Allele and Genotype Frequencies of the ABO Blood Group System in a Palestinian Population

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ABSTRACT— ***The ABO blood group system is one of the most important blood type in clinical practice. Currently, there is no information on the ABO genotype and allele frequency from Gaza strip. Data from this study will be useful for workers and the field and for future clinical applications of ABO genotyping in our area.***

This study was conducted in order to determine the major ABO alleles' and genotypes' frequencies in a Palestinian population residing in Gaza Strip.

A four separate–reaction multiplex allele specific polymerase chain reaction (AS-PCR) was used to determine the ABO genotypes of 201 unrelated subjects whose DNA, extracted from peripheral blood, was subjected to genotyping.

The study revealed the following genotypes: A_1A_1 (n=3), A_1O_1 (n=24), A_1O_2 (n=25), A_1A_2 (n=4), A_2A_2 (n=2), A_2O_1 (n=13), A_2O_2 (n=2), B_1B_1 (n=5), B_1O_1 (n=26), B_1O_2 (n=14), A_1B (n=11), A_2B (n=4) , O_1O_1 (n=31) , O_1O_2 (n=26) and O_2O_2 (n=11), from which the deduced phenotypes were: A (n=73), B (n=45), AB (n=15) and O (n=68). The frequencies of the A_1 , A_2 , B_1, O_1, O_2 alleles were: 0. 174, 0.067, 0.162, 0.376 and 0.221, respectively. There was no deviation between genotype-derived phenotypes results and serologically determined phenotypes. Additionally, the genotype distribution was in Hardy-Weinberg equilibrium.

ABO genotyping must be considered when determining ABO phenotypes, in blood transfusion, tissue/organ transplantation, blood typing discrepancies and forensic/paternity testing investigations.

Keywords— ABO alleles; ABO genotypes; AS-PCR; Palestine.

1. INTRODUCTION

In clinical practice, the ABO blood group system is one of the most important since the A and B epitopes may provoke a strong immune reaction. With the introduction of blood typing and cross-matching techniques, blood transfusion became not only a simple but also a much safer procedure. Furthermore, although ABO typing reduced the occurrence of transfusion reactions, they still occurred, indicating the presence of other genetic differences in blood groups of importance in transfusion medicine, as well as in the later emerging field of tissue/organ transplantation.

The human *ABO* locus is stretching over 18 kb of genomic DNA on chromosome 9q34.1-q34.2 [1-4] and consists of 7 exons, ranging in size from 28 to 688 base pairs (bp) and 6 introns with 554 to 12982 bp [5-7]. Exon 7 contains most of the largest coding sequence whereas, exon 6 contains the deletion found in most O alleles [8]. The frequency of the usual ABO phenotypes (A_1, A_2, B, A_1B, A_2B and O_1) varies between different populations [9]. The three major alleles (A_1, B, O_1) were first cloned and sequenced by Yamamoto et al [10].

Molecular genetic studies of the human ABO locus have demonstrated that ABO alleles have two critical single base substitutions in the last coding exon that result in amino acid substitutions responsible for the different donor nucleotide sugar substrate specificity between A- and B-transferases. Where A-transferase is α 1→3 N-acetyl-galactosaminyl transferase and the B-transferase is α 1→3 N-acetyl-galactosyl transferase [3, 4, 8,11].

Many mutations affecting the variety and specificity of the encoded glycosyl transferases have been identified. The nucleotide sequences of the A and B alleles are highly homologous (99%); the A^1 and B transferase alleles differ in seven base substitutions (at nucleotide positions 297, 526, 657, 703, 796, 803 and 930), but only four of these at positions : 526 (C to G: Arg → Gly), 703 (G to A: Gly → Ser), 796 (C to A: Leu → Met), and 803 (G to C: Gly → Ala) result in an amino acid change, while the substitutions 297, 657 and 930 are silent. The O^1 and A^1 transferase alleles are identical except for a single cytosine deletion at position 261 [3,11]. The loss of this single nucleotide results in a frame shift, producing a premature stop codon, leading to a truncated, enzymatically inactive protein of 115 amino acids [12].

