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Allele Frequencies and Molecular Genotyping of the ABO Blood Group System in a Kuwaiti Population

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Abstract

The phenotypic distributions of observed numbers of ABO blood groups in a Kuwaiti sample population of 18,558 subjects are 4962 (26.7%) with A, 4462 (24.1%) with B, 858 (4.6%) with AB, and 8276 (44.6%) with O. The calculated gene frequencies are 0.6678 for ABO*O, 0.1768 for ABO*A, and 0.1554 for ABO*B. Molecular genotyping of the ABO blood group system in a Kuwaiti sample population was determined using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The positions of nucleotides 258 and 700 of cDNA from A transferase were amplified by PCR. The amplified DNA was subjected to RFLP analysis to distinguish *A, B,* and *O* alleles. Blood samples of known ABO phenotype from 101 healthy unrelated Kuwaiti individuals (A, 29; B, 23; AB, 14; O, 35) were used. Two DNA fragments of the ABO locus were designed to be amplified by 2 pairs of primers. To identify the 258th nucleotide, a 199- or 200-bp DNA fragment was amplified by PCR and digested with *Kpn*I. For the 700th nucleotide, a 128-bp DNA fragment was amplified by PCR and digested with *Alu*I. By analyzing the electrophoresis patterns, ABO genotypes were conclusively determined by examining the DNA fragments. The ABO genotypes of the known 101 samples were as follows: *AA*, 4.30%; *AO,* 24.41%; *BB,* 4.16%; *BO,* 24.2%; *AB,* 8.46%; and *OO,* 34.65%. These results were confirmed statistically using the calculated frequencies of I^A, I^B, and I^O alleles. *Int J Hematol.* 2002;75:147-153.

Key words: Allele frequencies; Molecular; ABO; Kuwait

1. Introduction

The ABO blood group was the first major alloantigen system recognized in humans. Almost a century has passed since Landsteiner first observed the serological effects of ABO blood group incompatibility [1]. Red cell serology then underwent a renaissance during which the classification of blood group antigens continued apace, and the first examples of widespread human polymorphisms were defined. Recently, a different form of classification of human blood groups has been possible. More than 250 human blood group antigens have been classified into 23 blood group systems by the International Society of Blood Transfusion Working Party on Terminology for Red Cell Surface Antigens [2].

The ABO blood group system has proven to be one of the most conventional polymorphisms, versatile and long-lasting in forensic serology, blood transfusion, paternity testing, anthropologic investigation, genetic prophecy, and personality prediction because of the inherent stability of the antigens involved, the ability to readily obtain results from dried blood stains, and the availability of known blood types from the other records [3-6]. This system involves 3 antigens: A, B, and H. The H antigen is converted to A or B by $\alpha 1 \rightarrow$ 3-N-acetyl-galactosaminyl transferase (A transferase) or $\alpha 1 \rightarrow 3$ galactosyl transferase (B transferase), respectively. The A, B, and O alloantigens are coded by the *A, B,* and *O* genes, respectively, on the human chromosome 9 [7]. All the methods for determining ABO types are designed to detect antibody or antigen materials and have been widely adopted for several decades [8,9]. But even if these traditional methods are modified [10,11], they can detect only ABO phenotypes. In 1990, Yamamoto

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et al [12,13] determined the molecular basis of glycosyl transferase genes involved with the ABO system. They cloned and sequenced a complementary DNA (cDNA) encoding the A_1 transferase and subsequently cloned and analyzed both *B* and *O* allelic cDNAs as well as the amino acid residues conferring the different enzymatic activities. They found a single-base deletion (nucleotide position 261) in the *O* gene and 4 single-base substitutions (nucleotide positions 526, 703, 796, and 803) between the *A* and *B* genes. The coding sequence of the transferase is divided into 7 exons. Exons 6 and 7 encode the bulk of the enzyme (13% and 65%, respectively), including its active site. The residues critical for determining donor substrate specificity (ie, A versus B activity) are found at amino acid positions 266 and 268 [14,15]. After the elucidation of the 3 major alleles at the nucleotide level, demonstration of the molecular bases for several minor alleles, including A^1, A^2, A^3, A^{el}, *cis-AB, B,* $B^{(A)}$, B^3, O^1, O^{iv}, O^2, O^3, etc, soon followed [16-23].

Several polymerase chain reaction (PCR)-based techniques, such as restriction fragment length polymorphism (RFLP) [24], single-strand confirmation polymorphism [25], allele-specific PCR [26], denaturing gradient gel electrophoresis [27], amplified product length polymorphism [23], and others, have all taken advantage of altered restriction enzyme recognition sites caused by the substitutions to genotype the ABO locus for the *A, B,* and *O* alleles.

