsteiner and Wiener in 1941 (Rahman and Lodhi, 2004). Then, the discoveries of the ABO and Rh blood groups were an important achievement in the history of blood transfusion (Garratty *et al*., 2000).

2.2. ABO and Rh blood groups

The ABO and Rh blood group are two of the most notable blood groups in humans due to their importance and association with blood transfusion and used in the blood typing of humans (Khattak *et al*., 2008). Blood groups are classified 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).

Blood groups are genetically determined. Determination of ABO blood groups is done by detecting 'A' and 'B' antigens. In addition, known red cells are used to detect anti-A and anti-B in the serum, by a process called 'reverse' grouping (Dhot *et al.,* 2003). The law of Landsteiner's state that blood transfusions between any of the three blood groups, 'A', 'B' and 'O' will lead to a rejection of the blood and tissue by the recipient of the donor. That is, if a blood type 'A' gave blood to a 'B', the 'B' would not be able to receive the 'A's blood (Lialiaris *et al.,* 2011).

Rh blood group system is the second most important in blood transfusions after ABO. Currently, the Rh system consists of 50 defined blood group antigens out of which only five are important. RBC surface of an individual may or may not have Rh factor or immunogenic D-antigen. Accordingly, the status is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). In contrast to the ABO system, anti-Rh antibodies are, normally, not present in the blood of individuals with D-negative RBCs, unless the circulatory system of these individuals has been exposed to D-positive RBCs. These immune antibodies are immunoglobulin G (IgG) in nature and hence, can cross the placenta. Prophylaxis is given against Rh immunization using anti-D Ig for pregnant Rh-negative mothers who have given birth to Rh-positive child (Westhoff, 2004).

2.3. Collection and testing of blood samples

Collection of blood specimens should be carried out by trained person to avoid causing study participant discomfort or compromising the quality or quantity of the sample. The study participants should receive clear oral and/or written instructions, with information, for example, about fasting and avoidance of medications as necessary for the planned analyses (Holland *et al.,* 2003). For blood collection, standard protocols recommended by well-established organizations must be used. Blood collection tubes should be drawn in a specific order to avoid cross-contamination of additives. An important early decision in blood collection is whether to collect anti-coagulated blood (consisting of plasma, buffy coat, and RBCs) or coagulated blood (consisting of serum and RBC clot) (Caporaso, Vaught, 2002).

According to Dacie and Lewis, (2001), blood samples are collected from the people by finger-prick method and blood groups are examined by slide test haemagglutination method

using commercially available antisera; anti-A, anti-B and anti-D. Blood samples are mixed carefully with the antisera and rocked gently for 60 sec to observe agglutination. In case of doubt, the test is examined under a microscope or the results are confirmed by reverse grouping using known group A and B red cells. The other method is after giving informed consent for the people 2ml blood sample is drawn from the antecubital vein of each donor in a disposable syringe, and transferred immediately to a tube containing ethylene diamine tetra acetic acid (EDTA). ABO and Rh (D) blood grouping are done by test tube agglutination method forward and reverse both types. Forward blood grouping (cell grouping) is performed using commercially available (Bio-laboratories) standard antisera A, antisera B, and antisera D. Reverse blood grouping (serum grouping) is performed by test tube agglutination method with pooled known 'A', 'B' and 'O' cells. Final blood groups are confirmed only if both forward group (cell group) and reverse group (serum group) are identical. Rh (D) negative blood groups are confirmed by antiglobulin technique (Dacie and Lewis, 2006).

2.4. Frequency distribution of ABO and Rh blood groups

The frequency distribution of ABO and Rh blood groups varies worldwide and is not found in equal numbers in different races and even among ethnic groups. Apart from the spatial and ethnic variations, the ABO and Rh blood group frequencies may change temporally in a single population (Dhot *et al*., 2003; Pourfath *et al.,* 2004). Due to geographical variations, external environment and genetic factors there are variation in blood groups distribution. Racial and environmental factors have been described to affect the frequency of various blood groups in researchers conducted on various societies, including Bangladesh and Latin America (Shamim, 2002). The frequency of ABO and Rh varies; such contradictions are probably due to immensely different sample sizes, geographical environments and ethnic groups in the study populations. Moreover, it shows that specific ABO blood groups might be distributed in different regions of the world (Klug, 2002).

2.4.1. The frequency distribution of ABO and Rh blood groups in the world

The frequency of ABO and Rh blood groups varies greatly in different races and populations. In most populations, about 50% are group 'O', followed closely by group 'A', then with groups 'B' and 'AB' showing a much lower incidence (Franchini, Liumbruno, 2013). The most common

blood group in the studies conducted in Britain, the USA, Australia, Nigeria, and Saudi Arabia is 'O' group followed by 'A', 'B', and 'AB' (Patel *et al*., 2012; Rao, Shetty, 2014; Garratty, Glynn, 2004). The global data presented by Khattak *et al*., (2008) showed that the Rh factor data from the study found in all the populations surveyed, with a minor Rh-negative blood type representation with a range of 0% to 17%. These populations include Indians, Arabs, Bengalis, Africans, Chinese, Eskimos, Mexicans, and Americans. Moreover, most study in different countries further confirmed that Rh-positive blood group has the highest percentage frequency while Rh-negative has the lowest percentage frequency as observed among different ethnic groups (Nwauche and Ejele, 2004). Agarwal *et al*., (2013) carried out a study on automated analysis of blood groups in north Indian donor population and observed that the common blood groups in order of frequency were 'B', 'O', 'A', and 'AB'; 94.4% being Rh-positive. Globally, the frequency distribution of Rh (D) blood group is different, especially in Britain (17%) and USA (15%) where the frequency of Rh-negative blood group is reasonably high compared to other regions (Rao, Shetty, 2014). Generally, the majority of the people in the world have 'O' blood type (usually resulting from the absence of both 'A' and 'B' alleles) and the Rh-positive.

2.4.2. The frequency distribution of ABO and Rh blood groups in Africa

In Kenya, Uganda, Mauritania and Ethiopia most studies showed the predominant blood group to be 'O' and the least prevalent to be 'AB' (Akanmu *et al*., 2015; Apecu *et al*., 2016; Hamed *et al*., 2012;Tesfaye *et al*., 2015). However, there is regional variability; some studies show that in Western and Central Africa, the most predominant group was 'B' while in Eastern and Southern countries, blood group 'O' dominated (Hamed *et al*., 2012; Apecu *et al*., 2016). Study done at Iyola *et al.*, 2011 reported phenotypic blood group frequencies in Ilorin, north central Nigeria is 'A' 18.7%, 'B' 17.6%, 'AB' 5.6% and 'O' 58.1%. Another study among 7653 individuals in Ogbomoso, Oyo State, Nigeria, 50% had blood group 'O'; 22.9% blood group 'A'; 21.3% blood group 'B' and 5.9% blood group 'AB' (Bakare *et al*., 2006).

In the Guinea and Nepal, 95.90% and 96.70% belong to the Rh-positive blood groups respectively (Loua *et al*., 2007). Generally, most studies in African countries showed that the predominant blood group from ABO is type 'O' and the least common was 'AB'; and from Rh (D) blood group is Rh-positive.

