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Gene Frequencies of ABO and Rh (D) Blood Group Alleles in two Different Regions at the Northeastern of Libya

[(1)]Hameda Ali El-Moghrabi, [(1)]Ibrahim Al-Drussi , [(2)]Latifa Abdel-Hafid Jwieli , [(3)]Khalil El-Brghty

[(1)]Lecturer at Faculty of Arts and Sciences, Ghemins, The University of Benghazi. Libya.

[(1)] Professor at Faculty of Sciences, Department of Botany, The University of Benghazi. Libya

[(2)]Lecturer at Faculty of Arts and Sciences, Ghemins, The University of Benghazi. Libya.

[(3)]Postgraduate at Faculty of Sciences. The University of Benghazi. Libya

Abstract

Total number of 3000 individuals was collected from Ajdabiya and Ghemins, then analyzed. Phenotype O blood type has the highest frequency, followed by A and B, whereas the lowest prevalent blood group was AB. The overall phenotypic frequencies of ABO blood groups were O>A>B>AB. The allelic frequencies of O, A and B alleles for Ajdabiya's samples were O (0.6511528238), A (0.1839120439) and B (0.1649351323). The allelic frequencies for Ghemins's samples were O (0.5927337795), A (0.2273881627) and B (0.180). Individuals with a positive Rh factor were the most common in the target group of this study. 1269 samples from Ajadabia were positive (86.4%), while 231 samples were negative (15.4%). The positive Rh factor for Ghemins's samples was also most common 1307 (87.13%), whilst the negative Rh factor was 193 (12,8%). Chi-square test was performed to match the balance predictions for each blood group A, B, AB and O and the Rh factor. The predictions for each blood group A, B, AB and O in Ajdabiya were unbalanced, but they were balanced with the Rh factor. On the opposite, the predictions for each blood group A, B, AB and O in Ghemins were balanced, but they were unbalanced with the Rh factor.

Key words

ABO & Rhesus blood groups. Phenotype. Genotype. Allele. Gene frequency. Hardy-Weinberg equilibrium.

Introduction

There are more than 100 blood group systems with over 500 antigens in which the ABO system is the most studied group among humans (Dziczkowski and Anderson, 1998). The ABO blood group system was discovered by Austrian scientist, Karl Landsteiner, who found three different blood types A, B and O in 1900 from serological differences in blood called the Land- Steiner Law (Landsteiner, 1901). Forty years later both Karl Landsteiner and Weiner discovered the Rh blood group system (Landsteiner and Wiener, 1940). ABO and Rh blood groups are the most important blood groups despite the long list of several other blood groups discovered so far (Worlledge *et al*., 1974 & Seeley, Stephens and Tate,

1998). The ABO blood grouping is based on the presence or absence of A and B blood group antigens on the surface of red blood cells derived from inherited gene (Yamamoto, McNeill and Hakomori, 1995).

The ABO gene is located on chromosome 9 on the long arm of chromosome (9q34.1) (Al-Arrayed *et al*., 2001). Moreover, the ABO has seven exons, and is over 18 kilobases (kb) in length. The major coding region of ABO is found in exons six and seven (Chen *et al*., 2006). The Rh(D) gene encoding the Rh protein is located on chromosome 1(p34–p36) (Cartron, 1994).

Study of distribution of blood groups is important as it plays a vital role in blood transfusion, human evolution, anthropology and tracing ancestral relation of humans. Some blood groups have shown associations with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection and ABO& Rh incompatibility of new born (Skaik, and El-Zyan, 2006). The distribution of ABO blood groups varies in the different geographical and ethnic groups (Su *et al*., 2001). Race is thought to be one of the factors determining the level of ABO antibodies (Adewuyi and Gwanzura, 2001). Estimation of the allelic frequencies for genetic markers is very important in genetic studies. Also investigation of the concordance between observed and expected value based on the Hardy–Weinberg equilibrium (HWE) is strongly recommended by strengthening the reporting of Genetic Association studies (STREGA) (Little *et al*., 2009). This study aims to provide descriptive information about the population variance of genetic structure in two different regions, Ajdabiya and Ghemins that are located in the Northeastern of Libya, with regard to blood type and Rhesus factor.

Data and Method

Inclusion criteria

The society of this study consists of 3000 individuals with ages ranged from one day to eighty years, male and female, also included black and white races. Geographic area individuals were from two different regions Agadabia (1500 samples) and Ghemins (1500 samples), which are located in the Northeastern of Libya.

