

Original article

Gene Frequency and Prevalence of ABO and Rh D Blood Group Systems among Libyan Donors and Patients of Jabal Nafusa Region, Libya

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ABSTRACT

The ABO and Rh D/d blood group systems have significant medical and genetic importance. In Libya, several studies had been conducted on the distribution of the ABO and Rh D/d blood group systems in different regions of the country. However, there are many regions in Libya that have not yet been studied. Therefore, the aim of this study was to determine the gene frequency and prevalence of ABO and Rh D/d blood group systems among Libyan donors and patients of Jabal Nafusa region in Zintan, Alrujban, and Jadu cities north western of Libya; in addition to Tripoli city. This was a retrospective study that included only Libyans attending the Central Blood Bank in Zintan, Alrujban Clinic in Alrujban, Jadu Medical Analysis Laboratory in Jadu, and Al-Khadra Hospital in Tripoli. The ABO grouping and the Rh typing was done by the standard slide method using commercial blood grouping anti-A, anti-B and anti-D. Data was analyzed using descriptive statistics and Hardy–Weinberg equilibrium law. The overall results of this study show that the most prevalent ABO blood group was O (33.68%), followed by A (26.27%), B (21.31%), and AB (18.74%); which cluster into three categories: O>A>B>AB (Zintan and Alrujban cluster), A>O>B>AB (Tripoli cluster), O>AB>B>A (Jadu cluster). The highest prevalence of A⁺ (29.35%), B⁺ (19.73%) and A⁻ (8.74%), B⁻ (9.47%) was found in Tripoli and Jadu, respectively. Jadu had the highest prevalence of AB⁺ (14.86%) and AB⁻ (11.37%). The highest percentage of O⁺ (46.92%) and Rh D⁺ (91.64%), O⁻ (12.97%) and Rh d⁻ (42.56%) was observed in Zintan and Jadu, respectively. The occurrence of considerable percentages of O⁻ and AB⁻ blood groups in Jadu indicated significant medical implications for healthcare blood transfusion services. The gene frequency of ABO and Rh D for A, B, O, D⁺, and d⁻ alleles respectively are: 0.1795, 0.1025, 0.7180, 0.7109, and 0.2891 in Zintan; 0.2013, 0.0762, 0.7225, 0.5817, and 0.4183 in Alrujban; 0.1860, 0.1892, 0.6248, 0.3476, and 0.6525 in Jadu; 0.2693, 0.1885, 0.5422, 0.5660, and 0.4340 in Tripoli. Significant difference in ABO and Rh D allele frequencies was found in Jadu, which indicated that Jadu's population is not in Hardy–Weinberg equilibrium. This is the first study that documents the gene frequency for ABO and Rh D alleles and their prevalence of Jabal Nafusa region in Libya. The data in this study are of great importance to blood banks, blood transfusion services, genetics and anthropological studies in Libya.

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INTRODUCTION

The ABO and Rhesus (Rh) D/d blood group systems are the major alloantigen system recognized in humans, although, there are 45 recognized blood group systems containing 362 red cell antigens [1]. These two systems have significant important roles in various fields of science such as human population genetics, forensics, pathology, anthropology, blood transfusion, organ transplantation, hemolytic disease of the newborn, and for blood bank registers [2]. The ABO blood group system is under the control of a single gene that has multiple alleles. This gene is located on the long arm of chromosome 9 (9q34.1-34.2) [3]. The symbols I^A, I^B, and I^O are often used to designate these alleles. The I^A and I^B alleles are codominant amongst each other and both are dominant to the I^O allele (i.e. the I^O allele is recessive). The three alleles I^A and I^B, and I^O are responsible for exhibiting six genotypes: I^A/I^A (AA), I^A/I^O (AO), I^B/I^B (BB), I^B/I^O (BO), I^A/I^B (AB) and I^O/I^O (OO) yielding four phenotypes of blood groups: A, B, AB, and O. While, the Rhesus system is under the control of three pairs of closely linked allelic genes located on short arm of chromosome 1[4]. These alleles are responsible for the expression of more than 50 antigens [1]. However, only the D antigen is the most potent immunogenic antigen in the Rh blood group system. The D gene has two alleles (d and D) yielding two phenotypes of blood groups the Rh d- negative (dd) and the Rh D+ positive (DD and Dd) blood types respectively [5].

Many studies have been conducted worldwide across different ethnic populations and in different geographical regions to determine the prevalence, distribution, and gene frequency of ABO and Rh D blood group systems (Table 1). These studies have reported varying percentage in ABO and Rh blood group types, based on the phenotype of these groups (Table 1). The prevalence of blood groups A, B, and O throughout the world was reviewed by Goel et al., 2021[6].

Table 1. Phenotypic percentage (%) distribution of ABO and Rh D blood groups in several countries of the world.

Country	Blood System						Reference
	ABO				Rh D		
	A	B	O	AB	D+	d-	
Ethiopia	28.41	21.24	44.65	5.71	94.82	5.18	[7]
Nigeria	22.77	20.64	52.93	3.66	94.90	5.1	[8]
Somalia	26.50	11.27	60.30	1.93	96.49	3.43	[9]
Madagascar	22.61	29.66	41.60	6.13	98.90	1.1	[10]
Tanzania	24.4	19.1	52.4	4.0	95.3	4.7	[11]
Burkina Faso	22.5	28.5	43.3	5.6	92.24	7.76	[12]
Cameroon	27.38	11.79	55.51	5.32	95.82	4.18	[13]
Congo	21.6	15.4	60.5	2.5	98.4	1.6	[14]
Kenya	24.25	18.75	51.70	5.5	95	5	[15]
Mauritania	17.74	12.04	70.20	0.02	94.23	5.77	[16]
Morocco	32.86	15.80	46.80	4.53	91	9	[17]
Algeria	30.14	16.62	47.52	5.72	91.8	8.1	[18]
Egypt	35.12	23.12	31.94	9.7	91.78	8.22	[19]
Sudan	30	14	51	5	97.4	2.6	[20]
Saudi Arabia	26.70	19.09	50	4.21	91.81	8.19	[21]
Bahrain	18.67	24.49	53.4	3.44	92.82	7.18	[22]
Qatar	27.6	20.4	45.4	6.5	-	-	[23]
UAE	24	22.8	48.4	3.1	91.1	8.9	[24]
Oman	24.06	19.29	50.47	3.62	91.65	8.35	[25]
Yemen	34.41	8.43	55.54	1.71	80	20	[26]
Iraq	23.11	21.45	48.03	7.41	88.56	11.44	[27]
Kuwait	26.7	22.77	34.65	13.86	-	-	[28]
Jordan	36.82	18.62	37.44	7.12	88.73	11.27	[29]
Lebanon	42	13.86	37.48	6.84	88.45	11.55	[30]
Syria	46.25	13.13	37.5	3.12	-	-	[31]
Gaza	33.1	21.3	38.1	7.5	89.3	10.7	[32]
China	30.5	29.4	30.4	9.7	98.98	1.02	[33]
Sri Lanka	11	39	46	4	99	1	[34]
India	22.03	30.92	39.17	7.88	95.96	4.04	[35]
Pakistan	22.9	31.7	40.4	5.1	93.1	6.9	[36]
Bangladesh	27	34	28	10	99	1	[37]
Iran	28.48	24.71	40.21	6.6	92.38	7.62	[38]
Turkey	39.69	18.63	33.62	8.06	88.44	11.56	[39]

