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Frequency of ABO, Rh Blood Group Alleles Among Oromo, Amhara and Wolayita Ethnic Group Students in Robe Secondary, Preparatory and Zeybela Primary School, Bale, Ethiopia

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Abstract: The ABO and Rh blood groups are the most important blood groups despite the long list of several other blood groups discovered so far. The ABO and Rh blood groups varies worldwide and are not found in equal numbers even among ethnic groups. Therefore, this study aimed at having information on the frequencies of alleles, phenotypes and genotypes of ABO and Rh D blood groups among the major ethnic groups of Robe Secondary and Preparatory and Zebela Primary school students in Oromia region, Bale zone, Robe town. A total of 600 students were purposively selected and divided into 3 major ethnic groups i.e., Oromo, Amahara, and Wolayita, each consists of 200 students. Purposively sampled students were obtained on the basis of their willingness to participate by filling all their profile and signed on the consent agreement format. Differences in allelic, phenotypic and genotypic frequencies of the (ABO) and Rh D blood groups among the three ethnic groups of the students were observed. Blood group O and Rh (D) positive has highest allelic and phenotypic frequencies while blood group AB and Rh (D) negative has the lowest allelic and phenotypic frequencies in all the three ethnic groups. However, apart from the importance of ABO and Rh blood groups in blood transfusion practice, it is therefore imperative to have information on the distribution of these blood groups in any population group that comprise different ethnic groups.

Keywords: Allele, Ethnic Group, Frequency, Genotypes, Phenotypes, ABO Blood Group, Rh Blood Group

1. Introduction

The ABO and Rh blood groups are among the useful genetic markers in human population studies. They are the most well known and medically significant blood types in blood transfusion. The ABO system is the first polymorphism to be defined in human beings and was first describedby Karl Landsteiner in 1901 (Greenwell, 1997).

The Rhesus (Rh)D was in fact was the fourth blood group system to be discovered and latter ranked second to ABO system in terms of clinical importance (Ahmed *etal.,* 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. According to the presence of antigens and agglutinins, individuals are divided in to 4 major blood groups A, B, AB and O (Garratty *et al.,* 2000).

Blood carries several antigens with in it, which form the basis of its reactivity and hence it is not possible to mix the blood of all humans without initiating an immune reaction. Only the blood samples, which share the same antigenic

identity, do not initiate an immune response, and hence are termed as compatible. The utility of these antigens is not only for blood transfusion or organ transplantation, but have also been utilized in genetic research, anthropology and tracing of ancestral relation to human beings (Eastlund T, 1998).

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. It also plays a key role in medical treatment prior to blood transfusion and child birth. The blood group is determined by the genetic make-up of the alleles of a system.Estimates of genes frequency provide very valuable information on the genetic similarities or differences of different populations and to some extent on their ancestral genetic linkage, despite the cultural and religious differences (Meade, 1994).

Blood grouping has improved with the advent of monoclonal antibodies and the automation of testes. Although different advanced techniques, such as micro plate method, PCR based typing, FMC based typing, mini sequencing analysis, fluorescent immune micro plate technique, sandwich ELISA method, etc… are available for ABO genotyping, the Manual method has its own significance not only in blood typing but also measuring its genotypic frequency by Hardy- Weinberg law (Srikumary *et al.,* 1987).

Classification of the blood group was based on observation of the agglutination reaction between an antigen on erythrocytes and antibodies present in the serum of individuals directed against these antigens. Where no agglutination had occurred, either the antigen or the antibody are missing from the mixture. ABO and Rh blood group systems in humans are two important genetic markers that are routinely analyzed prior to blood transfusion and medical treatment. The ABO blood group system is governed by a single gene (the ABO gene) with three alleles (I^A, I^B and I^O), of whichI^A, and I^B alleles are co-dominant but both of them are dominant over the recessive alleles I^O in intra allelic interaction in diploid condition. The gene for ABO blood group is located on the long arm of the ninth chromosome9q34.1 of human genome (Amundadottir *etal.,* 2009)while that of Rh is located on the short arm of the first chromosome, 1p34-p36 (Cartron1994).

