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ORIGINAL RESEARCH ARTICLE

ABO and RH Blood Group Type Frequencies among Students from Different Ethnic Groups at Enchini Secondary School, West Shewa, Oromia Region, Ethiopia

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Abstract

The knowledge about ABO and Rh-D groups at individual and population levels is important for healthcare managements, clinical and genetic analyses. Ethiopia is working in archiving major events "*Kunets*" that have social and economic values. ABO blood groups and Rh-factor as biological characters and inheritance of genetic materials have major contributions to the "*Kunets*". The study aimed to determine ABO and Rh-D blood groups distribution frequencies among 108 Amhara, 72 Gurage and 238 Oromo students at Enchini Secondary School. Each participant's ABO and Rh-D blood types were determined by using agglutination reaction tests. In each study group, blood group O had the highest frequencies (38.39–41.18 %) whereas AB scored the least frequencies (6.30–10.18 %). Allele O scored the highest frequency (63.5–64.2%) and allele B scored the least frequencies (17.0–18.2%). Rh^+ had the higher frequencies (91.67–94.12 %). The highest frequencies for O type (41.18%) and Rh^+ (94.12%) were from Oromo students. The highest heterozygosity (54.65%) for ABO blood type was from Amhara students and the least (52.36%) was from Oromo students. All the differences between the observed and expected mean values of frequencies in the two blood system were not statistically significant at $\alpha = 0.05$. The result would serve as a reference by lawyers in paternity suits, by police in forensic science, by anthropologists in the study of different populations, and for other studies and future utilities in health care planning and other needs or "*Kunets*" in the studied area.

Keywords: ABO blood, allele frequency, genotype, phenotype, Rh-factor

Introduction

All human population shares the same blood group system but the frequency of specific blood type differs in population. The difference among human blood type groups are due to the presence or absence of certain protein molecules in the form of antibodies and antigens. In humans, about 36 blood group systems with about 700 antigens have been identified (Deshpande and Wadde, 2013). ABO blood group system is the first and Rh-D system is the second most significant blood group system in human blood transfusions and tissue transplantations. ABO and Rh-D antigens are specific to red blood cells (John *et al*, 2003).

A single gene which controls the development of ABO blood group type has three alleles: A, B, and O. The combination of these three alleles gives six genotypes: AA, AO, BB, BO, AB, and OO; and four blood group phenotypes: A, B, AB, and O. Alleles of ABO gene expressed either as codominant (A & B)

or complete dominant (A and B over O). The ABO gene encodes a glycosyltransferase that modifies the carbohydrate content of the red blood cell antigens. The *glycosyltransferase* encoded by allele “A” bonds α-N-acetylgalactosamine to the D-galactose end of the H antigen, produces antigen “A” and the enzyme encoded by allele “B” bonds α-D-galactose to the D-galactose end of the H antigen, produces antigen “B”. The gene mapped on chromosome 9q34.1-34.2 and consisted 7 exons ranging from 28 to 688 bp (Yamamoto, 2001).