Greece	39.95	16.03	38.77	5.25	91.41	8.59	[40]
Mexico	27.44	8.93	61.82	1.81	95.58	4.42	[41]

In Libya, several studies had been conducted on the distribution of the ABO and Rh D blood group systems in different regions of Libya (Table 2, Fig. 1A). The north western region includes the city of Sabratha [42], Zawia [43], Tripoli [44,] Almergab province, which comprise three cities: Zliten, khoms and Mesallata [45], Misurata [46], Bani Waleed [47], and Tarhuna [48]. The north eastern region includes the city of Ajdabiya [49], Benghazi [50], Albidya [51], and Derna [52]. The southern region includes Sebha city [53, 54]. The mid-central region of Libya represented by Aljufra province includes five cities: Waddan, Hun, Soknah, Zilla and Al-foha [55]. These studies have provided valuable data on the distribution of blood groups in Libya for better management of the regional and national availability of the blood supply.

Table 2. Phenotypic percentage (%) distribution of ABO and Rh D blood groups in several cities in Libya.

City	Blood System						Reference
	ABO				Rh D		
	A	B	O	AB	D+	d-	
Sabratha	34	18.13	43.19	4.68	83.53	16.47	[42]
Zawia	34.6	15.8	45	4.4	89.4	10.6	[43]
Tripoli	36.60	16.80	41.50	5.10	87.8	12.2	[44]
Almergeb	37.65	15.22	41.17	5.96	84.27	15.73	[45]
Misurata	36	18	38	8	85	15	[46]
Bani Waleed	31.7	17.7	43.6	7.0	72.2	23.8	[47]
Tarhuna	31.28	14.86	49.43	4.43	86.71	13.29	[48]
Ajdabiya	29.64	16.56	38.56	15.22	78.95	21.05	[49]
Benghazi	32.12	19.11	41.15	6.53	86.26	13.73	[50]
Albidya	30.168	23.43	37.44	8.96	83.92	16.08	[51]
Derna	28.24	24.36	39.50	7.90	89.4	10.6	[52]
Sebha	28	23	42	7	81.7	18.2	[53, 54]
Aljufra	26.8	21.6	46.2	5.4	85.2	14.8	[55]

However, these previously published studies are incomplete, because Libya is a large country with few different minor ethnic groups (i.e., Amazigh [Berber], Tuareg, Tubu) and there are many regions that have not yet been studied. Therefore, due to the lack of studies on gene frequency and distribution of the ABO and Rh D blood group systems in many regions of Libya, the present study was under taken. The gene frequency and prevalence of ABO and Rh D/d blood group systems was determined in the cities of Zintan, Alrujban, and Jadu of Jabal Nafusa region north western of Libya (Fig. 1); in addition to Tripoli city. The results of this study were compared with studies conducted previously in Libya and other countries.

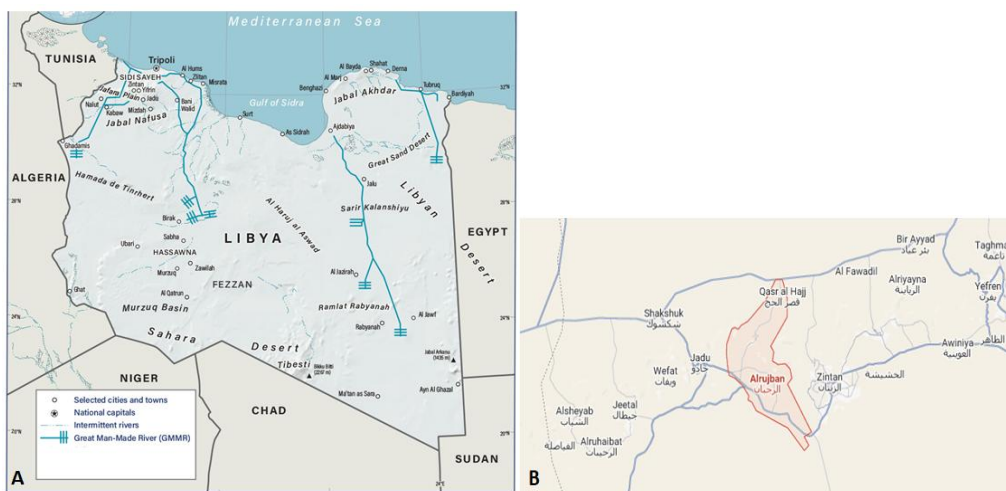


Figure 1. Map of Libya showing previously studied cities in the north western region (e.g., Zliten, khoms, Misrata, and Bani Waleed), in the north eastern region (Ajdabiya, Benghazi, Albidya, and Derna) and the southern region (Sebha city), as well as Zintan, and Jadu of Jabal Nafusa region (A). Location of cities in this study: Zintan, Alrujban, and Jadu (B). (Source google maps)