The ABO and Rh blood group alleles vary worldwide and are not found in equal numbers even among the same ethnic groups. Among African-Americans the distribution of ABO blood group is type O, 46%; A, 27%; type B, 20%; and type AB, 7%. AmongCaucasians in the United states, the distribution of type O, 47%; type A, 41%; type B, 9%; and type AB, 3%. Also, among Western Europeans, type O, 46%; type A, 42%; type B, 9% and type AB, 3% (L. Beckman, 2008).Table 1 shows the distribution of the ABO blood types along racial and ethnic lines.

Table 1. *Racial and ethnic distribution of ABO (without Rh) blood types.*

PEOPLE GROUP	O (%)	A (%)	B (%)	AB (%)
Aborigines	61	39	0	0
Abyssinians/Ethiopia	43	27	25	5
Ainu (Japan)	17	32	32	18
Albanians	38	43	13	6
Grand Andamanese	9	60	23	9
Arabs	34	31	29	6
Armenians	31	50	13	6
Asian (in USA - General)	40	28	27	5
Austrians	36	44	13	6
Bantus	46	30	19	5
Basques	51	44	4	1
Belgians	47	42	8	3
Blackfoot (N. Am. Indian)	17	82	0	1
Bororo (Brazil)	100	0	0	0
Brazilians	47	41	9	3
Bulgarians	32	44	15	8
Burmese	36	24	33	7
Buryats (Siberia)	33	21	38	8
Bushmen	56	34	9	2
Chinese-Canton	46	23	25	6
Chinese-Peking	29	27	32	13
Chuvash	30	29	33	7
Czechs	30	44	18	9
Danes	41	44	11	4
Dutch	45	43	9	3
Egyptians	33	36	24	8
English	47	42	9	3
Eskimos (Alaska)	38	44	13	5
Eskimos (Greenland)	54	36	23	8
Estonians	34	36	23	8
Fijians	44	34	17	6
Finns	34	41	18	7
French	43	47	7	3
Georgians	46	37	12	4
Germans	41	43	11	5

Source: - (L. Beckman, (2008).

Table 2. *ABO and Rh blood type donation showing matches between donor and recipient types.*

		Donors							
		O+	A+	B+	AB+	O- **	A-	B-	AB-
Recipients	O+	✔				✔			
	A+	✔	✔			✔	✔		
	B+	✔		✔		✔		✔	
	AB+ *	✔	✔	✔	✔	✔	✔	✔	✔
	O-					✔			
	A-					✔	✔		
	B-					✔		✔	
	AB-					✔	✔	✔	✔

* Type AB+ is the universal recipient: Although those with AB blood type may be referred to as universal recipients, in actuality, type AB+ blood is that of the universal recipient, whereas type AB- is not. This is an important distinction to make.

** Because A-, A+, B-, B+, AB-, AB+, O- and O+ individuals can all receive blood from donors of type O- blood, an individual with type O- blood is deemed universal donor. In similar manner, O+ is not the universal donor blood type.

As far as transfusion compatibility is concerned, it is not strictly as simple as matching A, B, and O groups. In other words, no individual will ever receive a blood transfusion

based on the ABO system alone. The Rhesus factor must also be considered. Together, the Rhesus factor and ABO blood grouping are the two most important compatibility factors to consider. (Mc Clelland DBL, 2001).

African Countries like 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 and/or 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 three major ethnic groups - Oromo, Amhara and Wolayita 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 usedto 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 of knowledge and serves as a reference material for another research of the same type or researches of different version of this topic carried in the zone or other places of our country.

Therefore the general objective of the study is to come up with document that shows differences on the phenotypic, genotypic and allelic frequencies of ABO and Rh blood group system among Robe Secondary and Preparatory and Zebela Primary School students belonging to the three ethnic groups living in the same geographical areawho were not interbreed.This research findings has multi-purposes future utilities for the health planners and also shows the common trends of the prevalence of various blood groups among the three ethnic group students.

Specific Objectives

a. To determine the frequency distribution of ABO and Rh blood group phenotypes among the Oromo, Amhara and Wolayita students of Robe Secondary and preparatory and Zebela primary school and compare it with the distribution of blood group phenotypes of the country, Ethiopia.
b. To determine the genotypic and allelic frequencies of ABO and Rh blood groups among the three ethnic groups under study.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in Oromia regional state, Bale zone, Robe town (Robe Secondary and Preparatory and Zebela Primary School which have got over 2752 students belonging to different ethnic groups. The town is located at an altitude of 2492 meter above sea level and 430Km to the southeast of Addis Ababa the capital city of Ethiopia, having an average annual rain fall of 1400mm. According to 2007 G.C. National house and population census report, Robe town has a total population of 57,385 from which 29,148 is male and 28,237 is female (Projected by the annual growth rate of 2.93% (CSAE, 2007).

