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The frequency of ABO and Rhesus blood groups phenotypes, genotypes from Sebha city of Libya

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Abstract The frequency of ABO and Rhesus blood groups vary geographically, ethnically and from one population to another. Some variations may even occur within one ethnic group and within one small country. This study was conducted to determine the frequency of ABO and Rhesus blood groups phenotypes, genotypes and alleles in Sebha city.A total of 527 samples were randomly collected and tested for ABO and Rhesus blood groups antigens using open slide methods. The frequencies of ABO and Rhesus blood groups phenotypes were expressed in percentages and the Hardy-Weinberg Law was used to determine allele and genotype frequencies.Results: Blood group (O) is the most frequented group (42%) of the totally, followed by group (A) (28%) and group B (23%). Least prevalent blood group was AB (7%). Rh D positive prevalence was 81.7% and 18.2% persons were Rh negative. The allele frequencies of IO, IA, and IB in the total sample were found to be, 0.649, 0.194, and 0.160 respectively. The order of ABO blood group allele frequencies was IO > IA > IB in the overall samples. ConclusionIn present study blood group O was the commonest followed by group A. the order of the frequencies of ABO blood group alleles is IO > IA > IB. Therefore, the findings of this study can be used as a baseline for the genetic diversity of the study area as a reference for Libyan ABO blood group phenotypes and genotypes studies.
Key Words:ABO blood-group system, Alleles frequencies, GenotypeFrequencies.

دراسة توزيع فصائل الدم و العامل ريسس وتكرار الطراز الجيني و الاليلات المحددة لنظام ABO في مدينة سبها

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الملخص يعتبر نظام فصائل الدم ABO من اهم أنظمة فصائل الدم ذات الاهمية السريرية في نقل الدم ، زراعة الأعضاء، فقر الدم الانحلالي الذاتي، والأثار المترتبة علي عدم التوافق بين فصيلة دم الم والجنين. تقع اليلات نظام فصائل الدم ABO علي الكروموسوم 9 وحتي الأن يوجد أكثر من 200 اليل تم التعرف عليها باستخدام فحوصات البيولوجيا الجزيئية. اجريت هذه الدراسة لتحديد تكرار النمط المظهري لفصائل الدم لنظام ABO والعامل ريسيس وكذلك معرفة التركيب الوراثي والتكرار الجيني في مناطق مختلفة في مدينة سبها اشتملت الدراسة علي 527 عينة عشوائية تم اختيارها من مناطق مختلفة في مدينة سبها. تم استخدام طريقة الشريحة الزجاجية وتم الإشارة الي تكرار النمط المظهري لكلا من نظام فصائل الدم ABO والعامل الريزوسي كنسبة مئوية، كما تم استخدام مبدأ هاردي واينبرج لتحديد التكرار الجيني والتركيب الوراثي من خلال نتائج هذه الدراسة تبين بأن فصيلة الدم O هي اكثر فصائل الدم تكرارا (42%) من أجمالي عدد العينات قيد الدراسة، يليها فصيلة الدم A (28%). في حين لوحظ بأن النمط المظهري للعامل ريسيس Rh+ (81.7%) كان أكثر تكرار بينما النمط المظهري لـــ Rh- كان أقل تكرار (18.2%). وكذلك أوضحت نتائج هذه الدراسة بأن التكرار الجيني لنظام فصائل الدم IO , IA, IB كان 0.649، 0.194 ، 0.160 علي التوالي وبالتالي فان ترتيب التكرار الجيني لنظام فصائل الدم كان كالتالي IO> IA>IB من أجمالي العينات المدروسة . بالإضافة الي ذلك فقد تبين عدم وجود فروق معنوية بين كلا من تكرار النمط المظهري والتكرار الجيني المتوقع .في الخلاصة تبين بأن فصيلة الدم O هي اكثر الفصائل تكرارا تليها الفصيلة B، وأن نتائج هذه الدراسة يمكن ان تستخدم كقاعدة بيانات اساسية للتنوع الوراثي في منطقة الدراسة وكذلك مرجع مهم لدراسة النمط المظهري والطراز الجيني لفصائل الدم في ليبيا.

