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ALLELIC FREQUENCY AND GENOTYPE DISTRIBUTION OF ABO AND RH BLOOD GROUP AMONG STUDENTS IN THE MAJOR TRIBAL COMMUNITIES AT GAMBELLA TEACHERS EDUCATION AND HEALTH SCIENCE COLLEGE, GAMBELLA REGIONAL STATE, ETHIOPIA. 2021

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The distribution of blood groups was different from place to place and may not be found similar even among same ethnic groups. Frequency distribution of blood groups is important for modern medicine, genetic research, anthropology, ancestral relations of humans and forensic science. Aim of this study was to determine the allelic, genotypic, and phenotypic frequency distribution of ABO and Rh blood groups among students in Gambella region of Ethiopia. For1000 Blood samples from five major tribal communities, blood groups were determined by agglutination method using commercially available anti-sera A, B, and Rh (D). Hardy-Weinberg equation was used to calculate percentage of the observed and expected allelic genotypic frequency. In the ABO system, majority was type O (48%) followed by A (25%), B (21%) and AB the least frequency (6%). In Rh system, majority were Rh+ (95.3%) and the rest were Rh-(4.7 %). The allelic frequencies of O (IO), B (IB) and A (IA) were 0.691, 0.171 and 0.144, respectively. While allelic frequencies of the Rh blood group of D and d were 0.783 and 0.217, respectively. Genotypic frequencies of OO, AA, AO, BB, BO, and AB were 0.477481, 0.029241, 0.236322, 0.020736, 0.199008 and 0.049248 respectively. While the genotypic frequency of DD, Dd and dd were 0.613089, 0.339822, and 0.047089 respectively. This 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.

INTRODUCTION

Background

Blood is the most important fluid for the transport of nutrients, hormones, and oxygen to the whole body (Cavalli and Feldman, 2003). Before 1900, it was thought that all blood was the same. This misconception causes fetal transfusion. After the discovery of ABO blood type in 1901 by Karl Landsteiner and AB blood type in 1902 by Adriano Sturli and Alfred von Decastello, people led to know that human blood is not the same. People have different blood groups depending upon the surface markers (antigen) found on the membrane of red blood cells (Dean, 2005). The antigen for the ABO blood group is a complex carbohydrate (oligosaccharides) and rhesus (Rh) factor is protein in nature (Dean, 2005). It was reported that the alleles for ABO blood type are present on chromosome 9 controlling four deferent phenotypes A, B, AB, and O while Rh factor which is governed by allele D an d present on chromosome number 1 have two phenotypes, positive (with antigen D) and negative (without antigen D). These both the ABO blood group and Rh factor have antigenic property on the surface of the membrane of red blood cells (Alimba *et al.,* 2010). Also the human red blood cells that carry antigen D are referred to as Rhesus positive (Rh+) while those without it are Rhesus negative (Rh-) (Conteras and Lubenko, 2001). The discovery of ABO and Rh blood groups has contributed immensely to blood banking services and transfusion medicine. They are useful in genetic studies of populations and resolving medico-legal issues like disputed parentage (Enosolease and Bazuaye, 2008). Some studies have also reported the association of ABO blood group with certain pathological conditions; for example, a higher prevalence of stomach cancer has been found among people with blood group A, persons with O blood group are more susceptible to malaria infection than non-O blood group persons (Akhigbe *et al.,* 2009). Both are of equal importance in clinical and forensic medicine. The human red blood cell contains different types polysaccharide antigens called agglutinogen. The A and B antigens are important complex oligosaccharide antigens on their external surface. Antibodies are produced in the blood plasma against these A and B antigens and continued to be produced throughout a person's life.

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 IA, IB, and IO. 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 discovery of Mendel's law of segregation and independent assortment has led to the foundation of population genetics. In 1908, British mathematician Godfrey H.

Hardy and German physician Wilhelm Weinberg independently discovered the relationship between gene and genotype frequencies, known as the Hardy-Weinberg (HW) principle, or HW equilibrium. HW principle has become a powerful research tool in both theoretical and applied research in population and quantitative genetics (Chen, 2010). A population is a set of organisms that belongs to the same species, live in a specific environment, affect each other and by mating, give fertile offspring (Jankowska *et al.,* 2011). Population genetics refers to the study of the distribution of genes or alleles in a population. It also refers to a study of the evolutionary changes within and between the ethnic groups (Milgroom, 2015; Grunewald *et al.,* 2017).

According to the HW Principle (Hartl and Clarke, 2007) which formally states that: 'If a genetic population is such that, organisms are diploid, sexual reproduction, generations do not overlap, mating is random, population size is significantly large, allele frequencies are equal in the sexes, and there is no migration, mutation, or selection, then the genotype frequencies in the population are given by weighted products of the allele frequencies. In the case of the one locus, two allele system have allele frequencies (A, a) or (p, q) give directly the genotype frequencies (AA, aa, Aa) $p + q + 2pq = 1$ (Bosco *et al.,* 2012). But deviation from the Hardy-Weinberg equilibrium itself strongly suggests that at least one of the assumptions is violated (Chen, 2010).

The need for blood group prevalence studies is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Platt *et al.,* 1985; Green *et al.,* 1995). Percentages of people belonging to these blood groups are different in different communities. Distribution of these blood groups is also different in different races. The frequencies of ABO blood groups vary from one population to another and time to time in the same region.

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; Nwaopara *et al.,* 2008; Pandey *et al.*, 2013; Getaneh and Mohammedaman, 2015; Kooffreh *et al.,* 2015). The different studies show 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).

The knowledge of distribution of ABO and Rhesus (Rh) blood groups at local and regional levels is helpful in the effective management of blood banks and safe blood transfusion services (Patel *et al.,* 2012). This knowledge about ABO is also 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).

The distribution of these two blood groups has been repeatedly investigated in various populations all over the world and their frequencies exhibited considerable variation in different geographic locations, reflecting the underlying genetic and ethnic diversity of human populations (Garratty *et al.,* 2004). This distribution of ABO and Rh groups are also varying very markedly in different part of the world and in different races (Khattak *et al.,* 2008). Among British, the ABO and Rhesus blood group is type A,

42%; type B, 8%; type O, 47%; AB, 3%; Rh +ve, 83% and Rh –ve, 17% (Giri *et al*., 2001). According to the Caucasians in the United State, the distribution is type A, 41%; type B, 9%; type O, 46%; type AB 4%; Rh +ve, 85% and Rh –ve, 15% (Giri *et al.,* 2001). In Malaysia the ABO and Rhesus distribution is type A, 24.9%; type B, 30.2%; type O, 38.3%; type AB, 2.8%; Rh +ve, 98.4 and Rh –ve, 1.6% (Sivananthan *et al,* 2013).