2.2. Sampling Procedure and Sample Size

Robe Secondary and Preparatory School and Zebela Primary School have 2752 students of which Robe Secondary and Preparatory school comprises of 1237 students while the later 1515 students enrolled for the academic year 2012/2013 G.C. The latter school was included in the research activity to increase the chance of getting students' of Amhara and Wolayita ethnic groups due to the fact that this school is the only primary school that uses Amharic language for media of education and hence the majority of Amhara and Wolayita ethnic group children attend their primary education in here. The study was conducted on 600 purposively sampled students comprising approximately 22% of the students' population within the two schools. The sample students were selected purposively so as to include equal number of students from the three ethnic groups:- Oromo, Amhara and Wolayita in the two schools. Thus, the sampled students were divided in to three equal groups each consisting of 200 students and stratified along ethnic lines. Purposively sampled students were obtained on the basis of their willingness to participate by filling all their profile and signed on the consent agreement format. The profile filled by the participant students were got acceptance by the researcher after the two school directors signed and stamped for its correctness. The information about ethnic groups was reapproved by directly asking the students during his or her actual participation. Students from parents of two different ethnic groups were not included in the research activity due to the difficulty in determining the ethnic group of such individuals.

2.3. Blood Sample Collection and Grouping Method

The ABO and Rh blood group test was performed by using standardized and packed lancet, to obtain blood from finger picks for 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. Standardized anti sera, Anti-A, Anti-B and Anti-D were obtained commercially from Robdan medical drugs and chemicals distributor in Robe-Bale.Blood groups and Rhesus factor were determined on the basis of 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.

2.4. Method of Data Analysis

The genetic structure of a population was determined by

the total number of all alleles (the gene pool) in the case of sexually interbreeding individuals; the structure was also characterized by the distribution of alleles in to genotypes. The genetic structure could be described in terms of allelic and genotypic frequencies (Russel, 2005). For this study, the frequency of the blood group genotypes was used to calculate the frequency of the ABO blood group alleles by using the extension of Hardy-Weinberg principle (HWP), and chi-square (χ^2) test was used during comparison of observed frequencies of ABO and Rh blood group with expected frequencies. (Chakraborty DP, 2010). Extension of the Hardy Weinberg law to loci with more than two alleles was used to analyze the genotypic and allelic frequencies based on Hardy–Weinberg equations. Chi-square goodness- of-fit statistic was calculated to compare observed and expected frequencies and to investigate heterogeneity.

When two alleles are present at a loci, the Hardy Weinberg law tells us at equilibrium the frequencies of the genotype is:-

$$p^2 + 2pq + q^2 = 1, \text{ which is the square of allelic frequencies } (p + q)^2 \quad (1)$$

This is the simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are sampled two at a time in to a diploid zygote (Daniel *et al.,* 2007).For this study three alleles are computed (A, B and O), with frequencies equal to p, q and r respectively. The frequencies of the genotype at equilibrium will be computed by the square of thee allelic frequencies.

$$(p+q+r)^2 = p^2\,(AA) + 2pq\,(AB) + q^2\,(BB) + 2pr\,(AO) + 2qr\,(BO) + r^2\,(OO)=1 \quad (2)$$

(Griffith *et al.,* 2008).

Gene frequency is calculated considering two alleles at the same locus for Rh system and three alleles at the same locus for ABO system using standard formulae of quantitative genetics. ABO allele frequencies were estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates were calculated as:

$$p = 1 - \sqrt{B+O},\ q = 1 - \sqrt{A+O},\ r = \sqrt{O} \quad (3)$$

where p, q, r denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O. A correction factor (d) will be calculated according to:-

$$d = 1 - p - q - r \quad (4)$$

The final allele frequencies were then calculated as follows:-

$$p1 = p\,(1 + d/2);\ q1 = q\,(1 + d/2);\ r1 = (r + d/2)\,(1 + d/2) \quad (5)$$

where p1, q1, and r1 denote corrected allele frequencies. Rh (D) allele frequencies were calculated according to the Hardy-Weinberg equation. Observed and expected genotype frequencies in Hardy-Weinberg were calculated on the basis of gene's frequency and Chi-square tests was done to test the independence and the goodness of fit for genotype frequencies (Chakraborty, 2010).