A second O allele (O^2) that lacks this deletion has been described, which has some of the B allele mutations and an additional mutation (G802A gly → arg), that presumably inactivates the enzyme [13,14]. The human blood group A^2 transferase encoded by the A^2 allele is characterized by a single base substitution at nucleotide 467 (C to T: Pro → Leu)

and a single cytosine deletion at position 1059. This deletion is thought to be critical and results in an additional domain at the carboxyl terminal of the mature protein [15].

The present study was carried out in order to determine the distribution of the major alleles and genotypes of the ABO locus in a group of Palestinians residing in Gaza strip. This is the first report on ABO genotyping in this population.

2. MATERIALS AND METHODS

A total of 201 unrelated subjects recruited from the Islamic University–Genetics Laboratory were included in this cross-sectional descriptive study. The study was approved by the local ethics committee and signed consent was obtained from all participants. ABO phenotyping of blood samples was carried by routine slide agglutination test using Anti-A and Anti-B reagents (Plasmatec, Monoclonal, UK). Genomic DNA was extracted from blood samples using Wizard DNA extraction kit (Promega, USA) following the manufacturer's instructions. PCR was performed using the primers described by Sung et al. (2009); [16]. Four allele specific PCR reactions were carried out for each sample. Primer nucleotide sequences and the primer combination for each reaction are listed in Table 1. For each reaction, 5 µl PCR master mix (Promega, USA), 2 µl deionized water, 1 µl (100 ng) DNA template and 0.5 µl of each allele specific primers (5 pmol) in one micro-tube were mixed. PCR was performed in a thermal cycler (Biometra, Germany). The cycling conditions were: an initial denaturation for 3 min at 95°C, followed by 35 cycles of 40s at 95°C, 40s at 58.5°C, 40s at 72°C and an additional 5 min at 72°C for final extension. Upon completion of PCR, the products were analyzed by electrophoresis on 2% ethidium bromide stained agarose gel.

Data analysis

The PCR product size was estimated by comparing it with DNA molecular size marker (50 bp ladder DNA) run on the same gel. Results were interpreted and the ABO genotypes were determined according to the collective pattern of PCR products obtained from the four reactions of each sample as illustrated in Table-2. Deviation of observed ABO genotype frequencies from Hardy-Weinberg equilibrium was investigated using Chi square test.

3. RESULTS

Serological ABO typing of blood samples

The slide agglutination test using A and B antisera showed that the ABO types of the 201 blood samples were distributed as: 73 (36.3%), 68 (33.8%), 45 (22.4%) and 15 (7.5%) for groups A, O, B, and AB, respectively.

ABO genotypes in the study population

Random mating with the six different alleles at the ABO locus, can produce 21 possible genotype combinations. Only 15 different genotype combinations were detected in the study samples since the cis-AB allele was not encountered in the study samples. The type and frequency of the recognized ABO genotypes is shown in Table 3.

Phenotypes of blood samples deduced fro genotypes

The distribution of phenotypes deduced from genotyping results were fully concurdant with those obtained by serological typing.

Allele frequencies

ABO allele frequencies were calculated from the genotype frequencies shown in Table 3 by counting method. For example, the frequency of A1 was calculated as:

3x2 + 24x1 + 25x1 + 4x1 + 11x1 / 201x2 = 0.174. The frequencies of the other alleles A_2 , B_1, O_1, O_2 alleles were: 0.067, 0.162, 0.376 and 0.221, respectively. The overall frequencies for the parent I^A, I^B, and I^O alleles would be 0.241, 0.162 and 0.597, respectively.

Hardy-Weinberg equilibrium of observed genotpyes

The calculated allele frequencies were used to compute the expected genotype frequencies and the results showed that the observed genotype numbers did not differ significantly from those expected under the assumption of Hardy-Weinberg equilibrium (Table 4).

