Allele Frequency and Molecular Genotypes of ABO Blood Group System in a Jordanian Population

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To determine the phenotypic, allelic frequencies and the genotypes of ABO blood groups in a Jordanian population, samples of 12215 randomly healthy Jordanian voluntary blood donors during the period 1998-2003 were taken from the National Blood Bank donor registry, Amman, Jordan. The results of the phenotypic distribution indicated that 4686 (38.36%) of the donors were type A, 4473 (36.62%) O, 2203 (18.04%) B and 853 (6.98%) AB. The gene frequencies were 0.6052 for F allele, 0.2607 for F allele and 0.1341 for F allele. Using PCR-RFLP technique, two separate segments of the transferase gene containing nucleotide 261 in exon 6 and nucleotide 703 in exon 7 of the ABO gene locus were amplified and their products were analyzed with two restriction enzymes (Kpnl and AluI). The electrophoresis patterns of 105 samples showed that ABO genotypes were AA: 6 (5.71%), AO: 35 (33.33%), BB: 1 (0.95%), BO: 14 (13.33%), AB: 10 (9.52%) and OO: 39 (37.14%).

Key words: ABO phenotypes, ABO genotypes, ABO gene frequency
INTRODUCTION

The blood group antigens are of clinical importance in blood transfusion, organ transplantation, autoimmune hemolytic anemia and fetomaternal blood group incompatibility. There is more than one systematic method for blood classification, one of these methods is the ABO system.

In human, the majority of cell types investigated have A, B, or O antigen on their surfaces. This includes some tissues like platelets (Brian et al., 2000), lung tissues (Clausen et al., 1990), intestinal mucosa (Takeya, 1990), mucous cells, epidermis, nervous receptors and vascular endothelium (Oriol et al., 1992).

The frequency of the usual ABO phenotypes (A, A, B, A, B, AB and O) varies between different populations (Mourant et al., 1976; Roychoudhuri and Nei, 1988). ABO allele frequency was determined in Asian, African and European population (Bandyopadhyay, 1994; Yip et al., 1995; Omotade et al., 1999; Varsahr et al., 2001; Al-Bustan et al., 2002).

Due to large number of alleles that could give similar phenotype but differ in genomic structure, there are several reasons for determining gene frequencies. These reasons include forensic medicine and paternity testing (Chester and Olsson, 2001; O'Keefe and Dobrovic, 1996; Ladd et al., 1996; Hashimoto and Nakamishi, 1993, Yang and Zhu, 1999, Sasaki et al., 1994) and linkage analysis (Yip et al., 1995).

Yamamoto et al. (1990a, b, 1992, 1993a-d) determined the eDNA sequences for human ABO blood group genes of three common alleles, A', B and O. Yamamoto et al. (1995) determined the genomic organization of the gene. The two major alleles, A' and B, differ in seven positions at nucleotide (nt) 297, 526, 657, 703, 796, 803 and 930. Four of these base substitution (nt) 526, 703, 796 and 803 result in amino acid substitutions residues 176, 235, 266 and 268 (Yamamoto et al., 1990a; Olsson and Chester, 1995).

There are three common O alleles. The O' allele differs from the A' allele by a single-base deletion at nt 261, which shifts the readings frame of the coding sequence and leads to the translation of enzymatically inactive protein (Yamamoto et al., 1990a). The O' variant (O') allele has not only the single-base deletion at nucleotide 261 but also another nine single-base substitutions when compared with the A' allele (Yamamoto et al., 1990a; Olsson and Chester, 1996). The O' allele does not possess the nucleotide 261 deletion found in O and O' alleles and differs from the A' allele in 4 nucleotide substitutions, 297, 526, 802 and 1096, resulting in two amino acid substitutions (Yamamoto et al., 1993d).

Restriction Fragment Length Polymorphism (RFLP) method has taken advantage of altered restriction enzyme recognition sites caused by nucleotide substitutions in the ABO locus of the A, B, or O alleles. This method used to differentiate between B and non-B alleles with or without multiplexing the PCR's (Yamamoto et al., 1990c; Lee and Chang, 1992; O'Keefe and Dobrovic, 1993; Stroncok et al., 1995).

