BioImpacts, **2012**, 2(4), 207-212
doi: 10.5681/bi.2012.016
http://bi.tbzmed.ac.ir/

Allelic Prevalence of ABO Blood Group Genes in Iranian Azari Population

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 07 May 2012
Revised: 10 June 2012
Accepted: 19 June 2012
ePublished: 25 June 2012

Keywords:
ABO Blood Groups
Genotyping
Multiplex Allele-Specific PCR
Iran

ABSTRACT

Introduction: ABO blood group system is the most important blood group in transfusion and has been widely used in population studies. Several molecular techniques for ABO allele's detection are widely used for distinguishing various alleles of glycosyl transferase locus on chromosome 9. ***Methods:*** 744 randomly selected samples from Azari donors of East Azerbaijan province (Iran) were examined using well-adjusted multiplex allele-specific PCR ABO genotyping technique. ***Results:*** The results were consistent for all individuals. The ABO blood group genotype of 744 healthy Azari blood donors was: 25.8% AA/AO ($_2$), 7.6% AO ($_1$), 1.6% BB, 11.3% B0 ($_1$), 10% AB, 9.3% 0($_1$)0($_1$) and 15.3%0($_1$)0($_2$). The highest genotype frequency belonged to O01/O02 genotype (15.3%) and the lowest frequency belonged to A101/A102 genotype (0.4%). ***Conclusions:*** The frequencies of ABO alleles didn't show significant differences between East Azerbaijan province population and that of other areas of the country. Meanwhile, statistical analysis of frequencies of A and B alleles between East Azerbaijan province population and neighbor countries showed significant differences whereas the frequency of allele O between them did not show significant difference (P>0.05).

Introduction

ABO blood group system was discovered by Landsteiner in 1900 (Landsteiner 1901). This system is one of the most significant blood groups in blood transfusion and organ transplantation (Martin *et al* 2001). ABO antigens are present on the surface of red blood cells as well as tissues and body fluids (Reid *et al* 2004; Brian *et al* 2000).Among such tissues, platelets (Brian *et al* 2000), intestinal mucosa (Takeya *et al* 1990), Lung tissue (Clausen *et al* 1990), epidermis, endothelium of vessels (Oriol *et al* 1992) and other body tissues can be referred to.

ABO gene locus is located on chromosome 9. It contains 7 exons that span more than 18 KB of genomic DNA (Yamamoto *et al* 1990, Yamamoto *et al* 1995). Exons 6 and 7 constitute 77 % of the coding region (Von Decastello and Sturli 1902, Crow 1993). One hundred different ABO alleles have been discovered so far (Yamaguchi 1973). The genomic sequence of known alleles have minor differences with prevalent alleles of ABO, and are mostly formed because of substations, hybrid alleles, base insertions, deletions and splice site mutation (Blumenfeld and Patnaik 2004, Olsson and Chester 2001). Prevalent alleles in Asian people are A101 (A^1), O01 (O^1), B 101 (B^1), A102 (A^{1v}) and O02 (O^{1v}) (Chen *et al* 2006). Typically A^1 is used as a reference allele in comparison of different alleles together (Landsteiner 1901).

Allele B is different from allele A^1 in seven single-base substitution in 703, 796, 803, 297, 526, 657, and 930 positions. Alleles A^1 and B differ in four amino acids' positions including Gly 235 Ser, Arg 176 Gly, Gly 268 Ala and Leu266 met, in which the position of amino acids 266 and 268 plays a major role in determining glycotransferans type from A^1 or B (Yamamoto and Hakomori 1990, Daniels 2009). Both alleles A101 (A^1) and A102 (A^{1v}) have phenotype A^1 (Yamamoto 2004) and their difference is in the substitution of nucleotide 467 (467 CCG > CTG) which results in substitution of Leucine in A^{1v} instead of Proline in allele A^1 (Shea

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2000). Allele A201 (A^2) has a single nucleotide deletion near the C-terminal region before the translation stop codon (Yamamoto *et al* 1992). This deletion in 1060 delC creates a reading-frame shift in coding sequence and therefore produces glycotransferase with 21 additional amino acids (Yamamoto 2001).

O01 or O^1 is the most prevalent type of allele O that has one single base (G) deletion in nucleotide 261. This deletion is responsible for reading-frame shift and creating a stop codon. As a result, a polypeptide is produced which lacks 116 amino acids and catalytic C-Terminal domain (Chen *et al* 2006, Yuch-ching *et al* 2006). Another allele O (O02 or O^{1v}), has 9 single-base substitutions in addition to single nucleotide deletion in the position 261 (Daniels 2009). Allele O03 (O_2) does not have single nucleotide deletion but is specified by 802G>A (268Gly>Arg) substation (Lee *et al* 2005, Yamamoto *et al* 1996).