الكلمات المفتاحية: نظام فصائل الدم، وتكرار الطراز الجيني، النمط المظهري لفصائل الدم.

Introduction

Variation in distribution of ABO and Rhesus phenotype between ethnic and geographic population is a well-documented fact. Blood groups are genetically determined and exhibit polymorphism in different populations[1].

At present, the International Society of Blood Transfusion (ISBT)[2] approves as 29 human blood group systems (Table I). The ABO blood group system consists of four antigens (A, B, O and AB). These antigens are known as oligosaccharide antigens, and widely expressed on the membranes of red cell and tissue cells as well as, in the saliva and body fluid.

Table I. ISBT Human Blood Group Systems

ISBT No	System name	ISBT Symbol	Locus	ISBT No	System name	ISBT Symbol	Locus	ISBT No	System name	ISBT Symbol	Locus
001	ABO	ABO	9	011	Yt	YT	7	021	Cromer	CROM	1
002	MNS	MNS	4	012	Xg	XG	X	022	Knops	KN	1
003	P	PI	22	013	Scianna	SC	1	023	Indian	IN	11
004	RH	RH	1	014	Dombrock	DO	12	024	OK	OK	19
005	Lutheran	LU	19	015	Colton	CO	7	025	Raph	RAPH	11
006	Kell	KEL	7	016	Landsteiner Wiener	LW	19	026	John Milton Hagen	JMH	15
007	Lewis	LE	19	017	Chido/Rodger	CH/RG	6	027	I	I	6
008	Duffy	FY	1	018	H	H	19	028	Globoside	GLOB	3
009	Kidd	JK	18	019	Kx	XK	X	029	Gill	GIL	9
010	Diego	DI	17	020	Gerbich	GE	2				

ABO system consists of four main groups, A, B, AB and O which is determined on the basis of presence or absence of A and B antigens. These antigens are under control of three allelic genes, namely I*A*, I*B* and IO which determine blood groups. I*A* produces A antigen, I*B* produces B antigen whereas IO produces neitherI*A* and I*B* are mutant alleles and show co dominances with each otherbutboth are dominant over the wild type allele IO[3]. The three alleles canproduce six genotypes and four phenotypes of blood groups[4] which are:

Phenotype	Genotype
O	IO/IO
A	IA/IA, IA/IO
B	IB/IB, IB/IO
AB	IA/IB

The study of blood grouping is very important as it plays an important role in genetics, bloodtransfusion, forensic study, blood bank, organ transplantation and paternity test, and some groups may have association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh incompatibility and ABO incompatibility of newborn[5].

The frequency of ABO and Rhesus blood groups vary geographically, ethnically and from one population to another. Some variations may even occur within one ethnic group and within one small country. Genetic studies on the Sebha population are limited and generally restricted to analysis of classical markers due to Libya's modern political instability, thus this study was conducted to determine the frequency of ABO and Rhesus blood groups phenotypes, genotypes and alleles among different area of Sebha city.

Material & Methods

This cross-sectional study was performed over a period from December 2015 to March 2016, in theblood bank of Sebhamedical center.It included n= 527both males and females.The study protocol was approved by the local EthicsCommittee ofSebha University.

Determination of blood group

Blood grouping were done using open slide methods where a drop of blood sample from a sterile finger pricks were placed in three different places on clean glass slide followed by a drop of blood grouping reagents, anti-A, anti- B and anti-D. The reagents and the blood were mixed using clean stick, spread by moving gently the test slide back and forth, and checked for agglutination within one minute.

Statistical Analysis:

Allele frequencies were calculated under 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 group antigens to that expected under the Hardy–Weinberg equation. P values <0.05 were considered statistically significant.