Among students at Ladoke Akintola University Ogbomosho, the frequencies of A, B, AB and O blood groups were 21.30, 22.73, 2.85 and 53.12%, respectively, 93.32% were Rhesus positive while 6.68% were Rhesus negative (Akhigbe *et al,*2009).

China comprises more than 20% of the world's population. (Shao *et al*., 2002) Social-economic development and increased healthcare coverage have increased the demand for blood and its products in China. (Shi *et al.,* 2014a) Despite steady increases in total blood collections and voluntary non-remunerated donors, China faces challenges to its blood donation system (Shi *et al.,* 2014b). Nine per cent of Chinese donate blood, and more than 60% of donors are first-time donors. (Shi *et al.,* 2014c). Thus, data regarding the frequencies and ethnic distributions of ABO and RhD blood groups in the general population may help us to develop rational and evidence-based strategies for blood collection and management. However, studies that focused on the frequency and ethnic distribution of ABO and RhD blood groups in the general population have been scarce in China.

Thus, they conducted a large population-based study to investigate the distribution of ABO and RhD blood groups in different ethnic groups in the general population in China to provide reliable data for better development of rational strategies for blood collection and management.

African Countries such as Kenya and Nigeria that have ethnically diversified people like Ethiopia carried out many researches on ABO and Rh blood group testing and came up with useful information used in different health care practices associated with blood types. But; here in Ethiopia, due to long time prevailed law that prevents research on human beings and animals because of ethical and confidential case, there was little or no such type of research and research findings in the study area in the country. So, this study is significant in coming up with document that shows the phenotypic, genotypic and allelic frequencies of ABO and Rh (D) blood groups that plays a key role in genetic marker of the five major ethnic groups – Anyuak, Komo, Majang, Nuer and Opo that serves as a base line information in creating awareness as different ethnic groups living in the same geographical area necessarily who were not interbreed shows differences in their blood type frequencies and also it is used to reduce complication occurred during blood transfusion activities and hemolytic disease of the newborn (HDN). It also used in adding knowledge to the already existing body and serves as a reference material for another research of the same type or researches of different version of this topic carried in the Region or other places of our country.

Statement of the problem

Until the discovery of the ABO blood group over 100 years ago, all blood had been assumed to be same, and the often-tragic consequences of blood transfusion were not understood (Tekade *et al.,* 2011). Before 1901 the discovered of blood group, peoples were dying because there is no expert knowing that if you mix blood from two different blood types, the blood can clump, which may be caused the fatal. Many scholars carried out research in different countries like Nigeria, India, Ethiopia, America, and others on the frequency distribution of ABO and Rh blood groups, until this time, no research was conducted on Gambella Teacher Education and Health Science College. So not known

ancestral relationship between their parent, no documented data used for genetic research and for clinical aspects, maternal mortality, and hemolytic disease for infants, and lack of knowledge about the blood type and awareness about blood transfusion and the determinants of blood group frequency in this region have not been studied properly. Blood group is genetically determined and exhibit polymorphism in different populations. A total of 30 human blood group systems are now recognized by the international society of blood transfusion (ISBT) of which the ABO and rhesus (Rh) are the most important in clinical practice (Jaff, 2010; Tekade *et al.,* 2011). The distribution of ABO and Rh blood types has been investigated in several populations around the globe. The blood plays more roles than one might expect, it is involved in respiration, nutrition, waste elimination, thermoregulation, immune defense, water and acid-base balance, and internal communication (Atire, 2015). Human body carries about four-to-six-liter blood in it which is made up of red blood cells, white blood cells and platelets in liquid called plasma is 90% water but contain proteins, hormones and waste products. Blood is made up of 60% plasma and 40% blood cells.

Due to the above reason, there is a need to determine ABO and rhesus blood group frequency and allelic distribution in a multiethnic area of Gambella region and to explore possible genetic, racial and environmental factors influence as determinants of major blood groups. Because the blood transfusion continues to play an important role in modern health care, even to satisfy the need for a safe and efficient blood supply, the focal points of this study, is the investigation and documentation of the distribution pattern frequency of blood group ABO and Rh, in a representative sample of the Gambella population, and the comparison of the results to the ones obtained from a previous study. So no known knowledge about the food preference, therefore there is a need to determine ABO and rhesus blood group frequency and allelic distribution in a multiethnic area of Gambella region and to explore possible genetic, racial and environmental factors influence as determinants of major blood groups. in order for a blood transfusion to be safe and effective It is essential for the donor and the recipient to have a blood types that goes together not only retain active donor but also to continuously recruit new donor to replace those who retire from donation. The study was also providing an unbiased national wide socio-demographic description of blood donors to allow blood banks to take this information in to consideration of awareness to the people to know about their blood type testing as well as selecting compatible blood products when designing strategies for future blood donor transfusion based on the agglutination and retention recruitments. Knowing your blood type can be very crucial in medical emergence and interesting insight in to your healthy. Therefore, people with the blood type A can safely get group A blood, and people with blood group B can receive group B blood called cross matching and just to be compatible. This made the information available for the purpose of blood transfusion and other blood related activities to reduced corresponding problems.

General Objective

To determine the Allelic and Genotypes frequencies distribution of ABO and Rh (D) blood groups system among five ethnic groups in Gambella teacher's education and health Science College.

Specific Objectives

- ✓ To determine the phenotypic frequency of ABO blood types among the indigenous students at Gambella teacher's education and health Science College.
- ✓ To determine the phenotypic frequency of Rh blood types among ethnic group at

Gambella teacher's education and health Science College.

- ✓ To determine the phenotypic frequency distribution of ABO and Rh blood groups students in Gambella Teaching Education and health Science College.
- ✓ To determine the genotypic and allelic frequencies of ABO and Rh blood groups among the five ethnic groups students in Gambella teacher education and health Science College under this study.