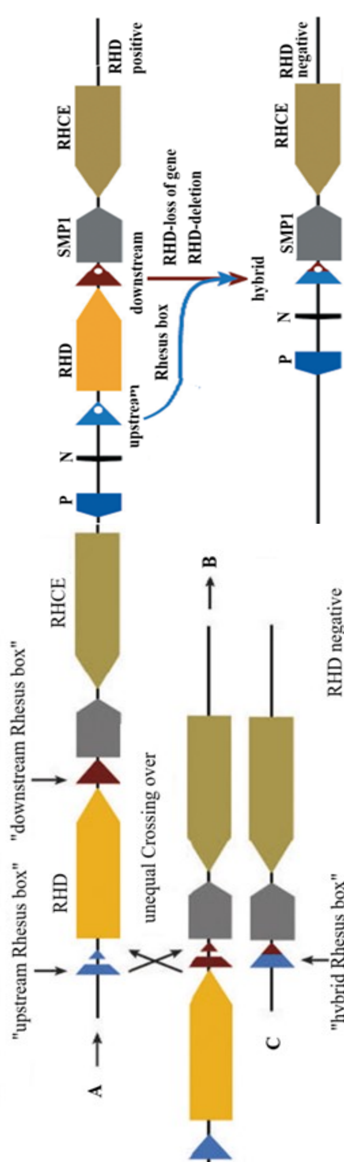

Figure 1. The occurrence of recombination between an upstream and a downstream Rhesus box on two different chromosomes (“A” and “B”). When the two crossed strands separate (from A over the recombination site to B), the DNA at the RH gene site completely lacks the RHD gene (C). Therefore, the recombination with unequal crossover resulted deletion of the Rh-D gene in one of the chromosomes (chromosome B) and individuals with homozygous for this haplotype (C) are Rh-D-negative. [The diagram is redrawn from Flegel (2007)].

In O allele, exon 6 at 261 position lacks a guanine nucleotide and results in a loss of enzymatic activity because of a frame-shift mutation and results in premature termination of the translation (Yamamoto *et al*., 1990). Therefore, the H antigen remains unchanged in O type blood group.

Allele A and B are co-dominant and individuals with AB alleles have no antibody for individuals with any type of ABO blood group. But AB individuals have antigen to any individual who receive blood from them. O individuals have no antigens but have antibody, therefore transfusion blood from them to others is clinically safe, while AB individuals do not have antibodies to individuals with any type of blood group. Therefore, for safe blood transfusion the recipient must not be able to produce Anti-A or Anti-B antibodies that correspond to the A or B antigens. When a serum containing antibody A is added to a blood from somebody is coagulating, the blood group type of the individual is either A or AB. When a serum containing antibody B is added to a blood from an individual is coagulating, the blood group type is either B or AB. If a blood from somebody does not coagulate neither with antibody A nor with antibody B, the blood group type of the individual is O.

Red blood cells which react with anti-D serum are termed as Rh-positive (Rh^{+}) otherwise Rh-negative (Rh^{-}). The expression of Rh-D gene in red blood cells is not a matter of being dominant, recessive or codominant of the alleles but it is the absence or presence of the gene in an individual genome (Fig.1). Rh-D antigen is a trans-membrane protein with loops expressed at the surface of red blood cells. They used for CO_2 and/or ammonia transportation across the plasma membrane (Endeward *et al.*, 2008). Different studies were conducted to determine the distributions of ABO blood group and Rh-factor in different nations and ethnic groups. Frequency of ABO and Rh blood groups vary worldwide (Nwauche and and Ejele, 2004; Beckman, 2008; Agrawal

et al., 2014; Rubeai, 1975). Several but fragmented studies on the distribution of ABO and Rh-factor were conducted on different Ethiopian ethnic groups (Megbaru, 2014; Kassahun *et al.*, 2015; Teklu and Shiferaw, 2016; Nigusu and Yohannes, 2017; Golassa *et al.,* 2017). All of the researchers reported that the distributions of ABO blood group and Rh-factor are not in equal numbers even within a nation from different ethnic groups.

The blood group distribution and frequency studies are multipurpose, as besides their importance in inheritance, their relation to disease and environment is being increasingly sought for modern medicine. It is, therefore, imperative to have information on the distribution of these blood groups in any population group that comprises different ethnic group, (Rai *et al*., 2009).

There have been no sufficiently documented data on the distribution pattern and frequency of ABO and Rh-D blood group phenotypes, genotypes and alleles of Oromo, Amara and Gurage ethnic groups belonging to Enchini Secondary School.

The aim of this study was to investigate the distribution of phenotypes, genotypes, and the allele frequencies of ABO blood groups and Rh-Factors among Oromo, Amara and Gurage students enrolled at Enchini School in the year 2018.

Materials and Methods

Description of the Study Area

The study was conducted at Enchini Secondary School, which is located in Enchini town, Adea Berga district, West Shewa zone of Oromia Regional State, Ethiopia. Enchini town is located at 65 km northwest of Addis Ababa and found at 9°12N and 38°17E with altitude of 2693 m above sea level as its central point. Enchini Secondary School had a total of 1444 students, of which 766 were male and 678 were female students in the 2019 Academic Year. Out of this total number of students 1192 (82.55%), 108 (7.48%), 72 (4.99%), 24 (1.66%) and 48 (3.32%) were from the Oromo, Amahra, Gurage, Wolita, and otherethnic groups, respectively.

