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A Comparative Study of Prevalence, Phenotype and Genotype of ABO and Rh (D) Factor among Blood Donors from the Central Blood Bank in Benghazi, Libya

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Abstract: ***Background and Objective: Central Blood Bank of Benghazi where blood gathered as result of blood donation is stored and preserved for later use in blood transfusion as well as is the blood artery that covers the entire eastern region, down to the southeast and in some cases all over Libya, specifically: Sirte Cancer Hospital, Al - Maqrif Hospital in Ajdabiya, Ajdabiya Military Hospital, Al - Wehda Hospital, Derna, and Tobruk, and also Medical hospitals, Al - Bayda and Al - Kufra. This study was undertaken with the objective to provide data on the ABO / Rh (D) blood groups distribution and gene frequency among Blood Donors from the Central Blood Bank of Benghazi, Libya. Materials and Methods: The determination of ABO / Rh (D) blood groups was done according to the principle of sliding method. Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium and expressed as percentage. In addition allelic and genotypic frequency distributions of the blood group and Rh antigens were compared by using the Chi - square test as demonstrated in many projects which was published. Results: During study's the total number of male and female donors in Benghazi Blood Central was 80707 (Male = 7948, Female = 1159). The highest blood groups was mostly "O", then "A", "B" and the least one was "AB". Also the current studies of allele frequencies of blood groups revealed that the allele repeating for IO was the highest one, followed by the IA then the IB. While the homozygous type OO was the highest, then comes homozygous type AA and finally type BB. On contrary AO heterozygous type was the most common, then BO heterozygous type, final one heterozygous type AB. Conclusion: This study includes that blood type O is the most common, followed by group A, then group B, and finally group AB. The order of frequencies of ABO blood group alleles is IO > IA > IB. The study can be used as a basic database for genetic diversity and is considered as a reference for ABO studies in Benghazi and can be compared to other local and international studies of phenotypes, genotypes and equilibrium expectations for blood types.***

Keywords: Prevalence, Blood donors, allele, Phenotype, Genotype, Hardy–Weinberg equilibrium

1. Introduction

Blood is the most important body fluid, which is responsible for circulation of important nutrients, enzymes, and hormones all across the body, besides the most critical substance, oxygen. Blood is man's complete and unchangeable identity (Khan *et al.*, 2006). Organ transplantation, development of forensic medicine, genetic research and anthropology relies on the discovery of blood groups that are transmitted between different populations regardless of their ethnic origin (Story, 2003). In human beings, 19 blood groups systems with more than 200 antigens have been identified (Rehman et al., 2005) ABO blood type is an example of multiple allelism and the allele for A and B is co - dominant over O (Pasha and Kumar, 2009). 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). The ABO blood system is controlled by a gene encoding a glycosyl transferase that has three allelic forms: IA, IB and IO where the alleles IA and IB are codominant and both are dominant over IO (Almaguer and Betancourt, 2014) and four possible phenotypes: A, B, AB, and O (Choudhury et al., 2014). The discovery of Mendel's law of segregation and independent assortment has led to the foundation of population genetics. In 1908, British mathematician Godfrey H. Hardy and German physician Wilhelm Weinberg independently discovered the relationship between gene and genotype frequencies, known as the Hardy - Weinberg (HW) principle, or HW equilibrium. The most powerful research tool in both theoretical and applied research in population and quantitative genetics is the HW principle (Chen, 2010).

2. Materials and Methods

The study population included the blood donors in the Central Blood Bank Located in the Benghazi City, Libya. The study period was from January 2018 till December 2020. Blood testing for ABO, and Rh "D" typing was done over three years, for both genders. The determination of ABO and Rh "D" blood group was done according to the principle of slide method (Sultana et al., 2013). A clean and dry glass slide was divided into three sections. The sections were labeled as anti - A, anti - B and anti - D to identify the antisera. Place one drop of donor blood in center of each section of slide, and then add one drop of anti - A, anti - B