2.4.3. The frequency distribution of ABO and Rh blood groups in Ethiopia

In Ethiopia, the frequency distribution of the ABO and Rh blood group system is little explored. In 2012, a total of 441 students of Silte Secondary and Preparatory School, in Silte Zone, Ethiopia one study showed that the blood group 'O' was predominant that is 36.73% in Sodo, 40.14% in Silte and 46.26% in Meskan ethnic groups. In a recent small scale study in south central Ethiopia the proportion of Rh-negative blood group subjects ranged from 8–9% (Tesfaye *et al*., 2015). Another study done for consenting patients (for minors parental consent) visiting the Gambella hospital, southwestern Ethiopia from November to December 2013 showed that among native ethnic groups (Nuer and Anuak) and various ethnic groups ('highlanders'), the majority of the participants (41.20%) had blood type 'O' followed by type 'A' (39.96%) and 'B' (20.48%). Type 'AB' was the least frequent (3.34%). This study showed that the proportion of individuals having blood type 'O' or 'A' was significantly higher compared to those having type 'B' or 'AB'. The frequency of blood group 'O' among the natives was significantly higher compared to that among 'highlanders' (30.8%). The frequency distribution of the Rh within the ABO system is indicated in with the highest Rh negativity frequency occurring in blood group 'O'. The allelic frequencies of 'O', 'A' and 'B' were 0.6418, 0.2305 and 0.1277 respectively. While the frequency of allele 'O' among the Nilotics was 0.7100, for the 'highlanders' it was 0.5549. The frequencies of 'D' and d alleles among the Nilotics were 0.5800 and 0.4200 respectively. Among 'highlanders' the allelic frequency of 'D' was 0.5382, and that of the allelic frequency of 'd' was 0.4617 (Golassa *et al*., 2017). Study in Arba Minch Blood Bank, South Ethiopia in 2016 showed that from a total of 416 blood donors participants the distribution of ABO phenotypes, in decreasing order, was 'O' (175, 42.1%), 'A' (136, 32.7%), 'B' (87, 20.9%), and 'AB' (18, 4.3%). Most of them were Rh-positive (386, 92.8%) (Alemu and Mama, 2016). Larger scale fragmented and small scale fragmented studies from different parts of the Ethiopian country reported that comparable or similar results to the above (Zerihun *et al.,* 2011).

2.5. Knowledge on the frequency distribution of ABO and Rh blood groups

Information on the distribution of ABO and Rh blood groups, apart from their importance in blood transfusion practice, it is also useful in population genetic studies, researching population migration patterns as well as resolving certain medico legal issues, particularly of disputed

paternity cases. In modern medicines, besides their importance in evolution, their relation to diseases and environment is being increasingly becoming important (Khan *et al.,* 2009).

2.6. ABO blood groups and diseases association

The blood groups are widely being studied in relation to susceptibility to infectious as well as non-infectious diseases in different human populations. The ABO blood group system is known to be associated with diverse forms of cancer such as that of skin (Xie *et al*., 2010), epithelial ovarian (Gates *et al*., 2010) and gastric cancer (Anstee, 2010). Different diseases associate with blood groups have been investigated (Brecher & Hay, 2011), for example people with blood group 'O' have high risk of peptic ulcer (Alkout *et al*., 2000), people with group 'A' have a substantially increased risk for coronary heart disease (Wazirali *et al*., 2005). Among individuals with stomach and peptic ulcers, there is an excess of type 'O', whereas among those with cancer of the stomach, there is an excess of type 'A'. Not all type 'O' individuals have increased risks for peptic or stomach ulcers (Richard, 2003). Besides, persons of group 'A' are affected more frequently with coronary heart disease, ischemic heart disease, venous thrombosis and atherosclerosis, while it is low in people with blood group 'O' which is stated to have protective effect against these diseases (Khan *et al*., 2009). Also identification of the Rh-system is important to avoid a potential risk of erythroblastosis fetalis. Due to its medical importance in relation to different diseases, pursuing a line of investigation on the ABO and Rh blood group systems have been of significance for years. It is well known that these blood group systems are of great importance in blood transfusion and organ transplantation (Chandra and Gupta, 2012), and have a paramount importance that the donor blood cells match those of the recipient; otherwise, donor blood cells may be destroyed by antibodies present in the plasma of the recipient (Jassim, 2012).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Saja Secondary and Preparatory School (Grade 9 -12), Yem Special Woreda. Yem special Woreda is one of the Woreda in the Southern Nations, Nationalities, and Peoples' Regions of Ethiopia. It is situated in the north-western apex of SNNPR and is located between 7° 57′N to 8° 02′N latitude and 37° 40′E to 37° 61′E longitude with an elevation ranges from 1967 to 2859 meters above sea level. The topography of the woreda is characterized by rolling mountains, long gorgeous land, steeply sloppy areas and flat to undulating plateaus. In general, the physiographic features of the woreda are framed by Laba peaks in central part and by Gibe River in the east part of the woreda. This woreda is bordered on the West and North by the Oromia region, and separated from Gurage on the Northeast and Hadiya on the east by the Omo River.

The study school was found in Saja town. Saja is the administering center of Yem special woreda and is located 241 kms from Addis Ababa. This town found with an elevation of 1942 meters above sea level (Figure 1) (Source: Yem Special Woreda Cultural and Tourism Office). The school was chosen for easy of logistic and infrastructure and also the researcher knows the area very well, and collaboration and willingness to support the researcher was also obtained from the school principal, teachers, students, health professionals and other people working in the study area.

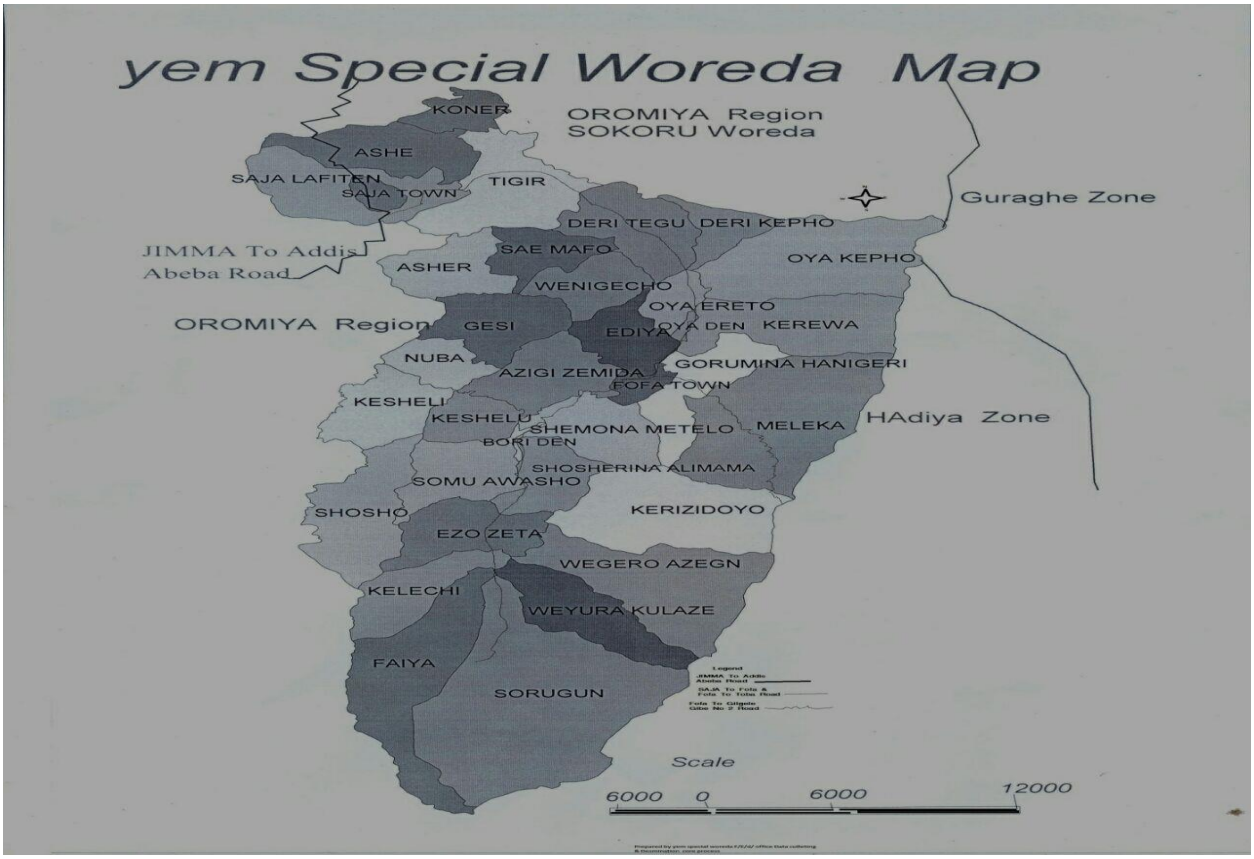


Figure 1. Map of Yem Special Woreda with the location of Saja town

3.2. Study design and period

A convenient non probability sampling study design was employed to determine the frequency distribution of ABO and Rh blood groups among students who were registered in the Saja Secondary and Preparatory School during the period of 2012 E.C.