ABO and Rh blood groups tests

A glass slide or a pillar of white porcelain is divided into three parts, where a drop of donated or received blood is mixed with each part of Anti-A, Anti-B and Anti-D antagonists separately. The pattern of hemagglutination or hematopoiesis can be seen visually. Through which the blood type ABO and rhesus D (RhD) can be determined. The test ends in five to ten minutes, and it is inexpensive, also requires a very small amount of blood test reagents. Further, it is an insensitive and only beneficial method in matching the initial BG to obtain an early result. This test cannot be performed for weak or rare reactive antigens. Furthermore, the results of which are difficult to interpret, additional to a low titer of Anti-A or Anti B antagonists can lead to false or false negative results (Malomgre and Neumeister, 2009).

Statistical method

Data were analyzed using SPSS-25 for the frequency and percentage. Allele frequencies were calculated by applied the assumption of Hardy–Weinberg equilibrium and expressed as percentages. Chi-square test was used to compare observed allelic and genotypic frequency distributions of the blood groups and Rh antigens to that expected under the Hardy–Weinberg (Saadat, 2015).

Results

This study included two different regions in the Northeastern of Libya, Agadabia and Ghemins. The results in Agadabia region, ABO & Rh blood group prevalence were determined in a total of 1500 individuals attended the city's medical analysis laboratory. The number of males is 756 (50.4%) and the number of females is 744 (49.6%). The blood group distribution and gender for a random sample of the population in Agadabia are shown in table (1) and figure (1). The varieties differ according to the Rh factor, of which the positive for the Rhesus factor, which is the most common 1269 (84.6%) and the negative for the Rhesus factor, which is the least 231 (15.4%) (Table 2) & (Figure 2). The frequency of the ABO and Rh blood alleles were determined as follows O (0.6511528238), A (0.1839120439), and B (0.1649351323), the frequency of genotypes, this is evident in Table (3).

The prevalence of blood type ABO & Rh were determined in the area of Ghemins, where the blood groups were collected from families living in Ghemins area directly through the official identification paper and a questionnaire form. Some families were recognized by ABO and Rh blood groups tests, which their results were in total of 1500 individuals, and the number of males 705 (47%), while the number of female distribution varies 795 (53%) (Table 4) & (Figure (3). According to the Rh factor, the positive Rh factor was the most common 1307 (87.14%) while the negative Rh factor was the least 193 (12.86 %) (Table 5) and (Figure 4). The allelic frequencies for Ghemins's samples were O (0.5927337795), A (0.2273881627), and B (0.180). Individuals with a positive Rh factor were the most common in the target group of this study, the frequency of genotypes, is evident in Table (6). Represents a comparison between the observed values and the expected values for ABO and Rh factor, Allelic frequencies and Goodness-of-fit χ^2 for ABO, Rh blood type among the targeted individuals in the study Ajdabiya and Ghemines is shown in table (7).

Table (1) Blood group and gender for a random sample of a population in Agadabia.

Blood group Gender	A	B	AB	O	**Total**
Female	211	170	55	308	744(49.6%)
Male	199	161	68	328	756(50.4%)
Total	410(27.3%)	331(22%)	123(8.2%)	636(42.4%)	1500(100%)

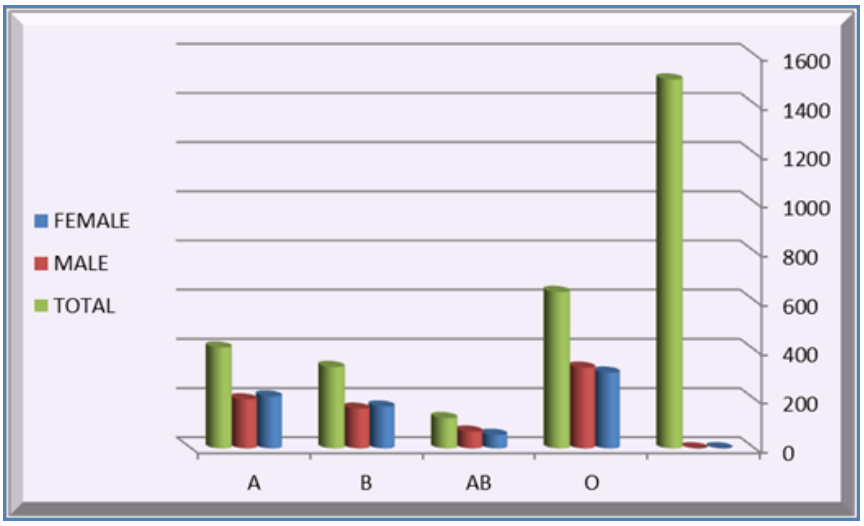

Figure (1) Blood group and gender for a random sample of a population in Agadabia.

