

Биохимия, генетика и молекулярная биология / Biochemistry, Genetics and Molecular Biology

Научная статья

Original article

DOI: [10.12731/2658-6649-2026-18-1-1441](https://doi.org/10.12731/2658-6649-2026-18-1-1441)EDN: [ALDAXS](#)

UDC 575.174:612.118.221



Determination of ABO group alleles in the Iraqi population

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Abstract

Background. The classification of blood types is considered one of the most significant pioneers. Four different phenotypes, i.e., A, B, AB, and O, were specified based on whether antigen A and/or B were present on the surface of the erythrocytes.

Purpose. To investigate the frequency of blood groups among healthy individuals using a sequencing method and to compare the findings with the corresponding traditional serological method.

Materials and methods. Thirty blood samples were collected from healthy volunteers who attended Southern Technical University between October 2023 and February 2024. Blood groups were investigated using both serology and genetic testing.

Results. The majority of volunteers carry blood group A, while the minority with group AB is 6.7%. Blood group A has four genotypes, including A¹A¹, A¹O¹, A²O¹, and A¹O², while the BO¹, O¹O¹ and A¹B genotypes represent B, O and AB, respectively. Genetic analysis of sequences showed that the O¹O¹ allele spotted the homozygous deletion of n261 in exon 6, corresponding to the O⁺ phenotype. Additionally, five SNPs, nt703, 796, 803, 1061, and 1096, of exon 7 sequences were analysed to identify single-nucleotide polymorphisms (SNPs) of ABO alleles.

Conclusion. Investigating blood group using a sequence is clinically important for improving patient safety. It supplies supportive information regarding antigen differences.

Keywords: ABO gene; blood group system; gene polymorphisms

For citation. Almyah, M. K., Chmagh, A. A., Al-laeiby, A. I. E., & Yassen, S. T. (2026). Determination of ABO group alleles in the Iraqi population. *Siberian Journal of Life Sciences and Agriculture*, 18(1), 170–183. <https://doi.org/10.12731/2658-6649-2026-18-1-1441>

Introduction

Karl Landsteiner developed a method in 1901 that allows grouping of blood according to its agglutination patterns. Based on the presence or absence of antigen A and/or B on the surface of erythrocytes, four phenotypes were identified: A, B, AB, and O [1]. Consequently, according to the inherited pattern of the antigen on the erythrocyte surface and interaction with specific antibodies, blood groups were defined. Blood groups were exploited as markers in transfusions and transplantations. Improper blood group transfer not only resulted in the development of blood agglutination but also death. Moreover, it was demonstrated that the impact of blood groups is associated with miscarriage, failure in transplantation, and death. Later, studies investigated the blood group in terms of carbohydrate structure and biosynthesis. In this sense, molecular biology and gene analysis methods were implemented to find out the association of gene polymorphisms with blood group alloantigenicity [1-3].

The term *blood group genotype* denotes the prediction of blood group phenotypes based on the analysis of specific nucleotide sequences in DNA. Red blood cell surface carbohydrate antigens A and B are produced by the enzyme transferase, which is encoded by the ABO gene. The *ABO* was identified on chromosome 9, which contains seven exons. The majority of proteins (77%) are encoded from exons 6 and 7. Two alleles, A and B, were characterized, and they showed variation in four amino acids. The identification of new *ABO* alleles, such as *A1*, *A2*, *A3*, *Ael*, *Aint*, *Am*, *Aw*, *Ax*, *cis-AB*, *B(A)*, *B*, *B3*, *Bel*, *Bw*, *Bx*, and *O*, was facilitated [3]. Blood groups have been shown to be associated with disease susceptibility, such that individuals with the O⁻ blood group show higher susceptibility to diseases than individuals with blood group O [4]. On the other hand, a previous study suggested an association between the hypertension condition and ABO¹ (G>T)(rs495828) genetic polymorphism [5].

The current study aims to investigate the ABO genotype among young people in the Basrah population. Moreover, our genotype data has been registered in the public database. Many previous studies were carried out using different methods, such as PCR-RFLP analysis, multiplex-PCR, two-dimensional PCR, and sequencing analysis [6-9].

Materials and methods

Samples collection

Thirty peripheral blood samples were collected from healthy student volunteers who attended Southern Technical University between October 2023 and February 2024. After an explanation of the aims of the study, consents were obtained from all

volunteers. All formal agreements were obtained from the scientific committee of the Department of Medical Laboratory Techniques, Southern Technical University. Blood samples were stored in the Department of Medical Laboratory Techniques at Southern Technical University at 4 C° for further analysis.