3.3. Study population and participants

Blood samples for this study were obtained from students of Saja Secondary and Preparatory School. The total numbers of students of this school in 2012 E.C were 813 (355 Males and 458 Females) (Source: Saja Secondary and Preparatory School record office). The participants were included 232 (101 were males and 131 were females). All blood donor students for ABO and Rh blood groups test were based on volunteers. The two most numerous ethnic groups in this school were the students of Yem and Oromo people.

3.4. Sample size of the study

The required sample size (n) was determined by using population proportion formula of Cochran, (2004).

The Cochran formula is:

$$n_o = Z^2pq/e^2$$

Where:

e = the desired level of precision (i.e. the margin of error) = 5% = 0.05

p = the (estimated) proportion of the population = 0.5

q = 1 – p = 0.5

z = found in a Z table = 95% confidence level gives z-values = 1.96

$$n_0 = ((1.96)^2 (0.5) (0.5)) / (0.05)^2 = 384$$

Modification for the Cochran formula for sample size calculation in smaller populations' was calculated in the above formula but for larger populations by using the following equation:

$$n = n_o/1+ ((n_0 – 1)/N))$$

Here,

n_0 = Cochran's sample size recommendation = 384

N = population size (target population) = 813

n = the new, adjusted sample size

$$n = 384 / 1+((384-1) / 813)) = 261$$

3.5. Methods of data collection

This study was conducted at Saja Secondary and Preparatory School from September to November 2012 E.C. Socio-demographic data was collected from all participants. The form was developed in order to capture data on socio-demographic characteristics including student code, sex, age and ethnicity. Students who are volunteers to participate were included as prospective donors of blood sample by filling informed consent form. After collecting the written informed consent form, the Saja Primary hospital laboratory technicians began to ready all the necessary materials to collect and test blood samples from all participants. To comply with the requirement that blood sample donors be genuine, each student was interviewed for his/her ethnicity during

blood sample collection. Ethnicity was excluded students whose ethnic groups were small numbers. Confidentially of the data were maintained.

3.5.1. Blood samples collection

Blood samples were taken from 233 students. Selection of students was volunteers for their benefit to know their own ABO and Rh blood groups. Blood samples were collected by finger prick method. By a total of six laboratory technicians (two laboratory technicians per day), three drops (60 micro liters) of blood samples were taken from each study participant on clean glass slides by needle bite on the tip of index or middle finger with a sterile disposable lancet after cleaning the fingertip with 70-90% ethyl alcohol. Blood samples collection were done in the Saja Secondary and Preparatory School.

3.5.2. ABO and Rh blood groups testing and typing

The collected blood samples were tested for ABO and Rh blood groups by slide-test haemagglutination method using antisera. For the ABO and Rh groups test, a drop of blood from each student was placed on a clean open slide in three places. A drop of each of the antisera; anti-A, anti-B and anti-D (manufactured by Tulip Diagnostics Ltd. India) was placed on a clean glass slide and mixed well with a mixing stick uniformly with each blood sample and rocked the slide gently back and forth for 60 sec to observe agglutination. Blood groups were determined on the basis of agglutination. Presence of agglutination indicates the presence of the corresponding blood group or blood drops exhibiting a clotting reaction with the test sera were considered positive for that particular blood grouping reagent. To determine ABO blood grouping, the person's blood type was 'A' if agglutination occurred with the anti-A test serum; similarly, the blood type was 'B' if agglutination occurred with the anti-B test serum. The blood type was 'AB' if agglutination occurred with both the test serums; blood type 'O' if no agglutination occurred in either case. Similarly, Rh typing was done by mixing the blood sample with an anti-D serum. If the blood cells clump together in response to the anti-D serum, it indicates that the blood was Rh-positive. If no clumping occurs, the blood was determined to be Rh-negative. Each reaction and corresponding blood types for each blood donor students was recorded and subjected to pooling and statistical analysis.

3.6. Data Analysis

The collected data was entered in to Microsoft Excel and analyzed using statistical package for social sciences (SPSS) software version 20 and Microsoft excel addition program. The frequency and percentage of each variable was calculated and 95% confidence intervals were also evaluated using Fisher exact (clopper – Pearson) for the proportions of each blood group to the total available sample. The phenotypic frequencies distribution of ABO and Rh blood groups was expressed in percentage and allele frequencies estimated under the assumption of HWE.
An association between variables was considered statistically significant only if P value ≤ 0.05 at 95% confidence level. P value is the usual test for the dependence, i.e., if the p value less or equal to 0.05 the variables are dependent or they have some relation. Hence, p, q, r, v and u represent the allelic frequencies of allele for 'A', 'B', 'O', Rh positive (D) and Rh negative (d), respectively. Allele frequencies were calculated under the assumption of HWE and prevalence was expressed as percentages (Klug *et al.*, 2012). Their frequencies were calculated under Hardy-Weinberg assumptions as:-

<u>Calculation of expected phenotype frequency:-</u>

- Calculation of 'O' allele frequency (r):

  r^2 = frequency of O phenotype,

  $r = \sqrt{r^2}$

- Calculation of 'A' allele frequency (p):

  $p = \sqrt{\text{frequency of A phenotype} + \text{frequency of O phenotype}} - r$

  i.e. p + q + r = 1, $(p + q + r)^2 = 1^2$

  $p^2 + 2pr + r^2 = (p+r)^2 = \sqrt{(p2 + 2pr + r2)} - r$

  $p = \sqrt{(p^2 + 2pr + r^2)} - r = \sqrt{f(A) + f(O)} - r$

- Calculation of 'B' allele frequency (q):

  q = 1- (p+r)

- Calculation of 'd' allele frequency (u)

  u^2 = frequency of d phenotype,

  $u = \sqrt{frequency\ of\ d\ phenotype}$

- Calculation of 'D' allele frequency (v):

  v+u=1, v = 1 - u

To calculation of genotype frequency:-

AA =p^2, AO = 2pr, BB =q^2, BO = 2qr, OO =r^2, AB = 2pq, DD =v^2, Dd = 2vu, dd = u^2

Based on the HWE the frequencies of the O, A, B, and AB phenotypes are r^2, (p^2 + 2pr), (q^2 + 2qr), and 2pq, respectively.