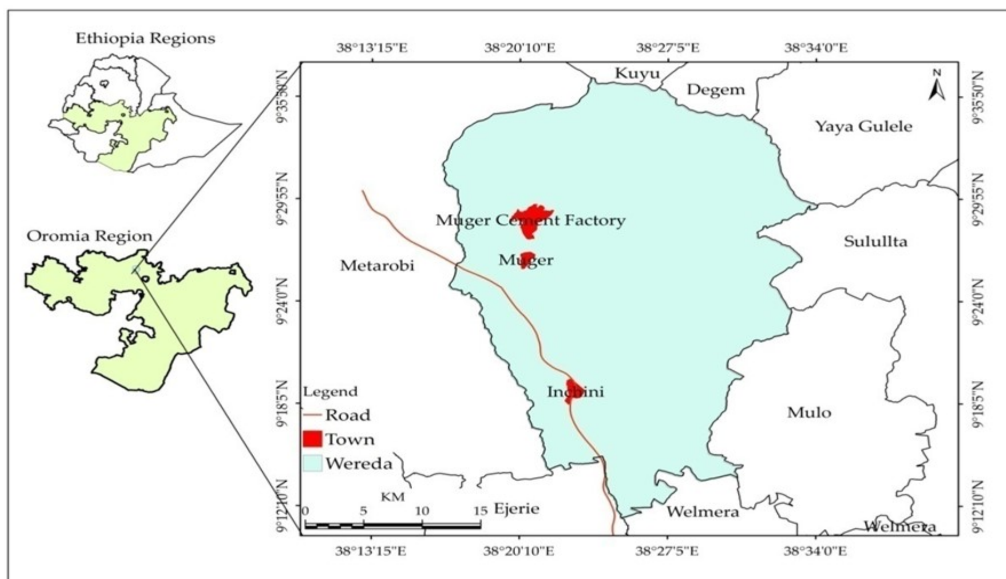

Figure 2. Location map of the study area. Source: *Ethiopian GIS* (www.ema.gov.et)

Sampling

The participants (418 students) selected for the study were from three ethnic groups, whose ages were ranging from 14 to 20 years. From these 418 students, 252 were male and 166 were female students. The randomly selected 20% of the participants who voluntarily participated in the study were from the Oromo, Amhara and the Gurage ethnic groups. Since the population sizes of students from other ethnic groups were low they were not included in the study.

Data collection

All the participants were informed about the aims and objectives of the study, as well as the blood grouping procedures. Written consent was obtained from the participants whose ages were 18+ but parents' consent was obtained for those who were under 18. Particulars of each participant were taken in a data collection sheet.

Laboratory Investigations

After aseptic washing with 70% ethyl alcohol, blood samples were collected on grease free clean slide from left ring finger tip with the help of a sterile lancet. Determination of ABO blood group and Rh (D) blood group was done by slide test. For ABO and Rh-D grouping, commercial monoclonal anti-sera: anti-A, anti-B and anti -D (Genuine Biosystem, India) and properly stored (at about 4 ^{o}C) were used. Four drops of fresh blood sample from a student were placed at four distinct places on a sterile and clinically standard plastic slide with six circles (each with 2.5 cm diameter) and then a drop (approximately 40 µL with 1:256 dilution) of one of the three anti-sera (anti-A, anti-B and anti-D approximately 40 µL) reagent was added to each blood sample in that order and the fourth blood sample left free for control. The first, the second and the third mixtures of anti-serum and blood were stirred and mixed thoroughly using clean toothpicks and rotate gently for about 1 to 1.5 minutes at room temperature (24°C). Blood group types were determined on the basis of agglutination reaction. If clumping or clotting occurs in the test blood upon exposure to the A, B, and D anti-sera, the test showed the sampled bloods from the students contain the A, B, and D antibody, respectively. If all antisera cause clotting to the drop they added, the blood group type of the individual is AB-positive, and if neither of the anti-sera cause clotting, the blood type of the individual is "O-negative". If the addition of anti-D serum resulted clotting of the blood, the blood sample is termed as Rh-positive (Daniels, 2002; Rai and Kumar, 2010).