Research Questions

- ✓ What is frequency distribution of ABO and Rh blood group of major communities in Gambella town?
- ✓ What is the genotypic and allelic frequencies of ABO and Rh blood of major communities in Gambella town?
- ✓ What is the genotypic frequency of Rh blood group among indigenous people at Gambella teacher's education and health Science College?
- ✓ What is the genotypic frequency of ABO blood group among the major tribal communities at Gambella teacher education and health science?

Significance of the study

The research findings had multi-purposes for the health planners in the study area. It shows the common trends of the prevalence of various blood groups among the major community in Gambella. It will also provide information about ABO and Rh blood group distribution of indigenous people in the study area. The study is significant regarding the inventory management of blood bank and transfusion services for those who need blood transfusion.

However, this study is significant in coming up with document that shows the phenotypic genotypic and allelic frequencies of ABO and Rh (D) blood groups that plays a key role in genetic marker of the five major ethnic groups Nuers, Anyuak, Majang, Opo, Komo.

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.

In order to ensure adequate supply of the most medically useful blood types the blood banks would require timely information concerning the distribution and frequency of blood groups among the five major tribal communities in Gambella region particularly in Gambella town. The knowledge of the distribution of Rhesus antigen in a population is critical in managing a transfusion service in study area such as antenatal serology, paternity testing as well as selecting compatible blood products, there is the lack of published data in Gambella describing the distribution of ABO and Rh among blood donor and this information is scarce in Ethiopia as a whole, it also a helpful for effective management of blood banks and safe blood transfusion service in Ethiopia particularly in Gambella region.

Limitation of the study

This study was limited to Gambella town especially in Gambella teacher education and health Science College. Moreover, the study was limited in content focusing only ABO and RH blood group not related with other traits. The other limitation was smaller sample size because of the financial problem. The blood donation data in the Region was not available properly.

MATERIALS and METHODS

Description of the Study Area

The study was conducted in Gambella Regional State, Gambella Town, (Gambella Teachers Education and health Science College) which have got over 1000 students belonging to different indigenes groups. The state is in the South-western part of Ethiopia about 777 km away from Addis Ababa, the capital city of Ethiopia. It has been situated in the lowlands of Baro Akobo River Basin between latitudes $6^0$22' to $8^0$37' North and longitudes $33^0$10' to 35^0 50' East. It is in the south-western Ethiopian lowlands bordering the Republic of South Sudan from the west, the Oromia Regional State from the north-east and the Southern Nations Nationalities and Peoples Regional State (SNNPRS) from the south-east. The average temperature is 27°c–33°c and the annual rainfall amount ranges from 900mm-2200mm (Wondachew and Muchie, 2017). The region experiences unimodal rainfall characterized by heavy rainfall from May to October and low precipitation from November to April (Lema, *et al.,* 2017). According to the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the population of Gambella Region has a total number of 307,096, of which 159,787 are men and 147,309 are women; among these, the population size of urban inhabitants is 77,925 or 25.37% of the population, while the remaining 229,171, or 74.63 % live in rural areas (CSA, 2007). The Gambela Region is mainly inhabited by various indigenous Nilotic ethnic minority populations, Nuer 47%, Anyuak 21%, Majang 4%, Opo 0.32% and komo 0.07% as well as some Omotic groups keficho 5.04% shakicho 2.27%), Afro-Asiatic populations (Amhara 8.42%, Oromo 4.83%, Kambatta 1.44%, Tigray 1.32%), and other ethnic groups predominantly from southern Ethiopia 4.86% (All highlander = 28%).

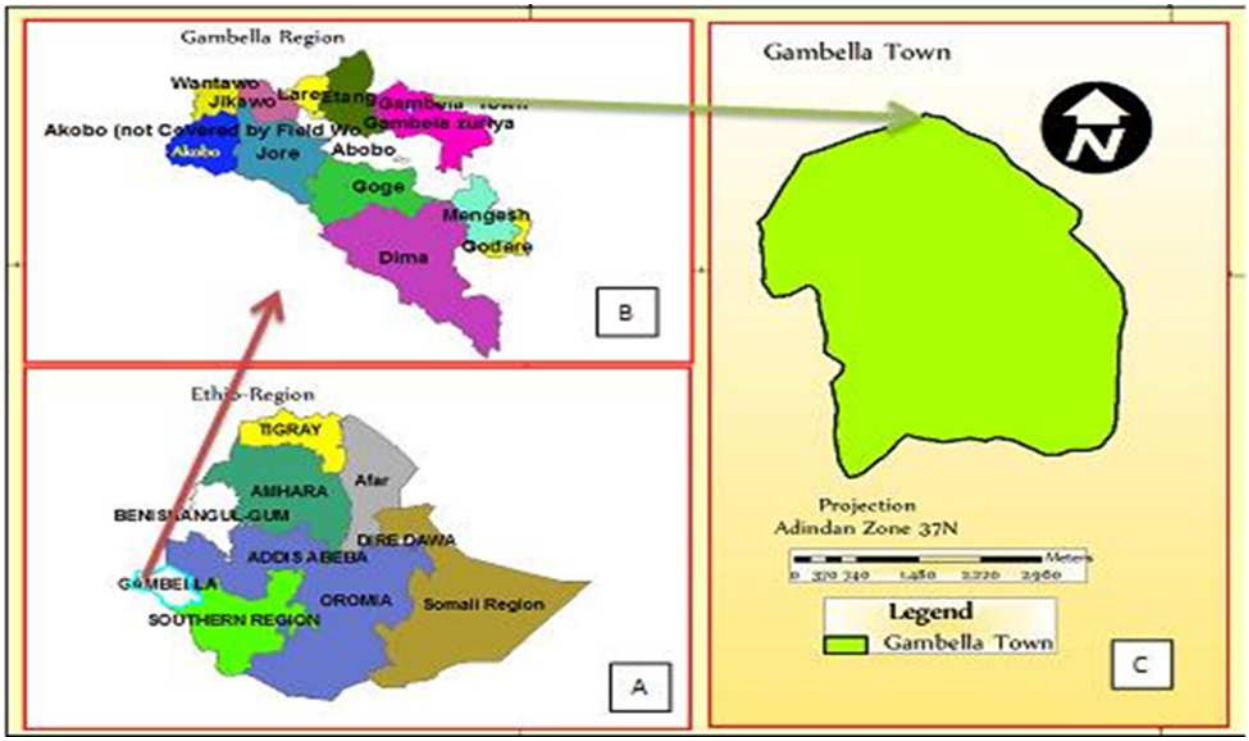

Figure 1: Map of Gambella region of Ethiopia (source; 2007 census).