4. DISCUSSION

Since the first delineation of the molecular basis of the ABO blood group by Yamamoto et al. [11, 17], it has become possible to determine the ABO genotypes using molecular methods without the need for family investigations. ABO genotyping is commonly used in cases of an ABO discrepancy between cell typing and serum typing, as well as in forensic practices for personal identification and paternity testing [16].

Multiplex allele-specific PCR (the technique we used here) has advantages over PCR-RFLP in terms of cost effectiveness, reaction time and simplicity of handling. It is a rapid method that is based on detecting four single nucleotide polymorphisms at nucleotides 261, 297, 796, and 803 of the ABO locus. One major drawback of this

technique, however, is the preferential amplification of one target sequence over another that can in turn lead to bias in template-to-product ratio. This phenomenon can be monitored by including the right concentration of a suitable internal control in each reaction mixture.

The frequency of the alleles in our study population were 0.597 for the I^O, 0.241 for the I^A allele and 0.162 for the I^B allele. This distribution, and as shown in Table 5, is comparable to the distribution reported for populations from Iran, Iraq and Jordan [18-20]. This result may imply that those populations descended from common ancestors or founders with comparable ABO alleles' frequencies. Moreover, the trend $I^O > I^A > I^B$ observed in the present study is consistent with that reported in many world populations [21]. Apart from Kuwait and Bahrain (which showed $I^O > I^B > I^A$, a trend that also could be due to the genotypes of founders of those populations) all the populations depicted in Table 5 presented this trend. Still all the populations have the I^O allele as the predominant one.

According to our results it was found that the highest frequency of A phenotype (64/73,87.67%) had the *AO* genotype, while (9/73,12.33%) were A phenotype homozygotes (*AA*). Forty samples with phenotype B were recognized as heterozygous (*BO*) and 5 samples with phenotype B recognized as homozygous (*BB*). On the other hand, (68/201, 33.8%) were homozygous (*OO*) and 15 (7.46%) samples were *AB* heterozygotes. The high heterozygosity observed in this study is mainly due to the high frequency of the I^O allele (0.597) in our population as compared to that of I^A (0.241) and I^B (0.162) alleles. Additionally, the samples investigated here were collected from unrelated individuals and that allowed for the random assortment of the alleles according to their frequencies in the population. This is further confirmed by finding that the observed genotypes did not deviate from Hardy-Weinberg equilibrium.

Regarding the observed genotypes (Table 3), the highest frequency belonged to O_1O_1 genotype with a frequency of 15.4% (31/201) and the lowest frequency belonged to A_2A_2 and A_2O_2 genotypes with a frequency of 1.0% (2/201) each. In addition, 145 (72.14%) out of all the samples were heterozygous and 56 samples (27.86%) were homozygous.

In our population the calculated frequencies of the O_1 and O_2 alleles were 0.376 and 0.221, respectively. These values are consistent with other studies where they also reported higher prevalence of the O_1 allele [18,22,27].

The method employed here for blood group genotyping discriminates A_1, A_2, O_1, O_2, and *B* alleles. All expected alleles and allelic combinations were observed in this group except the *cis-*ABO_1 allele and its pertinent genotypes. The *cis-*ABO_1 allele was not observed in this study. It is a rare allele which was reported in Korea (0.0354%) among blood donors [23]. This allele also was not detected in the Kuwaiti or Iranian populations.

The present study determined, for the first time in Gaza strip, the frequency of the prevalent ABO alleles namely, A_1, A_2, B_1, O_1 and O_2 and their pertinent genotypes. It is valuable to have molecular genotyping ability of the ABO blood system in our area as it will provide a supplement to serological typing and should be useful in cases of typing discrepancies, tissue/organ transplntation and forensic investigations particularly when RBCs are not available.