Molecular genetic analysis

Genomic DNA (GDNA) was isolated following the instructions provided by the kit manufacturer, obtained from Geneaid/Taiwan. GDNA products were separated onto 0.8% agarose gels stained with ethidium bromide, and their quantity and quality were measured using a nanodrop spectrophotometer. Targeted DNA (exon 6 and 7) was amplified following the protocol described by Martin and Detzel [10]. The successful PCR was detected after separation on a 1% agarose gel stained with ethidium bromide. The successful PCR products were then sent for sequencing, and the resulting sequences were analysed using Codon Code Aligner v. 7.1.2 software to identify the position of SNPs.

Results

The participants' age ranged from 19 to 39 y.o., with a mean age of 23 y.o. All the donors were healthy individuals with no medical issues, except for two participants, one with insulin resistance and the other with iron deficiency anaemia (IDA). All the participants were interrogated to obtain previous information and all the agreements were obtained from the donors after explaining the aim of the study. Additionally, formal agreements were secured from the University to achieve the current work.

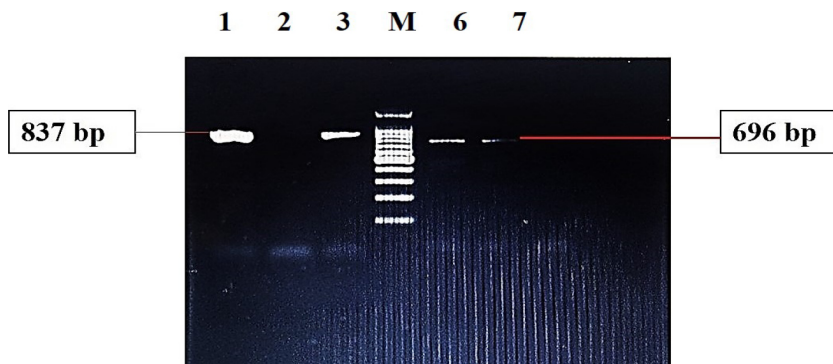


Fig. 1. Agarose gel electrophoresis of the ABO gene. The PCR products were separated on a 0.8% agarose gel stained with ethidium bromide. Amplified PCR sizes were 837 bp and 696 bp for exon 7 and exon 6, respectively. Lane 1,3 are PCR products of exon 7, while Lane 6 and 7 show PCR products of exon 6. Lane 2 represents the control; Lane M is the ladder.

Exons 6 and 7 of the *ABO* gene were amplified using oligonucleotide primer pairs. The PCR products were separated on a 1% agarose gel stained with ethidium bromide and then sent for sequencing. The size of the PCR product was 837 bp and 696 bp for exon 7 and exon 6, respectively (Fig. 1). Table 1 shows the frequency of blood groups among participants. The majority of individuals carry the A+ blood group phenotype with different genotypes, including 2 (6.66%) A¹A¹, 5 (16.7%) A¹O¹, and 1 (3.3%) A¹O². Also, 2 (6.66%) of the individuals with A- carry the A¹O¹ allele. Data showed that the maximum frequency of the allele was 10 (33.33%) among individuals with the O⁺ phenotype. The frequency of BO¹ allele was 7 (23%) in the individuals who showed B⁺ and B⁻ phenotypes 2 (6.7%).

Table 1.

Frequency of genotype and phenotype of blood group in the participants

Blood group	Genotype	Frequency (%)	Phenotype	Total (%)
A+	A ¹ A ¹	2(6.66)	10(90.9)	11(36.7)
	A ¹ O ¹	5(16.66)		
	A ² O ¹	2(6.66)		
	A ¹ O ²	1(3.33)		
A-	A ¹ O ¹	1(3.33)	1(9.1)	7(23.33)
B+	BO ¹	6(20)	6(85.7)	
B-	BO ¹	1(3.33)	1(14.3)	
O+	O ¹ O ¹	10(33.33)	10(33.33)	
AB+	A ¹ B	2(6.66)	2(6.67)	
Total		30(100)		

The serological analysis method was used to identify blood groups. As shown in Table 1, the majority of the participants were in the A group; among them, 90.9% were A⁺, and one individual was A⁻. In the B group, similarly, 85.7% were B⁺, and 14.3% were B⁻. Then, 10 out of 30 samples were O⁺, and only 2 samples were AB⁺.