Sample size determination

The blood collections were taken from 1000 voluntary students that were divided into five major ethnic groups i.e., Nuer, Anyuak, Majang, Komo, and Opo Peoples and randomly selected. Automatically purposively sampled students comprising with in the college in order to fill the consent form. The profile filled by participants was got acceptance by the researcher after the Dean and his committee signed and stamped for correctness indicated at (Appendix 9). For Calculated sample size the researcher used Hard-Weinberg equilibrium formula at 5% level of significance and considered ABO/ Rh blood group.

Blood Sample Collection and Grouping Method

An interview based on structured questionnaire was used for those individuals available during data collection to collect sociodemographic data such as name, sex, and, ethnicity, first and second language, and district event date of collection data and the objective of this study were explained to the participants. The data was collected from a total of 1000 individuals from this 415 were females and while 585 were males. From those variables we were used only sex and Ethnicity for this manuscript and a check list was used to collect primary data from individual voluntary participants. The ABO and Rh blood group test was performed by using standardized and packed lancet, to obtain blood from each sample students. Blood samples were taken from finger picks, and open slide method of testing for ABO and Rh (D) blood groups was followed (Bhasin *et al.,* 1995). Then, it was placed on a clean slide in three places anda drop of one of the Anti-sera that is antibody coated, Anti-A, Anti-B and Anti-D was added to each of an individual's blood samples and mixed using a glass rod. Blood groups and Rhesus factor were determined based on agglutination, and recorded as blood group A+, B+, AB+, and O+ and A-, B-, AB-, and O-. The blood samples were collected and tested by qualified laboratory technicians using the standard clinical procedure at Gambella Teachers Education and Health Science College that are summarizing in the following steps necessary.

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

Data Analysis

All statistical analyses were done by Microsoft words excel. Frequency table were used for summarizing key findings. Chi-square test was also used to compare observed and expected value. 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. The formula is found below;

- ✓ "A" stands for dominant allele and represented by "p";
- ✓ "a" stands for recessive allele and represented by "q";
- ✓ $P + q = 1$ (1)
- ✓ $(p + q)^2$ =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.

- ✓ $p + q + r = 1$. (3)
- ✓ $(p + q + r)^2 = 1$. 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., IA, IB, and IO, and their frequencies were represented by p, q, and r, respectively.
- The frequencies were calculated as follow:

r=√O=Allele IO
p = 1 - √ (B + O) = Allele IA
q = 1 - √ (A + O) = Allele IB
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 IAIA, q^2 is the genotypic frequency of IBIB, 2pq is the genotypic frequency of IAIB, 2pr is the genotypic frequency of IAIO, 2qr is the genotypic frequency of IBIO and r^2 is the genotypic frequency of IOIO 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 = √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

Chi-square tests will be done to test the independence and the goodness of fit for genotype frequencies (Chakra borty, 2010). The Chi-square (χ2) test statistic is then:

X^2=Obs-Exp/Exp^2

Ethical clearance

Ethical clearance was obtained from Hawassa University, College of Natural and Computational Sciences, Faculty of Biology department of Biology Ethical Clearance Committee obtained on 11/03/2021. Informed consent was obtained also from the study subjects.

RESULT AND DISCUSION

Phenotypic frequency distribution of ABO blood groups

As the result in (Table 1) illustrates that the distribution pattern of A, B, AB, and O blood groups among the total of 1000 studied subjects were 255(25.5 %), 209 (20.9%),58 (5.8%) and 478(47.8%) respectively. In this distribution the male participant of blood were 162 (16.2%), 130(13%), 36 (3.6%) and 257(25.7%), for A, B, AB, and O blood group respectively, whereas, in females, were 93(9.3%), 79(7.9%), 22 (2.2%) and 221 (22.1%) 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).

In this study the sampled population distribution regard to ABO blood phenotype, blood group O was the first most common prevalent (47.8%), and type A (25.5%) was the second most prevalent followed by B (20.9%), while the least blood type in the studied subject was AB (5.8%). 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 was conducted in 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). However, current pattern is similar to the Caucasians in the United State and Uyo, in Nigeria the distribution of type A is, 41%; type B, 9%; type O, 46%; type AB 4%; (Giri *et al.,* 2011), and in Uyo, Nigeria record showed that blood group O was the most common with 56.10%; followed by blood group A, 25.07 %; blood groups B was 16.4%; type AB, 2.45%, and also for the blacks in the United States, the distribution is type O, 46%; type A, 27%; type B, 2% and AB, 7% (Seeley *et al*., 1998). This result was also similar to the studies that had been conducted by 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 and Mohammadaman, 2015 and Habitie, 2018 showed similar result in terms of the distribution of blood A and B.