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Table 1: Allele-specific PCR primers used for ABO genotyping

PCR reaction	Primer pair	Fragment size (bp)	Allele specificity
1	261G: 5′-GCAGTAGGAAGGATGTCCTCGTGTTG-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′ 467C: 5′-CCACTACTATGTCTTCACCGACCATCC-3′ 803G: 5′-CACCGACCCCCCGAAGATCC-3′	205	*A101, A102, B101, cis-ABO1*
		381	*A101, O01, O02*
2	297A: 5′-CCATTGTCTGGGAGGGCCCA-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′ 467C: 5′-CCACTACTATGTCTTCACCGACCATCC-3′ 803C: 5′-CACCGACCCCCCGAAGATCG-3′	164	*A101, A102, O01, cis-ABO1*
		381	*B101*
3	261A: 5′-GCAGTAGGAAGGATGTCCTCGTGTTA-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′ 467T: 5′-CCACTACTATGTCTTCACCGACCATCT-3′ 803G: 5′-CACCGACCCCCCGAAGATCC-3′	205	*O01, O02*
		381	*A102*
4	297G: 5′-CCATTGTCTGGGAGGGCCCG-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′ 467T: 5′-CCACTACTATGTCTTCACCGACCATCT-3′ 803C: 5′-CACCGACCCCCCGAAGATCG-3′	164	*B101, O02*
		381	*cis-ABO1*

Table-2. The interpretation table used for ABO genotyping

PCR fragments in bp in the four reaction tubes				ABO genotype
Reaction 1	Reaction 2	Reaction 3	Reaction 4	
381	164	205	-	O_1O_1
381	-	205	164	O_2O_2
205	381	-	164	B_1B_1
381	164	205	164	O_1O_2
205 + 381	164 + 381	205	164	B_1O_1
205 + 381	164 + 381	-	164	A_1B_1
205 + 381	164	381	-	A_1A_2
205 + 381	164	205 + 381	164	A_2O_2
205 + 381	164	205	-	A_1O_1
205 + 381	164	-	-	A_1A_1
205 + 381	164	205	164	A_1O_2
205 + 381	381	205	164	B_1O_2
205	164	381	-	A_2A_2
205 + 381	164	205 + 381	-	A_2O_1
205	164 + 381	381	164	A_2B_1

Table 3: The frequency of ABO genotypes in the study population

Genotype	Frequency	%
A_1A_1	3	1.5
A_1O_1	24	11.9
A_1O_2	25	12.4
A_1A_2	4	2.0
A_2A_2	2	1.0
A_2O_1	13	6.5
A_2O_2	2	1.0
B_1B_1	5	2.5
B_1O_1	26	12.9
B_1O_2	14	7.0
A_1B	11	5.5
A_2B	4	2.0
O_1O_1	31	15.4
O_1O_2	26	12.9
O_2O_2	11	5.5
Total	**201**	**100.0**

Table 4 : ABO observed genotypes as compared to expected genotypes

Genotype	Observed		Expected		p-value
	Number	%	Number	%	
AA	9	4.5	12.6	6.3	0.230
AO	64	31.8	58.3	29	0.476
BB	5	2.5	5.8	2.9	0.720
BO	40	19.9	39.6	19.7	0.950
AB	15	7.5	17.08	8.5	0.592
OO	68	33.8	67.6	33.6	0.961
Total	201	100	201	100	

Table 5: Frequency of ABO alleles in Gaza Strip as compared to other populations

Population	Allele frequencies			Reference
	I^A	I^B	I^O	
Gaza Strip	0.241	0.162	0.597	Current study
Kuwait	0.1338	0.1676	0.6986	[22]
Bahrain	0.141	0.157	0.704	[24]
Iraq	0.212	0.177	0.6611	[19]
Jordan	0.270	0.130	0.60	[20]
Saudi Arabia	0.1663	0.1197	0.714	[25]
Egypt	0.188	0.149	0.663	[26]
Sudan	0.192	0.140	0.668	[26]
Iran	0.23974	0.18147	0.57879	[18]