METHODS

This was a descriptive retrospective study and included only Libyan nationals attending the Central Blood Bank in Zintan, Alrujan Clinic in Alrujan, Jadu Medical Analysis Laboratory in Jadu, and Al-Khadra Hospital in Tripoli. Data on blood group types were collected from Laboratory Medical Records (i.e. registration logbook for blood group typing results) from individuals of both genders and of different ages for the year 2018. Permission to data access of Laboratory Medical Records was granted from health authorities to view the records of ABO and Rhesus D blood typing results for donors and patients and use them as data for this study. The confidentiality of the data was maintained throughout the study. Informed consent was not required from the study participants; hence the study was a retrospective examination of Laboratory Medical Records for ABO and Rh D blood group typing results. The total number of registered individuals taken from each city was 861 (Zintan), 240 (Alrujan), 3430 (Jadu), and 988 (Tripoli). The ABO grouping and the Rh typing was done serologically by the standard slide method using commercial blood grouping anti-sera: anti-A, anti-B and anti-D monoclonal antibodies for agglutination test. Data was recorded and then analyzed using descriptive statistics and Hardy-Weinberg equilibrium law; the results were presented in the form of tables.

The phenotype frequencies of the ABO (A, B, O, AB) and Rh D (D^+ , d^-) blood group systems were determined as percentages of observed numbers (i.e. phenotype frequency was determined by the ratio between the number of individuals of particular blood group type and the total number of individuals multiplied by 100). Gene frequency of the ABO and Rh D blood group alleles was calculated using Hardy-Weinberg equilibrium law related to population genetic studies. The formulas " $p + q + r = 1$ " and " $a + b = 1$ " represent the gene frequency for **A** (p), **B** (q), **O** (r), Rh **D**⁺ (a), and Rh **d**⁻ (b) alleles; the expansion of these formulas " $p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = 1$ " and " $a^2 + 2ab + b^2 = 1$ " represents the phenotype frequency of the **O** (r^2), **A** (p^2), **B** (q^2), **AB** ($2pq$), Rh **D**⁺ (a^2), Rh **d**⁻ (b^2) phenotypes. Genotypes AO, BO, and Rh Dd phenotype frequencies are represented by $2pr$ and $2qr$, and $2ab$, respectively. Therefore, the allele frequency of O, A, B, Rh D⁺, and Rh d⁻ can be calculated from the observed ABO and Rh d⁻ phenotype frequency by the following equations:

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

$$q = \sqrt{\text{frequency of B phenotype} + \text{O phenotype}} - r \qquad b = \sqrt{\text{frequency of Rh d}^- \text{ phenotype}} \qquad a = 1 - b$$

The calculated allele frequency by these equations is called the observed or unadjusted (uncorrected) allele frequency. The sum of ABO or Rh D allelic frequencies should be equal to one (if the observed frequencies of the phenotypes had no deviation from the expected values). However, the sum of the allele frequencies can deviate from one. Therefore, if $(p+q+r) \neq 1$, correction by deviation (D) should be performed. The deviation (D) can be calculated as follows: $D = 1 - (p+q+r)$; then the expected allelic frequencies can be estimated from corrected allelic frequencies by the following equations:

$$p_c = (1+D)^{1/2} \qquad q_c = (1+D)^{1/2} \qquad r_c = 1 - (p_c + q_c)$$

Where p_c , q_c , and r_c represent the corrected or adjusted allele frequency. The phenotype and genotype frequency, recalculated from the corrected observed allele frequency is called expected phenotype frequency and expected genotype frequency. The expected phenotype frequency of A = $p_c^2 + 2p_c r_c$, expected genotype frequency of A A = p_c^2 , expected genotype frequency of AO = $2p_c r_c$. The expected phenotype frequency of B = $q_c^2 + 2q_c r_c$, expected genotype frequency of BB = q_c^2 , expected genotype frequency of BO = $2q_c r_c$. The expected phenotype frequency of O = r_c^2 , expected genotype frequency of OO = r_c^2 . The expected phenotype frequency of AB = $2p_c q_c$, expected genotype frequency of AB = $2p_c q_c$.

Additionally, the Chi-square (χ^2 , goodness-of-fit) test was performed to compare observed and expected gene allele and phenotypic frequency distributions of the ABO blood group system under the Hardy-Weinberg equilibrium law. The calculated Chi-square p value determined the significance of deviation of ABO blood phenotypes based on allele frequencies distribution in the studied cities. Chi-square p value of less than 0.05 ($P < 0.05$) was considered to be statistically significant (i.e. population is not in Hardy-Weinberg equilibrium and there is significant difference between observed and expected ABO allele frequencies). With ABO, it is statistically feasible to do a chi-square test because there is one degree of freedom (number of phenotypes (A, B, O, AB) - number of alleles (p, q, r), $df = 4 - 3 = 1$). However, for Rh D blood group system the chi-square test cannot be investigated because we have no degree of freedom (i.e. two phenotypes, positive and negative blood types, corresponding to two alleles Rh D⁺ and Rh d⁻).

RESULTS

This study presents new statistics on gene frequency and phenotypic prevalence of ABO and Rh D blood group systems in Jabal Nafusa north-western region of Libya. This study will serve as a reference for future studies and contributes new data to existing knowledge status of ABO and Rh D blood group types in Libya.

In this study, the total number of individuals surveyed for their blood group type was 5519, which were taken from previously recorded data (i.e. Laboratory Medical Records) on blood group types for the cities of Zintan, Alrujban, Jadu of Jabal Nafusa region and Tripoli city representing the north western region of Libya. The overall total (combined) results of this study show that the O blood group had the highest prevalence (33.68%), followed by A (26.27%), B (21.31%), and AB (18.74%) (Table 3, Fig. 2A). The percentage of Rh D⁺ was higher than Rh d⁻ in all the studied cities, the combined percentage of Rh D⁺ was 68.11% and 31.89% for Rh d⁻ (Table 3, Fig. 2B).

Table 3. The phenotypic percentage (%) distribution of ABO and Rh D blood group types in the cities of Zintan, Alrujban, Jadu and Tripoli.