ABO genotyping studies are mainly used in ABO discrepancy, Forensics, organ transplantation, and family studies (Olsson and Chester 1996). It is a valuable supplementary test for the blood grouping of acceptor and donor and can be helpful in determining subsidiary subgroups and revealing weak A and B antigens (Shea 2000, Olsson and Chester 1995, Olsson and Chester 1996).

Several PCR-based strategies for ABO genotyping are developed using PFLP (Lee and Chang 1992), allele-specific PCR (AS-PCR) (Uogozzoli and Wallace 1992), single-strand conformation polymorphism (SSCP) (Akan *et al* 1996) and amplified product length polymorphism (APLP) (Watanabe *et al* 1997). PCR using Allele-specific primers is a robust tool to differentiate between alleles due to single-base substitution_or deletion. This method is neither time-consuming nor costly compared to enzymatic digestion or nucleotides' hybridization (Sung *et al* 2009).

East Azerbaijan province is the largest and most populated province of the north west of Iran. The province covers an area of 45,491 km^2 (about 2.8 % of the area of the country) (Rahman 2010). According to the population census of 2006, the province of east Azerbaijan has a population of 3,603,456 million people which is equal to 5.11 % of the population of the country (http://www.ostan-as.gov.ir).

So far, no studies have been carried out about genotypic frequency over ABO alleles on Azari people of East Azerbaijan. In this research, genotype frequencies of ABO alleles were determined using AS-PCR method.

Materials and methods

744 samples were randomly collected from Azari donors who referred to blood transfusion organization of East Azerbaijan province. Phenotypes of samples were determined using serological test. Cell typing was carried out using Anti-A, Anti-B (Iranian Blood Transfusion Organization) and reverse typing was carried out using A1 and B cells. Genomic DNA was extracted by NaCl saturated solution (5 Molar solution) and chloroform from blood leukocytes (Iranpur-Mobarakeh and Esmailizadeh 2010). Briefly, 1ml cell lyses buffer (Tris HCl 1mM, Sucrose 11%, Mgcl2 5mM, Triton 1%) was added to 750ul EDTA collected blood sample in 2ml micro-tube. White blood cells collected using 3 min centrifuge and lysed using nuclease buffer (Tris HCl 10mM, SDS 1%, EDTA 10mM, and sodium citrate 10mM) in room temperature for 10 min. DNA was precipitated using 110ul Nacl (5M) and 600ul chloroform in 13000rpm for 15 min followed by ethanol precipitation. PCR was performed using primers listed in table 1 in 4 micro-tubes (Sung *et al* 2009). For each PCR, 5 µl master mix (Cinnagen, Tehran, Iran), 2 µl deionized water, 1 µl DNA templet and 0.5 µl of each allele specific primers (5 Pmol) in one micro-tube were mixed. PCR was performed in a thermal cycler (SENSQUEST, Germany). The cycling conditions were: The first denaturation for 3 min at 95°C, followed by 35 cycles of 40s at 95°C, 40s at 61°C, 40s at 72°C and additional 5 min at 72°C for final extension.

In each PCR, GAPDH gene specific primers (Forward: GAAGATGGTGATGGGATTTC and reverse: GAAGGTGAAGGTCGGAGT) were used with the following sequence as the internal controller that produces a 600bp fragment. After the reaction, the PCR mixture (10 µL) was electrophoresed on a 3% agarose gel, stained with ethidium bromide and visualized using Transluminator (UVTEC- UK) device.

Results

Phenotypes of blood samples

The phenotypes of the 744 blood samples in this study were A (33.5%), B (23%), AB (10.1%) and O (33.5%).

Blood group genotyping

Multiplex AS-PCR reaction consists of four independent PCR reactions. In the first reaction, PG ABO1-PG ABO9 pair primers were used to produce 205 bp fragment. They recognized Cis-ABO1, B101, A101 and A102 alleles whereas second pair primers, PG ABO5-PG ABO8, recognized alleles A101, O01 and O02 by producing 381bp fragment. The second reaction consisted of 2 pair primers PGABO3-PGABO9 and PGABO5-PGABO7 which produced 164bp and 381bp respectively. PGABO3-PGABO9 primers amplified A101, A102, O01 and cis-AB01alleles and PG ABO5-PG ABO7 primers were used for recognition of B101 allele.