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

$$x^2 = \sum \frac{(O_i - E_i)^2}{E_i} \quad (6)$$

Ethical clearance of the study design was obtained from Bale zone Health Office after it was revised and got acceptance by the health management committee of the office.

3. Results, Discussions and Conclusion

3.1. Phenotypic Frequencies of ABO and Rh Blood Group Among Oromo, Amhara and Wolayita Ethnic Groups in the Study Area

Table 3 represents the phenotypic percentage distribution of ABO and Rh blood group of Oromo, Amhara and Wolayita ethnic group students in Robe secondary and preparatory and Zebela Primary school of 200 blood samples tested from each ethnic groups in this study are O (42%), A (28%), B (25%) and AB (10%) for Oromo ethnic group, O (43%), A (29%), B (23%) and AB (10%) for Amhara ethnic group and O (44.5%), A (27%), B (24%) and AB (4.5%) for Wolayita ethnic group.

With respect to Rhesus93.5% of the population sampled from Oromo ethnic group were Rh (D) +vewhile 6.5% were Rh (D)-ve and 94.5% population sampled from both Amhara and Wolayita ethnic groups were Rh (D) +ve while 5.5% were Rh (D)-ve.

Table 2. *Phenotypic Distribution of ABO and Rh Blood Group Systems among the three ethnic groups (Oromo, Amhara and Wolayita).*

Study Group	ABO Blood Grouping System					Rh (Rhesus Blood Grouping System	
	O	A	B	AB	Total	Rh+	Rh-
Oromo	84 (42)	56 (28)	50 (25)	10 (5)	200	187 (93.5)	13 (6.5)
Amhara	86 (43)	58 (29)	46 (23)	10 (5)	200	189 (94.5)	11 (5.5)
Wolayita	89 (44.5)	54 (27)	48 (24)	9 (4.5)	200	189 (94.5)	11 (5.5)
Over all	259 (43.17)	168 (28)	144 (24)	32 (4.83)	600	565 (94.17)	35 (5.83)

Values in parentheses represent phenotype percentage occurrence
Source: Own survey data, 2013

3.2. Estimating Genotypic and Allelic Frequencies of ABO and Rh Blood Group Among Oromo, Amhara and Wolayita Ethnic Groups in the Study Area

Table 4 represents the allelic and genotypic frequencies of ABO and Rh blood group of Oromo, Amhara and Wolayita ethnic group students in Robe secondary and preparatory and Zebela Primary school. The allelic frequencies of ABO blood group for Oromo ethnic group was O = 0.6540, A = 0.1821 and B was 0.1639. The allelic frequencies of ABO blood group for the Amhara ethnic group was also O = 0.6600, A = 0.1881 and B was 0.1519. Similarly O = 0.6772, A = 0.1729 and B was 0.1549 for Wolayita ethnic groups.

With respect to Rhesus blood grouping system, the allelic frequency of Oromo ethnic group was 0.745 for D and 0.255 for d. And the allelic frequency was found to be 0.765 for D and 0.235 for d for both Amhara and Wolayita ethnic groups.

Table 4 also shows the frequencies of the various genotypes in the ABO and Rh systems. So that OO = 0.4277, AA = 0.0332, AO = 0.2382, AB = 0.0597, BB = 0.0269 and BO = 0.2144 in Oromo ethnic group. It follows the same patterns of distribution in both Amhara and Wolayita ethnic groups. The frequency of the genotypes for Rh blood group in Oromo ethnic group were 0.5550 for DD, 0.380 for Dd and 0.065 for dd and 0.5852 for DD, 0.3596 for Dd and 0.0552 for dd both for Amhara and Wolayita ethnic groups.