3.7. Ethical consideration

The study was conducted following ethical clearance approval from the Research and Ethical Review Board of College of Natural Sciences, Jimma University. At the beginning of the study, the objectives of the study was explained to the Yem Special Woreda Education Office, school principal, school teachers and Saja Primary hospital. After permission obtained from the school director and other concerned bodies the research was carried out by giving information about ABO and Rh blood groups to convince students to voluntarily donate blood for ABO and Rh blood groups test by entering each student class together with voluntary people who are fluent speak in the local language. Students who are volunteers to participate were included as prospective donors of blood sample. Oral and written informed consent were obtained from all participants in which the participant expresses willingness to give blood sample through the student's signature or parent/guardian signature if the student is under the age of 18 years. Blood samples collection and testing were done in Saja Secondary and Preparatory School using sterile and disposable materials. Only laboratory technicians were taken the blood samples and all activities in laboratory test were done by health personnel of Saja primary hospital.

4. RESULTS

4.1. Phenotypic frequencies of ABO and Rh blood groups

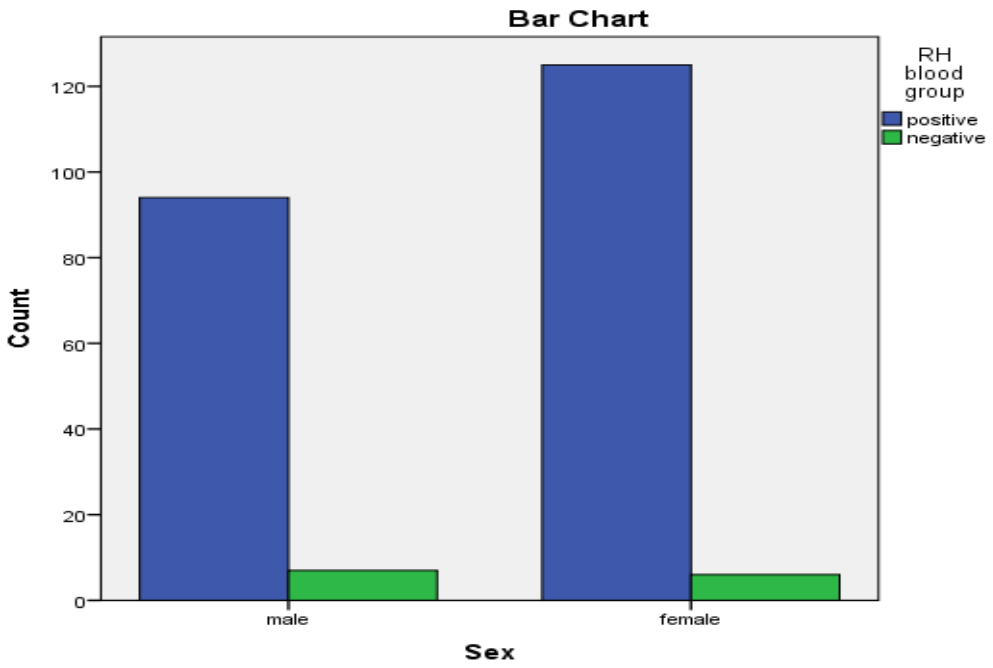
From a total of 813 students of Saja Secondary and Preparatory School, 233 students were volunteers to donate blood for ABO and Rh blood groups test from both sexes. Only one student of other ethnic group was found and rejected. Due to this 232 students were accepted for this research. Out of 232 students, majority of them were female 131 (56.5%) and males were 101 (43.5%) between the ages of 15 and 24. The frequency distribution of ABO blood group with 95% CI was tabulated in Table 1 below. From the ABO blood group, the most common blood group was 'O' (34.9% with 95% CI 28.8 – 41.0) followed by 'A' (30.2% with 95% CI 24.3 – 36.1), 'B' (26.3% with 95% CI 20.6 – 32.0) whereas 'AB' (8.6% with 95% CI 5.0 – 12.2), contributes minimum of the study subjects. There was no significant difference in the distribution of blood group 'A' and 'B' but, there was significant difference in blood groups 'O' and 'AB'. The ABO blood group frequency distribution also varies across sexes. Among the female students, blood group 'O' was the highest frequency, 52 (39.7%), followed by blood group 'A' 40 (30.5%), 'B' 25 (19.1%), and 'AB' 14 (10.7%). Likewise, among the male students, blood group 'B' was the highest with a frequency of 36 (35.6%), followed by blood group 'A' 30 (29.7%), 'O' 29 (28.7%) and 'AB' 6 (5.9%).

Table 1. Frequency distribution of ABO blood group among students of Saja Secondary and Preparatory school, 2012 E.C

ABO blood group	Sex				Total		95% CI
	Males		Females				
	Count	%	Count	%	Count	%	
O	29	28.7%	52	39.7%	81	34.9%	28.8 – 41.0
A	30	29.7%	40	30.5%	70	30.2%	24.3 – 36.1
B	36	35.6%	25	19.1%	61	26.3%	20.6 – 32.0
AB	6	5.9%	14	10.7%	20	8.6%	5.0 – 12.2
Total	101	43.5%	131	56.5%	232	100%	-

The frequencies of Rh blood group are shown in Figure 2 below. The Rh positive and Rh negative blood group distribution varies among participants. 93.1% of the male and 95.4% of the female students had Rh positive whereas 0.1% of the males and 4.6% of the females had Rh negative blood groups. Females had higher Rh positive than males. Most of the female and male students were found to have Rh positive blood group 219 (94.4%) and Rh negative was found to have 13 (5.6%). Overall, Rh positive is commonest among both males and females.

Figure 2. Frequency distribution of Rh blood group of the participants, 2012E.C.



As shown in Table 2 below, the Rh positive and Rh negative frequency distribution varies among the four ABO blood groups. With respect to Rh blood group system among the population studied, blood group 'O' Rh positive was the most common with frequency of 32.8% and 'AB' Rh positive was lest common (7.8%), whereas among the Rh negative students, blood group 'O' Rh negative and 'B' Rh negative were the most frequent (2.2%). Generally, among the blood donors for ABO and Rh test, the blood groups were distributed in the following order O+ve > A+ve > B+ve > AB+ve > O-ve= B-ve >AB-ve >A-ve.

The frequency distribution of blood groups according to Rh phenotype is Rh positive female pattern was found to be 'O' > 'A' > 'B' > 'AB', which is similar to overall ABO blood groups pattern but male pattern was found to be 'B' > 'A' > 'O' > 'AB'. The frequency of Rh negative

patterns in female and male were found to be ‘O’ > ‘A’ = ‘O’ = ‘AB’ and ‘B’ > ‘O’ > ‘AB’ > ‘A’ respectively. In male participants’ blood group ‘B’ Rh positive and in female participants’ blood group ‘O’ Rh positive was the most common. The least common blood group in males was ‘A’ Rh negative, and in females were ‘A’ Rh negative, ‘B’ Rh negative and ‘AB’ negative. There was no similar relation pattern in the distribution of blood groups between sexes (χ^2 for Rh = 0.596, P = 0.440).