The amplified PCR products were sequenced, and homology was compared with the counterpart sequences deposited in the public database at the Gene Bank. Sequencing was deposited in the NCBI public database, as shown in Table 2.

Table 2.

List of genotypes identified in this study and their accession numbers in the NCBI

Locus	Genotype	Accession number
Exon 7	A ¹ A ¹	LC800609
Exon 7	A ¹ B	LC800610

Exon 7	BO ¹	LC800611
Exon 7	A ¹ O ¹	LC800612
Exon 7	A ² O ¹	LC800613
Exon 7	A ¹ O ²	LC800614
Exon 7	O ¹ O ¹	LC800615
Exon 7	O ¹ O ¹	LC800616

Exon 6 was amplified using oligonucleotide primer pairs, sequenced, and compared with the public database at the Gene Bank. The sequence of the O phenotype was compared with the consensus O¹O¹ allele at nucleotide 261 in exon 6, as shown in Fig. 2. Homozygous deletion of G-G (-/-) at position nt261 produces an inactive protein (null genotype), as shown in Fig. 3A. Meanwhile, homozygous insertion of G-G (+/+) nucleotides refers to the identification of blood group alleles as A¹A¹, A¹B, or A¹O² (Figure 3B). The O¹O¹ allele highlighted the homozygous deletion of n261 in exon 6, corresponding to the O+ phenotype.

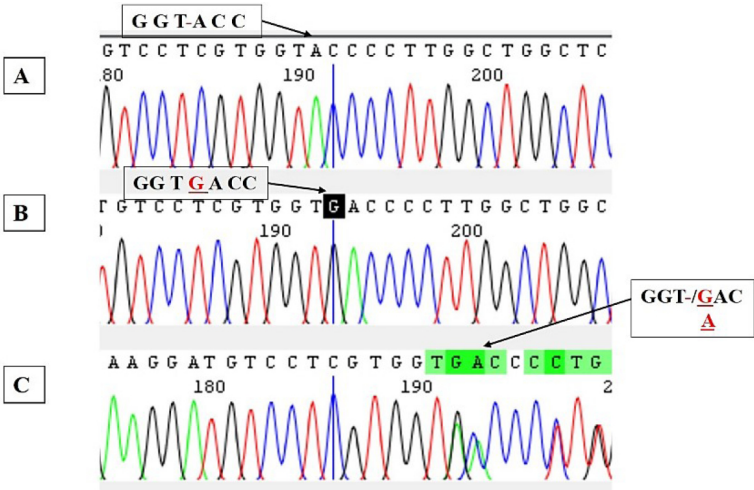


Fig. 2. Sequence of exon 6 at nt 375-1155. A. Homozygous allele refers to the null genotype G-G deletion (-/-) GGT-ACC (O¹O¹). B. Homozygous insertion of the G-G (+/+) GGTGACC genotype, referring to A¹A¹, A¹B, or A¹O² alleles. C. Heterozygous genotype G-G (+/-) GGT-/GAC represents A¹O¹, BO¹, or A²O¹ alleles. Black arrows point to the SNP (at position 621 in the reference sequence)

Five SNPs, nt703, 796, 803, 1061, and 1096, of exon 7 sequences were analyzed to identify single-nucleotide polymorphisms (SNPs) of ABO alleles.

In Fig. 3, the position nt703 was analyzed to differentiate among ABO alleles. Our results indicate that heterozygous AG genotypes are carried by individuals with BO¹ or A¹B genotypes. Accordingly, it was observed that the A¹A¹, A¹O¹, A²O¹, and A¹O² genotypes carry homozygous (G-G) individuals.