Table (2) Rh blood group and gender for a random sample of a population in Agadabia.

Rh blood group Gender	Rh +	Rh -	Total
Female	615	129	744 (49.6 %)
Male	654	102	756 (50.4%)
Total	1269(84.6%)	231 (15.4%)	1500 (100%)

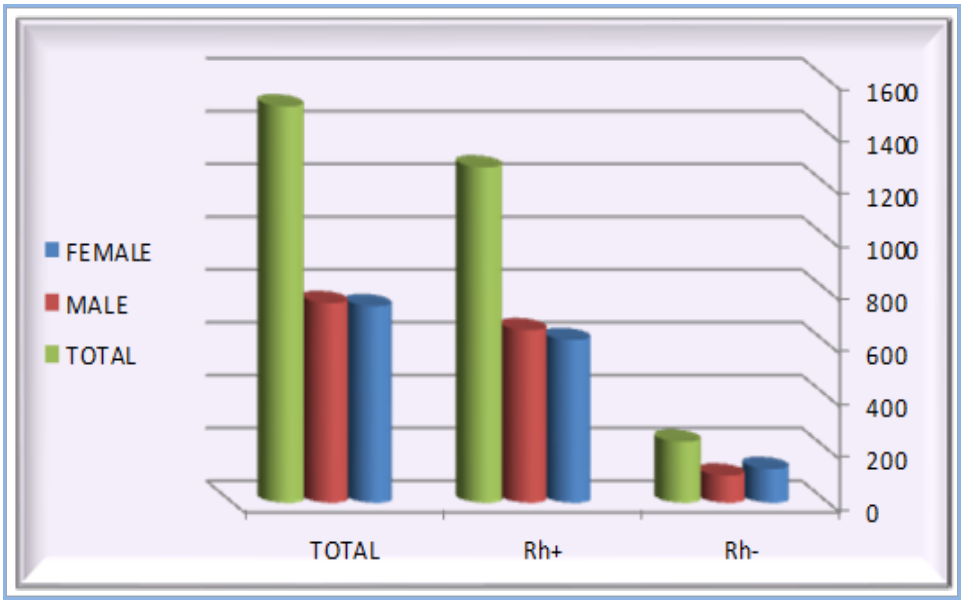

Figure (2) Rh blood group and gender for a random sample of a population in Agadabia.

Table (3) Gene frequencies of ABO and Rh blood group alleles for a random sample of a population in Agadabia

Study population	Blood group system	GENE (allele)	Frequency of alleles	Genotype	Frequency of genotype	Phenotype & Frequency of Phenotype
Agadabia	ABO	O	0.65115282	OO	0.424	O 42.4%
		A	0.183912043	AA	0.0338236398	A 27.3%
				AO	0.2395096898	
		B	0.164935132	BB	0.02720359789	B 22.06%
				BO	0.2147959543	
				AB	0.06066711458	AB 8.2%
	Rhesus	D	0.607571662	DD	0.3691433252	Rh(D) +ve
				Dd	0.4768566743	Rh(D) +ve 84.6%
		d	0.392428337	Dd	0.1539999997	Rh(d) –ve 15.4%

Table (4) Blood groupand gender for a random sample of a population in Ghemins.

Blood group Gender	A	B	AB	O	Total
Female	230	213	73	279	795 (53%)
Male	252	160	45	248	705 (47%)
Total	482(32.13%)	373(24.86%)	118(7.88%)	527(35.13%)	1500(100%)