Table 1. Primers used for ABO genotyping

PCR reaction	Primer pair	Fragment size (bp)	Allele specificity
1	PG-ABO1: 5'-GCAGTAGGAAGGATGTCCTCGTGTTG-3' PG-ABO9: 5'-AGACCTCAATGTCCACAGTCACTCG-3'	205	*A101, A102, B101, cis-AB01*
	PG-ABO5: 5' -CCACTACTATGTCTTCACCGACCATCC-3' PGABO8: 5' -CACCGACCCCCCGAAGATCC-3'	381	*A101, O01, O02*
2	PG-ABO3: 5' -CCATTGTCTGGGAGGGCCCA-3' PG-ABO9: 5' -AGACCTCAATGTCCACAGTCACTCG-3'	164	*A101, A102, O01, cis-AB01*
	PG-ABO5: 5' -CCACTACTATGTCTTCACCGACCATCC-3' PG-ABO7: 5' -CACCGACCCCCCGAAGATCG-3'	381	*B101*
3	PG-ABO2: 5' -GCAGTAGGAAGGATGTCCTCGTGTTA-3' PG-ABO9: 5' -AGACCTCAATGTCCACAGTCACTCG-3'	205	*O01,O02*
	PG-ABO6: 5' -CCACTACTATGTCTTCACCGACCATCT-3' PG-ABO8: 5' -CACCGACCCCCCGAAGATCC-3'	381	*A102*
4	PG-ABO4: 5' -CCATTGTCTGGGAGGGCCCG-3' PG-ABO9: 5' -AGACCTCAATGTCCACAGTCACTCG-3'	164	*B101, O02*
	PG-ABO6: 5' -CCACTACTATGTCTTCACCGACCATCT-3' PG-ABO7: 5' -CACCGACCCCCCGAAGATCG-3'	381	*cis-AB01*

In the third reaction, pair primers PG ABO2-PG ABO9 were selected to produce 205 bp fragment for amplification of O02 and O01 Alleles whereas PG ABO6-PG ABO8 pair primers recognized A102 allele by producing 381 bp fragment. Finally in the fourth reaction PG ABO4-PG ABO9 pair primers were applied to produce 164 bp fragments and recognize O02 and B101 alleles and PGABO6-PGABO7 pair primers to produce 381 bp fragment. They also recognized Cis-ABO1 allele. Only 15 different genotypic combinations recognized out of 21 recognizable combinations amongst 744 samples were (Fig. 1). The frequency of ABO recognized genotypes belonging to Azari donors in East Azerbaijan province are shown in Table 2. All genotyping results were compatible with the determined phenotypes by serological method. The observed genotyping data did not have a significant difference with the expected genotypes under the assumption of Hardy-Weinberg equibrium (Table 3).

Table 2. The frequency of recognized genotypes using multiplex ASPCR method for donors of Azari people in East Azerbaijan

Genotype	Frequency	Percent
A101/A101	09	01.2
A101/A102	03	00.4
A101/B101	30	04.0
A101/O01	18	02.4
A101/O02	105	14.1
A102/A102	24	03.2
A102/B101	45	06.0
A102/O01	39	05.2
A102/O02	51	06.9
B101/B101	12	01.6
B101/O01	84	11.3
B101/O02	78	10.5
O01/O01	69	09.3
O01/O02	114	15.3
O02/O02	63	08.5
Total	**744**	**100.0**

Table 3. Expected genotypes and observed ones in East Azerbaijan

Phenotype	Genotype	Observed		Expected		P-Value
		Number	Percent	Number	Percent	
A	AA	036	04.8	042.757	5.747	0.76
	AO	213	28.6	206.460	27.75	0.87
B	BB	012	01.7	024.499	3.293	0.65
	BO	162	21.8	156.240	21.00	0.86
AB	AB	075	10.0	064.735	8.701	0.80
O	OO	246	33.1	249.230	33.499	1.00
Total		**744**	**100**	**744**	**100**	