Results

In the present study 527 samples were taken, the study conducted over a period from December 2015 to March 2016in the blood bank of Sebhamedical center.Among general population of Sebha city blood group O showed highest prevalence with 222 (42.12%) persons. It was closely followed by group A with 149 (28.27%) persons. Group B and AB were in 120 (22.77%) and 36 (6.83%)persons respectively(Table II).Inclusive of all ABO blood groups, Rh D positive prevalence was 81.7% (431),as compared to Rh negative group which contributes only 18.2% (96) of the study subjects.

Table II: distribution of ABO and Rh phenotype among blood donors of Sebha city

Blood Group	Rh + ve	Prevalence (%)	Rh-ve	Prevalence (%)	Total (No)	Prevalence (%)
A	123	23.3%	26	4.93%	149	28.27%

B	88	16.69%	32	6.07%	120	22.77%
O	189	35.86%	33	6.26%	222	42.12%
AB	31	5.88%	5	0.94%	36	6.83%
TOTAL	431	81.7%	96	18.2%	527	99.99

Based on the Hardy-Weinberg principle at equilibrium the frequencies of the genotypes become p2 + 2pq + q2 =1, which is the square of the allelic frequencies (p + q) ^{2}. This is a simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are inherited two at a time into a diploid zygote. The three alleles of ABO blood group which are IA, IB and IO are represented as p, q and r, respectively in which:
p is the frequency of allele A,
q is the frequency of allele B and
r is the frequency of allele O.
Therefore the genotypic frequencies were represented by trinomial expansion as (P+q+r) ^{2} = p2 + 2pq + q2 + 2pr + 2qr + r2=1 (TableIII)[6], where:
P2 is the frequency of genotype I^AI^A
q2 is the frequency of genotype I^BI^B
2pq is frequency of genotype I^AI^B
2pr is frequency of genotype I^AI^O
2qr is the frequency of genotype I^BI^O
r2 is the frequency of genotype I^OI^O

Table III. Represent phenotype and genotype frequency of ABO groups

O R	B Q	A P	Frequency	
AO Pr	AB Pq	AA P^2	A	p
BO Qr	BB q^2	AB Qp	B	q
OO r^2	BO Rq	AO Rp	O	r
AB 2pq	OO r^2	BB/BO q^2 + 2qr	AA/AO P^2 + 2pr	

Table IV. Phenotype, genotype frequency, donor phenotype number among blood donors of Sebha city

Expected Frequency	Observed frequency	Donor phenotype Number	Genotype frequency	Genotype	Phenotype
p= 0.194	0.2827	149	P^2 + 2pr	IA/IA,IA/IO	A
q= 0.160	0.2277	120	q^2 + 2qr	IB/IB, IB/IO	B
0.0625	0.0683	36	2pq	IA/IB	AB
r = 0.649	0.4213	222	r^2	IO/IO	O
	1	527	(p+q+r)2		

Observed frequency of blood groups was calculated as follow:

Observed frequency of blood group A = = 149\5270.2827

Observed frequency of blood group B = \527 = 1200.2277

Observed frequency of blood group O = 527= 222\0.4213

Observed frequency of blood group AB = 36\527=0.0683

Calculations of ABO allele frequency were as follow:
For A allele frequency (p),
p = 1 -√B+O
$P = 1 - \sqrt{0.2277 + 0.4213} = 0.194$
For B allele frequency (q)
q = 1 - √A+O
$q = 1 - \sqrt{0.2827 + 0.4213} = 0.160$
For O allele frequency
r = √O
$r = \sqrt{0.4213} = 0.649$
For AB allele frequency
$2pq = 2 \times 0.194 \times 0.160 = 0.0625$
The calculated gene frequencies are 0.194 for IA (P), 0.160 for IB (q) and 0.649 for IO. In population from Sebha city O (r) recorded the maximum frequency followed by A (p), and B (q) (Table IV).
Frequencies of RhD blood group alleles D and d are represented as p and q respectively in which p is frequency of allele D and q is frequency of allele d (Table V) . Using Hardy- Weinberg equation, at equilibrium the frequencies of the genotype were represented as (p + q) ^{2} = p^2 + 2pq + q^2 =1, where p^2 is frequency of genotype DD, 2pq is frequency of genotype Dd and q^2 is frequency of genotype dd[7].