City	Rh Blood Type	ABO Blood Group Type				
		A n (%)	B n (%)	O n (%)	AB n (%)	Total
Zintan	Rh D ⁺	230 (26.71%)	126 (14.63%)	404 (46.92%)	29 (3.36%)	789 (91.63%)
	Rh d ⁻	20 (2.32%)	10 (1.16%)	42 (4.87%)	0 (0%)	72 (8.36%)
	Total	250 (29.03%)	136 (15.79%)	446 (51.80%)	29 (3.36%)	861
Alrujban	Rh D ⁺	61 (25.41%)	27 (11.25%)	107 (44.58%)	3 (1.25%)	198 (82.5%)
	Rh d ⁻	19 (7.91%)	1 (0.41%)	21 (8.75%)	1 (0.41)	42 (17.5%)
	Total	80 (33.33%)	28 (11.66%)	128 (53.33%)	4 (1.66%)	240
Jadu	Rh D ⁺	460 (13.41%)	450 (13.11%)	550 (16.03%)	510 (14.86%)	1970 (57.43%)
	Rh d ⁻	300 (8.74%)	325 (9.47%)	445 (12.97%)	390 (11.37%)	1460 (42.56%)
	Total	760 (22.15%)	775 (22.59%)	995 (29.00%)	900 (26.23%)	3430
Tripoli	Rh D ⁺	290 (29.35%)	195 (19.73%)	230 (23.27%)	87 (8.08%)	802 (81.17%)
	Rh d ⁻	70 (7.08%)	42 (4.25%)	60 (6.07%)	14 (1.41%)	186 (18.82%)
	Total	360 (36.43%)	237 (23.98%)	290 (29.35%)	101 (10.22%)	988
Overall Total (combined)	Rh D ⁺	1041 (18.86%)	798 (14.46%)	1291 (23.39%)	629 (11.40)	3759 (68.11%)
	Rh d ⁻	409 (7.41%)	378 (6.85%)	568 (10.29%)	405 (7.34%)	1760 (31.89%)
	Total	1450 (26.27%)	1176 (21.30%)	1859 (33.68%)	1034 (18.73%)	5519

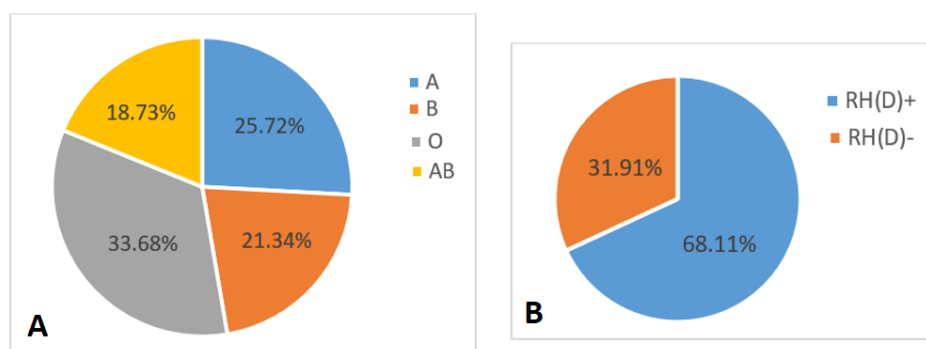


Figure 2. Pie chart showing the overall total (combined) phenotypic percentage (%) distribution of ABO (A) and Rh D (B) blood groups in this study.

The phenotypic percentage distribution of ABO blood groups according to Rhesus blood type is shown in table 3. The highest prevalence of A⁺ blood group was found in Tripoli (29.35%), followed by Zintan (26.71%), Alrujban (25.41%), and Jadu (13.41%). The overall total prevalence of A⁺ was 18.86%. While, the highest prevalence of A⁻ was observed in Jadu (8.74%), followed by Alrujban (7.91%), Tripoli (7.08%), and Zintan (2.32%). The overall total prevalence of A⁻ was 7.41%. The highest prevalence of A phenotype (i.e., A⁺ and A⁻) was observed in Tripoli (36.43%), followed by Alrujban (33.33%), Zintan (29.03), and Jadu (22.15%). The overall total of A phenotype in the studied cities was 26.27% (Table 3).

The highest prevalence of B⁺ blood group was observed in Tripoli and Zintan 19.73% and 14.63%, respectively, followed by Jadu (13.11%), and Alrujban (11.25%). The overall total of B⁺ was 14.46%. Whereas, the highest prevalence of B⁻ blood group was in Jadu (9.47%), followed by Tripoli (4.25%), Zintan (1.16%), and Alrujban (0.41%). The overall total of B⁻ blood group was 6.85%. Tripoli showed the highest percentage (23.98%) of B phenotype blood group, followed by Jadu (22.59%), Zintan (15.79%), and Alrujban (11.66%). The overall total of B phenotype in the studied cities was 21.30% (Table 3).

Furthermore, the highest percentage of O⁺ blood group was seen in Zintan (46.92%), followed by Alrujban (44.58%), Tripoli (23.27%), and Jadu (16.03). The overall total prevalence of O⁺ blood group was 23.39%. The highest percentage of O⁻ blood group was seen in Jadu (12.97%), followed by Tripoli (6.07%), Alrujban (8.75%), and Zintan (4.87%). The overall total prevalence of O⁻ was 10.29%. Alrujban city exhibited the highest prevalence of blood group O (53.33%) followed by Zintan (51.80%), Tripoli (29.35%), and Jadu (29%). The overall total O phenotype in the studied cities was 33.68% (Table 3).

Moreover, the highest prevalence of AB⁺ blood group was observed in Jadu (14.86%), followed by Tripoli (8.08%), Zintan (3.36%), and Alrujban (1.25%). The overall total prevalence of AB⁺ blood group was 11.40%. AB⁻ was highest in Jadu (11.37%), followed by Tripoli (1.41%), and Alrujban (1.25%). No AB⁻ cases were observed in Zintan city (Table 3). Jadu showed the highest prevalence of AB phenotype (26.23%), followed by Tripoli 10.22%, Zintan (3.36%), and Alrujban (1.66%). The overall total of AB phenotype in the studied cities was 18.73% (Table 3).