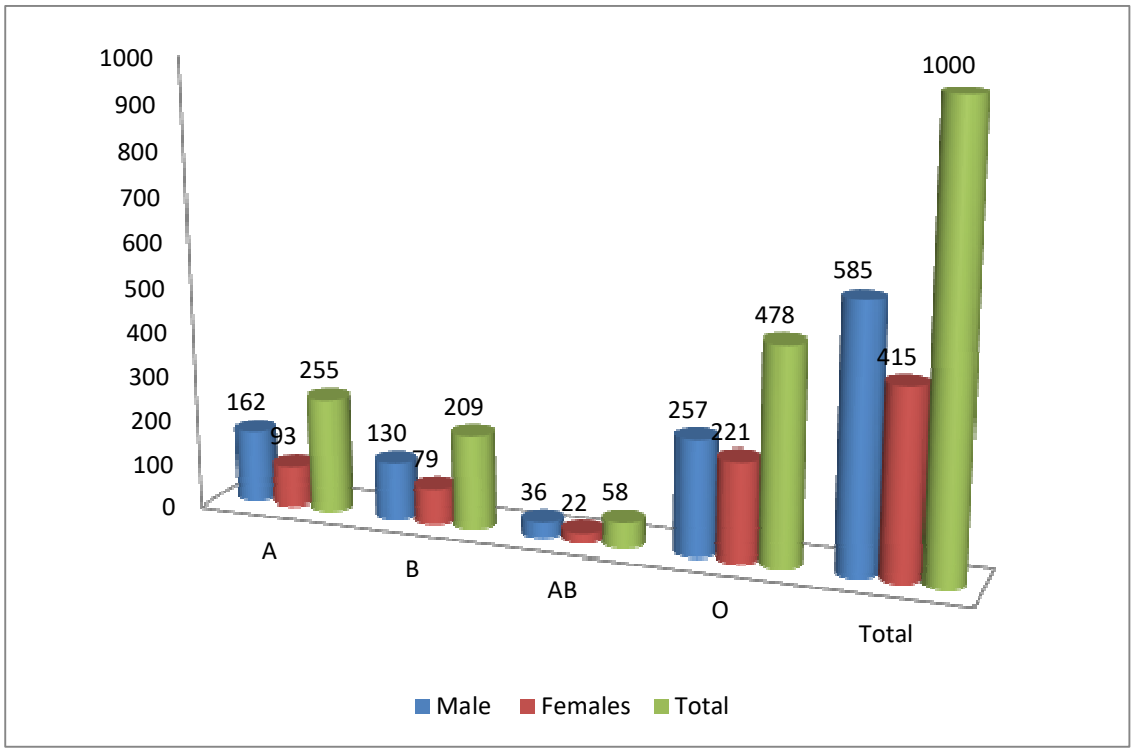

Figure 2: Phenotypic Distribution of ABO blood group based on sex among the students of five ethnic groups

Table 1: Phenotypic frequency distribution of ABO among students of major ethnic group based on sex respondents.

Study groups	A	B	AB	O	Phenotype	Males	Females	Sum
Anyuak	55(5.5%)	52(5.2%)	10(1%)	111(11.1%)	O>A>B>AB	146(14.6%)	82(8.2%)	228(22.8%)
Komo	31(3.1%)	21(2.1%)	11(1.1%)	51(5.1%)	O>A>B>AB	58(5.8%)	56(5.6%)	114(11.4%)
Majang	54(5.4%)	49(4.9%)	11(1.1%)	102(10.2%)	O>A>B>AB	112(11.2%)	104(10.4%)	216(21.6%)
Nuer	73(7.3%)	54(5.4%)	18(1.8%)	125(12.5%)	O>A>B>AB	179(17.9%)	91(9.1%)	270(27%)
Opo	42(4.2%)	33(3.3%)	8(0.8%)	89(8.9%)	O>A>B>AB	90(9%)	82(8.2%)	172(17.2%)
Overall	255(25.5%)	209(21.9%)	58(5.8%)	478(47.8%)	O>A>B>AB	585(58.5%)	415(41.5%)	1000(100)

Phenotypic frequency distribution of Rh blood groups

From all sampled population 953(95.3%) of them were Rh positive. The remaining 47 (4.7%) of them were Rh negative. As distribution pattern of Rh blood group showed that among 585 male individuals 558 (55.8%) and 27 (2.7%) were found for Rh-positive and Rh-negative respectively, while in 415 female individuals 395 (39.5%) and 20 (2%) were Rh-positive and Rh negative respectively. that is given in (Table 2).

In this study the distribution of Rh positive was higher prevalent than Rh negative blood. From all sampled populations 95.3% of them were Rh positive and the remaining 4.7% 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. The current study was similar to studied that has been conducted 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). This study also same with the study that had been conducted by (Getaneh and Muhammadaman, 2015) in Arba Minch, Ethiopia observed that most of the populations were Rh+ (92.8%) and Rh- were (7.2) 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.

The gap between these studies with previous studied is due to the distribution of percentage of Rh+ and Rh- like in Tanzania was Rh+ 98% and Rh- was also 2% compared to current studies the Rh+ was 95.3% while Rh- was 4.7%.

Table 2: Distribution of Rh blood group among students based on ethnic group and sex respondent at Gambella, Ethiopia.

Study group	Rh+	Males	Females	Rh -	Males	Females
Anyuak	216(21.6%)	139(13.9%)	77(7.7%)	12(1.2%)	7(0.7%)	5(0.5%)
Komo	108(10.8%)	55(5.5%)	53(5.3%)	6(0.6%)	3(0.3%)	3(0.3%)
Majang	209(20.9%)	109(10.9%)	100(10%)	7(0.7%)	3(0.3%)	4(0.4%)
Nuer	254(25.4%)	168(16.8%)	86(8.6%)	16(1.6%)	11(1.1%)	5(0.5%)
Opo	166(16.6%)	87(8.7%)	79(7.9%)	6(0.6%)	3(0.3%)	3(0.3%)
Overall	953(95.3%)	558(55.8%)	395(39.5%)	47(4.7%)	27(2.7%)	20(2%)