Table V.Allele frequencies of RhD blood group phenotypes among blood donor of Sebha city

Expected frequency	Observed frequency	Prevalence (%)	Donor phenotype Number	Genotype frequency	Genotype	Rhesus
0.5733	0.8178	81.7%	431	P=1-q	DD	Rh^+
0.4267	0.1821	18.2%	96	q=√p	Dd	Rh^-
1	1		527			Total

Calculation of D allele frequency I^D(p)
p +q = 1
p= 1- q
$p = 1 - 0.4267 = 0.5733$

Calculation of d allele frequency I^d(q)
q^2= frequency of the d phenotype
q=√ Rh^-
$q = \sqrt{0.1821} = 0.4267$

Thus, based on Hardy- Weinberg law the frequencies of the Rh genotype were calculated as:

$$(p+q)2 = p2 + 2pq + q2 = 1$$

$$(0.5733 + 0.4267)^2$$

$$= (0.5733)^2 + 2(0.5733 \times 0.4367) + (0.4267)^2$$

$$0.182 + 0.50 + 0.32 = 1$$

The Chi Square test for Goodness to fit between the observed and expected phenotype in case of ABO blood group was .0077 and the result is not significant at p=>0.05

$$Chi - square(x^2) = \sum \frac{(\mathrm{Of} - \mathrm{Ef})^2}{\mathrm{Ef}}$$

X2 = chi- square
∑ = Sigma
Of = Observed frequency
Ef = Expected frequency
X2 calculated
X2 table

$$X2 = \frac{(\mathrm{O} - \mathrm{E})^2}{\mathrm{E}} + \frac{(\mathrm{O1} - \mathrm{E})^2}{\mathrm{E}} + \frac{(\mathrm{O2} - \mathrm{E})^2}{\mathrm{E}} + \frac{(\mathrm{O3} - \mathrm{E})^2}{\mathrm{E}}$$

$$X2 = \frac{(0.2827 - 0.1944)^2}{0.1944} + \frac{(0.2277 - 0.1609)^2}{0.1609} + \frac{(0.4213 - 0.6490)^2}{0.6490} + \frac{(0.0683 - 0.0625)^2}{0.0625}$$

X^2 cal= 0.1465

X^2 tableα (0.01), df (1) = 6.635

According to our results it was found thatthe value of X^2 calculated is less than X^2 table thus we can conclude that there were no significant difference between observed and expected frequency. This provides that the ABO genotypes of the randomly collected samples were in good agreement with molecular analysis data.

Also the population was at Hardy- Weinberg genetic equilibrium, these data also indicated that Hardy-Weinberg Law can be used to reflect the percentages of the major blood groups in any population and can be used to compare data among different populations.

Discussion

Frequency of ABO and Rh blood groups vary worldwide and are not found in equal numbers even among different ethnic groups. As ABO blood group system is of autosomal inheritance controlled by a single gene at chromosome 9q34 [8], thus the frequency of blood groups is not different in both sexes, so we have not divided study group on the basis of male and female. In addition, individual has the same blood group throughout his life; therefore no categorization of donors according to age was done.

Among general population of Sebha city Blood group O showed highest prevalence with 222 (42.12%) persons. While bloodgroup AB was the least common blood group 36 (6.83%). Many other studies have shown that blood group O was the most common blood group and bloodgroup AB was the least common blood group in different populations and ethnic groups (Table VI) [9], [10], [11],[12], [13], [14], [15], [16].

However, the results of this study do not agree with the results from some Asian countries whereblood group B has the highest frequency in some and blood group A in the others. For example several previous studies [17], [18], [3], [19]reported high incidence of B blood group in Indianpopulation. In addition, other previous studies [20],[21]also reported high incidence of B blood group in Pakistan populations. Thehighest frequency of A blood group was documented in Jordan populations[6]and Palestine populations [22].