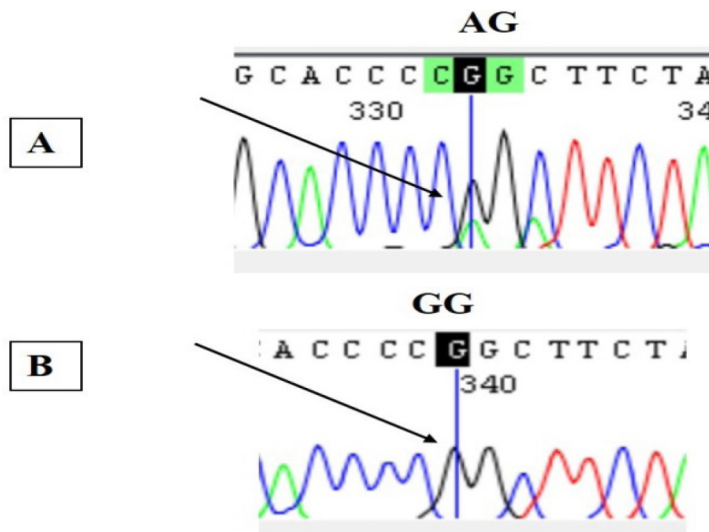


Fig. 3. Sequence of exon 7 at nucleotide position 703. A. An individual carries the heterozygous allele AG/GA representing the BO¹ or A¹B of ABO genotypes. B. Homozygous G-G in the same position produces A¹A¹, A¹O¹, A²O¹, or A¹O² genotypes. Arrows point nt703 SNP

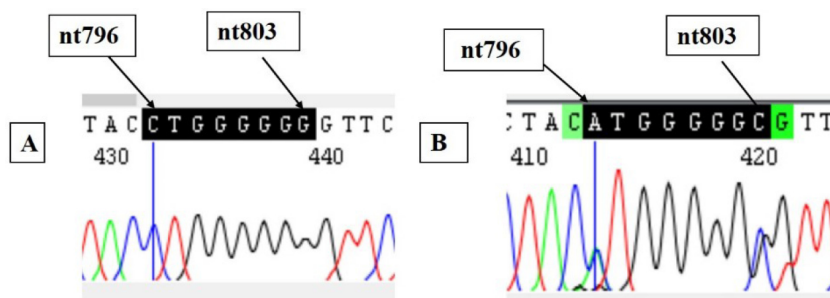


Fig. 4. The two closely related SNPs at exon 7, nt796 and nt803. A. An individual with the C-C allele (C=Leu) at nt796 and the G-G allele (G=Gly) at nt803 carries the A¹O¹ genotype. B. Individual identified as having the A¹B genotype when possessing the A-A allele at nt796 and the GC allele at nt803

At exon 7, two closely related SNPs were included: nt796 and nt803. It was demonstrated that by examining these two positions, it could be predicted that individuals carry the C-C allele (C=Leu) at nt796 and the G-G allele (G=Gly) at nt803, and identify them as the A¹O¹ genotype. Meanwhile, the two alleles, the A-A allele at nt796, and the GC allele at nt803, were identified as the individual A¹B genotype (Fig. 4).

Further analysis of exon 7 at nt11061 (Fig. 5) could identify individuals with the A²O¹ genotype when C/G is deleted at nt11061.

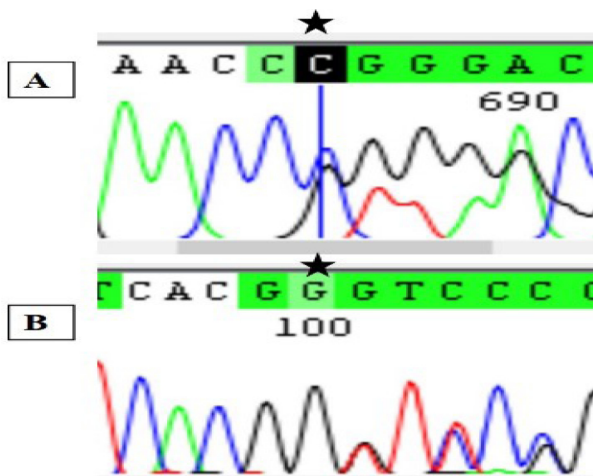


Fig. 5. Deletion in the nt1061 exon 7. A. Sequences with a forward oligonucleotide primer pair; B. Reverse primer pair. A star indicates the deletion position

Another position at exon 7 was analyzed in the nt1096; the presence of G-G in the nt1096 represents the wild type, while the alternation of nucleotides in the same locus to G-A indicates heterozygous A¹B and A¹O² genotypes (Fig. 6).

Individuals with homozygous deletion in the nt261 at exon 6 carry the O¹O¹ allele and have the O blood group. Many loci were scrutinized in exon 7 to identify genotypes and blood groups. Individuals with the BO¹ genotype, which produces the B blood group, have a heterozygous allele in exon 6, nt261 and three positions in exon 7, including nt703, nt796 and nt1096. No SNPs were identified in the examined loci, neither exon 6 nor exon 7, referring to the A¹A¹ genotype, while the A¹O¹ genotype has a single SNP at exon 6 represented by a heterozygote (+/-) at exon 6. Also, a single SNP was identified at exon 7 in the nt1096 producing A¹O² heterozygote (G-A). The A²O¹ genotype has two SNPs,

one showing a heterozygous allele at exon 6 (+/-), as well as, heterozygous deletion in nt1061 at exon 7. The A¹B genotype has four SNPs in nt703, nt796, nt803 and nt1096 at exon 7.