Data Analysis

Excel spread sheet and statistical package SPSS version 23 were used for the analyses. Using Hardy-Weinberg Equilibrium Equation blood group phenotypes, genotypes and alleles frequencies were determined. Under H -W equilibrium, the following equations and representations were used:

"A" stands for dominant allele and its frequency is represented by "p";

"a" stands for recessive allele and its frequency is represented by "q";

$p + q = 1$ (1)

$(p + q)^2 = 1^2$;

which implies $p^2 + 2pq + q^2 = 1$ (2)

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

Following the same principle and notion the ABO blood group type alleles also represented by p, q, and r.

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

$(p + q + r)^2 = 1^2$. This gives

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

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

Chi-square (χ^2) tests using the statistical package SPSS ver. 23 were conducted and statistically significant differences were determined at $p < 0.05$.

Chi-square test $(\chi^2) = \sum \frac{(o_i - e_i)^2}{e_i}$ (5)

Observed and expected hemizygosities of the studied population and each ethnic group was determined using the extension of Hardy-Weinberg Equilibrium equation (equation 8) for loci with more than two alleles. Since we were dealing with three allelic ABO gene, the analysis to determine allelic frequencies needs the use of quadratic equation solution:

$$aX^2 + bX + c = 0 \text{ is } X = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

However, to minimize the points of confusion in choosing the true set values, the authors used Bernstein's equation (1930) cited in Kalmes and Hurt (2002) which simplifies the calculations:

$$p = 1 - (F[B] + F[O])^{1/2}$$
$$q = 1 - (F[A] + F[O])^{1/2} \quad (6)$$
$$r = (F[O])^{1/2}$$

Then, if p+q+r is different from 1, correction by the deviation

$D = 1 - (p + q + r)$ was made:

$$p' = p(1 \pm D/2)$$
$$q' = q\,(1 \pm D/2) \quad (7)$$
$$r' = (rD/2)\,(1 \pm D/2)$$

Expected heterozygosity (He) at Hardy-Weinberg Equilibrium was calculated assuming the occurrence of all the three alleles of ABO gene in equal frequency (p = q = r = 1/k where k is the number of alleles) using:

$$He = 1 - \sum_{n=1}^{n} p_i^{\,2}, \quad (8)$$

where p_i^2 = genotype frequency of expected homozygotes and He = 0.666 for ABO gene considering 3 alleles (Ayele and Kelele, 2016).

Results

Table 1. Distribution of ABO and Rh blood group Phenotypes from the three ethnic groups

Blood Groups	Amhara (n = 108(25.84%))			Gurage (n = 72(17.22%))			Oromo (n = 238(56.94))			Sex: M (n = 252 (60.29))			Sex: F(n = 66 (39.71))			Total (n = 418 (100%))		
	Rh⁺	Rh⁻	Total	Rh⁺	Rh⁻	Total	Rh⁺	Rh⁻	Total	Rh⁺	Rh⁻	Total	Rh⁺	Rh⁻	Total	Rh⁺	Rh⁻	Grand Total
A	28	0	28	18	2	20	62	4	66	65	4	69	43	2	45	108	6	114(27.27%)
B	23	3	26	17	1	18	56	3	59	65	3	68	34	1	35	96	7	103(24.64%)
AB	9	1	10	5	0	5	14	1	15	16	1	17	11	2	13	28	2	30(7.18%)
O	41	3	44	26	6	29	92	6	98	92	6	98	65	8	73	159	12	171(40.91)
Total	101	7	108	66	6	72	224	14	238	238	14	252	153	13	166	391	27	418 (100%)

Table header: Ethnic groups and sex by blood group types and Rh-Factor frequencies

The order of frequencies for the four phenotypes from the total studied population was “O” > “A” > “B” > “AB”.