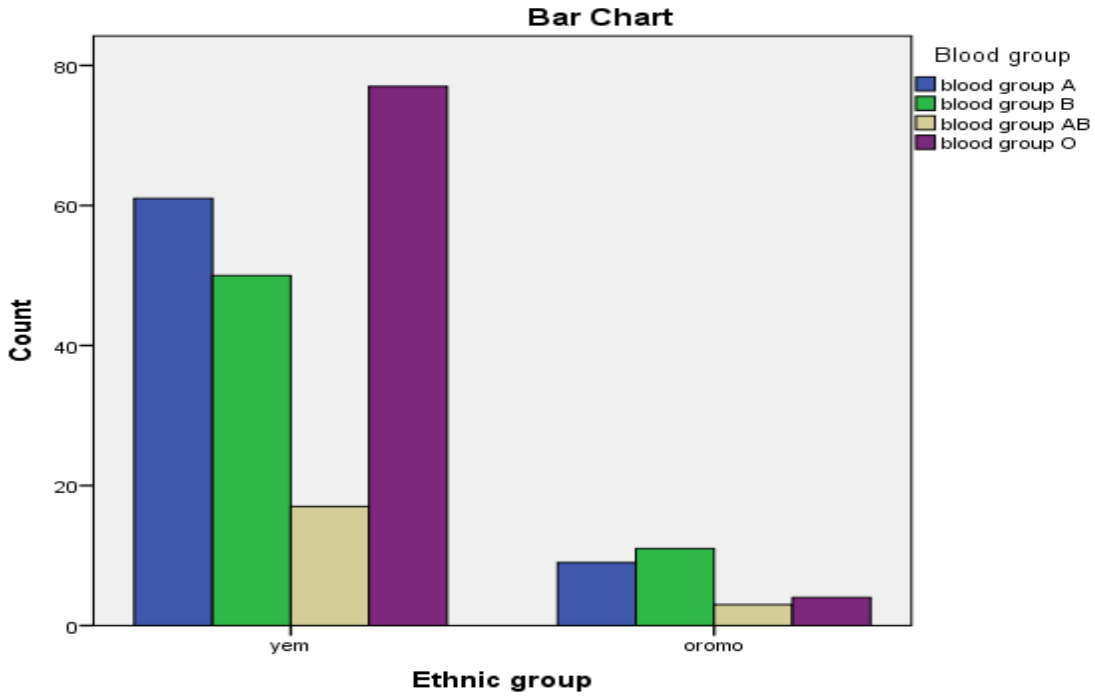
Table 2. Frequency distribution of ABO and Rh blood groups with respect to gender

ABO and Rh blood groups	Males	Females	Total	Prevalence rate	95% CI
O+	27 (26.7%)	49 (37.4%)	76	32.8	26.8 – 38.8
O-	2 (2%)	3 (2.3%)	5	2.2	0.3 – 4.1
A+	30 (29.7%)	39 (29.8%)	69	29.7	23.8 – 35.8
A-	0 (0%)	1 (0.8%)	1	0.4	(-0.4) – 1.2
B+	32 (31.7%)	24 (18.3%)	56	24.1	18.6 – 29.6
B-	4 (4%)	1 (0.8%)	5	2.2	0.3 – 4.1
AB+	5 (5%)	13 (9.9%)	18	7.8	4.4 – 11.3
AB-	1(1%)	1 (0.8%)	2	0.9	(-0.3) – 2.1
Total	101 (43.5%)	131 (56.5%)	232	100	-

4.2. Phenotypic frequencies of ABO and Rh blood groups of each ethnic group

Out of 232 students, 205 Yem and 27 Oromo ethnic groups were found in this study as shown in Figure 3 below. There was found to be differences in frequency distribution pattern of the ABO blood groups among the two ethnic groups of the students. From 205 Yem ethnic group blood group ‘O’ (37.6%) was found to be highly distributed and from 27 Oromo ethnic group blood group ‘B’ (40.7%) was found to be highest. The least common blood group was blood group ‘AB’ representing 8.3% of Yem ethnic group and 11.1% of Oromo ethnic group participants. There was no similar pattern relation between the blood group and the ethnic groups (χ2 for ABO = 6.282, P = 0.099).

Figure 3. Phenotypic frequency distribution of ABO blood group of each ethnic group



There was a great difference in numbers of students found in each ethnic group. Table 3 below showed that the variations in the frequency distribution of blood group among the two ethnic groups followed the different pattern. The percentage distribution of the ABO blood group and ethnic groups varies significantly based on the Rh blood group. The ABO blood group distribution based on Rh in Yem ethnic group pattern was found to be 'O' > 'A' > 'B' > 'AB'. In Oromo ethnic group pattern was found to be 'A' > 'B' > 'O' > 'AB'. The frequency distribution of Rh negative is very small or rare in the two ethnic groups.

Table 3. ABO blood group frequency distribution of each ethnic group based on Rh factor

Ethnic groups	Rh blood group	ABO blood group system				Total
		O (%)	A (%)	B (%)	AB (%)	
Yem	Positive	72(93.5%)	60(98.4%)	48(96%)	15(88.2%)	195(95.1%)
	Negative	5(6.49%)	1(1.6%)	2((4%)	2(11.8%)	10(4.9%)
	Total	77(37.6%)	61(29.8%)	50(24.4%)	17(8.3%)	205(100%)
Oromo	Positive	4(100%)	9(100%)	8(72.7%)	3(100%)	24(88.9%)
	Negative	0(0%)	0(0%)	3(27.3%)	0(0%)	3(11.1%)
	Total	4(14.8%)	9(33.3%)	11(40.7%)	3(11.1%)	27(100%)

4.3. Estimation of allelic frequencies of ABO and Rh blood groups

The present study was calculated the allele frequency of ABO and Rh blood groups in students of each ethnic group by using the statistical methods based on the extension of the HWE as shown in table 4. The calculated/estimated allele frequency for Yem ethnic group was found to be 0.6132 for O (r), 0.2075 for A (p), and 0.1793 for B (q). The Rh status, 95.1% was Rh positive while 4.9% was Rh negative. This gave the allelic frequencies as 0.7787 and 0.2213 for 'D' and 'd' respectively. The calculated allelic frequency for Oromo ethnic group was 0.3848, 0.3090 and 0.3062 for 'O', 'A' and 'B' respectively. On the Rh status, 88.9% were Rh positive while11.1% were Rh negative. This gave the allelic frequencies as 0.6668 and 0.3332 for 'D' and 'd' alleles, respectively.

Table 4. Allelic frequencies of ABO and Rh blood groups of each ethnic group

Ethnic group	Allele/ Gene	Allelic frequency	Genotype	Genotypic frequency	Phenotype	Phenotypic frequency (%)
Yem	O(r)	0.6132	OO	0.3761	O	37.61%
	A(p)	0.2075	AA	0.0435	A	4.35%
	B(q)	0.1793	AO	0.2541	A	25.41%
			BB	0.0321	B	3.21%
			BO	0.2118	B	21.18%
			AB	0.083	AB	8.3%
	D	0.7787	DD	0.6063	Rh +ve	60.63%
			Dd	0.3447	Rh +ve	34.47%
	d	0.2213	dd	0.0490	Rh -ve	4.90%
Oromo	O(r)	0.3848	OO	0.1481	O	14.81%
	A(p)	0.3090	AA	0.0954	A	9.54%
	B(q)	0.3062	AO	0.2379	A	23.79%
			BB	0.0937	B	9.37%
			BO	0.3137	B	31.37%
			AB	0.1111	AB	11.11%
	D	0.6668	DD	0.4446	Rh +ve	44.46%
			Dd	0.4444	Rh +ve	44.44%
	d	0.3332	dd	0.1110	Rh -ve	11.1%

4.4. Observed and expected phenotype frequencies of ABO and Rh blood groups

Table 5 below showed that the comparison between observed and expected values for both ABO and Rh blood groups of the two ethnic groups separately. For Yem ethnic group the observed phenotypic frequency for ‘O’, ‘A’, ‘B’, ‘AB’, ‘D’ and ‘d’ were 0.3761, 0.2976, 0.2439, 0.0829, 0.9510 and 0.0490 while the expected frequency value were 0.3760, 0.2975, 0.2520, 0.0744, 0.9510 and 0.0490 respectively. For Oromo ethnic group the observed frequency for ‘O’, ‘A’, ‘B’, ‘AB’, ‘D’ and ‘d’ were 0.1481, 0.3333, 0.4074, 0.1110, 0.889 and 0.1110 while the

expected frequency value were 0.1481, 0.3333, 0.3294, 0.1892, 0.889 and 0.1110 respectively. It was found that the observed and expected phenotype frequency distribution pattern and proportion of individuals having ABO blood group slightly differ from those expected under HWE for both ethnic groups. The distribution and proportion of individuals having Rh blood group for all the two ethnic groups did not differ from those expected under HWE.