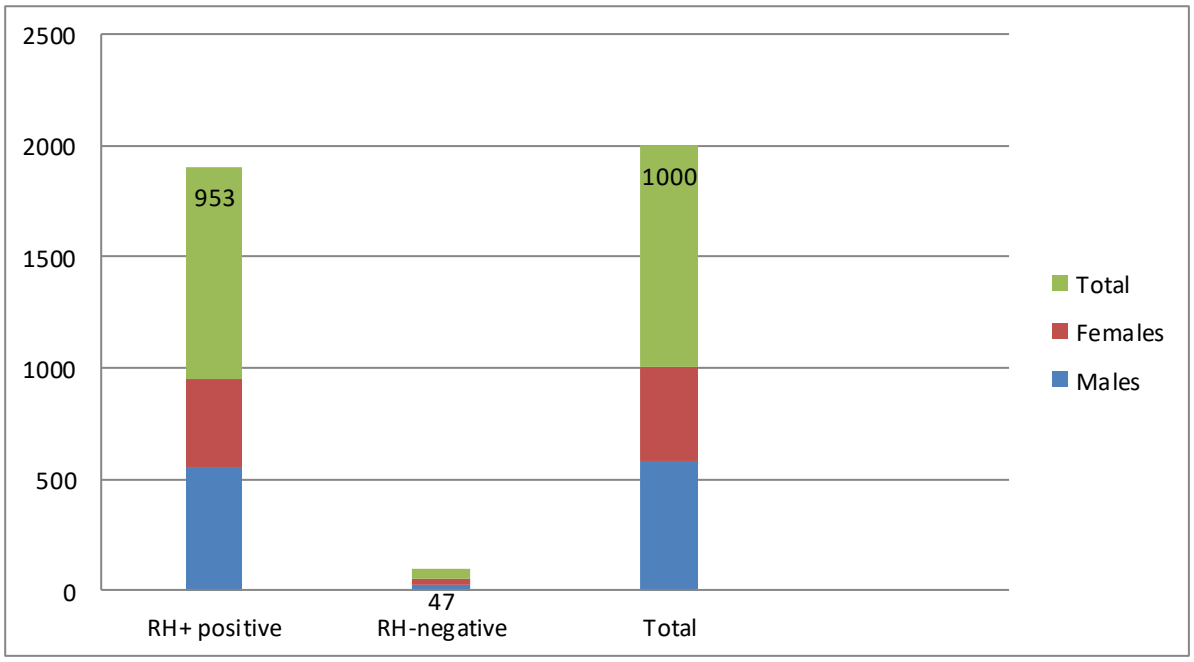

Figure 3: Frequency distribution of Rhesus factor based on sex among five major tribal.

Pooled phenotypic frequency distribution of ABO and Rh blood groups

ABO and RhD frequency distribution also varies with any ethnic groups of human population. In this study it was observed that O is the predominant with frequency of 48%, followed by A, with frequency of 25%, blood group B, is 20% and AB is the least percentage frequency wit 6%. According to the Rh system, 95.3% were Rh positive and 4.7% were Rh negative. Among blood group O+ was found to be the most common 44.5% which is followed by blood group A+ with percentage frequency of 24.9%, blood group B+ is 20.1% and AB+ is the least percentage frequency of 5.8%. As among the Rh negative subject O- was the most frequency of 3.3%, followed by blood group B-, 0.8, A-blood group 0.6%, and lack of AB- in both gender among the overall and each ethnic group as shown in Table 3 and was showed in figure 4.

The current study showed that the majority of donors were male while female was found to be the most common minority of blood donors whereas in this study blood group O+ was found to be the most common (44.5%), followed by A+ (24.9%), B+ (20.1%) and AB+ (5.8%) was the least common among the blood donors, in case of Rh negative subjects, blood group O- was the most frequent (3.3%), followed by blood group B- (0.8%), A- (0.6%) and blood group AB- was not tested among the all donors. This study was same to the study that has been conducted by Hussain *et al.,* 2013 had reported that, out of the 724 subjects tested 613 (84.67%) subjects were Rh+ve and 111 (15.33%) subjects were Rh-ve. This study it is also similar to the study conducted by Patel *et al.*, 2012, had reported that looking at the rhesus grouping, on male's sex, 28.78% were Rh Positive O blood type and remaining 1.98% was Rh Negative O blood type.

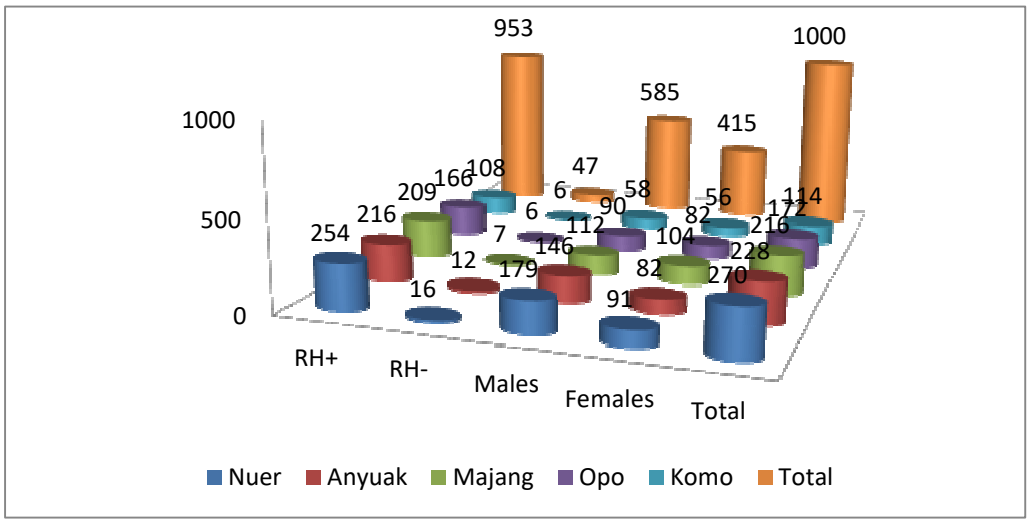

Figure 4: The distribution of Rh Blood D based on ethnic groups among the five major tribal.

Table 3: Distribution of ABO blood group/Rhesus factor based on ethnicity and gender.

Characteristic	ABO blood group and Rhesus factor								
Ethnicity	Sex	A+	A-	B+	B-	AB+	O+	O-	Sum
Anyuak	M	37	1	32	-	8	62	6	146
	F	17	-	18	2	2	40	3	82
Komo	M	18	-	10	-	2	25	3	58
	F	12	1	10	1	9	22	1	56
Majang	M	33	1	28	1	8	40	1	112
	F	20	-	20	-	3	57	4	104
Nuer	M	46	2	36	2	16	70	7	179
	F	24	1	16	-	2	44	4	91
Opo	M	24	-	20	1	2	41	2	90
	F	18	-	11	1	6	44	2	82
Overall tribes		249	6	201	8	58	445	33	1000

Estimation of ABO blood group and Rh (D) alleles

Results of allelic frequencies of ABO and Rh blood groups are listed in (Table 4). The allelic frequencies of the ABO blood group of r (IO), p (IB) and q (IA) were 0.691, 0.144 and 0.171, respectively (IO> IA> IB) while allelic frequencies of the Rh blood group of D and d were 0.783 and 0.217, respectively. In this study, blood group O allele is highly distributed and B allele is the least distributed. For the Rh blood group, there were a higher proportion of Rh (D) +ve individuals than the Rh−ve.