From the studied population, 60.29% were male students and the rest 39.71% were females. Although the total proportion of female students was lower than the proportion of male students (1.52:1), females outweighed in scoring the higher number in AB⁻ (2/3) and O⁻ (8/14) blood groups. The total numbers for Rh⁻ from each gender were almost the same: 14, which was with 1:1.08 ratio for female: male ratio (Table 1). Blood group O from all ethnic groups scored the highest frequency (40.91%) and among the three groups, the Oromo students scored the highest frequency (41.17%) whereas the least frequency (40.28%) was from Gurage students. Blood group AB was with the least frequency (7.18%) and among the three groups Oromo students scored the least AB blood group frequency (6.30%) (Table 1). Rh-positive students accounted 93.54%. The order of the three studied groups for Rh-positive was Oromo (94.12%) > Amhara (93.52%) > Gurage (91.67%). Individuals with “A” blood type from Amhara and with “AB” blood type from Gurage students were monomorphic for Rh-factor phenotye, consisted only Rh-positive blood type.

Table 2. Phenotypic, allelic and genotypic frequencies of ABO blood group and Rh-factor

Studied group	Blood group type	Observed Phenotypic Number and Frequency	Genotype	Corrected Allelic frequency	Genotypic frequency
Amhara Students (n = 108)	A	A = 28 (0.259)	AA	p = 0.193	P^2 (P x P) = 0.193 x 0.193 = 0.037
			AO	---	2Pr = 2 x 0.193 x 0.635 = 0.245
	B	B = 26(0.241)	BB	q = 0.182	q^2 (q x q) = 0.182 x 0.182 = 0.033
			BO	---	2qr = 2 x 0.182 x 0.635 = 0.231
	O	O = 44(0.407)	OO	r = 0.635	r^2 = (r x r) = 0.635 x 0.635 = 0.403
	AB	AB = 10(0.093)	AB	---	2pq = 2 x 0.193 x 0.182 = 0.07
	Rh^+	Rh^+ = 101(0.9352)	DD	D = 0.7454	DD = 0.556
	Rh^-	Rh^- = 7(0.0648)	Dd		Dd = 0.38
			dd	d = 0.2546	dd = 0.065
					Heterozygosity for ABO gene = 54.65%
Gurage Students (n = 72)	A	A = 20(0.278)	AA	p = 0.191	P^2 (P x P) = 0.191 x 0.191 = 0.037
			AO	---	2Pr = 2 x 0.191 x 0.635 = 0.243
	B	B = 18(0.25)	BB	q = 0.174	q^2 (q x q) = 0.174 x 0.174 = 0.03
			BO	---	2qr = 2 x 0.174 x 0.635 = 0.221
	O	O = 29(0.403)	OO	r = 0.635	r^2 = (r x r) = 0.635 x 0.635 = 0.403
	AB	AB = 5(0.069)	AB	---	2pq = 2 x 0.191 x 0.174 = 0.067
	Rh^+	Rh+ = 66(0.9167)	DD	D = 0.7114	DD = 0.506
	Rh^-	Rh^- = 6 (0.0833)	Dd		Dd = 0.411
			dd	d = 0.2886	dd = 0.083
					Heterozygosity for ABO gene = 53.1%
Oromo Students (n = 238)	A	A = 66(0.277)	AA	p = 0.188	P^2 (P x P) = 0.188 x 0.188 = 0.035
			AO	---	2Pr = 2 x 0.188 x 0.642 = 0.241
			BB	q = 0.170	q^2 (q x q) = 0.170 x 0.170 = 0.029
	B	B = 59(0.248)	BO	---	2qr = 2 x 0.170 x 0.642 = 0.218
	O	O = 98(0.412)	OO	r = 0.642	r^2 = (r x r) = 0.642 x 0.642 = 0.412
	AB	AB = 15(0.063)	AB	---	2pq = 2 x 0.188 x 0.170 = 0.064
	Rh^+	Rh+ = 224(0.9412)	DD	D = 0.7575	DD = 0.574
	Rh^-	Rh^- = 14(0.0588)	Dd		Dd = 0.367
			dd	d = 0.2425	dd = 0.059
					Heterozygosity for ABO gene = 52.31%
Total studied population (n = 418)	A	A = 114(0.273)	AA	p = 0.189	P^2 (P x P) = 0.189 x 0.189 = 0.036
			AO	---	2Pr = 2 x 0.189 x 0.639 = 0.242
	B	B = 103(0.246)	BB	q = 0.173	q^2 (q x q) = 0.173 x 0.173 = 0.028
			BO	---	2qr = 2 x 0.173 x 0.639 = 0.221
	O	O = 171(0.409)	OO	r = 0.639	r^2 = (r x r) = 0.639 x 0.639 = 0.408
	AB	AB = 30(0.072)	AB	---	2pq = 2 x 0.189 x 0.173 = 0.065
	Rh^+	Rh^+ = 391(0.9354)	DD	D = 0.7458	DD = 0.556
	Rh^-	Rh^-= 27(0.0646)	Dd		Dd = 0.379
			dd	d = 0.2542	dd = 0.065
					Heterozygosity for ABO gene = 52.80%