In general, the overall ABO blood group frequencies in this study cluster into three categories: O>A>B>AB (Zintan and Alrujban cluster), A>O>B>AB (Tripoli cluster), O>AB>B>A (Jadu cluster). Furthermore, the highest percentage of Rh D⁺ was observed in Zintan (91.64%), followed by Alrujban (82.5%), Tripoli (81.17%), lowest in Jadu (57.43%); whereas, the highest Rh d⁻ was observed in Jadu (42.56%), followed by Tripoli (18.82%), Alrujban (17.5%), and Zintan (8.36%) (Table 3).

In addition, gene frequency of the observed and expected blood group alleles of ABO and Rh D blood group systems in the studied cities of Zintan, Alrujban, Jadu and Tripoli are shown in Table (4). For the cities of Zintan and Alrujban, the highest ABO expected gene frequency was for blood group O, followed by A, then B with allele frequencies of 0.7180, 0.1795, 0.1025, and (0.7225), (0.2013), (0.0762), respectively; the expected gene frequency of Rh D alleles was 0.7109 (Zintan) and 0.5817 (Alrujban) for Rh D⁺ allele, 0.2891 (Zintan) and 0.4183 (Alrujban) for Rh d⁻ allele. Whereas, in Tripoli city the highest ABO expected gene frequency was for blood group A, followed by O, then B with an allele frequency of 0.2693, 0.5422, 0.1885, respectively. The expected gene frequency of Rh D alleles was 0.5660 for Rh D⁺ allele and 0.4340 for Rh d⁻ allele. For the city of Jadu, the highest ABO expected gene frequency was for the O blood group (0.6248), followed by B (0.1892), then A 0.1860; the expected gene frequency of Rh D alleles was 0.3476 for Rh D⁺ allele and 0.6525 for Rh d⁻ allele. In general, the highest expected O allele frequency was observed in Alrujban city (0.7225), the highest expected A allele frequency was observed in Tripoli city (0.2693), the highest expected B allele frequency was observed in Jadu city (0.1892). The highest Rh D⁺ and Rh d⁻ expected allele frequency was observed in Zintan city (0.7109) and Jadu city (0.6525), respectively (Table 4).

Table 4. Gene frequency of observed (Obs) and expected (Exp) blood group alleles of ABO and Rh D blood group systems in the studied cities of Zintan, Alrujban, Jadu and Tripoli.

City	Gene Frequency									
	ABO Blood Group System						Rh D Blood Group System			
	Alleles						Alleles			
	A (p)		B (q)		O (r)		D+ (a)		d- (b)	
Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	
Zintan	0.2903	0.1795	0.1579	0.1025	0.5180	0.7180	0.9164	0.7109	0.0836	0.2891
Alrujban	0.3333	0.2013	0.1166	0.0762	0.5333	0.7225	0.8250	0.5817	0.1750	0.4183

Jadu	0.2215	0.1860	0.2259	0.1892	0.290	0.6248	0.5743	0.3476	0.4257	0.6525
Tripoli	0.3643	0.2693	0.2398	0.1885	0.2935	0.5422	0.8117	0.5660	0.1883	0.4340
Total (combined)	0.2627	0.2002	0.2131	0.1665	0.3368	0.6333	0.6811	0.4353	0.3189	0.5647

Additionally, the expected frequencies of the various genotypes and phenotypes in the ABO and Rh D systems in the studied cities of Zintan, Alrujban, Jadu and Tripoli were calculated and presented in Table 5. For example, the frequency of AA genotype was 0.0322 (Zintan), 0.0405 (Alrujban), 0.0346 (Jaduo), and 0.0726 (Tripoli); while that of AO genotype was 0.2578 (Zintan), 0.2909 (Alrujban), 0.2324 (Jadu), and 0.2921 (Tripoli). Hence, the percentage of homozygous AA among those who are blood group A was 3.2% (Zintan), 4.0% (Alrujban), 3.5% (Jaduo), and 7.3% (Tripoli) (Table 5). This indicates percentage of heterozygous AO are more prevalent in the studied cities. Comparable inferences can be made for blood group B, and for Rh D⁺ among DD and Dd individuals. The genotype frequencies of overall total Rh blood group combined were 0.1895 for DD, 0.4916 for Dd and 0.3189 for dd (Table 5).

The Chi-square (χ^2 , goodness-of-fit) test and P value were calculated for the observed and expected ABO blood group phenotypes in the studied cities of Zintan, Alrujban, Jadu and Tripoli (Table 6) using expected ABO allele frequencies (Table 4, 5). There was no significant difference between the observed and expected ABO blood group phenotypes in the cities of Zintan ($P > 0.50$), Alrujban ($P > 0.10$), and Tripoli ($P \geq 0.95$) as determined by the p value (Table 6). However, significant difference between the observed and expected ABO blood group phenotypes was observed in Jadu city, $P > 0.0001$; this indicates that the population is not in Hardy–Weinberg equilibrium and there is significant difference between observed and expected ABO allele frequencies in Jadu city.

Table 5. Genotypic and phenotypic expected frequencies of ABO and Rh D blood group systems in the studied cities of Zintan, Alrujban, Jadu and Tripoli.

Genotype/ Phenotype Expected Frequency		City				
		Zintan	Alrujban	Jadu	Tripoli	Combined
ABO Blood Group System						
Genotype	AA	0.0322	0.0405	0.0346	0.0726	0.0401
	AO	0.2578	0.2909	0.2324	0.2921	0.2536
Phenotype	A	0.2900	0.3314	0.2670	0.3647	0.2937
Genotype	BB	0.0105	0.0058	0.0358	0.0355	0.0277
	BO	0.1471	0.1101	0.2364	0.2044	0.2108
Phenotype	B	0.1577	0.1159	0.2722	0.2399	0.2385
Genotype	OO	0.5155	0.5220	0.3904	0.2939	0.4012
Phenotype	O	0.5155	0.5220	0.3904	0.2939	0.4012
Genotype	AB	0.0368	0.0308	0.0704	0.1016	0.0666
Phenotype	AB	0.0368	0.0308	0.0704	0.1016	0.0666
Rh D Blood Group System						
Genotype	DD	0.5054	0.3384	0.1208	0.3205	0.1895
	Dd	0.4110	0.4866	0.4534	0.4912	0.4916
Phenotype	D	0.9164	0.8250	0.5742	0.8117	0.6811
Genotype	dd	0.0836	0.1750	0.4257	0.1883	0.3189
Phenotype	d	0.0836	0.1750	0.4257	0.1883	0.3189

Table 6. Observed (Obs) and expected (Exp) ABO blood phenotypes, Chi square test and P value in the studies cities of Zintan, Alrujban, Jadu, and Tripoli.