There have been no previous studies on ABO allele frequencies comparison with their genotyping in the Jordanian population. Therefore, the aims of this study were to determine the phenotypic and allelic frequencies in the Jordanian population using Hardy-Weinberg Law, also to determine the genotype of the ABO blood groups using PCR-RFLP molecular analysis and finally to compare the molecular analysis data with those obtained from the statistical estimation of the ABO gene frequency.

MATERIALS AND METHODS

Estimation of the ABO phenotypes and gene (Alleles) frequencies: To estimate the phenotypes and gene frequencies for the ABO blood groups among Jordanian population, sample of 12215 randomly apparently healthy voluntary Jordanian blood donors was taken from the National Blood Bank donor registry, Amman, Jordan. The sample represents donors during the period of 1998 to 2003. The gene (alleles) frequencies p, q and r were calculated according to Hardy-Weinberg Law \[ p^2(1^A 1^B) + 2pr (1^A 1^O) + q^2 (1^O 1^O) + 2qr (1^O 1^B) + 2pq (1^A 1^B) + r^2 (1^O 1^O) \].

Molecular genotyping of the ABO blood groups

Sample collection: The molecular genotyping study was performed on blood samples taken from the National Blood Bank, Amman, Jordan. The samples size was 105 apparently healthy Jordanian blood donors. All blood samples were collected from the blood donors by drawing peripheral venous blood into ethylenediaminetetraacetic (EDTA) tubes under septic conditions.

Blood group serology: ABO blood group serology was performed at the National Blood Bank using commercially available monoclonal Anti-A, Anti-B, reagents according to the National Blood Bank standard operating procedures.

DNA extraction: DNA extracted from peripheral blood leukocytes according to a modified salt-out method (Miller et al., 1988). DNA quality and quantity were determined according to (Sambrook and Russell, 2001).
Fig. 1: Schematic description of the location of oligonucleotide primers, restriction sites for KpnI at 261 and AluI at 703 and sizes of the PCR products of the first fragment 468 bp and for second fragment 289 bp

Table 1: Sequences of primer pairs used in the amplification of ABO gene locus

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer location</th>
<th>Primer sequence 5' - 3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO-F1</td>
<td>Intron 5 at 280 bp</td>
<td>GGG AGG GTT GTG TCC TAT CTC</td>
</tr>
<tr>
<td>ABO-R1</td>
<td>Intron 6 at 60 bp</td>
<td>AAT GTC CAC AGT CAC TCG CC</td>
</tr>
<tr>
<td>ABO-F2</td>
<td>Exon 7 at 503 bp</td>
<td>GCA GCT GTC AGT GCT GGA G</td>
</tr>
<tr>
<td>ABO-R2</td>
<td>Exon 7 at 792 bp</td>
<td>GTA GAA ATC GCC CTC GTC CTT</td>
</tr>
</tbody>
</table>

Amplification of ABO gene locus by PCR: The DNA samples obtained from salting-out method were amplified by using two sets of primers that were designed manually from the consensus sequence of A' allele cDNA nucleotide sequences [Genbank Acc. No AF 014105] (Table 1). The first set includes the primer pair ABO-F1 and ABO-R1 that was used for the amplification of 468 bp DNA fragment, which contains the nucleotide 261. The second set includes the primer pair ABO-F2 and ABO-R2 that was used to amplify a 289 bp DNA fragment, which contains the nucleotide 703 (Fig. 1).

A master mix of 1000 μL containing: 200 μM of dNTP, 35 units of Taq DNA polymerase (Promega), 200 μM of each primer (Alpha DNA), 1x PCR reaction buffer (10 mM Tris-HCl, pH 9.0 at 25°C, 50 mM KCl, 0.1% Triton X-100 and 1.5 mM MgCl₂, Promega) was prepared. For each PCR reaction, 22.5 μL of the master mix and 250 ng of DNA template were used for amplification.

A total of 35 PCR cycles of amplification was carried out in thermal cycler (iCycler, Bio-Rad), but the conditions were varied with each set of primers: for the primer pair ABO-F1 and ABO-R1, the amplification condition was as follows: after initial denaturation for 2 min at 95°C, 35 PCR cycles of amplification were carried out as follows: denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, extension at 72°C for 30 sec, with final extension for 3 min at 72°C. The amplification condition for the primer pair ABO-F2 and ABO-R2 was as follows: after initial denaturation for 2 min at 95°C, 35 PCR cycles of amplification were carried out as follows: denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec, extension at 72°C for 30 sec, with final extension for 3 min at 72°C.