Among countries surrounding Libya, A group predominance has been reported from Egypt[23] and O group predominance from Morocco, Algeria and Tunisia[24](Table VI).

In this study, the frequencies were found to be in the order of IO> IA > IB and IO =0.649, IA = 0.194 and IB = 0.160 for ABO blood groups alleles and their genes, respectively. This is a common feature of Libyan population that is confirmed from previous studies.Other study[25] from Butajira, south Ethiopia, high allelic frequency of O blood group was reported which is in an agreement with our finding.

This distribution, and as shown in Table IV, is comparable to the distribution reported for populations from Nigeria [26] , Iraq, Jordan [27], [28]Kuwaiti [29], and Palestine[22]. Distribution of Rh phenotype in present study is shown in Table V. The frequency of allele D and d of RhD blood group were 0.57 and 0.42 respectively in the total sample. Previous study conducted by AlSuhaibani et al [30]reported that allele D was seen to be concentrated in the Domah region of Saudi Arabia. It was also observed to be more prevalent at the South-Eastern and South-Western tips of the Arabian Peninsula, i.e., Yemen and Oman, respectively. Allele d was observed to be most prevalent in Lebanon and Jordan.

The Hardy-Weinberg law states that both allele and genotype frequencies will remain constant from generation to generation in an infinitely large, interbreeding population in which mating is at random and there is no selection, migration and mutation. Under conditions of Hardy-Weinberg equilibrium expected genotype frequencies may be derived from population allele frequencies.

According to our results it was found that the value of X^2 calculated is less than X^2 table thus we can conclude that there were no significant difference between observed and expected frequency. This proved that the ABO genotypes of the randomly collected samples were in good agreement with molecular analysis data. The Chi Square test for Goodness to fit between the observed and expected phenotype data was non-significant difference. Also the population was at Hardy- Weinberg genetic equilibrium, these data also indicated that Hardy-Weinberg Law can be used to reflect the percentages of the major blood groups in any population and can be used to compare data among different populations.In conclusion, the present study reports the distribution of ABO phenotypes and genotypes in a group of individuals from Sebha city of Libya.

Blood group O dominated the study population followed by A, B and AB, respectively. In the RhD blood group system RhD positive blood group has the highest frequency while RhD negative blood the lowest frequency. The allele frequencies of A, B and O indicted O > A > B. No statistically significant differences were found between the frequencies of observed and expected genotypes .This proved that the ABO genotypes of the randomly collected samples were in Hardy-Weinberg equilibrium data. The distribution of the ABO genotypes in Sebha population is similar to that of many other populations.

Table VI. Distribution of ABO phonotype in different countries

Population	A	B	O	AB
Present study	28.27%	23%	42.12%	7%
Palestine [22]	36.3%	22.4%	33.8%	7.5%
Saudi Arabia [9]	30.8%	24.3%,	37.4%,	7.5%
Jourdan [6]	38.36%	18.04%	36.62%	6.98%
Iraq [10]	28.7%	13.8	49.9%	7.6%
Kuwait [29]	26.7%	24.1%	44.6%	4.6%
Ethiopia [12]	30.29%	22.76%	40.50%	6.45%
Nigeria [13]	22.77%,	20.64%	52.93%,	3.66%
Egypt [23]	36.9%	23.2%	30.7%	9.2%
Morocco [14]	31.47%	15.15%	49.01%	4.35%
Algeria [15]	39.28%	12.84 %	44.04 %	3.84 %
Tunisia [24]	37.64%	22.55%	59.27%	6.71%
India [1]	22.17%)	35.42%	33.55%	8.17%
African-Americans [12]	27%	20%	46%	7%
United State (Caucasians)[12]	41%	9%	47%	3%
Western Europeans [12]	42%	9%	46%	3%

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