ABO Blood Phenotypes		City			
		Zintan	Alrujban	Jadu	Tripoli
A	Obs	250	80	760	360
	Exp	249.69	79.54	915.81	360.32
B	Obs	136	28	775	237
	Exp	135.78	27.68	933.65	237.02
O	Obs	446	128	995	290

	Exp	443.85	125.28	1339.07	290.37
AB	Obs	29	4	900	101
	Exp	31.68	7.39	241.47	100.3
Chi-square Test	χ^2	0.2379	1.561	1937.80	0.0046
	P-value	>0.50	0.10>P<0.25	>0.0001*	≥ 0.95

DISCUSSION

The present study is the first study that documents the prevalence of the ABO and Rh D phenotypes, gene and allele frequencies and expected genotypes in the cities of Zintan, Alrujban, and Jadu of Jabal Nafusa region north western of Libya; in addition to Tripoli city. Gene frequency data on ABO and Rh D systems are very important because phenotype frequencies can be easily determined. Gene and allele frequencies provide a reference percentage about ABO and Rh D blood group types in a given population. This information can then be applied in practice for better planning of blood donation campaign sites and blood donor recruitment according to blood bank transfusion medicine needs and requirements. Additionally, the availability of this data is useful for the integration management of blood banks across the country and will aid in the establishment of a national database for the geographical distribution of blood group types in Libya. The national database can then provide data for doctors and medical personal workers in blood bank centers, medical analysis laboratories, and hospitals, allowing them to determine where they can acquire rare blood group types for transfusion therapies in emergency and trauma medical situations. Furthermore, gene and allele frequencies information have anthropological significance and could be used to decipher the genetic diversity of a particular population when there is no genetic information about the population genetic structure.

The results of this study show that the most prevalent ABO blood group was O (33.68%), followed by A, B, and AB blood groups (26.27%, 21.30%, and 18.73%, respectively); hence, clustering blood group prevalence in this study as O> A> B> AB. This result is in agreement with similar previous studies conducted in Libya (Table 2). Similar results were also reported from several studies conducted in African and Arab countries (Table 1); although there are differences in the percentages of ABO blood groups reported. These include: Ethiopia [7], Somalia [9], Tanzania [11], Cameroon [13], Congo [14], Kenya [15], Mauritania [16], Morocco [17], Algeria [18], Sudan [20], Saudi Arabia [21], Qatar [23], UAE [24], Oman [25], Yemen [26], Iraq [27], Kuwait [28], and Jordan [29] (Table 1). However, several countries showed different O> A> B> AB cluster for ABO blood groups. The cluster O>B>A>AB includes Madagascar [10], Bahrain [22], Burkina Faso [12], Sri Lanka [34], India [35], and Pakistan [36] (Table 1). The A>O>B>AB cluster includes Egypt [19], Lebanon [30], Syria [31], Turkey [39], Greece [40] (Table 1) and several European countries [55]. While, most oriental Asian countries show B > O > A > AB cluster [6]. None the less, many studies have documented that the O blood group remains to be the most prevalent blood group globally, reaching 80% in certain ethnic populations of South America [6]; while, the AB blood group is the least prevalent (1.8%) among different ethnic populations [41]. The variation in the distribution of the ABO blood group has been attributed to genetic diversity as well as geographic and regional variations of studied populations.

Additionally, this study showed that the percentage of Rh D+ was higher than Rh d- in Tripoli, Zintan, Alrujban, and Jadu. This result is consistent with previous studies conducted in different regions of Libya (Table 2) and other parts of the world (Table 1). However, the combined percentage of Rh D+ (68.11%) and Rh d- (31.89%) (Table 3, Fig. 2B) varies greatly with percentages reported in Libya and elsewhere in the world. For example, previous studies in Libya reported lowest percentage of Rh D+ 72.2% and 78.95% in Bani Waleed [47] and Ajdabiya [49], respectively (Table 2). While, the highest reported percentage (89.4%) of Rh D+ in Libya was observed in Zawia [43] and Derna [52] (Table 2). On the other hand, the highest reported percentage of Rh d- (23.8% and 21.055%) in Libya was observed in Bani Waleed [47] and Ajdabiya [49], respectively. Lowest Rh d- (10.6%) was observed in Zawia [43] and Derna [52] (Table 2). At the international level, Sri Lanka [34], Bangladesh [37], and China [33] show the highest percentage of Rh D+ (99%); while the lowest Rh D+ found in Yemen 80% [26] and Turkey 88.44% [39] (Table 1). Whereas, the highest prevalence of Rh d- was reported in Basque population [56] and Berbers of Morocco in the range of 50–60% [57], Gambela, Ethiopia (19.37%) [58], Yemen (20%) [26], Turkey (11.56%) [39], Lebanon (11.55) [30], and Iraq (11.44%) [27] (Table 1). The reason for the high percentage range variation in this study is due to Jadu's blood group data, which showed a prevalence of Rh d- of 42.56% that is twice as high as in Alrujban (17.5%) and Tripoli (18.82%), and 5 times more than Zintan (8.36%) (Table 3). Therefore, the percentage of Rh D+ and Rh d- in this study and not including Jadu's blood group data, is 85.64% and 14.36%, respectively; which is similar to some previous studies done in Libya (e.g. Aljufra (14.8) [55], Almergeb 15.73% [45], Albiyda (16.08%) [51], Sabratha (16.47%) [42] (Table 2). Rh d- occurs in about 15% of Caucasians in Europe, Canada, and the USA [59].