The amplified DNA products were then run on a 2% agarose gel in 1X TBE buffer at 150 volts. The molecular size of the amplified DNA products was determined by comparison with DNA marker (100 bp DNA ladder, Invitrogen life technologies, Cat. No 15628-019). The bands were then visualized under the ultraviolet light and photographed using Gel Documentation system (Bio-Rad Laboratories 2000 Alfreed Nobel Drive Hercules CA 94547-9980).

Restriction Fragment Length Polymorphism (RFLP): The restriction enzymes that used in this study were KpnI and AluI. KpnI enzyme was used to detect the deletion in the nucleotide 261 (G - A) that detect O' and O" alleles, while AluI enzyme was used to detect the substitution in the nucleotide 703 (G - A) that detect B allele. The digested DNA fragments were run on 2% agarose gel in 1X TBE buffer at 150 volts. The molecular size of the digested amplified DNA samples was determined by comparison with DNA marker 100 bp DNA ladder.

Comparing the data of molecular genotyping with ABO genotype frequency: The molecular analysis data was compared with those obtained from the statistical estimation of the ABO gene frequency by using chi square test.

RESULTS

Phenotypic and allele frequencies

ABO phenotype frequency: The sample used for estimating the allele frequencies of the ABO blood groups consisted of 12215 randomly apparently healthy Jordanian voluntary blood donors that were taken from the National Blood Bank donor registry, Amman, Jordan. The ABO blood groups phenotypes for this sample were 4686 (38.36%) A, 4473 (36.62%) O, 2203 (18.04%) B and 853 (6.98%) AB (Table 2).

ABO gene (allele) frequencies: The ABO gene locus is controlled by three different alleles I⁺, I⁻ and I⁻. The frequencies of the I⁺, I⁻ and I⁻ alleles were calculated according to the Hardy-Weinberg Law of equilibrium based on data present in Table 2. According to Hardy-Weinberg Law the population should consist of: p² (I⁺ I⁻) + 2pq (I⁻ I⁻) + q² (I⁻ I⁻) + 2q² (I⁺ I⁻) + r² (I⁻ I⁻).

Based on this formula, the allele frequencies of the ABO blood groups in the population was as follows:
Table 2: Phenotypes, genotypes, genotypic frequencies, donors phenotypic numbers, phenotypic percentage and phenotypic frequencies of 12215 healthy Jordanian blood donors during the period 1998-2003

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Genotypes</th>
<th>Genotypic frequencies</th>
<th>Donors phenotypic numbers</th>
<th>Phenotypic percentage</th>
<th>Phenotypic frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(F⁺)</td>
<td>P⁺2P⁻</td>
<td>4486</td>
<td>35.33</td>
<td>0.3026</td>
</tr>
<tr>
<td>B</td>
<td>(F⁻)</td>
<td>P⁻2P⁺</td>
<td>2003</td>
<td>16.34</td>
<td>0.1094</td>
</tr>
<tr>
<td>AB</td>
<td>(F⁺)</td>
<td>P⁺2P⁺</td>
<td>855</td>
<td>6.85</td>
<td>0.0600</td>
</tr>
<tr>
<td>O</td>
<td>(F⁻)</td>
<td>P⁻2P⁻</td>
<td>4473</td>
<td>36.62</td>
<td>0.3163</td>
</tr>
<tr>
<td>Total</td>
<td>(p²+q²)</td>
<td>(p²+q²)²</td>
<td>12215</td>
<td>100</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Fig. 2: Gel electrophoresis of PCR products obtained by using the primer pair (ABO-F1 and ABO-R1 primers). Lane 1: DNA marker (M); lanes 2 and 3: A phenotype; lanes 4 and 5: B phenotype; lanes 6 and 7: AB phenotype; lanes 8 and 9: O phenotype

Fig. 3: Gel electrophoresis of PCR products obtained using the primer pair (ABO-F2 and ABO-R2). Lane 1: DNA marker (M); lanes 2 and 3: A phenotype; lanes 4 and 5: B phenotype; lanes 6 and 7: AB phenotype; lanes 8 and 9: O phenotype

the frequency of the F allele (p) = 0.6052, the frequency of the F allele (q) = 0.2607 and the frequency of the F allele (q) = 0.134.