Table 4. *Allelic and Genotypic frequencies of ABO and Rh blood group for the three ethnic groups (Oromo, Amhara and Wolayita).*

Study Group	Allele	Freq.	Genotype	Freq.	Phenotype	Freq.
Oromo	O	0.6540	OO	0.4277	O	42
	A	0.1821	AA	0.0332	A	28
			AO	0.2382	A	
	B	0.1639	BB	0.0269	B	25
			BO	0.2144	B	
	-	-	AB	0.0597	AB	5
			DD	0.5550	D	93.5
	D	0.745	Dd	0.3710	D	
	d	0.255	dd	0.0650	d	6.5
Amhara	O	0.6600	OO	0.4356	O	43
	A	0.1881	AA	0.0354	A	29
			AO	0.2483	A	
	B	0.1519	BB	0.0231	B	23
			BO	0.2005	B	
	-	-	AB	0.0571	AB	5
	D	0.765	DD	0.5852	D	94.5
			Dd	0.3596	D	
	d	0.235	dd	0.0552	d	5.5
Wolayita	O	0.6722	OO	0.4519	O	44.5
	A	0.1729	AA	0.0299	A	27
			AO	0.2324	A	
	B	0.1549	BB	0.0240	B	24
			BO	0.2082	B	
	-	-	AB	0.0536	AB	4.5
	D	0.765	DD	0.5852	D	93.5
			Dd	0.3596	D	
	d	0.235	dd	0.0552	d	6.5
Over all	O	0.6621	OO	0.4384	O	43.17
	A	0.1810	AA	0.0328	A	28
			AO	0.2397	A	
	B	0.1569	BB	0.0246	B	24
			BO	0.2078	B	
	-	-	AB	0.0568	AB	4.83
	D	0.7585	DD	0.5753	D	94.17
			Dd	0.3664	D	
	d	0.2415	dd	0.0583	d	5.83

Source: Own survey data, 2013

3.3. Statistical Method to Test the Goodness-of-Fit

Table 5 represents the observed proportions of ABO and Rh individuals in the studied population when compared with expected proportions. It also shows the chi-square (χ^2) and probability (p) value for all the three ethnic groups separately and for the overall student population sampled in the study.

Table 5. *Observed versus expected frequency of ABO and Rh Blood groups among individuals sampled from the three ethnic groups (Oromo, Amhara and Wolayita).*

StudyGroup	ABO Blood system					Rh Blood System				
	Blood group	Obs. No	Obs. Freq. (%)	Exp. Freq(%)	Exp. No	Blood Group	Obs. No	Obs. Freq. (%)	Exp. Freq.(%)	Exp. No
Oromo	O	84	42	0.4356	85.54	Rh(D)	187	0.745	0.9350	186.995
	A	56	28	0.2713	54.28	Rh(d)	13	0.255	0.0650	13.005
	B	50	25	0.2413	48.26					
	AB	10	5	0.0579	11.94					
$\chi^2 = 0.4601$, $p < 0.95$										
Amhara	O	86	43	0.4356	87.12	Rh(D)	189	0.765	0.9448	188.96
	A	58	29	0.2837	56.74	Rh(d)	11	0.235	0.0552	11.04
	B	46	23	0.2236	44.72					
	AB	10	5	0.0571	11.43					
$\chi^2 = 0.2556$, $p > 0.95$										
Wolayita	O	89	44.5	0.4519	90.38	Rh(D)	189	0.765	0.9448	188.96
	A	54	27	0.2623	52.46	Rh(d)	11	0.235	0.0552	11.04
	B	48	24	0.2322	46.45					
	AB	9	4.5	0.0536	10.72					
$\chi^2 = 0.3965$, $p \leq 0.95$										
Overall	O	259	43.17	0.4384	263.04	Rh(D)	565	0.7585	0.9417	565.02
	A	168	28	0.2725	163.50	Rh(d)	35	0.2415	0.0583	34.98
	B	144	24	0.2324	139.44					
	AB	29	4.83	0.0568	34.08					
$\chi^2 = 1.082$, $p \leq 0.8$										

Source:- Own survey data, 2013

3.4. Discussions

As it is indicated in Table 3, the phenotypic distribution of ABO and Rh (D) blood group among Oromo, Amhara and Wolayita ethnic group students in Robe secondary and preparatory and Zebela primary school in Bale zone, Ethiopia. My data revealed that the ABO blood group frequencies in Oromo ethnic group was found in the order O > A > B > AB (42%, 28%, 25% and 5%) respectively among 200 students purposively sampled. The order of ABO blood group frequencies observed in Oromo ethnic group holds true for both Amhara and Wolayita ethnic groups in which O > A > B > ABand their frequencies were 43%, 29%, 23%, 5% and 44.5%, 27%, 24% and 4.5% respectively among 200 students purposively sampled for each ethnic groups. When compared with other reports from similar studies, the researcher's data is consistent with previous findings from other parts of the world including Ethiopia: - O (43%) > A (27%) > B (25%) > AB (5%) (L. Beckman, 2008).