Table 5. Comparison of Observed (Obs.) and expected (Exp.) phenotype frequencies of ABO and Rh blood groups among students of each ethnic group, 2012E.C

Ethnic group	Blood group	Phenotype	Observed frequency	Genotype	Expected frequency
Yem	ABO	O	0.3761	OO	0.3760
		A	0.2976	AA	0.2975
				AO	
		B	0.2439	BB	0.2520
				BO	
		AB	0.0829	AB	0.0744
	Rh	Rh +ve	0.9510	DD	0.9510
				Dd	
		Rh -ve	0.0490	dd	0.0490
Oromo	ABO	O	0.1481	OO	0.1481
		A	0.3333	AA	0.3333
				AO	
		B	0.4074	BB	0.3294
				BO	
		AB	0.1110	AB	0.1892
	Rh	Rh +ve	0.889	DD	0.889
				Dd	
		Rh -ve	0.1110	dd	0.1110

5. DISCUSSION

In the present study, the collected data showed that ABO blood group frequencies were found in the order 'O' > 'A' > 'B' > 'AB' (34.9%, 30.2%, 26.3% and 8.6% respectively). This study also revealed that the most common blood group was 'O' and the least common was 'AB'. This study finding is similar with other studies from USA (Garratty and Glynn, 2004), Mauritania (Hamed *et al*., 2012), Iran (Torabizade *et al*., 2016), Iraq (Saleh and Abood, 2016), Nigeria (Iyola *et al.*, 2011), Kenya (Akanmu *et al*., 2015), Uganda (Apecu *et al*., 2016), and Ethiopia (Zerihun *et al.*, 2011; Tesfaye *et al*., 2015; Alemu and Mama, 2016; Zerihun and Bekele, 2016) where they reported the same order. However, this study result is slightly different from study done in Madagascar (Saad, 2016) and Guinea (Saleh and Abood, 2016) as they reported 'O' > 'B' > 'A' > 'AB'. This study result is also not in congruent with data from India (Chandra and Gupta, 2012) and Bangladesh (Verma *et al.,* 2016) where prevalence of 'B' is highest followed by 'O', 'A' and the least was 'AB'. Like this study, all the above national and international studies have shown that the least common blood group was 'AB'.

According to Swamy *et al.,* 2012, the study conducted in India showed that most of the studies in India, there was large number of male blood donor than female blood donor participants. The main reasons behind it were lack of education, social taboo, cultural habits, lack of motivation, and fear of blood donation. In this study, it was found that higher contribution of female blood donors compared to male blood donors for ABO and Rh blood groups test.

The present study showed that the most common Rh blood group was Rh positive (94.4%) and the least common was Rh negative (5.6%), which is similar result found in the above mentioned countries such as USA, Mauritania, Iran, Iraq, Nigeria, Kenya, Uganda, and Ethiopia. Similarly, in the global data presented by Khattak *et al.,* (2008) showed that the Rh factor data from the study found in all the populations surveyed, with blood group Rh positive had the highest percentage frequency while Rh-negative had the lowest percentage frequency. These populations include Indians, Arabs, Bengalis, Africans, Chinese, Eskimos, Mexicans, and Americans. Moreover, the distribution of the Rh blood group in these populations are all consistent, with Rh positive being the more dominant allele and Rh negative expression being almost negligible.

In the present study, the prevalence of blood group 'O' Rh positive is more frequent in females (37.4%) as compared to males (26.7%), while blood group 'B' Rh positive is more frequent in males (31.7%) as compared to females (18.3%). However, some other studies reported that blood group 'A' Rh positive to be the most frequent among males, while blood group 'B' Rh positive was the highest frequency among females. This shows that inheritance of ABO and Rh blood groups are not sex-linked (Nwauche and Ejele, 2004 and Raja *et al*., 2016).
The ABO blood group distribution based on Rh in Yem ethnic group pattern was found to be 'O' > 'A' > 'B' > 'AB', but in Oromo ethnic group pattern was found to be 'A' > 'B' > 'O' > 'AB'. This study results that indicate there is no similar pattern relation of the ABO and Rh blood groups to sexes and ethnic groups ($P > 0.05$). Moreover, frequency distribution pattern of the ABO blood group types differs significantly between the sexes and ethnic groups.

The phenotypic and allelic frequencies of ABO and Rh blood groups vary widely across different races and geographical areas of the world. Few studies have been done across Ethiopia to find the variation. The present study was also calculated the allelic frequency of ABO and Rh blood groups by following Hardy–Weinberg equation. The allelic frequencies for alleles O(r), A(p), B(q), D (v) and d (u) from this study were 0.6132, 0.2075, 0.1793, 0.7787 and 0.2213 for Yem ethnic group; and 0.3848, 0.3090, 0.3062, 0.6668 and 0.3332 for Oromo ethnic group respectively. This is comparable to the allelic frequencies study from Tunisia, Mauritania, Morocco, Cameroun and Ethiopia (Said *et al.,* 2003; Hamed *et al.,* 2012; Benahadi *et al.,* 2013; Ndoula *et al.,* 2014 and Tesfaye *et al.,* 2015) but slightly different reports were recorded from Madagascar, Guinea and Bangladesh because prevalence of allele 'B' was greater than allele 'A', though 'O' was still the highest allele (Dewan, 2015; Randriamanantany *et al.*, 2012 and Loua *et al.,* 2007). Moreover, comparing the allelic frequencies for the 'A', 'B', 'O', 'D' and 'd' alleles for these populations reinforce the positive distribution pattern association. The allele frequencies of ABO and Rh blood groups found in other studies. The actual distribution of ABO and Rh blood groups did not differ significantly from the calculated gene frequencies.

Hardy–Weinberg shows that the allelic and genotypic frequencies will remain stable from generation to generation, provided that there is no mutation, no migration, no stochastic effects or genetic drift and no natural selection in a very large population with random mating. Violation of any of these assumptions can result to evolutionary change in terms of allelic frequency

distribution (Minelli *et al*. 2007; Klug & Cummings, 2002; Mayo, 2008). According to Tesfaye *et al*., 2015, the study done in SNNPR region in three ethnic groups (Sodo, Silte and Meskan) of Silte Zone, Ethiopia being showed that the observed and expected phenotypic frequency value for 'O', 'A', 'B', 'AB', 'D' and 'd' of each ethnic groups. In similar manner, this study also showed that the observed phenotypic frequency for 'O', 'A', 'B', 'AB', 'D' and 'd' were 0.3761, 0.2976, 0.2439, 0.0829, 95.1% and 4.9% while the expected frequency value were 0.3760, 0.2975, 0.2520, 0.0744, 95.1% and 4.9% respectively in Yem ethnic group; and in Oromo ethnic group the observed frequency were 0.1481, 0.3333, 0.4074, 0.1110, 0.889 and 0.1110 while the expected frequency value were 0.1481, 0.3333, 0.3294, 0.1892, 0.889 and 0.1110 respectively. This study showed that the observed frequency distribution of ABO blood group slightly different from the calculated/ expected frequencies that resulting to some population not exhibiting HWE and are therefore evolving. This study results also showed that '*O'* gene frequency is higher than that of '*A'* or '*B'* followed by '*A'* then '*B'* and indicated the global predominance of '*A'* gene over '*B'*. This finding is in agreement with Al-Bustan *et al*., 2002.