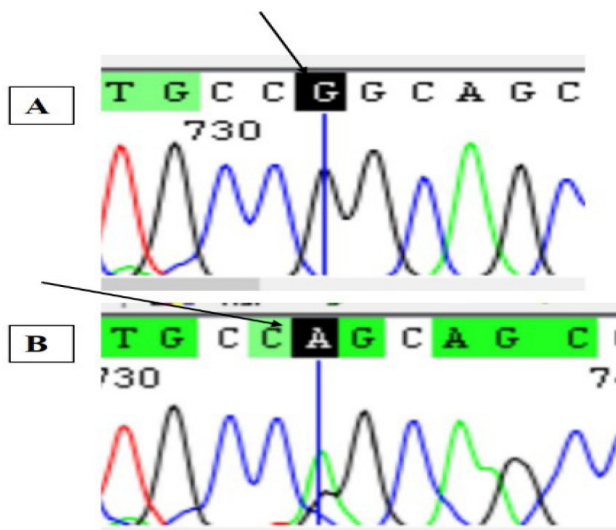


Fig. 6. Nucleotide polymorphism in nt1096 at exon 7. A. Sequences of individuals with wild-type genotype (G-G). B. Heterozygous G-A allele representing the A¹B, and A¹O² genotypes. Arrows point nt1096

Table 3.

Single-nucleotide polymorphisms in different loci of the ABO gene at Exons 6 and 7

ABO genotype	Exon 6	Exon 7				
	G 261	nt703	nt796	nt803	nt1061	nt1096 exon 7
BO ¹	-/+	AG	AC	CG	CC	AG
O ¹ O ¹	-/-	GG	CC	GG	CC	GG
A ¹ A ¹	+/+	GG	CC	GG	CC	GG
A ¹ B	+/+	GA	CA	GC	CC	GA
A ¹ O ¹	-/+	GG	CC	GG	CC	GG
A ² O ¹	-/+	GG	CC	GG	- C	GG
A ¹ O ²	+/+	GG	CC	GG	CC	GA

nt = nucleotide, + = nucleotide present, - = nucleotide deletion

The variation in the SNPs among blood groups produced different genotypes that can be used to differentiate among blood groups properly.

Discussion

This study was designed to investigate the genetic diversity of the ABO genotype among the Basrah city population using the sequencing method. Blood samples were collected from thirty young, healthy individuals. Two exon regions, including exons 6 and 7, were selected to identify the genetic variation among populations.

Although previous studies in Iraq were mostly done using PCR-RFLP analysis to investigate ABO genotypes, our current study uses a specialized sequencing method for examining a precise set of SNP loci, which makes our results deeper and more accurate [6]. Rare mutations may occur in a particular population [3, 11]. Another advantage of genotypic characterization over phenotypic characterization for clinical purposes is its ability to detect weak antigen expression [8]. The determination of ABO blood group system genotypes in the population is crucial owing to their clinical, evolutionary and forensic importance [2]. No database has been found for genotypes of blood groups concerning the Iraqi population, except for the single allele [8]. All these reasons encourage us to conduct this study.

The sequencing method has been shown to provide accurate analysis for genetic variation and can store data for future analysis using more advanced tools. Our results found that it could predict blood groups by sequencing analysis compared with the genotype results of the phenotypic serological methods. These results are consistent with previous work, which indicated the accuracy of SNP analysis, resulting in the identification of a blood group [3].

According to the phenotypes, 36.7% of the subjects belong to the A blood group, being the highest percentage among other groups. The O group, on the other hand, accounts for more than half of the total percentage of 33.33%; and the B group has an approximate percentage of 23.33; and lastly, the AB group, which constitutes 6.67%. Our result is consistent with previous findings that found the majority of samples were from group A, while Saleh *et al.* and Eissa found that the prevalence of the O group was higher than other blood groups [12,13]. Similarly to our results, in Saudi Arabia, the A group is more prevalent than other groups [7]. In addition, an early study conducted in Iraq found that the predominant blood group was the A, followed by B, AB, and O, respectively [6]. In accordance with our results, the frequency of blood groups in the Jordanian population was 38.36% in the A group, 36.62% in the B group, followed by 18.04% in the B group and 6.9% in the AB group [14].