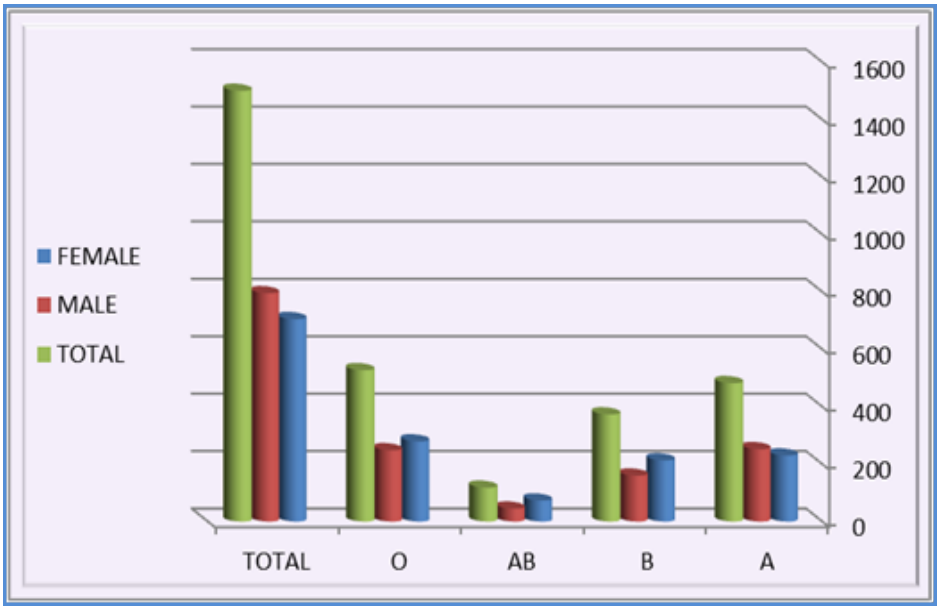

Figure (3) Blood group and gender for a random sample of a population in Ghemins.

Table (5) Rh blood group and gender for a random sample of a population in Ghemins.

Rh blood group / Gender	Rh +	Rh -	**Total**
Female	667	128	795 (53%)
Male	640	65	705 (47%)
Total	1307(87.14%)	193 (12.86%)	1500 (100%)

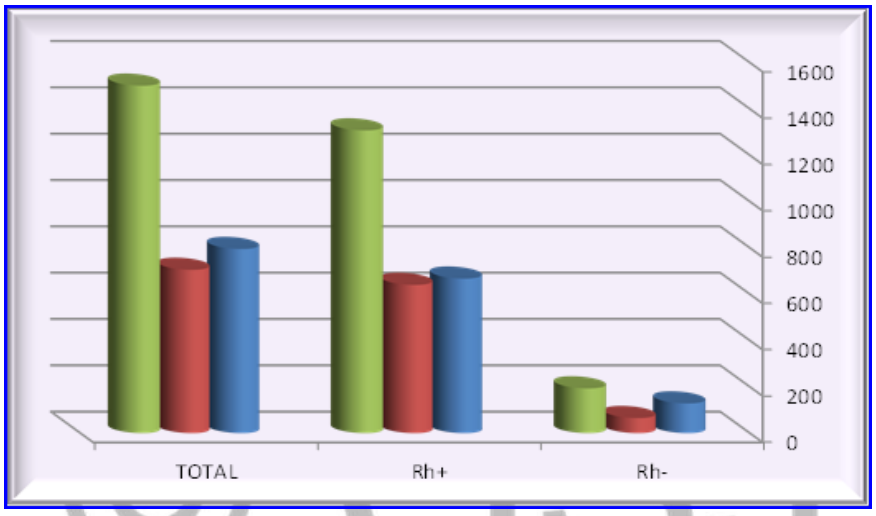

Figure (4) Rh blood group and gender for a random sample of a population in Ghemins

Table (6) Gene frequencies of ABO and Rh blood group alleles for a random sample of a population in Ghemins.

Study population	Blood group system	GENE (allele)	Frequency of alleles	Genotype	Frequency of genotype	**Phenotype & Frequency of Phenotype**
Ghemins	ABO	O	0.59273377	OO	0.351	O 35.13 %
		A	0.22738816	AA	0.0517	A 32.13 %
				AO	0.2695	
		B	0.180	BB	0.0324	B 24.86%
				BO	0.2133	
				AB	0.078	AB 7.866%
	Rhesus	D	0.641298	DD	0.4112631	Rh(D) +ve 87.14 %
				Dd	0.46006718	Rh(D) +ve
		d	0.35870	dd	0.12866569	Rh(d) – ve 12.86%

Table (7) Allelic frequencies and Chi Square -Goodness- of -Fit Test for ABO, Rh blood type among the targeted individuals in the study, Ajdabiya and Ghemines.