In this study the overall population allelic frequencies distribution O (0.691), is the commonest genes and B (0.144) was the least common allelic in case of Rh D, Rh+ positive was found to be the highest alleles (0.783) and the Rh- was very least allele d (0.217). So the pattern of allelic frequencies was IO>IA>IB. The current study was similar to study that has been reported in Oromia National Regional State (Amsalu, and Daniel, 2017) the allelic frequencies of O (0.64), A (0.21) and B (0.15); D (0.73), and (0.27), the pattern of allelic frequencies was $I^{O}>I^{A}>I^{B}$ (Kassahun *et al*., 2015). This study was also the same to study that was conducted 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), But the slightly different reports were recorded from Madagascar, Guinea and Bangladesh, because prevalence of allele B was greater than A allele, thought O still was the highest allele (Randriamanantany, *et al.,* 2012; Loua, *et al*., 2007; Dewan, *et al*., 2015) Shown in (Table 8).

Table 4: Frequency distribution of allele A (p), B (q) and O(r) among students.

Study groups	Overall tribe	Anyuak	Komo	Majang	Nuer	Opo
A	0.171	0.155	0.205	0.163	0.186	0.158
B	0.144	0.147	0.152	0.150	0.144	0.127
O	0.691	0.698	0.668	0.687	0.680	0.719
RhD	0.783	0.771	0.771	0.821	0.757	0.813
Rhd	0.217	0.229	0.217	0.179	0.243	0.187

Genotypic frequencies of ABO and Rh blood groups among donors

The overall result of the genotypic frequency in this study were OO, AA, AO, BB, BO, AB was 0.477481, 0.029241, 0.236322, 0.020736, 0.199008 and 0.049248 respectively. In case of Rh factor DD, Dd and dd was 0.613089, 0.339822 and 0.047089 respectively, OO is the most common genotype and BB was found to the lowest genotypic frequency, the pattern of genotypic frequency OO>AO>BO>AB> AA>BB. In case of Rh (D) the genotypic frequency distribution was the highest in DD and dd was least genotypic frequency. Indicated in (Table 5).

In this study the overall genotypic frequency OO was the most common frequency with the genotype of 0.477481 and BB was the least common with the genotypes frequency of 0.020736. In case of Rhesus factor the genotypes frequency of DD is the highest than Dd and dd was the least common.

This study was closely related to study that has been conducted by Amsalu and Daniel, 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).

Table 5: Genotypic frequency distribution of ABO/RhD blood groups among participants.

Phenotype	Genotype	Frequency	Overall	Anyuak	Komo	Majang	Nuer	Opo
A	AA	P^2	0.029241	0.024025	0.042436	0.026896	0.034596	0.024964
B	AO	2Pr	0.236322	0.21638	0.27604	0.225336	0.25296	0.227204
AB	BB	q^2	0.020736	0.021609	0.023104	0.022801	0.020736	0.016129
O	BO	2qr	0.199008	0.205212	0.20368	0.207474	0.19584	0.182626
Rh+ve	AB	2pq	0.049248	0.04557	0.062624	0.049528	0.053568	0.040132
Rh-ve	OO	r^2	0.477481	0.4987204	0.4489	0.471969	0.4624	0.516961
	DD	P^2	0.613089	0.594441	0.594441	0.674041	0.573049	0.660969
	Dd	2pq	0.339822	0.353118	0.353118	0.304658	0.367902	0.304062
	dd	r^2	0.047089	0.052441	0.052441	0.032041	0.059049	0.034969

Table 6: Chi square test among each indigenous group ($\alpha = 0.05$).

Study group	df	Table X^2 value	Calculated X^2hyp1	Calculated X^2hyp2	Significance
Anyuak	1	3.841	1.6 *	0.0017*	
Komo	1	3.841	0.015*	3.5*	
Majang	1	3.841	5.4 **	0.04*	
Nuer	1	3.841	3.6*	1.4*	
Opo	1	3.841	2.4*	0.27*	
Overall	1	3.841	15.53**	2.35*	

N.B: **= Significance and *=Not significant whereas $X^2$1 is hypothesis chi-square one and $X^2$2 is also hypothesis chi-square two.

Table 7: Comparative analysis of phenotypic distribution of ABO and Rhesus antigens in different population**.**

Population	O	A	B	AB	Rh(D)+	Rh(D)-	References
Anyuak	11.1	5.5	5.2	1	21.6	1.2	This study
Komo	5.1	3.1	2.1	1.1	10.8	0.6	This study
Majang	10.2	5.4	4.9	1.1	20.9	0.7	This study
Nuer	12.5	7.3	5.4	1.8	25.4	1.6	This study
Opo Overall	8.9 47.8	4.2 25.5	3.3 20.9	0.8 5.8	16.6 95.3	0.6 4.7	This study This study
White non-Hispanic in US	45.20	39.70	10.90	4.10	82.7	17.30	Garratty, *et al.,* 2004.
Hispanic in US	56.50	31.10	9.90	2.50	92.7	7.30	Garratty, *et al.,* 2004.
Black non-Hispanic in US	50.20	25.80	19.70	4.30	92.9	7.10	Garratty, *et al.,* 2004.
Asian in US	39.80	27.80	25.40	7.10	98.3	1.70	Garratty, *et al.,* 2004.
NorthAmerican Indian in US	54.60	35.00	7.90	2.50	90.3	9.70	Garratty, *et al.,* 2004.
All donors in US	46.60	37.10	12.20	4.10	85.4	14.60	Garratty, *et al.,* 2004.
Turkey	29.70	38.20	14.40	6.80	89.6	10.40	Kayiran, *et al.,* 2012.
Northern India	29.10	21.73	39.84	9.33	95.71	4.29	Chandra and Gupta, 2012.
Mauritania	49.10	28.28	18.56	4.05	94.23	5.77	Hamed, *et al.,* 2012.
Morocco	46.80	32.86	15.80	4.53	91	9.00	Chandra and Gupta, 2012.
Cameroun	48.62	25.07	21.86	4.45	96.32	3.68	Ndoula, *et al.,* 2014.
Madagascar	41.60	22.61	29.66	6.13	98.9	1.10	Randriamanantany, *et al.*, 2012.
Guinea	48.88	22.54	23.86	4.72	95.94	4.06	Loua, *et al.*, 2007
Ethiopia	43.08	28.11	23.35	5.44	92.06	7.94	Tesfaye, *et al.,* 2015.
Bangladesh	28.00	27.00	34.00	10.00	99	1.00	Dewan, *et al.*, 2015.
Colombia	37.00	51.18	8.66	3.14	91.33	8.66	Causil-Vargas, *et al.,* 2016.