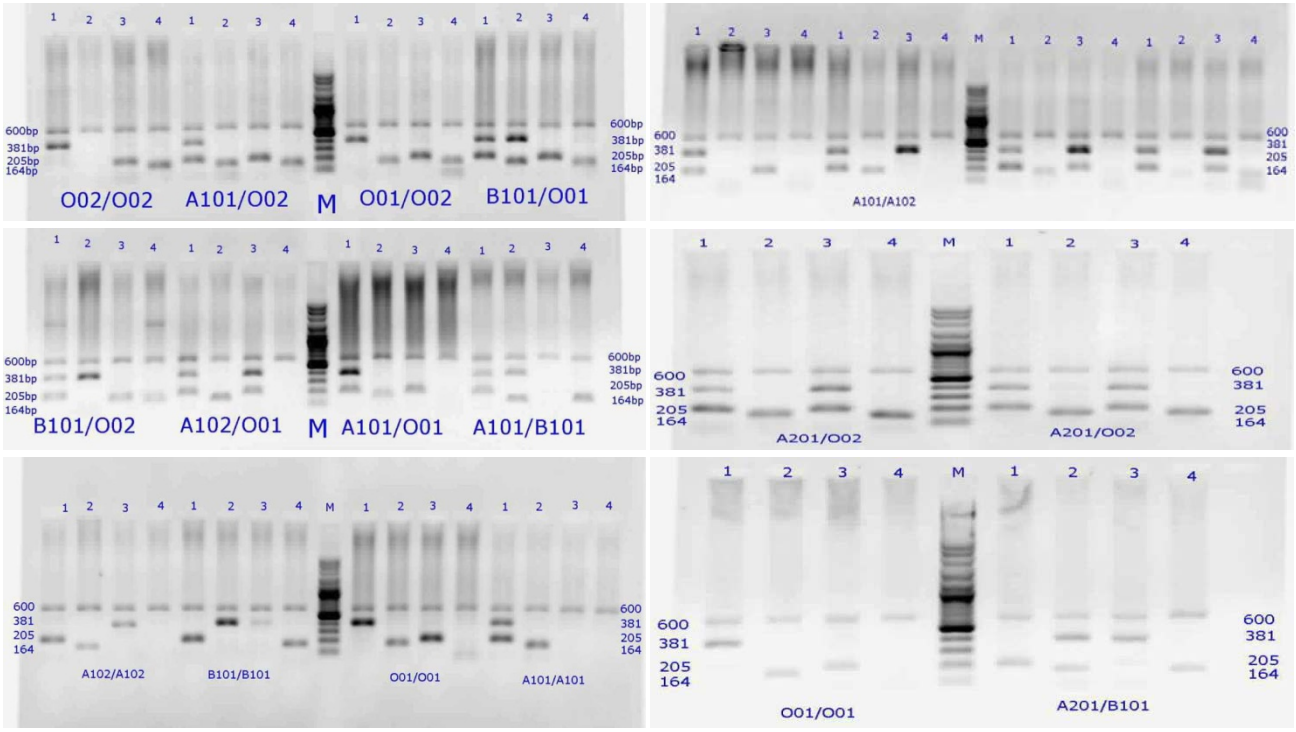
Table 4. Frequency of ABO gene Alleles in East Azerbaijan in comparison to neighbor countries and Iran

Population	Allele frequencies			Reference
	p^A	q^B	r^O	
East Azerbaijan	0.23974	0.18147	0.57879	Current study
Bahrain	0.14100	0.15700	0.70400	Al-Aarrayed *et al* 2001
Egypt	0.18800	0.14900	0.66300	Khalil *et al* 1989
Iran	0.22230	o.16950	0.60820	Walter *et al* 1991
Iraq	0.21200	0.17700	0.66110	Tills *et al* 1983
Jordan	0.27000	0.13000	0.60000	Irashaid *et al* 2002
Kuwait	0.13380	0.16760	0.69860	Mokhtar and Yunus 2008
Saudi Arabia	0.16630	0.11970	0.71400	Bashwar *et al* 2001
Sudan	0.19200	0.14000	0.66800	Khalil *et al* 1989

Table 5. Comparison of phenotypic percentages of ABO types in East Azerbaijan Province between 2001 and 2002

Blood group	A	B	AB	O
2001	36.24	21.25	8.62	33.91
Current study	033.5	23.00	10.1	33.50
P-Value	0.41	0.62	0.18	0.36

Fig. 1. The electrophoresis pattern of recognized genotypes using the multiplex ASPCR method .The figure of electrophoresis shows 15 different genotypes. 600 bp size band as internal control exists in all reactions. M columns are marker ladder. The numbers show PCR reaction tube number.