There have been no previous studies on genotyping of ABO alleles and their frequencies in the Kuwaiti population. The State of Kuwait is an Arab country located northwest of the Arabian Gulf. The surface area spans 17,818 km^2 with a population of 2.275 million (2001 census, Ministry of Planning, State of Kuwait). The population comprises approximately 37.8% Kuwaitis and 62.2% expatriate workers of various nationalities, the majority of whom are Arabs and Asians. Kuwait is an oil-producing country that is highly urbanized. Because of their homogeneity and close social structure, residents of Kuwait appear to be exposed to a relatively common environment. Therefore, this environmental uniformity will be useful in determining whether there are any ethnic variations distinguishing this population. The present paper describes the result of a study on the allele frequencies and molecular genotyping of the ABO blood groups in a Kuwaiti population using PCR-RFLP linked to the major alleles A, B, and O.

2. Materials and Methods

2.1. Allele Frequencies

The sample used for estimating the allele frequencies of the ABO blood groups consisted of 18,558 randomly selected healthy Kuwaiti blood donors. The information about their ABO data was taken from the computer service in the Central Blood Bank of Kuwait. Maximum likelihood estimates of gene frequencies in the ABO system were calculated, estimating gene frequencies from observed phenotype frequencies and the equations for Hardy-Weinberg equilibrium. Standard errors and the chi-square test for goodness of fit of observed and expected (using estimated values) phenotype numbers were also calculated.

Table 1.
Sequences of Primers Used for PCR Amplification of ABO Gene Locus*

Primer	Name	Sequence	Length
ABO-1	A7500	5′-CACCGTGGAAGGATGTCCTC-3′	20
ABO-2	A7501	5′-AATGTCCACAGTCACTCGCC-3′	20
ABO-3	A7502	5′-TGGAGATCCTGACTCCGCTG-3′	20
ABO-4	A7503	5′-GTAGAAATCGCCCTCGTCCTT-3′	21

*PCR indicates polymerase chain reaction.

2.2. DNA Samples

EDTA blood samples were collected from 101 healthy unrelated Kuwaiti blood donors from the Kuwait Central Blood Bank. Four different ABO phenotypes (A, 29; B, 23; AB, 14; O, 35) were determined by the routine serological typing method. The preparation of genomic DNA from leukocytes of the blood samples was performed using the standard phenol-chloroform extraction method [28] and a commercially available DNA extraction kit (Nucleospin, Biogene Limited, Cambs, UK). Extracted DNA samples were then diluted to approximately 100 ng/μL.

2.3. Amplification of DNA Fragments

The standard method of DNA amplification by PCR described by Lee and Chang [24] for the ABO gene locus was used with some modifications for optimization. Two DNA fragments of the ABO locus were designed to be amplified by 2 pairs of primers as described in Table 1 (synthesized by Oswel, Southampton, UK). Primers ABO-1 and ABO-2 were used for amplification of the 200-bp fragment including the 258th nucleotide. Primers ABO-3 and ABO-4 were used for amplification of the 128-bp fragment including the 700th nucleotide. Amplification was accomplished in 100 μL of reaction mixture containing 50 ng of extracted DNA, 10 μL of PCR reaction buffer II (final concentration: 10 mM Tris-HCl, pH 8.3 at 25°C, 50 mM KCl, 3.5 mM $MgCl_2$, and 0.1% wt/vol gelatin), 200 μM of each dNTP, 2 units of ampli-Taq gold DNA polymerase (Perkin-Elmer, Boston, MA, USA), and 15 pmol of each primer. A total of 35 cycles of amplification were carried out, but the conditions were varied with each set of primers. For primers ABO-1 and ABO-2, denaturation was carried out for 2 minutes at 94°C, primer annealing for 2 minutes at 55°C, and extension for 3 minutes at 72°C. For primers ABO-3 and ABO-4, denaturation was carried out for 2 minutes at 94°C, primer annealing for 2 minutes at 58°C, and extension for 3 minutes at 72°C. The amplified products were checked by electrophoresis in 2% agarose gel (Pharmacia, Uppsala, Sweden) for 2 hours at 150 V and direct visualization of the products with ethidium bromide under UV light.

2.4. RFLP Analysis

The amplified products of primers 1 + 2 were digested with *Kpn*I, and products of primers 3 + 4 were digested with *Alu*I. Ten microliters of each PCR amplified product was digested with 5 units of either of the restriction enzymes *Kpn*I or *Alu*I (Oswel) for 1 hour. The digested amplified

DNA was then run on a 3% Nusieve 3:1 agarose gel in TBE buffer (0.89 M Tris-HCl, 0.089 M borate, 0.002 M EDTA, pH 8.0) at 50 V for 1 hour. The gel was stained for 15 minutes in 0.5 μg/mL ethidium bromide, and the bands were visualized by UV light. A 100-bp ladder (Pharmacia) was used as a size marker for estimation of fragment sizes.