ABO genotype frequency: The genotypic frequencies for the ABO blood groups of the 12215 Jordanian donors calculated based on the estimation of gene frequencies according to Hardy-Weinberg Law. The estimated genotype percentages were: 6.797% AA, 31.556% AO, 1.798% BB, 16.231% BO, 6.991% AB and 36.627% OO.

Molecular Genotyping of ABO:
Blood phenotype of the studied sample: In this part of study, the sample consisted of 105 apparently healthy unrelated voluntary Jordanian blood donors. The ABO blood group phenotypic distribution of this sample was as follows: 41 A, 39 O, 15 B and 10 AB.

Amplification of the ABO gene locus by PCR: The samples were amplified using two sets of primers (Table 1). The first set includes the primer pair ABO-F1 and ABO-R1 was used to amplify a fragment of the 468 bp DNA fragment that contains nucleotide 261 (Fig. 2). The second primer pair ABO-F2 and ABO-R2 was used to amplify a fragment of the 289 bp DNA fragment that contains nucleotide 793 (Fig. 3).
DISCUSSION

Phenotypic and allele frequencies: The distribution of ABO phenotypes and their percentages among the 12,215 Jordanian voluntarily blood donors revealed that ABO phenotypes were: 4685 (38.36%) A, 4473 (36.62%) O, 2203 (18.04%) B and 853 (6.98%) AB. The ABO alleles frequencies of the I', I' and I alleles were calculated using the statistical methods based on Hardy-Weinberg Law of equilibrium. The estimated allele frequencies were 0.6052 for O, 0.2607 for A and 0.1341 for B. These results showed that O gene frequency is higher than that of A or B followed by A then B and indicated the global predominance of A gene over B. These findings are in agreement with (Al-Bustan et al., 2002; Varsah et al., 2001; Omote and et al., 1999; Revazov et al., 1983; Tills et al., 1983; McArthur and Penrose, 1951).

The estimated genotype frequencies were: 6.797% AA, 31.556% AO, 1.798% BB, 16.231% BO, 6.991% AB, 36.627% OO according to Hardy-Weinberg Law, the order of the estimated genotype frequencies were: OO, AO, BO, AB, AA and BB.

Molecular genotyping of ABO: The PCR products of the primer pair ABO-F1 and ABO-R1 were digested with the enzyme Kpn I and the pattern of this digestion had the following forms: when digestion was complete, the 468 bp DNA fragment yielded two fragments, 295 and 173 bp, which indicated the presence of an OO homozygote alleles; these fragments were used as an O allele marker. When digestion was partial, it would yield three fragments, 468, 295 and 173 bp DNA fragments; these represent AO or BO heterozygote alleles. However, with no digestion it would yield one fragment (468 bp) and no O allele was confirmed and the genotypes AA, AB, BB were possible (Fig. 4).

The PCR products of the primer pair ABO-F2 and ABO-R2 were digested with the enzyme Alu I that cleaves B around nucleotide 703 and the pattern of this digestion had the following forms: when digestion was complete, the 289 bp DNA fragment yielded two fragments, 201 and 88bp, which indicated the presence of an BB homozygote alleles; these fragments were used as a B allele marker. When digestion was partial, it would yield three fragments, 289, 201 and 88 bp DNA fragments; these represent AB or BO heterozygote alleles. But with no digestion, it would yield one fragment (289 bp) and no B allele was confirmed and the genotypes AA, AO, OO were possible (Fig. 5). If the two DNA fragments were not digested with both enzymes Kpn I or Alu I, then homozygote allele AA must be assigned to these fragments.

Restriction fragment length polymorphism: The PCR products of the first fragment obtained using the primer pair ABO-F1 and ABO-R1 primer pair were digested with Kpn I (Fig. 4). The PCR products from the second fragment obtained by using primer pair ABO-F2 and ABO-R2 were digested with Alu I (Fig. 5).