For example, inBritain, the percentage frequencies of the ABO bloodgroup were 47%,42%, 9%, and 3.0% for O,A, B, and AB blood groups respectively (L. Beckman, 2008). Findings reported from India (USA-General) also showed that the percentage frequencies are 79%, 16%, 4%, and 1% for O, A, B, and AB blood groups respectively (L. Beckman, 2008). See Table 1. In the Northern part of Nigeria, (Kulkarni *et al.,* 1985) obtained frequencies of 46.6%, 29.95%, 23.05% and 4.4% for blood group O, A, B and AB respectively and frequencies of 55.3%, 25.3%, 16.7% and 2.7% in the order O > A > B > AB were also obtained among 150 students of Cell Biology and Genetics at the University of Lagos, Nigeria (Adeyemo and Soboyejo, 2006). Among the Caucasians in the United States of America, the frequency of blood group O, A, and AB are 47%, 41%, 9% and 3% respectively (L. Beckman, 2008).

However; my findings seem to deviate from the results obtained by L. Beckman, 2008 on the phenotypic frequencies of blood group antigens from Ainu (Japan) where ABO blood group frequency occurredin the order A = B (32%) > AB (18%) > O(17%). It also seem not to agree with the results obtained from Swat district in Pakistan where the percentage frequencies were A=27.92%, B= 32.40%, O = 29.10% and AB= 10.58% in which B > O > A > AB (Khattak *et al.,* 2008). It is also not consistent with ABO phenotypic frequency of Bororo (Brazil) in which 100% of the population are O blood groups (L. Beckman, (2008).

Results shows along ethnic line, 93.3% of the oromo ethnic group (consisting 55.5% of DD individuals and 37.995% Dd individuals) were phenotypically Rh +ve while 6.5% were Rh -ve. In the case of Amhara and Wolayita ethnic groups 94.5% (consisting 58.5% of DD individuals and 35.96% Dd individuals) were phenotypically Rh +ve while only 5.5% were Rh –ve. These findings show that in all the three ethnic groups- Oromo, Amhara and Wolayita, the proportion of Rh -ve is far lower than for Rh +ve. The findings are consistent with reports from previous similar studies among different sets of Nigerian population where the Rh(D) positive was found to behigher in the population sampled than the Rh (D)negative (Kulkarni *et al.,* 1985,Ahmed and Obi, 1998;Omotade *et al.,* 1999; Ahmed *et al.,* 2004;Ahmed *et al.,* 2007; Jeremiah and Odumody,2005, Bakare *et al.,* 2006, Akhigbe *et al.,* 2009, Adeyemo and Soboyejo, 2006) see Table 3.The results, however; differ from the work reported by Yousaf and colleagues where the

population sampled among Bahawalpur division of Pakistan population were all Rh D positive (Yousaf *et al.,* 1988). It also disagrees to that of Salmon *et al.* 1988 and Njoku *et al.,* 1996 who reported rhesus positive values of 100% for Eastern Highlands of Papua Guinea and Nigeria, respectively. In addition it is dissimilar to that in Indians with a preponderance of the Rh(d) of 89.7% over theRh(D) gene of 10.3%(Thangaraj *et al.,* 1992).

The frequencies of the various genotypes of the ABO and Rh systems in Table 4 shows that OO = 0.4277, AA= 0.0332, AO = 0.2382, AB = 0.0597, BB= 0.0269 and BO = 0.2144 in Oromo ethnic group. It follows the same patterns of distribution in both Amhara and Wolayita ethnic groups. Blood group A and B shows co-dominance to each other and dominant to O. For example, in Amhara ethnic group, the frequency of AA genotype was 0.0354 while AO genotype was 0.2483. Thus, among those who are blood group A, 13.17% were homozygous AA, while about 86.83% were heterozygous AO. Similar deduction can be made for blood group B and for Rh +ve among DD and Dd individuals in all the three ethnic groups.

The frequency of the genotypes for Rh blood group were 0.5550 for DD, 0.380 for Dd and 0.065 for dd in Oromo ethnic group and 0.5852 for DD, 0.3596 for Dd and 0.0552 for dd both for Amhara and Wolayita ethnic groups.

Hence, it is possible to say that the highest percentage of blood group A and B are found in heterozygous genotypes AO and BO. Blood group O and homozygous dominant Rh(D) records the highest frequencies while the co dominant blood group AB and homozygous recessive Rh(d) genotypes record the least genotype frequencies in all the three ethnic groups under this study.