and anti - D to each drop of blood and mixed by using a separate the aid of glass rods. Continuing mixing by tilting the slide for 60 sec to check for agglutination. Immediately the agglutination results were recording after mixing time was done. The agglutination in blood drop A was considered as group A, and agglutination in blood drop B as group B. The agglutination in both drops was considered as group AB, and if both blood drops were not agglutinated, it was considered as group O. The agglutination in rhesus blood drop was considered as rhesus positive and non - agglutination as rhesus negative. Allele frequencies were calculated under the assumption of Hardy–Weinberg equilibrium and expressed as percentages (Dabholkar, 1999). Statistical analysis was performed by using the SPSS software, Version 23. Chi - square test was used to compare observed allelic and genotypic frequency distributions of the blood group and Rh antigens to that expected under the Hardy–Weinberg (Iyiola, Igunnugbemi and Belloa, 2012).

3. Result

Table 1: Statistics of the Central Blood Bank of Benghazi for the year 2018

Blood Group	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
O+	745	572	741	724	679	560	785	651	699	705	633	685
O -	198	97	122	149	119	132	135	146	109	107	134	129
A+	698	541	635	578	558	608	675	542	546	569	550	503
A -	130	75	93	124	72	95	120	97	98	90	95	80
B+	388	310	372	331	324	320	421	412	360	330	336	279
B -	62	55	61	52	47	51	63	51	54	57	60	34
AB+	152	127	107	108	120	141	125	108	136	137	130	122
AB -	23	13	17	16	16	19	20	36	11	14	20	25
Total	2396	1790	2148	2082	1935	1926	2344	2043	2013	2009	1958	1857

Table 2: ABO and Rh blood group distribution rate for 2018 in the Central Blood Bank of Benghazi

	ABO blood group and Rh-D phenotypes										Total
2018	O+	O -	A +	A -	B+	B -	AB+	AB -	Rh+	Rh -	
Frequency	**8179**	**1577**	**7003**	**1169**	**4183**	**647**	**1513**	**230**	**20878**	**3623**	24501
Percentage	33.3823%	6.4364%	28.5720%	4.7712%	17.0727%	2.6407%	6.1752%	0.9387%	85.2128%	14.7871%	100%
	O = 39.8187 %		A = 33.34324%		B = 19.7134%		AB = 7.1139 %				

Table 3: The statistic of the Central Blood Bank of Benghazi at the year of 2019

Blood Group	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
O+	683	613	757	900	543	764	832	706	953	809	819	885
O -	124	96	126	160	112	163	128	106	186	167	93	160
A+	481	508	600	704	442	621	706	538	785	686	581	754
A -	112	79	82	103	68	105	117	100	111	99	102	132
B+	329	304	348	464	252	357	489	316	432	434	386	402
B -	47	49	44	72	48	87	49	44	79	68	57	62
AB+	123	131	107	152	94	155	187	126	157	145	134	184
AB -	9	14	9	16	11	32	32	23	23	22	27	30
Total	1908	1794	2073	2571	1570	2284	2549	1959	2726	2430	2199	2609

Table 4: The percentage of ABO and Rh blood groups distribution for 2019 in the Central Blood Bank of Benghazi

2019	ABO blood group and Rh-D phenotypes										Total
	O+	O -	A +	A -	B+	B -	AB+	AB -	Rh+	Rh -	
Frequency	9264	**1**621	**7**406	**1**210	4522	706	**1**695	**2**48	**22887**	**3**785	26672
Percentage	34.7330%	6.0775%	27.7669%	4.5365%	16.9541%	2.6469%	6.3544%	0.9298%	85.809%	14.19%	100%
	O = 40.8105 %		A = 32.3034%		B = 19.601%		AB = 7.2842%				

Table 5: The Central Blood Bank of Benghazi statistics for the year 2020

Blood Group	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
O+	941	857	749	745	805	1144	1004	834	723	827	863	881
O -	1164	125	138	136	169	207	129	149	135	125	132	172
A+	826	686	630	617	656	1107	724	677	628	684	649	637
A -	114	111	100	90	123	142	131	98	88	90	95	103
B+	442	396	386	345	338	611	407	388	396	450	399	351
B -	91	67	61	63	45	88	78	54	51	49	44	44
AB+	143	143	148	127	124	229	135	129	112	136	105	143
AB -	30	20	13	17	22	27	16	25	20	25	18	23
Total	2751	2405	2225	2140	2282	3555	2624	2354	2153	2386	2305	2354