Table 2 revealed that the number of individuals from the overall studied students with *AA, AO, BB, BO, OO, and AB* genotypes were 15(3.6%), 101(24.2%), 12(2.8%), 92(22.1%), 171(40.9%) and 27 (6.5%), respectively. The number of "*DD*" and "*Dd*" expressed as Rh-positive and "*dd*" as Rh-negative genotype individuals were 232(55.6%), 159(37.9%) and 27(6.5%), respectively. There was no statistically significant difference between any of these calculated and observed values at $\alpha = 0.05$.

The highest heterozygosity for ABO gene genotypes (54.65%) and Rh-D gene genotypes (41.06%) were from Amhara and Gurage students, respectively whereas the least heterozygosity for ABO gene genotypes (52.31%) and for Rh-D gene genotypes (36.7%) both were from Oromo students. Heterozygosity for ABO gene genotype from the overall studied population was 52.80% whereas for Rh-D-gene genotype was 37.92%. Therefore, the difference between homozygosity and heterozygosity was higher in D-gene than ABO gene.

A (p), B (q) and O(r) allelic frequencies from the overall studied students were 0.191, 0.174 and 0.639, respectively (Table 2). Since the sum of the three alleles frequencies was 1, corrections were made (equation 7) and the corrected frequencies were p =0.189; q = 0.173 and r = 0.639. The following Chi-Square (χ^2) test conducted to check whether there is significant differences between observed and expected allelic, genotypic and phenotypic frequencies of ABO blood group for overall studied population:

(1) Allelic frequencies:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.189-0.333)^2}{0.333} + \frac{(0.173-0.333)^2}{0.333} + \frac{(0.639-0.333)^2}{0.333}$$

$$\Rightarrow 0.0623 + 0.0769 + 0.2812$$

$\chi^2 = \underline{0.4204}$; $df = 2$; and $p = 0.8104$ *at* $\alpha = 0.05$.

(2) Genotypic frequencies:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.0357-0.111)^2}{0.111} + \frac{(0.2415-0.222)^2}{0.222} + \frac{(0.0299-0.111)^2}{0.0.111} + \frac{(0.4083-0.111)^2}{0.111} + \frac{(0.2211-0.222)^2}{0.222} + \frac{(0.0654-0.222)^2}{0.222}$$

$$\Rightarrow 0.0511 + 0.0017 + 0.0593 + 0.0000 + 0.1105 + 0.7963$$

$\chi^2 = 1\underline{.0189}$; $df = 5$; and $p = 0.9610$ *at* $\alpha = 0.05$.

(3)Phenotypic Freq.

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.2772-0.333)^2}{0.333} + \frac{(0.2510-0.333)^2}{0.333} + \frac{(0.0654-0.222)^2}{0.222} + \frac{(0.4083-0.111)^2}{0.111}$$

$$\Rightarrow 0.0094 + 0.0202 + 0.1105 + 0.7963$$

$\chi^2 = \underline{0.9346}$; $df = 3$; and $p = 0.8166$ at $\alpha = 0.05$.

Therefore, there was no statistically significant difference (at $\alpha = 0.05$) between observed and expected allelic, genotypic and phenotypic frequencies.