	Agadabia	*Ghemins*
ABO blood group		
Allelic frequencies		
A	0.183912043	0.22738816
B	0.164935132	0.180
O	0.65115282	0.59273377
Observed values		
A	410	482
B	331	373
AB	123	118
O	636	527
Excepted values		
A	410	481.95
B	362	372.9
AB	91	117
O	636	527
The calculated χ^2 value	χ^2 =13,9073	χ^2=0.00
Rh blood group		
Allelic frequencies		
D	0.607571662	0.641298
d	0.392428337	0.35870
Observed values		
Rh+	1269	1307
Rh-	231	193
Excepted values		
Rh+	1269	1320
Rh-	231	192
The calculated χ^2 value	χ^2 =0.00	χ^2 =5.328

Discussion

The study determined the distribution of the ABO, Rhesus blood group phenotypes and genotypes in the two different study groups Agadabia & Ghemins, blood groups O were the highest blood groups 42.4% in Agadabia, 35.13% in Ghemins followed by blood group A 27.3% in Agadabia, 32.13% in Ghemins and B 22.06 % in Agadabia, 24.8% in Ghemins respectively and the lowest frequency is that of AB blood type which were 8.2% in Agadabia, 7.8% in Ghemins. The distribution of ABO blood group varies from one population to another, ABO frequency, among Libyans of Fazzan Province blood group was the most frequent group O 48.2%, the least frequent group was group AB 4.9% (Salih *et al*., 2005).

ABO frequency in ALzawia was blood group O48.9% followed by blood group A 33.1%, B 12.8% and AB 5.2% (Fayrouz, Farida and Irshad, 2012). ABO frequency, in the western part of Libya found that blood group O 48.9% was the highest, followed by A 33.1% B12.8% (Noor and Eldin, 2013). In the blood bank of the central hospital in Al-Bayda 37.44%, 23.43%, 30.17%, and 8.96%, and blood types O, A, B, and AB respectively (Saad, 2016). In Bani Waleed O blood group was the most common 43.6%, followed by A 31.7%, B 17.7%, and finally, AB blood group 7.0% (Ameigaal and Ageel, 2019). In addition, studies in other Arab countries such as Saudi Arabiain, Jazan, found that ABO frequency were group O 62%, A27.6%, B 1.7% and AB 7,8% (Abdullah, 2010). In Najran, ABO frequency most common blood group is O 69.47%, and the next common group is A 24.3%, B 4.04% and AB 2.19% were the least respectively (Al-Noaemi and Daghriri, 2018). In other countries, for instance Caucasians, the USA, the distribution for blood group O was 47%, A 41%, B 9% and AB 3% (Seeley, Stephens and Tate, 1998). In Lagos, Nigeria, blood group O was 55.3%, A 25.3%, B 16.7% and AB 2.7% (Adeyemo, Soboye and Omolade, 2006).

Frequency of ABO blood groups and ethnic distribution in Greater–Accra region of Ghana, group O is, the least compare with AB (Doka *et al*., 2019). A phenotype distribution of 37.4%, 30.8%, 24.3%, and 7.5% was found for blood groups O, A, B, and AB (Mohamed *et al*., 2016). Several studies found that the commonest blood group as B followed O, A and AB (Rahman and Lodhi, 2004; Khan *et al*., 2004; Rajshree and Raj, 2013 and Nazli *et al.*, 2015). These regional differences may be are explained by genetic mapping and the varying origins of diverse ethnic groups (Bodmer, 2015).

The distribution of Rhesus D also differs among human groups, in this study the distribution of rhesus factor was 1269 samples from Agadabia were positive (84.6%). 231 Rh negative samples were the least (15.4%). The individuals with a positive Rhesus factor, 615 of them are females and 654 of them are males. The individuals with a negative Rhesus factor, 129 are females and 102 are males. In Ghemins region where there are 1307 individuals (87.14%). The individuals with a positive Rhesus factor, 667 of them are females and 640 of them are males, while the individuals with a negative

Rhesus factor are 193 individuals (12,86 %), 128 of them are females and 65 are males. The results confirmed that the positive Rhesus factor has the highest recurrence rate in the regions of Agdabiya and Ghemins, while the negative Rhesus factor is the lowest ratio (Tables 2, 5), (Figures 2, 4). this result agreed with several previous studies in different places (Akbas, Aydin and Cenani, 2003); Nwauche and Ejele, 2003; Salih *et al*., 2005; Anees, Jawad and Hashmi, 2007; Khattak *et al*., 2008); Abdullah ,2010; Fayrouz, Farida and Irshad, 2012; Noor and Eldin, 2013; Saad, 2016; Al-Noaemi and Daghriri, 2018, Doka *et al*., 2019 and Ameigaal, and Ageel, 2019).