Table 6: The distribution of ABO and Rh blood groups in the Central Blood Bank of Benghazi / the year 2020

2020	Rh-D phenotypes and ABO blood group										Total
	O+	O -	A +	A -	B+	B -	AB+	AB -	Rh+	Rh -	
Frequency	10373	1781	8521	1285	4909	735	1674	256	25477	4057	29534
Percentage	35.122%	6.03033%	28.85149%	4.3509%	16.6215%	2.4886%	5.6680%	0.8667%	86.2632%	13.736%	100%
	O = 41.1523 %		A = 32.1178%		B = 19.1101%		AB = 6.534%				

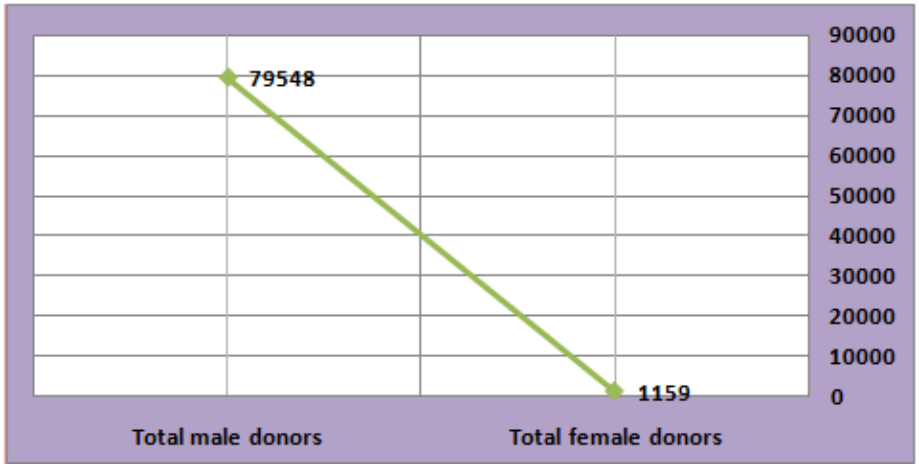

Figure 1: The total number of male and female blood donors in the Central Blood Bank of Benghazi during period of study

The figure shows that there were more male individuals blood donors than females. The total number of donors the year 2018 reached a total of 24.501 donors, including 24.234 males and 267 females. While the number of donors in 2019 have went up to 26.672 donors, (26.217 males, and 455 females). But in 2020 rise to 29.534, of who 29.079 are males and 437 are females.

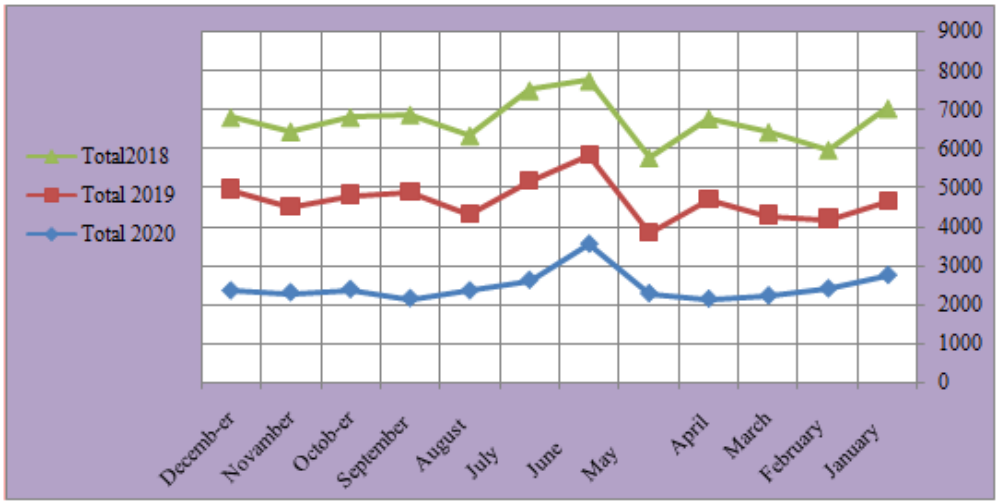

Figure 2: The total number of blood donors in the Central Blood Bank of Benghazi during period study months 2018, 2019 and 2020