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The present study was determined and provided information on the frequency distribution of ABO and Rh blood groups and their corresponding allelic proportion among students of Saja Secondary and Preparatory School. Based on data gathered for the ABO and Rh blood groups, the most common ABO blood group was "O" (34.9%) followed by 'A' (30.2%), 'B' (26.3%) and 'AB' (8.6%); and Rh positive was found in 94.4% but Rh negative was found in only 5.6%. Blood group 'O' and Rh positive had the highest frequencies while blood group 'AB' and Rh negative had the least frequencies. The frequency distribution of ABO and Rh blood groups varied and was not found in equal numbers in each ethnic group. The observed frequency of ABO blood group in each ethnic group (Yem and Oromo) was slightly different from the expected/ calculated frequencies. There was no positive distribution relation pattern between ABO blood groups with sexes and ethnic groups.

6.2. RECOMMENDATIONS

The current study determines the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School; Based on the findings of the research study the following recommendation forwarded.

- It is necessary to have information on the frequency distribution of ABO and Rh blood groups in any population. So more research needs to be conducted on with the aim of creating awareness to test and know ABO and Rh blood groups.
- This study only determines the frequency distribution of ABO and Rh blood groups in the students of Saja Secondary and Preparatory School. It is recommended that further surveys in different parts of Ethiopia in order to determine frequency distribution of ABO and Rh blood groups.
- Every individual be ABO and Rh blood grouped at birth since the antigens are naturally presence on the surface of RBC. ABO and Rh blood groups of individual indicated on local identity cards, driving licenses and school or office identity cards will be of remarkable use in case of acute hemorrhage or anaemia when urgent transfusion of cross filed blood is required.
- The data obtained in the present study and several other studies of different areas of Ethiopia have to be important contribution for policy making and policy implementation to face the future health challenges in the region and nation.

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APPENDEX-I: ABO and Rh blood groups test informed consent form

- The purpose of this research is to determine the frequency distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, Yem special woreda, Southern Ethiopia.

This written informed consent form is used to allow a health person to perform ABO and Rh blood test. This test determines the frequency distribution of ABO and Rh blood groups in the study area. The test is made using a blood sample taken from the voluntary blood donor student by laboratory technician. The form affirms that the students donate blood for ABO and Rh test voluntary and the participant students have the right to request to see the results of the test. By completing below, I acknowledge that I am a student of Saja Secondary and Preparatory School and understand all information present in this form, consent to it and authorize to donate blood for ABO and Rh blood test. I confirm the written informed consent given based upon the following signature.

Name of person being tested: _____

Code number: _____

Age: _____

Sex: _____

Signature: _____

Date: _____

APPENDIX –II: የደም ምርመራ ለማድረግ የወላጅ ወይም የአሳዳጊ እና የተማሪ ፈቃድ መጠየቂያ ፎርም/Informed consent form/

i. የወላጅ ወይም የአሳዳጊ የደም ምርመራ ለማድረግ መጠየቂያ ፎርም/Informed consent/

የወላጅ ወይም የአሳዳጊ ፈቃድ መጠየቂያ ፎርም መሙላት የእርሶን ልጅ የደም ምርመራ ለማድረግ ነዉ። ይህ የደም ምርመራ የሚከናወነዉ በሰለጠነ የሳጃ የመጀመሪያ ደረጃ ሆስፒታል ባለሙያ ነዉ። ይህ የደም ምርመራ የሚጠቅመዉ የእርሶ ልጅ የደም አይነት ለመለየትና ለልጅዎ ለማሳወቅ ነዉ። የደም ምርመራዉ የሚከናወነዉ ያለምንም ክፍያ /በነፃ/ ነዉ። እኔ _____ የተባልኩት የተማሪ _____ ወላጅ ወይም ሕጋዊ አሳዳጊ ስሆን ልጄ የራሱን/ የራሷን የደም አይነት ማወቅ ጥሩ ስለሆነ የደም ምርመራዉን ፈቅጃለዉ።

ፊርማ _____

ቀን _____

ኮድ _____

ii. የደም ምርመራ ለማድረግ የተማሪ ፈቃድ መጠየቂያ ፎርም/Informed consent/

ይህን ፎርም መሙላት የአንተን/ የአንቺን ደም ምርመራ ለማድረግ ነዉ። ይህ የደም ምርመራ የሚከናወነዉ በሰለጠነ የሳጃ የመጀመሪያ ደረጃ ሆስፒታል ባለሙያ ነዉ። ይህ የደም ምርመራ የሚጠቅመዉ የአንተን/ የአንቺን የደም አይነት ለመለየትና ለራስህ/ ለራስሽ ለማሳወቅ ነዉ። የደም ምርመራዉ የሚከናወነዉ ያለምንም ክፍያ /በነፃ/ ነዉ። እኔ _____ የተባልኩት በ2012 ዓ.ም በሳጃ 2ኛ ደረጃና መሰናዶ ት/ቤት የ _____ ክፍል ተማሪ ስሆን የራሴን የደም አይነት ማወቅ በጣም ጥሩ ስለሆነ የደም ምርመራ ማድረግ እፈልጋለዉ።

ስም _____

ፆታ _____

ዕድሜ _____

ፊርማ _____

ቀን _____

APPENDIX –III: List of the distribution of ABO and Rh blood groups among students of Saja Secondary and Preparatory School, 2012 E.C.

Student code	Sex	Age	Ethnic group	ABO blood group	Rh blood group	Remark
001	F	19	Yem	O	Positive	
002	M	20	Oromo	B	Negative	
003	M	19	Yem	A	Positive	
004	M	19	Yem	A	Positive	
005	M	19	Yem	B	Positive	
006	M	19	Yem	O	Positive	
007	M	19	Yem	B	Positive	
008	M	19	Yem	A	Positive	
009	M	19	Yem	O	Positive	
010	M	19	Yem	O	Positive	
011	M	19	Yem	A	Positive	
012	F	19	Yem	O	Positive	
013	M	19	Yem	A	Positive	
014	M	19	Oromo	A	Positive	
015	F	19	Yem	A	Positive	
016	M	19	Oromo	B	Negative	
017	F	19	Yem	B	Positive	
018	F	19	Yem	B	Positive	
019	F	19	Oromo	B	Positive	
020	F	19	Yem	O	Positive	
021	F	19	Yem	O	Positive	

022	M	24	Yem	A	Positive	
023	F	18	Yem	O	Negative	
024	F	17	Yem	AB	Positive	
025	F	17	Yem	O	Positive	
026	F	18	Oromo	O	Positive	
027	F	17	Yem	AB	Positive	
028	F	17	Yem	B	Positive	
029	F	17	Yem	B	Negative	
030	F	18	Oromo	AB	Positive	
031	F	18	Yem	A	Positive	
032	F	16	Yem	O	Positive	
033	F	17	Yem	O	Positive	
034	F	17	Yem	B	Positive	
035	F	17	Yem	B	Positive	
036	F	18	Yem	AB	Positive	
037	F	18	Yem	A	Positive	
038	F	17	Yem	A	Positive	
039	F	17	Yem	O	Positive	
040	F	18	Yem	B	Positive	
041	F	16	Yem	O	Positive	
042	F	16	Yem	A	Positive	
043	M	16	Yem	A	Positive	
044	F	15	Yem	A	Positive	
045	F	16	Yem	O	Positive	