In this research it was observed that the general trend of blood type ABO was O> A> B> AB in both Agadabia and Ghemins. Furthermore, the blood group O was the most common phenotype, whereas group AB was the least frequent among the studied population of the both gender. The frequencies of the genotypes of the Rhesus factor in Agadabia were (DD and Dd) 0.3691 and 0.4768 Rh negative (dd) 0.1539. Moreover, in Ghemines found that Rh (D) positive was the most phenotype, frequencies of the genotypes of the Rhesus factor were (DD and Dd) 0.411 and 0.460. Rh are negative (dd) 0.128, Rh (D) turned out to be the most phenotype.

Chi-square test for conformance with the equilibrium expectations for both ABO and Rh blood groups, what were statistically significant differences between observed and expected recurrences of blood groups using a Chi square at the level of significance < 0,05 and degree of freedom 3 was tested what were there statistically significant differences between observed and expected frequencies Rh factor positive and negative using Chi-Square to determine statistical significance at P- value of >0,05 and freedom score of 1 for both study areas. The decision to Agadabiya region test was as follows:

Firstly: ABO blood groups were compare with calculated value (χ^2) and the theoretical value of the Chi square, since the calculated value (χ^2) of (13,9073) is greater than (7.815) tabular (theoretical) value, so we reject the zero hypothesis and accept the alternative hypothesis. There are statistically significant differences between the observed frequencies and the expected frequencies. This is not agreeing with the equilibrium expectations.

Secondly: rhesus factor compares the calculated value with the theoretical value of the Chi Square, since the calculated value of (0) is less than the value of the tabular (theoretical) of (3.84) so the null hypothesis is accepted, and the alternative hypothesis is rejected. That meaning it does not exist. There are significant statistical differences between the observed frequencies and the expected frequencies. That consistent with the equilibrium predictions for the rhesus factor.

The decision of Ghemins region test was as follows: ABO blood groups were compared with calculated value (χ^2) and the theoretical value of the Chi square, since the calculated value of (0) is less than the value of the (theoretical) tabular (7,815), so the zero hypothesis is accepted, and the alternative

hypothesis is rejected. There are no significant statistical differences between the observed frequencies and the expected repetitions. That agrees with the equilibrium predictions for blood groups. The calculated value was compared with the theoretical value of the Chi square, since the calculated value of (5.328) is greater than the value of the (theoretical) tabular (3.84). For this reason, the zero hypothesis is rejected, and the alternative hypothesis is accepted. That meaning there are a significant Statistical differences between observed frequencies and expected frequencies. That is not agreeing with the equilibrium expectations for the Rh factor.

Several studies have referred to the ABO and RH blood system balance predictions tests of which, the calculated Chi-Square value for ABO blood group was 0.4078 which has the P value is between 0.95 and 0.90 with 3 degrees freedom (P > 95%). The results indicated that there was no significant difference between observed and expected values in the distribution of ABO blood groups. Regarding the Rh blood group, there was also no significant difference between observed and expected values (Goodness of fit $X2$ = 0.0119, df = 1, P > 95%) (Fufa and Debelo, 2019). The calculated Chi-Square value for total studied population was 5.10 with P < 0.05 with 3 degrees of freedom, this demonstrates that there is genetic variability and polymorphism as regards ABO and Rh blood group among the population sampled (Regasa and Gudeta, 2019).

Allele O was more frequent in Agdabiya (0.65115282), and in Ghemins (0.59273377) (Table 3, 6). Allele D was more frequent in Agdabiya (0.607571662), and in Ghemins (0.641298) (Table 3, 6). The Mexican population allele O was more frequent in Puebla (0.8623), in Estado de Mexico (0.8407), and in San Luis Potosi (0.8214). For the Rhesus group allele D was more frequent in Puebla (0.8700), in San Luis Potosi (0.8666), and in Estado de Mexico (0.8523) (Canizalez-Román *et al.*, 2018)

Conclusion

This database can provide data for workers in blood centers, medical analysis laboratories, and hospitals, enabling them to determine where they can obtain the blood types needed for medical interventions. This information also contributes to knowledge of the genetic makeup of the target population in the study, as there is no genetic information about their genetic makeup, and this information can be It has major implications in various fields of medicine and population studies.

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