The graph demonstrates that in the year 2018, the highest number of blood donors in the Central Blood of Benghazi was in January and lowest in February. In 2019, the largest number of blood donors was in September and lowest was in May. While during 2020, the most donors were in June and least was in April.

Table 7: Gene frequencies of ABO and Rh blood group alleles for the year 2018 among blood donors of the Central Blood Bank in Benghazi

	Number blood donors	Genotype	Genotype frequency	Phenotype	Allele	Allele Frequency
ABO	8172	IA/IA, IA/IO	0.05035003634 , 0.2831873702	A	IA	0.2243881377
	4830	IB/IB, IB/IO	0.020906444262, 0.1824794893	B	IB	0.1445906035
	1743	IA/IB	0.06488883229	AB		
	9756	IO/IO	0.3981878291	O	IO	0.6310212588
Rh	20878	DD,	0.3787902052	Rh+	D	0.6154593449
		Dd	0.4733382794			
	3623	dd	0.1478715154	Rh -	d	0.3845406551

Table 8: Gene frequencies of ABO and Rh blood group alleles for the year 2019 among blood donors of the Central Blood Bank in Benghazi

	Number blood donors	Genotype	Genotype frequency	Phenotype	Allele	Allele Frequency
ABO	8616	IA/IA, IA/IO	0.04675815517, 0.2762772376	A	IA	0.216236341
	5228	IB /IB , IB/IO	0.02100528683, 0.1851743075	B	IB	0.1449320076
	1943	IA/IB	0.06267829867	AB		
	10885	IO/IO	0.4081058708	O	IO	0.6388316514
Rh	22887	DD	0.3884925848	Rh+	D	0.6232917333
		Dd	0.4688184348			
	3785	dd	0.149091182	Rh -	d	0.3767082667

Table 9: Gene frequencies of ABO and Rh blood group alleles for the year 2020 among blood donors of the Central Blood Bank in Benghazi

	Number blood donors	Genotype	Genotype frequency	Phenotype	Allele	Allele Frequency
ABO	9806	IA/IA, IA/IO	0.04874851254, 0.2832755952	A	IA	0.2207906532
	5644	IB /IB , IB/IO	0.01896312377, 0.1766783831	B	IB	0.1377066584
	1930	IA/IB	0.06080868612	AB		
	12154	IO/IO	0.4115256992	O	IO	0.6415026884
Rh	25477	DD	0.396105738	Rh+	D	0.6293693182
		Dd	0.466527154			
	4057	dd	0.1373671023	Rh -	d	0.3706306818

Table 10: Observed and expected frequencies of ABO blood group distribution among blood donors of the Central Blood Bank in Benghazi

ABO blood group	**2018**	**2019**	**2020**
Allelic frequencies			
A	0.2243881377	0.216236341	0.2207906532
B	0.1445906035	0.1449320076	0.1377066584
O	0.6310212588	0.6388316514	0.6415026884
Observed values			
A	8172	8616	9806
B	4880	5228	5644
AB	1743	1943	1930
O	9756	10885	12145
Excepted values			
A	8085	8615	9480
B	4949	5227	5611
AB	1470	1867	1920
O	9555	10882	12109
The calculated χ^2 value	$\chi^2 = 58$	$\chi 2 = 3.09$	$\chi^2 = 11.5$

Table 11: Observed and expected frequencies of Rh blood group distribution among blood donors of the Central Blood Bank in Benghazi

Rh blood group	2018	2019	2020
Allelic frequencies			
D	0.6154593449	0.6232917333	0.6293693182
d	0.3845406551	0.3767082667	0.3706306818
Observed values			
Rh+	20878	22887	25477
Rh -	3623	3785	4057
Excepted values			
Rh+	15079.38	16624.437	18587.7
Rh -	9421.63	10047.5	10946.20
The calculated χ^2 value	$\chi^2 = 5798.5$	$\chi^2 = 6262.5$	$\chi^2 = 6889.2$

4. Discussion

There are differences in the distribution of AB and Rh (D) blood groups between different populations. It was noted during the three years study that blood type O is the most common among the donors of the Central Blood Bank of Benghazi. This result is consistent with what researchers have found in other studies at the local and global levels.