Individuals who have the gene were designated as either *DD* or *Dd* and those who have no the gene were designated as *dd* (see Fig. 1). Therefore, expected phenotypic, allelic and genotypic frequencies for the Rh-D gene are depend on the probability of each of the two alleles. D + d = 1; and $(D+d)^2 = 1^2 \Rightarrow D^2 + 2Dd + d^2 = 1$, which implies $D^2 + 2Dd + d^2 = 0.25 + 0.50 + 0.25 = 1$. Observed phenotypic frequencies for Rh-positive and Rh-negative individuals from the studied population were 93.54% and 6.46%, respectively. In terms of genotype, Rh^+ individual were DD + Dd and Rh^- were dd. The d allele had a frequency of 0.2542 which

is determined from d = √0.0646. The frequency of D allele was 1-d which is 1-0.2542 ⇒ D = 0.7458. Therefore, observed genotypic frequencies were determined to be 0.5562, 0.3792 and 0.0646, respectively from $D^2 + 2Dd + d^2$.

The following Chi-Square (χ^2) test was conducted to check whether there is significant difference between observed and expected allelic, genotypic and phenotypic frequencies for Rh-D gene:

(1) Allelic frequencies:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.7458-0.5)^2}{0.5} + \frac{(0.2542-0.5)^2}{0.5}$$

$$\Rightarrow 0.1208 + 0.1208$$

χ^2 = 0.2416; df = 1; and $p = 0.6231$ *at* $\alpha = 0.05$.

(2) Genotypic frequencies:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.5562-0.25)^2}{0.25} + \frac{(0.3792-0.5)^2}{0.5} + \frac{(0.0646-0.25)^2}{0.25}$$

$$\Rightarrow 0.0292 + 0.0047 + 0.3750$$

χ^2 = 0.4089; df = 2; and $p = 0.8151$ *at* $\alpha = 0.05$.

(3) Phenotypic Freq. $\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} \Rightarrow \frac{(0.9354-0.75)^2}{0.75} + \frac{(0.0646-0.25)^2}{0.25}$

$$\Rightarrow 0.0458 + 0.1375$$

χ^2 = 0.1833; df = 1; and $p = 0.6685$ *at* $\alpha = 0.05$.

All the Chi-Square (χ^2) tests showed that there were no statistically significant differences between observed and expected mean values. That is the observed differences had no genetic base and the studied ethnic groups met the necessary conditions to keep their Rh-factor controlling gene alleles at equilibrium.

Discussion

When the general order of frequency for the ABO phenotype (O > A > B > AB) from the present study compared with Atire (2015), Teklu and Shiferaw (2016), Kassahun *et al*. (2015) and Amsalu and Daniel (2019) reports, it was consistent with these reports. Similar general formula for the order were reported from other parts of the world like Guinea: O (48.88%) > A (22.54%) > B (23.86%) > AB (4.72%) (Seltsam *et al*., 2003). At ethnic group level Atire (2015) reported the distributions of A, B, O and AB phenotypes from SNNP (Southern Nation Nationality People) students were 35.9% (14O) = 35.9% (14B) > 25.64% (10A) >2.56% (2AB); from Gambela students were 40% (2O) = 40% (2B) > 20% (1A) >0% (0AB); and from Tigray students were 50% (5O) > 30% (3A) > 20% (2B) > 0% (0AB) which are different from the present finding. The source of variation between the present and Atire (2015) report

could be the presence of considerable sample size variation. Atire (2015) subjects from Gambela and Tigray were 5 and 9, respectively that were with less than 10 in numbers and all were non-AB blood group type. It had also been reported in several studies (Falusi *et al*., 2000; Adeyemo and Soboyejo, 2006; Kassahun *et al*., 2015) that there were variations in ABO blood group among different ethnic groups. Many other studies from the other parts of the world had shown that blood group "O" was the most common blood group and blood group "AB" was the least common blood group in different ethnic groups and nations (Nwauche and Ejele, 2004; Beckman, 2008). From Beckman's (2008) report, which represented the different nations in the world, Ethiopians had 45% "O" and 5% "AB" type blood group. The findings of the present research differed from the results obtained from Bannu region in Pakistan (B>A> O> AB) (Khan *et al*., 2009); from Swat district in Pakistan (B >, O >, A > AB) (Khattak *et al*., 2008); from Bangladesh (B > O > A > AB) (Haque *et al*., 2013); and from Latur (India) (B > O > A > AB) (Deshpande and Wadde, 2013) showing that blood group type B was with highest frequency. It was not the present report but also other Ethiopian ethnic group based studies showed exchangeable orders for the ABO gene phenotypes frequencies. Ayele *et al.* (2014) reported the frequencies of blood group types A and O from Merawi (Ethiopia) had 38% and O 36% and from Adet (Ethiopia) 30% and O 42%, respectively. Such and other differences among nations could be the reflection of the degree of prevalence of hereditary risk factors associated with ABO blood group type (Qi *et al.,* 2010; Cusack *et al.,* 2013), and the studied populations from different nations could have very distant evolutionary relations. Therefore, the blood group type frequency distribution is not only based on ethnic groups but also geographical proximity and environmental factors such as infectious diseases.