046	F	16	Yem	O	Positive	
047	F	18	Yem	O	Positive	
048	F	17	Oromo	A	Positive	
049	F	16	Yem	AB	Positive	
050	F	16	Yem	O	Positive	
051	M	17	Yem	A	Positive	
052	F	18	Yem	O	Positive	
053	F	16	Yem	A	Positive	
054	F	18	Yem	B	Positive	
055	F	16	Yem	B	Positive	
056	F	16	Oromo	O	Positive	
057	F	16	Oromo	A	Positive	
058	F	16	Yem	A	Positive	
059	F	16	Yem	A	Positive	
060	F	17	Yem	O	Positive	
061	F	16	Yem	A	Positive	
062	F	17	Yem	A	Positive	
063	F	16	Yem	O	Positive	
064	M	18	Oromo	B	Positive	
065	F	16	Yem	B	Positive	
066	M	16	Yem	A	Positive	
067	M	19	Oromo	B	Negative	
068	M	19	Yem	O	Positive	
069	M	19	Yem	O	Positive	

070	M	21	Yem	O	Positive	
071	M	17	Gurage	O	Positive	Reject
072	M	22	Yem	O	Positive	
073	M	18	Yem	B	Positive	
074	M	18	Yem	B	Positive	
075	M	21	Oromo	B	Positive	
076	F	16	Oromo	B	Positive	
077	F	19	Yem	O	Positive	
078	M	17	Yem	AB	Positive	
079	M	17	Yem	B	Positive	
080	M	17	Yem	O	Positive	
081	M	16	Yem	B	Positive	
082	M	17	Yem	A	Positive	
083	M	16	Yem	A	Positive	
084	M	16	Yem	A	Positive	
085	M	17	Yem	O	Positive	
086	M	17	Yem	A	Positive	
087	M	17	Yem	A	Positive	
088	M	17	Yem	B	Positive	
089	M	17	Yem	A	Positive	
090	F	17	Yem	A	Positive	
091	F	17	Yem	A	Positive	
092	M	23	Yem	O	Positive	
093	F	24	Yem	A	Positive	

094	F	22	Yem	O	Positive	
095	F	22	Yem	O	Positive	
096	M	21	Yem	A	Positive	
097	M	24	Yem	O	Positive	
098	M	24	Yem	O	Negative	
099	M	19	Yem	O	Positive	
100	M	19	Oromo	A	Positive	
101	M	19	Yem	B	Positive	
102	M	21	Yem	O	Positive	
103	M	19	Yem	O	Positive	
104	M	19	Yem	B	Positive	
105	M	19	Oromo	A	Positive	
106	M	19	Yem	B	Positive	
107	M	19	Yem	O	Positive	
108	F	17	Yem	O	Positive	
109	F	16	Yem	A	Positive	
110	F	18	Yem	O	Positive	
111	F	17	Yem	O	Negative	
112	F	17	Yem	O	Positive	
113	F	17	Yem	O	Positive	
114	F	17	Yem	A	Positive	
115	M	17	Yem	O	Positive	
116	M	18	Yem	O	Positive	
117	M	18	Yem	B	Positive	

118	F	16	Yem	O	Negative	
119	F	17	Yem	O	Positive	
120	F	16	Yem	B	Positive	
121	M	17	Yem	A	Positive	
122	F	18	Oromo	O	Positive	
123	F	18	Yem	B	Positive	
124	F	18	Yem	O	Positive	
125	F	19	Yem	A	Positive	
126	F	16	Yem	A	Positive	
127	F	17	Yem	B	Positive	
128	M	16	Yem	O	Positive	
129	F	18	Yem	O	Positive	
130	F	18	Yem	A	Positive	
131	F	18	Yem	O	Positive	
132	M	16	Oromo	B	Positive	
133	F	17	Yem	B	Positive	
134	F	17	Yem	A	Positive	
135	F	17	Yem	O	Positive	
136	M	17	Yem	B	Positive	
137	M	17	Yem	A	Positive	
138	F	17	Yem	A	Positive	
139	F	18	Yem	B	Positive	
140	F	18	Yem	AB	Positive	
141	M	16	Yem	B	Positive	

142	M	18	Yem	B	Positive	
143	F	18	Yem	O	Positive	
144	F	18	Yem	A	Positive	
145	M	19	Yem	B	Positive	
146	F	19	Yem	O	Positive	
147	F	19	Yem	O	Positive	
148	F	19	Yem	O	Positive	
149	F	19	Yem	B	Positive	
150	F	19	Yem	A	Positive	
151	F	19	Yem	A	Positive	
152	F	19	Yem	AB	Positive	
153	M	19	Yem	B	Positive	
154	M	19	Oromo	B	Positive	
155	M	19	Oromo	A	Positive	
156	M	19	Yem	B	Positive	
157	M	19	Yem	O	Positive	
158	M	19	Yem	O	Positive	
159	M	19	Yem	O	Positive	
160	M	19	Oromo	B	Positive	
161	M	19	Yem	B	Positive	
162	F	19	Yem	A	Positive	
163	F	19	Yem	A	Positive	
164	F	19	Yem	O	Positive	
165	F	19	Yem	B	Positive	

166	M	19	Yem	A	Positive	
167	M	19	Yem	A	Positive	
168	F	20	Yem	O	Positive	
169	F	20	Yem	A	Positive	
170	M	19	Oromo	A	Positive	
171	M	19	Yem	B	Positive	
172	F	19	Yem	O	Positive	
173	M	19	Oromo	B	Positive	
174	F	19	Yem	O	Positive	
175	M	19	Yem	AB	Positive	
176	M	19	Yem	A	Positive	
177	M	19	Yem	O	Positive	
178	F	17	Yem	O	Positive	
179	F	18	Yem	A	Positive	
180	F	17	Yem	B	Positive	
181	F	17	Yem	A	Positive	
182	F	17	Yem	O	Positive	
183	F	17	Yem	A	Positive	
184	F	17	Yem	B	Positive	
185	M	16	Yem	B	Positive	
186	F	16	Yem	AB	Positive	
187	F	18	Yem	AB	Positive	
188	F	18	Yem	O	Positive	
189	F	18	Yem	A	Positive	

190	F	18	Oromo	AB	Positive	
191	F	18	Yem	A	Positive	
192	F	18	Yem	A	Negative	
193	M	18	Oromo	AB	Positive	
194	F	16	Yem	B	Positive	
195	F	18	Yem	O	Positive	
196	M	17	Yem	B	Positive	
197	F	18	Yem	B	Positive	
198	F	18	Yem	A	Positive	
199	F	18	Yem	AB	Positive	
200	F	18	Yem	AB	Positive	
201	M	17	Yem	AB	Positive	
202	M	18	Yem	B	Negative	
203	F	18	Yem	A	Positive	
204	F	18	Yem	O	Positive	
205	F	18	Yem	A	Positive	
206	M	18	Oromo	A	Positive	
207	F	18	Yem	B	Positive	
208	F	18	Yem	O	Positive	
209	M	23	Yem	O	Negative	
210	M	19	Yem	B	Positive	
211	M	24	Yem	AB	Negative	
212	M	17	Yem	B	Positive	
213	F	17	Yem	O	Positive	

214	M	19	Oromo	O	Positive	
215	M	24	Yem	O	Positive	
216	M	22	Yem	B	Positive	
217	M	23	Yem	O	Positive	
218	M	21	Yem	O	Positive	
219	M	19	Yem	O	Positive	
220	F	18	Yem	O	Positive	
221	M	19	Yem	A	Positive	
222	F	19	Yem	A	Positive	
223	M	19	Oromo	A	Positive	
224	F	19	Yem	AB	Positive	
225	F	18	Yem	A	Positive	
226	F	18	Yem	AB	Negative	
227	M	17	Yem	A	Positive	
228	F	17	Yem	B	Positive	
229	M	24	Yem	AB	Positive	
230	F	16	Yem	O	Positive	
231	M	19	Yem	B	Positive	
232	M	19	Yem	B	Positive	
233	F	16	Yem	O	Positive	