The overall phenotypic frequencies of ABO blood groups were O>A>B>AB (Sakal et al., 2019)., (Ameigaal and Ageel, 2019)., (Asteal, 2021)., (Al - Noaemi and Daghriri.,

2018)., (El - Moghrabi et al., 2020)., (Matough et al., 2019)., (Enosolease and Bazuaye, 2008)., (Joshi *et al.*, 2020)., (Kemishi, Nasef and Alkimeeshi, 2020) and (El - Moghrabi et al., 2021). In contrast, blood group B is the commonest (Chishti et al., 2012), (Butt et al., 2016) (Rehman & Shi, 2021) another study, blood group A is also the commonest (Ullah & Ahmad, 2015), (Hanania, Hassawi and Ihaidrs, 2007).

The Rh blood group is name for the rhesus monkey. This group is determined by genes called (D) which has two alleles D and d. Additional other alleles a person may have anyone with genotype DD or Dd, has D antigens on his or her RBCs and is classified as Rh - positive Rh+ (Saladin, 2003). The results showed that the frequency of D allele responsible for the Rh+ phenotype was dominant over the d allele responsible for the Rh - phenotype in the all years of this study, as is the case in the predominance of the Rh^{+} phenotype over the Rh - phenotype, and this explains the role of the genotype in the manifestation of the phenotype (Table 7, Table 8 & Table 9). Rh (+ve) remains higher than Rh (- ve) worldwide. The frequencies of IA, IB, and IO alleles were calculated using the Hardy - Weinberg law of equilibrium. The calculated gene frequencies for blood donors during year 2018 are (0.2243881377) for IA (p), (0.1449320076) for IB (q), and (0.6388316514) for IO (r). In the year 2018, O (r) re - cords the highest value, followed by A (p) and B (q), (O > A > B).

The homozygous types were as follow:
OO (0.3981878291), AA (0.05035003634) and BB (0.020906444262). The heterozygous types were AO (0.2831873702), BO (0.1824794893%) and AB (0.06488883229) (Table 7). The calculated gene frequencies for blood donors during year 2019 were (0.216236341) for IA (p), (0.1449320076) for IB (q), and (0.6310212588) for IO (r). Also in the year 2019, O (r) re - cords the highest value, followed by A (p) and B (q), (O> A > B).

The homozygous types were as follows: OO (0.4081058708), AA (0.04675815517) and BB (0.02100528683) (Table 8). The heterozygous types were AO (0.2762772376), BO (0.1851743075) and AB (0.06267829867). The calculated gene frequencies for blood donors during 2020 are (0.2207906532) for IA (p), (0.1377066584) for IB (q), and (0.6415026884) for IO (r). Likewise in the year 2020, O (r) re - cords the highest value, followed by A (p) and B (q), (O> A > B). The homozygous types were as follows: OO (0.4115256992), AA (0.04874851254) and BB (0.01896312377). The heterozygous types were AO (0.2832755952, BO (0.1766783831) and AB (0.06080868612) (Table 9). The estimated frequency of ABO blood groups in the world is O (40 - 45%), A (35 - 40%), B (4 - 11 %) and AB (0 - 2%) (Adamo, 2002), the high frequency of blood type O is due to the fact that many people have heterozygous genotype A or B, and have the O allele at the same time as gene A or B, through in this study, AO, BO genotypes represented high values over the years 2018, 2019 and 2020. Note Tables (7, 8 and 9).

The chi - square was used to test whether the distribution of blood groups agrees during the years of study, or is there a discrepancy in the distribution. The comparison was made between (2018 and 2019), (2019 and 2020) and (2018 and 2020).