Discussion

Gene frequencies for the polymorphism of blood groups are mostly considered as racial_markers (Daniels 2002). The majority of phenotypic frequencies are obtained through calculation although in some cases molecular methods are used (Daniels 2009). So far, no genotypic studies on ABO blood types have been carried out in East Azerbaijan province and among Azari people. In this study, the genotype of 744 samples of Azari donors was determined in East Azerbaijan using multiplex AS-PCR method. None of the B101/Cis ABO 1, A101/Cis ABO1, A102/ Cis ABO1, O01/CisABO1, O02/Cis ABO1 and Cis ABO1/Cis ABO1 genotypes was recognized. Cis-AB allele is so rare and among donors of west of Korea, it is 0.0354% (Cho *et al* 2004). Interpretation of AS-PCR products of electrophoretic patterns revealed 15 different genotypes. Thirty-six samples with phenotype A were recognized as homozygote, 213 samples with phenotype A as heterozygote, 12 samples with phenotype B as homozygote B, 162 samples with phenotype B as heterozygote B genotype, 75 samples with phenotype AB as heterozygote AB genotype and 24 samples with phenotype O as homozygote O genotype. Unfortunately since there was no available data about ABO blood group genotyping in the population discussed above, it was impossible to compare the frequency of ABO alleles with previous studies; however, the comparison of observed genotypes (Resulted from genotypic studies) and expected ones (Resulted from Hardy-Winberg equibrium) clarified that there was no significant difference between them (Table 3).

The highest frequency belonged to O01/O02 genotype with a frequency equal to 114 (15.3%) and the lowest frequency belonged to A101/A102 genotype with a frequency equal to 3 (0.4%). What's more, 177 samples (23.79%) out of all were for homozygote ABO alleles and 567 samples (76.2%) for ABO alleles were heterozygote.

Based on genotyping data, the ABO phenotypes were determined and their percentages among 744 samples being studied were as the following: A (33.5%) 249 people, B (23%) 171 people, O (33.5%) 249 people and AB (10.1%) 75 people. ABO alleles' frequencies, I^O, I^A and I^B, were calculated using statistical methods and Hardy-Weinberg equibrium. The frequencies of alleles were estimated as I^O=0.57879 for allele O, I^A=0.23974 for allele A and I^B=0.18147 for allele B. The results specified that the frequency of gene O is higher than A and B and Also the frequency of gene A is higher than B. The highest frequency of O (>0.7) belongs to USA and some parts of Africa and Australia. The frequency of allele A in Europe is relatively high (0.25-0.55). It is higher than 0.45 in the south of Australia and in some indigenous American tribes is higher than 0. The frequency of B in Europe varies from 0.15 in east to

<0.05 in the Netherlands and France. The highest frequency of B in Asia is 0.2-0.3 while there is almost no frequency in aboriginal American tribes (Daniels 2009). Comparing the frequency of three main alleles (I^A,I^B, I^O) obtained from the results of current study with published data from regional countries and previous studies related to Iran (Table 4) (Walter *et al* 1991, Mokhtar and Yunus 2008, Bashwar *et al* 2001, Al-Aarrayed *et al* 2001, Tills *et al* 1983, Irashaid *et al* 2002, Khalil *et al* 1989), it was specified that the frequency of alleles in Kuwait and Bahrain is as $r^O > q^B > P^A$ even though it is as $r^O > p^A > q^B$ in other countries and also in Iran and East Azerbaijan. Statistically, the frequency of allele p^A among the population of east Azerbaijan and the countries of Kuwait (P<0.001), Saudi Arabia (P=0.03), Bahrain (P=0.028) and Egypt (P=0.048) are significantly different. Moreover, the frequency of allele q^B between the population of East Azerbaijan and Saudi Arabia are statistically different (p=0.04). Regarding r^O there was no significant difference between east Azerbaijan and aforementioned countries (P>0.05). None of these 3 alleles have significant differences with their frequencies in Iran, Iraq and Jordan (P>0.05) (Table 4).

Comparing the results of phenotypic frequency of present study with Poorfathollah *et al* (2004), no significant difference was observed (P>0.05) (Table5).

This study determined the exact phenotypic frequency of ABO blood groups in Azari people of East Azerbaijan also the frequencies of prevalent ABO alleles such as A101, A102, B101, O01 and O02 for the first time. Considering the shifts in ABO phenotypes, alleles and genotypes in different ethnic groups, recognition of rare alleles requires more studies in order to determine the exact frequency of ABO gene and ABO genotypes in the population of East Azerbaijan.

Ethical issues

All samples are collected by written permission of Azari blood donors.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgment

This work was funded in part by grants from Research Center of Iranian Blood Transfusion Organization and Research Vice-Chancellorship of Tabriz University of Medical Sciences. East Azerbaijan Blood Transfusion Center Head quarter is gratefully acknowledged for kind cooperation.

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