Table 8: Frequency distribution of allele A (p), B (q) and O (r) among in different population.

Population	A(p)	B (q)	O(r)	References
Anyuak	0.155	0.147	0.698	This study
Komo	0.205	0.152	0.668	This study
Majang	0.163	0.150	0.687	This study
Nuer	0.186	0.144	0.680	This study
Opo	0.158	0.127	0.719	This study
Overall	0.171	0.144	0.691	This study

Tunisia	0.195	0.120	0.685	Said, *et al.,* 2003.
Mauritania	0.177	0.120	0.702	Hamed, *et al.,* 2012
Morocco	0.209	0.108	0.684	Benahadi, *et al.,* 2013.
Cameroun	0.161	0.142	0.698	Ndoula, *et al.,* 2014.
Madagascar	0.156	0.199	0.645	Randriamanantany, *et al.,* 2012.
Guinea	0.147	0.155	0.698	Loua, *et al.,* 2007
Ethiopia	0.189	0.157	0.655	Tesfaye, *et al.,* 2015.
Bangladesh	0.211	0.259	0.531	Dewan, *et al.,* 2012

CONCLUSION

In this study, the percentage frequency distribution of blood group O is the highest and the least percentage frequency is that of blood group AB. Rh (D) positive has the highest percentage frequency while Rh-negative has the lowest percentage frequency as observed among the five ethnic groups. There is also a similar trend in overall student population sampled in which blood group O (47.8%) > A (25.5%) > B (20.9%) > AB (5.8%) and Rh (D) 95.3% is by far greater than Rh (d) which scores 4.7%. With respect to allelic frequencies, allele O records the highest frequencies followed by A allele while allele B records the least frequencies. In the case of Rhesus factor allele D has a frequency distribution far higher than d allele in all the five ethnic groups under this study. This information would be useful to the geneticists and clinicians particularly in the planning of blood transfusion programs. Larger sample sizes, at different area are also being needed to investigate the frequency distribution of ABO/Rh blood groups to make strong generalizations.

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Appendix 1: Expected number of each of the four blood groups genotypes under the two hypotheses.

Blood	Observed	Exp No of Genotypes	Obs-Exp	(Obs-Exp)^2/Exp
	Hyp1 Overall Tribes			
O	478	1000(0. 829)2(0.856)^2 = 503.5	-25.5	1.29
A	255	1000(0.313)(0.856)^2 = 229.4	25.6	2.86
B	209	1000(0.267)2(0.829) = 183.5	25.5	3.54
AB	58	1000(0.313)(0.267) = 83.6	-25.6	7.84
	Hyp2			
O	478	1000(0.685)^2 = 469.22	8.78	0.16
A	255	1000((0.171)^2 + 2(0.171)(0.685)) = 263.51	-8.51	0.27
B	209	1000((0.144)^2 + 2(0.144)(0.685)) = 218.02	-9.02	0.37
AB	58	1000(2(0.171)(0.144)) = 49.25	8.75	1.55
Anyuak				
A	55	228(0.285) (0.854)^2=47.4	7.6	1.22
B	52	228(0.27)(0.845)^2=44	8	1.45
AB	10	228(0.285)(0.27) =17.5	-7.5	3.21
O	111	228(0.845)2(0.854)^2=119	-8	0.54
A	55	228(0.155)^2+228X2(0.155)(0.699)=54.9	0.1	0.000018
B	52	228(0.146)^2+228X2(0.146)(0.699)=51.4	0.6	0.0007
AB	10	228X2(0.155)(0.146)=10.3	-0.3	0.00087
O	111	228X2(0.699)^2=111.4	-0.4	0.00014
komo				
A	31	114(0.37)(0.848)^2=30.3	0.7	0.016
B	21	114(0.281)(0.79)^2=20	1	0.05
AB	11	114(0.37)(0.281)=12	-1	0.08
O	51	114(0.79)2(0.848)2=51.2	-0.2	0.000078
A	31	114(0.21)2+114X2(0.21x0.64)=35.7	-4.7	0.62
B	21	114(0.15)^2+114x2(0.15x0.64)=24.4	-3.4	0.47
AB	11	114x2(0.15x0.21)=7.2	3.8	2.00
O	51	114(0.64)^2=46.7	4.3	0.4
Majang				
A	54	216(0.301)(0.85)^2=47	7	1.04
B	49	216(0.278)(0.836)^2=42	7	1.17
AB	11	216(0.301)(0.278)=18	-7	2.72
O	102	216(0.836)2(0.85)^2=109	-7	0.45
A	54	216(0.16)^2+216x2(0.16x0.69)=53.2	0.8	0.01
B	49	216(0.15)^2+216x2(0.15x0.69)=49.6	-0.6	0.00072
AB	11	216x2(0.16x0.15)=10.4	0.6	0.03
O	102	216(0.69)^2=102.8	-0.8	0.00062
Nuer				
A	73	270(0.34)(0.856)^2=67.3	5.7	0.48
B	54	270(0.267)(0.81)^2=47.3	6.7	0.95

AB	18	270(0.267)(0.34)=25	-7	1.96
O	125	$270(0.856)^2(0.81)2=130$	-5	0.19
A	73	270(0.19)2+270x2(0.19x0.666)=78	-5	0.32
B	54	$270(0.144)^2$+270x2(0.144x0.666)=57.2	-3.2	0.18
AB	18	270x2(0.19x0.144)=14.8	3.2	0.7
O	125	$270(0.666)^2=120$	5	0.2
Opo				
A	42	$172(0.29)(0.87)^2=38$	4	0.42
B	33	$172(0.24)(0.84)^2=29$	4	0.55
AB	8	172(0.24)(0.29)=12	-4	1.33
O	89	$172(0.87)2(0.84)^2=92$	-3	0.09
A	42	$172(0.16)^2$+172x2(0.16x0.71)=43.5	-1.5	0.05
B	33	172(0.13)2+172x2(0.13x0.71)=34.5	-1.5	0.06
AB	8	172x2(0.16x0.13)=7	1	0.14
O	89	$172(0.71)^2=87$	2	0.02