The phenotype frequencies of Rh-D gene in the present report was in line with other reports on different Ethiopian ethnic groups previously studied (Yasin, 2013; Atire, 2015; Teklu and Shiferaw, 2016; Kassahun *et al*., 2015). However, it was different from Atire (2015) report which showed 100% Rh-D^+ from Tigray ethnic groups and this could be taking insufficient number of subjects (5) from Tigray ethnic group.

Allele frequencies in a population can remain the same from generation to generation as well as among ethnic groups if the assumptions for Hardy-Weinberg Equilibrium (HWE) meet. The absence of significant differences between the observed and expected frequencies of blood group typing system parameters may indicate the studied ethnic groups were under the conditions necessary to satisfy the HWE such as non-selective marriage and unrestricted movement from region to region and settled in the new destinations. The results from the present report showed that the gene (D) controlling Rh-factor had higher rate of fixation towards Rh-positive or ***D*** allele. In other words the absence of the gene (D) which is expressed as ***d*** allele is the result of the occurrence of deletion mutation (Fig. 1) but it may not be a current phenomenon. The differences between observed and expected mean values from the different parameters used to analyse the different parameters used to analyse the phenotypic, genotypic and allelic characteristics of ABO and Rh-D system controlling genes may not have genetic base. Rubeai (1975) reported that it seems adjacent populations differ only slightly in the frequencies of particular alleles and these differences tend to increase with the distances separating the populations.

Worldwide frequencies of A, B, O, and AB blood groups vary from nation to nation. O varies from 9% (Grand Andamanese) to 100% (Bororo in Brazil); A varies from 0% (Bororo in Brazil) to 82% (Blackfoot – N. Am. Indian); B varies from 0% (Bororo in Brazil) to 41% (in Kalmaks); and AB from 0% (Bororo in Brazil) to 18% (Ainus – Japan) (Beckman, 2008). It also varies from ethnic group to ethnic group and even within an ethnic group. Although the observed differences were not statistically significant, different findings were reported on the frequ-

ency distribution of ABO and Rh-factor groups among Ethiopians. Megbaru (2014) reported 41.5% O and 5.5% AB; Teklu and Shiferaw (2016) reported 43.1% O and 3.5% AB; Nigusu and Yohannes (2017) reported 43.2% O and 4.8% AB, and Golassa *et al.* (2017) reported 41.20% type O and 3.3% type AB. The research by Golassa *et al.* (2017) was dealing on two groups: Nilotic and Highlander. The dominant blood type in the native Nilotics was type O (50.4%) and in Highlanders was blood type A (44.1%). All research reports based on Ethiopian groups showed that O was the most common and AB was the least common blood type. The result from the present study was in line with most reports.

Conclusion

In the studied population, the proportion of blood group O individuals was dominant. In terms of Rh-D the proportion of Rh-D^+ individuals was dominant. In the event of blood transfusion and cell/tissue/organ transplantation the studied population was more advantageous, because individuals with blood type O are most readily available as universal donors. However, the large proportion of Rh-D^+ blood group would counterbalance the advantage from having largest proportion of O individuals, because Rh-D^+ is not universal donor. The result would serve as a reference in health care planning and other needs or "Kunets".

Ethical considerations and conflict of interest

An authorization to carry out the study was obtained from Adea Berga District ethics committee. All the information that was obtained about the subjects was kept confidential. The authors declared that they have no conflict of interest.

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