Statistical hypotheses (2018 and 2019):

H0: The distribution of blood groups in the Central Blood Bank for the year 2018 is consistent with the distribution of blood groups in the year 2019.

H1: The distribution of blood groups in the Central Blood Bank for 2018 does not agree with the distribution of blood groups in 2019 in the same place. When the chi - square table is used to set the tabular value df = 3, α = 0.05, its value is X2 = (7.815). The calculations of chi value X2 = (13). It appears from the outputs that the calculated chi value X2 = (13) is greater than the tabular chi value X2 = (7.815). This value X2 = (13) means that is statistically significant, meaning there are statistically significant differences between the two groups, depends on this results we will reject the null hypothesis and therefore the blood type distribution in 2018 and 2019 is different.

Statistical hypotheses (2019 and 2020):
H0: The blood groups distribution rate for 2019 in the Central Blood Bank coordinated with 2020.
H1: In 2019, the blood groups distribution in the Central Blood Bank does not agree with the distribution of blood groups for the year 2020. The calculated chi value X2 = (4.4). These results show that the calculated chi value X2 = (4.4) is less than the tabular chi value X2 = (7.815). This value X2 = (4.4) means that is no statistically significant, consequently there are not statistically significant differences between the two groups. Based on these results we will reject the alternative hypothesis and therefore the blood type distribution in 2019 and 2020 is the same.

Statistical hypotheses (2018 and 2020):
H0: The distribution of blood groups in the Central Blood Bank for 2018 and 2020 is consistent.
H1: The distribution of blood groups in the Central Blood Bank for the year 2018 does not agree with the distribution of blood groups in the Central Blood Bank for the year 2020 .
When the chi - square table is used to set the tabular value df = 3, α = 0.05, its value is X2 = (7.815). The calculated chi value X2 = (1124). These data clearly show that the calculated chi value X2 = (1124) is greater than the tabular chi value X2 = (7.815).
This value X2 = (1124), means that is statistically significant, meaning that there are statistically significant differences between the two groups we will reject the null hypothesis and therefore the blood type distribution in 2018 and 2020 is different.

Given (Table 10), chi - square of Goodness - of – fit were using for equilibrium predictions for blood groups at the degree of freedom = 3, significance level 0.05, and the results were as follows:

The year 2018 is unbalanced as the calculated chi value X2 = (58) is greater than the scheduled chi value X2 = (7.815).

For 2019 equilibrium forecasts that were balanced, the calculated chi value X2 = (3.09) was lower than the scheduled chi value X2 = (7.815).

2020 is unbalanced as the calculated chi value X2 = (11.55) is greater than the scheduled chi value X2 = (7.815).

Given (Table 11), the equilibrium predictions were by using chi - square Goodness - of - fit test blood Rh at a degree of freedom = 1, a significance level of 0.05.

The year 2018 is unbalanced as the calculated chi value X2 = (5798.5) is greater than the scheduled chi value X2 = (3.84). The year 2019 is unbalanced with the calculated chi value X2 = (6262.5) less than the tabulated chi value X2 = (3.84). The year 2020 is unbalanced because the calculated chi value X2 = (6889.2) is greater than the scheduled value chi X2 = (3.84).

5. Conclusion

The study of human genetics is one of the most important topics of recent studies and research. This research included an inventory of blood donor numbers, frequency of alleles, and genotypes of ABO and Rh blood groups for the Benghazi Central Blood Bank for three years, from January 2018 to December 2020.

In general, there are three different alleles called I^A, I^B, and i which are used to determine human blood type. Where the I^A and I^Balleles are codominant, while the i allele is recessive.

All studies clearly show that the overall phenotypic frequencies of ABO blood groups were O>A>B>AB.

Also, calculation of allele frequencies for blood groups revealed that the most frequent allele for the highest IO allele was, followed by the IA allele, and then the IB allele.

Interestingly, types A, B, AB and O are specific to human blood phenotypes. Accordingly, individuals with type A and type B can be either homozygous (I^AI^A or I^BI^B), or heterozygous (I^Ai or I^Bi).

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