Comparing molecular and ABO genotype frequency data: The distribution of ABO molecular genotypes based on the allele frequency was compared with the data obtained from the molecular analyses by using chi-square test (Table 3). The statistical analysis indicated that molecular data were in good agreement with the ratio calculated from the estimated gene frequencies of the ABO blood group system in the Jordanian population.

### Table 3: Observed and expected genotypes for the 105 samples used in chi-square test

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. of samples</th>
<th>Percentage</th>
<th>No. of samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>6</td>
<td>5.714</td>
<td>7.137</td>
<td>6.797</td>
</tr>
<tr>
<td>AO</td>
<td>35</td>
<td>33.333</td>
<td>33.254</td>
<td>31.556</td>
</tr>
<tr>
<td>BB</td>
<td>1</td>
<td>0.952</td>
<td>1.885</td>
<td>1.798</td>
</tr>
<tr>
<td>BO</td>
<td>14</td>
<td>13.333</td>
<td>17.042</td>
<td>16.231</td>
</tr>
<tr>
<td>AB</td>
<td>10</td>
<td>9.524</td>
<td>7.341</td>
<td>6.991</td>
</tr>
<tr>
<td>OO</td>
<td>39</td>
<td>37.143</td>
<td>38.458</td>
<td>36.627</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>100.000</td>
<td>105.000</td>
<td>100.000</td>
</tr>
</tbody>
</table>

($\chi^2 = 2.218, df = 5, \alpha = 0.10$)
From the combined digestion patterns of the two enzymes Kpn I and Alu I, the ABO genotypes were determined for all the 105 samples. Fourteen samples with (B) phenotype were recognized as heterozygous genotype (BO), one sample of (B) phenotype was recognized as homozygous genotype (BB), thirty five samples of (A) phenotype were recognized as heterozygous genotype (AO), six samples with (A) phenotype were recognized as homozygous genotype AA, thirty nine samples with (O) phenotype were recognized as homozygous genotype (OO) and ten samples with (AB) phenotype were recognized as heterozygous genotype (AB).

Only one sample with the phenotype O in the genotyping results appeared as a heterozygote, this indicated that one of these alleles was O and the other one could be another allele of O that don’t have the nucleotide 261 (G) deletion and it could be O; further analysis of this sample is necessary. The percentage of this allele was not determined before in the Jordanian population; the expected percentage according to this finding was 0.9524%. Further investigation is required to determine the accurate percentage of this allele. By comparing the expected percentage with other population, the O² allele was not found in Chinese, neither was reported in Japanese (Fukmori et al., 1996; Watanabe et al., 1997), nor Koreans (Kang et al., 1997), suggesting that this allele may be extremely rare or even does not exist in the Orientals. Interestingly, this allele was not found in Amerindians either. Although not very common, this allele was found in white Europeans at a frequency of 1.02% (Yip, 2000); previous studies reported different frequencies (1.7 to 3.7%) (Olsson and Chester, 1995; Nishimukai et al., 1996; Watanabe et al., 1997; Grunnet et al., 1994; Franco et al., 1995; Gassner et al., 1996; Pearson and Hessner, 1998).

In the present study, the PCR-RFLP molecular technique was used for genotyping of the ABO blood groups in the Jordanian population. By analyzing the electrophoresis patterns of the digested fragment, the phenotypes for the 105 sample were (A: 41, B: 15, AB: 10 and O: 39). The results of the ABO genotypes were AA: 6 (0.05714), AO: 35 (0.33333), BB: 1 (0.00953), BO: 14 (0.13333), AB: 10 (0.09524), OO: 39 (0.37143); similar findings were reported by (Inshaid et al., 2002). This result, differ from the ABO genotypes of the Kuwaiti population that have higher percentage of B phenotype (Al-Bustan et al., 2002). On the other hand, the Italian population has higher percentages of BB, AB genotypes and has lower percentages of AO genotype (Villa et al., 1996).

The chi-square test for goodness of fit between the observed and expected phenotypes data showed no significant differences. This proved that the ABO genotypes of the randomly collected samples were in good agreement with molecular analysis data. 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.

The molecular data that detect rare ABO subgroups could reflect the actual ABO genotypes in any population.

REFERENCES