Amplification of exons 6 and 7 and sequence analysis were utilized to identify approximately 80% of the coding sequence of the *ABO* gene. This method was utilized to identify the blood group genotypes.

In our results, the prevalence of individuals with the A¹ allele is higher than that of A². 6.7% of the individuals have the A²O genotype; the A² part refers to the fact that an individual inherited an A allele from one parent and an O allele from the other. The main difference between the A¹ and A² parts of the genotype is the expression of antigen A. It was found that A¹ is expressed more pronouncedly than A². Lehner et al. suggested that the differentiation between A¹ and A² reduces incompatible platelet transfusions owing to variation in A-antigen expression [15]. This result draws attention to the importance of genotype examination in clinical institutes.

The sequence analysis shows no difference between A¹O¹ and A²O¹ at exon 6; both are heterozygous (+/-), while A¹O² is homozygous (+/+). Previous work found that the difference between the A¹O¹ and A¹O² alleles is related to the mutation at nt467 within exon 7, leading to an alternate amino acid residue at codon 156 from proline to leucine (Pro156Leu) [16]. In Saudi Arabia, the prevalence of A¹O¹ and A¹O² alleles was 3.27% and 17.76%, respectively [7]. It is currently differentiated between the A¹O¹ and A¹O² alleles through exon 6. It was demonstrated that two specific alleles 467C>T and 1061delC at exon 7 were recorded in the A allele associated with A²O¹ [17].

23.3% of the samples had the BO¹ genotype, compared with previous results that were registered in Saudi Arabia when they found that the frequency of the B¹O¹ genotype was 11.2%. On the other hand, in the northeast Algerian population, the frequency of the B blood group was slightly higher than in Saudi Arabia at 15.93% [18].

Similarly to our findings, the BO¹ genotype shows an alteration in the position nt703 G<A and nt803 G<C [16]. In agreement with our results, it was demonstrated that the BO¹ genotype has polymorphisms at positions 796 (C>A) and 803 (G>C) in exon 7 [19].

The O¹O¹ genotype was characterized by a deletion in exon 6 at position G261, this mutation was previously highlighted by Nakamura and his co-workers [20]. A minority of samples (6.7%) have the A¹B genotype, marking a slight deviation, lower than previous results recorded in Saudi Arabia (7.5%) and Algeria (4.3%). In our results, the A¹B genotype is characterized by a single polymorphism at nt1096 in exon 7. A previous study found that in the Iraqi man sample, genetic variations influenced the expression of antigens A and B, involving the incompatibility of blood groups [8]. A small number of samples included in our study is one of the limitations of this study. A limitation of this study is that it was self-funded by the authors; therefore, the limitation of sample numbers is attributed to the high cost of sequencing and materials, as earning

financial support is difficult in Iraq. Because of the lack of financial support, this work was limited to a single geographic area (Basrah city). The diversity of group ethnicities across Iraq is broad, and scanning a vast population with different ethnicities, e.g., Arabic, Kurdish, Turkman, etc., may help to discover novel genotypes. The present study encourages further research by our team to investigate the association between blood group genotypes and chronic inherited diseases.

Conclusion

The investigation in the current study represents a novel contribution involving determining ABO genotypes in Iraq through the sequencing method. With respect to the selection of Basrah city, our findings reflect a preliminary overview of genotype distributions in the region. It was revealed that a higher frequency was observed in one blood group among four, whereas the minority was represented by the AB group. The appearance of the A¹O² genotype reflects the importance of applying genetic analyses to reduce incompatibility among blood groups. In addition, our comparisons to earlier studies show deviations in genotypes and blood group frequencies of diverse populations in the Arab region, probably a reflection of different ethnical backgrounds. These emphasize the need for more studies to uncover the complex relationship between genetics and race in the determination of the distribution of different blood groups.

Ethics Committee Conclusion. This study was approved by the ethical committee of the Department of Medical Laboratory Techniques, Southern Technical University, approval number: 386 in date 15.12.2024.

Conflict of interest information. The authors declare no conflict of interest.

Acknowledgements. We would like to express our gratitude to Southern Technical University and the students Fatima and Ali Al-Muhammad, who helped with the sample collection.

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Поступила 09.09.2025

После рецензирования 30.10.2025

Принята 20.11.2025

Received 09.09.2025

Revised 30.10.2025

Accepted 20.11.2025