Once again as it is indicated in Table 4 in all the three ethnic group students (Oromo, Amhara and Wolayita), the allelic frequencies of ABO blood group was occurred in the order O > A > B. It shows similar patterns of allelic frequencies with those documented from earlier studies among various segments of the world population including Ethiopia in which O (0.66) > A (0.1759) > B (0.1638). For instance similar study by Bakare *et al,* 2006 in Ogbomoso,South-west Nigeria,Omotade *et al,* 1999 among a healthy infant populationin Ibadan, Nigeria, Yan *et al,* 2005 on Chinesepopulations and Hussain *et al,* 2001 amongBalochistan in Pakistan all found the allelic frequenciesto occur in O > A > B order.

With respect to Rhesus factor, allele D is far higher in frequency than allele d in all the three ethnic groups and in the overall student population sampled.

As it is indicated in table 5, the application of extended Hardy-Weinberg principle for three or more alleles yields little variation in the observed and expected genotypic frequencies and numbers which serves as a base in determining the chi-square (χ^2) values that further used in determining the goodness-of- fitness. Hence, the chi-square value for Oromo ethnic group is 0.4601 with $p \leq 0.95$, for Amhara ethnic group $\chi^2 = 0.2556$ with $p > 0.95$, for Wolayita ethnic group $\chi^2 = 0.3965$ with $p \leq 0.95$ and for the overall student population sampled, the chi-square value is 1.082 with $p \leq 0.8$ values. With determined degree of freedom 3 and 0.05 significance level the researcher's result is acceptable.

3.5. Conclusion

In this study, the percentage frequency distribution of blood group O is the highest with percentage frequency of 42%, 43% and 44.5% in Oromo, Amhara and Wolayita ethnic groups respectively, followed by blood group A (28%, 29% and 27%) and blood group B (24%, 23% and 24%), and the least percentage frequency is that of blood group AB (5%, 5% and 4.5%) in the three ethnic groups. Moreover, this study further confirmed that Rh (D) positive has the highest percentage frequency while Rh-negative has the lowest percentage frequency as observed among the three ethnic groups. There is also a similar trend in overall student population sampled in which blood group O (43.17%) > A (28%) > B (24%) > AB (4.83%) and Rh(D) 94.17% is by far greater than Rh(d) which scores 5.83%. With respect to allelic frequencies, allele O records the highest frequencies (0.6540, 0.66 and 0.6722) in Oromo, Amhara and Wolayita ethnic groups respectively. Followed by allele A (0.1821, 0.1881 and 0.1729) while allele B records the least frequencies (0.1639, 0.1519 and 0.1549). In the case of Rhesus factor allele D has a frequency distribution far higher than d allele in all the three ethnic groups under this study.

The implication of this finding is that blood type O is the most readily available blood group in the study area which is more advantageous for the population in the event of blood transfusion. The higher proportion of blood group O in the studied population is an advantage because some parts of Bale zone is a malaria epidemic zone hence individuals belonging to blood group O may be protected from severe malaria attack due to the mechanism of reduced resetting.

The dominance of O allele may be as a result of the fact that many As and Bs blood group have been heterozygous carrying O allele silently thereby maintaining O allele in the heterozygous population. For example, this finding shows that in Amhara ethnic group, the frequency of AA genotype was 0.0356 while AO genotype was 0.2483. Thus, among those who are blood group A, 13.17% were homozygous AA, while about 86.83% were heterozygous AO. Similar deduction can be made for O allele to be carried silently in BO heterozygous form in blood group B in all the three ethnic groups. However; it is the researcher's candid opinion that molecular characterization of O allele could assist in elucidating the possible causes of blood group O predominance in various populations.

To sum up results from this study show the phenotypic, genotypic and allelic frequencies of ABO and Rh blood group varies in different ethnic groups and hence, it serves as a key genetic markers in population studies. I have by these results presented in this study, provide information on the Phenotypic, genotypic and allelic frequencies of ABO and Rh blood group in Oromo, Amhara and Wolayita ethnic groups, Bale zone of Ethiopia hoping that it will serve as a reference

for other studies. I believed this study will further contribute to existing knowledge in this field and help in planning for future clinical challenges especially when it relates to blood transfusion, genetic counseling and HDN.

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