In addition, the results of the present study showed significant difference in the allele frequencies for the Rh D system. The combined expected allele frequencies for the Rh D system in the present study were 0.4353 for the Rh D+ allele and 0.5647 for the Rh d- allele, which are different from previous studies conducted in Libya; The reported Rh D allele frequencies are 0.6293 Rh D+ and 0.3706 Rh d- in Benghazi [50], 0.6076 Rh D+ and 0.3924 Rh d- in Ajdabiya [49], 0.6413 Rh D+ and 0.3587 Rh d- in Ghemins [49], and 0.5753 Rh D+ and 0.4267 Rh d- in Sebha [53]. The reason for this difference in Rh D allele frequency in this study is also due to Jadu's blood group data, which showed an allele frequency of 0.6525 for the Rh d- allele and an allele frequency of 0.3476 for the Rh D+ (Table 4). When Jadu's blood group data was removed, the obtained result for Rh D gene frequency is 0.6211 and 0.3789 for Rh D+ and Rh d-, respectively; which is similar to Rh D gene frequency observed in Benghazi, Ajdabiya, and Ghemins in the north eastern part of Libya [50, 49] Also, it is close to Rh D gene frequency reported in Iraq 0.65 for Rh D+ allele and 0.35 for Rh d- allele [60]. However, it is different from Rh D allele frequency in Saudi Arabia (0.7138 for Rh D+ allele, 0.2862 for Rh d- allele [21] and Oman (0.71 for Rh D+ allele, 0.29 for Rh d- allele) [25].

Furthermore, the combined expected allele frequencies for the ABO blood group system in the present study were 0.2002 for the A allele, 0.1665 for the B allele, and 0.6333 for the O allele. However, excluding Jadu's blood group data, the combined expected allele frequencies for the ABO blood group system were 0.2194 for the A allele, 0.1375 for the B allele, and 0.6431 for the O allele. These findings are similar to the results of previous studies conducted in Benghazi (0.2208 A allele, 0.1377 B allele, and 0.6415 for O allele), Ajdabiya (0.1839 A, 0.1649 B, 0.6511 for O allele), Ghemins (0.2274 A allele, 0.180 B allele, 0.5927 for O allele) north eastern part of Libya [50, 49], and Sebha (0.194 A, 0.160 B, 0.6511 for O allele) in southern part of Libya [53]. The expected allele frequencies for the ABO blood group system in this study are also similar to neighboring countries of north Africa: Algeria (0.198 A allele, 0.118 B allele, and 0.683 for O allele) [18], Tunisia (0.195 A allele, 0.120 B allele, and 0.685 for O allele) [61]; other countries such as Iraq (0.20 A allele, 0.14 B allele, and 0.64 for O allele) [60]. However, it is different from Egypt (0.254 A allele, 0.181 B allele, and 0.565 for O allele) [19], Jordan (0.260 A allele, 0.134 B allele, and 0.605 for O allele) [62], Bahrain (0.118 A allele, 0.134 B allele, and 0.73 for O allele) [22], and Saudi Arabia (0.1688 A allele, 0.1242 B allele, and 0.7070 for O allele) [21]. The higher incidence of the O allele in this study is consistent with the high occurrence of the O blood group, which is the most widespread blood group globally [6].

Moreover, regarding the ABO blood group phenotypes the results of this study showed a highly significant difference between observed and expected number of individuals in Jadu city only (Table 6) attributed to a decrease number of O (995), A (760) and B (775) to that expected (1339.07), (915.81) and (933.65) respectively; also, to an increasing number of AB individuals (900) in the study sample to that expected (241.47) according to Hardy-Weinberg equilibrium. Factors that interrupt the Hardy-Weinberg equilibrium of allelic and genotypic frequencies of the population include mutation, migration (i.e. gene flow), random genetic drift, and natural selection [63]. Therefore, the difference between observed and expected ABO blood phenotypes in Jadu city could be attributed to genetic factors and natural selection which is affected mainly by marriage traditions in Jadu. Marriage traditions in Jadu are considered non-random because people in Jadu marry only from their own Jadu kins but not necessarily consanguineous marriages; hence, they have maintained their distinct population structure and pure gene pool, along with the Amazigh identity and language for many centuries. In general, the prevalence of the ABO and Rh D blood groups in Jadu contrasts with Zintan, Alrujban, and Tripoli as well as previous studies in Libya. This is because Jadu city represents distinct genetic population Amazigh Jadu residing in Jabal Nafusa. More genetic studies are needed to clarify if this contrast is due to selecting pressure or genetic isolation. Furthermore, comparison of ABO and Rhesus blood group phenotypes results in this study (Table 3) and previous studies conducted in Libya (Table 2) showed highest prevalence of A+ in Tripoli (29.3%) (Table 3), Almerghab (32.2%) [45], followed by Sabratha (29.3%) [42], Zawia (29%) [43], and Benghazi 28.85%) [50]; lowest prevalence of A+ was in Jadu (13.41%) (Table 3) and Aljufera (22.8%) [55]. While, highest A- was reported in Almerghab province (5.43%) [45], Jadu (8.74%) (Table 3); the lowest A- was in Sebha (1.4%) [54], followed by Zintan 2.32% (Table 3), and Derna (2.6%) [52]. The highest prevalence of B+ blood group was reported in Derna 22% [52], followed by Tripoli (19.73%) (Table 3), Albiyda (19.29) [51], Aljufera (17.4% [55], and Benghazi (16.62%) [50]. The lowest prevalence of B+ blood group was reported in Bani Waleed (11.3%) [47], followed by Alrujban (11.25%) (Table 3), Almerghab (13.03%) [45], and Jadu (13.11%) (Table 3). Whereas, the highest prevalence of B- blood group was reported in Jadu (9.47%) (Table 3) and Bani Waleed (6.4%) [47]; the lowest in Alrujban (0.41%) (Table 3) and Sebha (0.90%) [53]. For blood group O+, the highest prevalence was reported in Zintan (46.92) (Table 3) and Sebha (45.4%) [54]. The lowest prevalence of O+ blood group was reported in Jadu (16.03%) (Table 3) and Bani Waleed (33.4%) [47]. On the other hand, the highest prevalence of O- blood group was reported in Jadu (12.97%), followed by Bani Waleed (9.9%) [47], Sabratha (9.2%) [42], and Alrujban (8.75%) (Table 3). The highest prevalence of AB+ blood group was reported in Jadu (14.86%) (Table 3), followed by Albiyda (8.19%) [51], and Derna (7.3%) [52]; lowest prevalence of AB+ was in Alrujban (1.25%), and

Zintan (3.36%) (Table 3), Zawia (3.7%) [43]. The highest prevalence of AB- blood group was reported in Jadu (11.37%) (Table 3) and Derna (6%) [52]; lowest in Aljufera (0.40%) [55], Arujban (0.41%) (Table 3). The aforementioned statistics show noticeable regional differences in the distribution of ABO and Rh D blood group systems in different regions of Libya so far studied, which could be due to the combined effects of natural selection, migration, and marriage traditions. These differences emphasize the need for more studies, especially among other ethnic groups (e.g. Zuwarah, Tuareg, and Tubu), to explain the specific genetic factors responsible for these variations. Further studies are needed to study molecular genotyping of ABO blood group system (i.e. ABO subtypes: A1, A2, A3, Ax, Ai, B3) and other Rhesus (Rh) antigens (e.g. C, E, c, and e) and Kell blood group systems; such studies are important for patients needing multiple transfusion therapy (e.g. hemoglobinopathy patients) to reduce the hazards related to alloimmunization.

CONCLUSION

This is the first study that documents the gene frequency for the ABO and Rh D alleles and their prevalence in the populations of Jabal Nafusa north-western region of Libya. This study is of great value since it adds a more comprehensive view of the overall prevalence and geographical distribution of the ABO and Rh D blood groups in Libya. The results of this study show that the most prevalent ABO blood group was O followed by A, B, and AB (O > A > B > AB), the percentage of Rh D+ was higher than Rh d- as shown by other previous studies conducted in Libya. However, the presence of high Rh d- in Jadu appeared to be different from all other regions in Libya. The occurrence of considerable percentages of O- (12.97%) and AB- (11.37%) blood groups in Jadu city indicated significant medical implications for healthcare blood transfusion services and blood donation programs due to their rare presence and short supply. O negative blood is considered the best choice for blood transfusion therapy in emergency and trauma situations when patient's blood group is not known. Hence, the data in this study are vital for the management of blood banks and transfusion services (i.e. aid in the selection and supply of matched rare blood for patients needing blood transfusion). This study also provides useful information for genetic and anthropological studies in Libya.

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Conflicts of Interest

The authors declare no conflicts of interest.

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التكرار الجيني وانتشار أنظمة فصائل الدم ABO و Rh D بين المتبرعين والمرضى الليبيين في منطقة جبل نفوسة، ليبيا

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المستخلص

تتمتع أنظمة فصيلة الدم ABO و Rh D بأهمية وراثية و طبية كبيرة. في ليبيا، تم إجراء العديد من الدراسات حول توزيع أنظمة فصائل الدم ABO و Rh D في مناطق مختلفة من البلاد. لكن هناك مناطق كثيرة في ليبيا لم تتم دراستها بعد. لذلك، كان الهدف من هذه الدراسة هو تحديد تردد الجينات وانتشار أنظمة فصائل الدم ABO و Rh D في الزنتان والرجبان وجادو في منطقة جبل نفوسة شمال غرب ليبيا؛ بالإضافة إلى طرابلس. كانت هذه دراسة استرجاعية شملت فقط الليبيين الذين يترددون على بنك الدم المركزي في الزنتان، وعيادة الرجبان في الرجبان، ومختبر جادو للتحاليل الطبية في جادو، ومستشفى الخضراء في طرابلس. تم إجراء تصنيف ABO وتصنيف Rh بطريقة الشريحة القياسية باستخدام الأجسام المضادة التجارية anti-A و anti-B و anti-D. تم تحليل البيانات باستخدام الإحصاء الوصفي وقانون توازن هاردي-وينبرج. أظهرت النتائج الإجمالية لهذه الدراسة أن فصيلة الدم ABO الأكثر انتشاراً هي O (33.68%)، تليها A (26.27%)، B (21.31%)، و AB (18.74%)؛ والتي تتجمع في ثلاث فئات: O>A>B>AB (مجموعة الزنتان والرجبان)، A>O>B>AB (مجموعة طرابلس)، O>AB>B>A (مجموعة جادو). وقد وجد أعلى معدل انتشار لـ A+ (29.35%) و B+ (19.73%) و A- (8.74%) و B- (9.47%) في طرابلس وجادو على التوالي. سجلت جادو أعلى معدل انتشار لـ AB+ (14.86%) و AB- (11.37%). وقد لوحظت أعلى نسبة من O+ (46.92%) و Rh D+ (91.64%) و O- (12.97%) و Rh d- (42.56%) في الزنتان وجادو على التوالي. إن وجود نسب كبيرة من فصائل الدم السالبة O و AB في جادو يمكن أن يكون له آثار طبية كبيرة على خدمات نقل الدم في الرعاية الصحية. الترددات الجينية لـ ABO و Rh D للأليلات A و B و O و D و d- على التوالي هي: 0.1795 و 0.1025 و 0.7180 و 0.7109 و 0.2891 في الزنتان؛ 0.2013 و 0.0762 و 0.7225 و 0.5817 و 0.4183 في الرجبان؛ 0.1860 و 0.1892 و 0.6248 و 0.3476 و 0.6525 في جادو؛ 0.2693 و 0.1885 و 0.5422 و 0.5660 و 0.4340 في طرابلس. تم العثور على فرق كبير بين ترددات الأليلات في جادو، مما يشير إلى أن مدينة جادو ليست في حالة توازن هاردي-واينبرج. هذه هي الدراسة الأولى التي توثق تردد الجينات ABO و Rh D وانتشارها في منطقة جبل نفوسة شمال غرب ليبيا. وتعتبر البيانات الموجودة في هذه الدراسة ذات أهمية كبيرة لبنوك الدم وخدمات نقل الدم والدراسات الوراثية والأنثروبولوجية في ليبيا.

الكلمات الدالة: فصيلة الدم ABO، فصيلة الدم Rh D، تردد الجينات، تردد الأليلات، جبل نفوسة، ليبيا