3. Results and Discussion

3.1. Phenotypes and Gene Frequencies

No previous data are available about the distribution of phenotypes and gene frequencies of the ABO blood

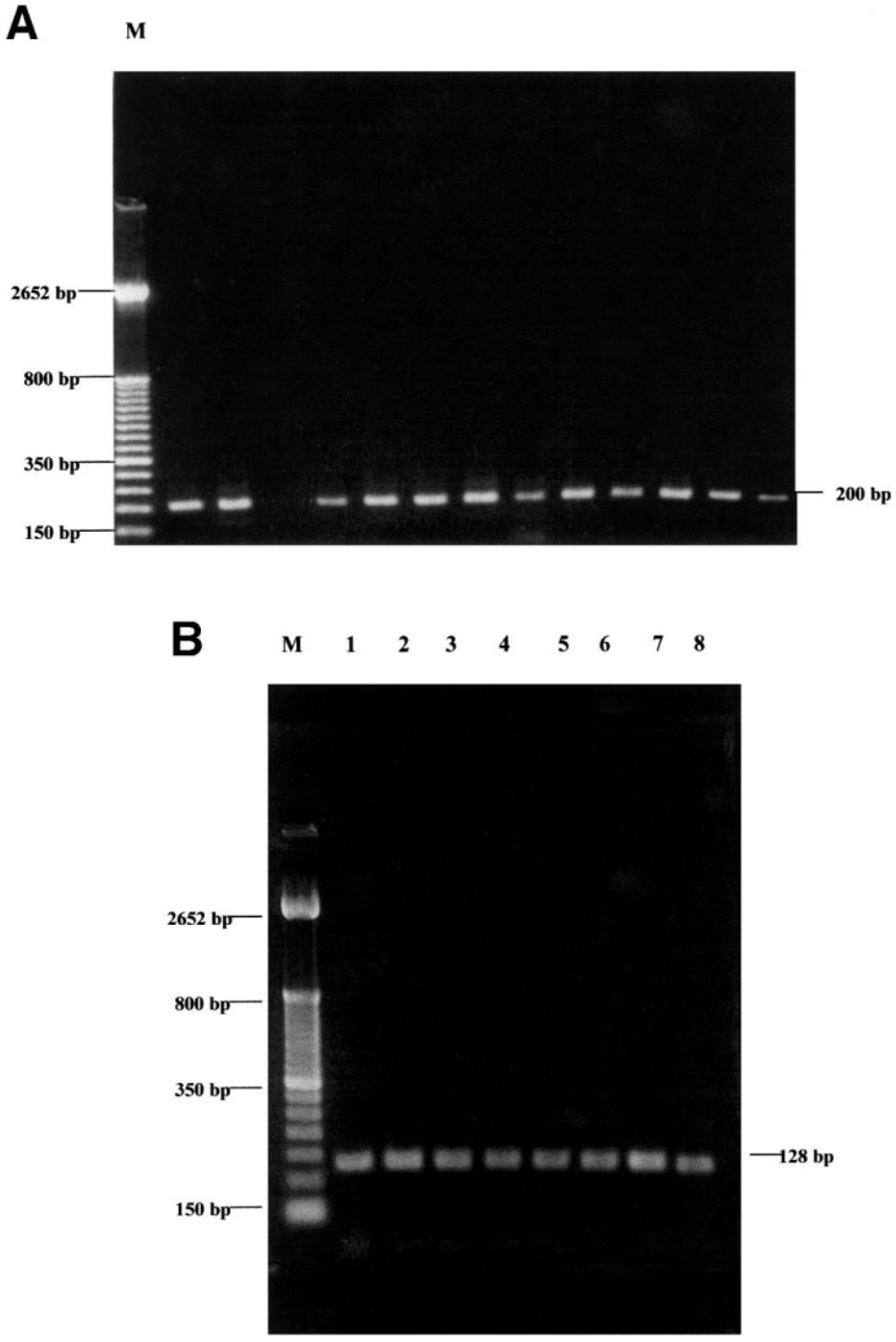

Figure 1. Polymerase chain reaction products of the ABO gene locus as visualized on a 2% agarose gel stained with ethidium bromide (10 mg/mL). A, The 200-bp amplification products using primers ABO-1 and ABO-2. B, The 128-bp amplification products using primers ABO-3 and ABO-4. (M is the lane with the 50-bp step DNA ladder)

Table 2.
ABO Genotype Interpretation by Restriction Enzyme Digestion of PCR Products*

	Possible Genotypes Predicted By:	
Restriction Enzyme Digestion	*Kpn*I	*Alu*I
Complete digestion	OO	BB
Half digestion	AO, BO	AB, BO
No digestion	AA, AB, BB	AA, AO, OO

*PCR indicates polymerase chain reaction.

Table 3.
Pattern of Restriction Fragments Visualized After Digestion of the PCR Product*

ABO Genotype	*Kpn*I	*Alu*I
OO	171, 28	128
AA	200	128
AO	200, 171, 28	128
BB	200	88, 40
BO	200, 171, 28	128, 88, 40
AB	200	128, 88, 40

*Restriction fragments sizes are in base pairs. PCR indicates polymerase chain reaction.

group system among the Kuwaiti population. According to the present study, distributions of observed numbers of ABO phenotypes and their percentages among 18,558 Kuwaiti subjects are 4962 (26.7%) A, 4462 (24.1%) B, 858 (4.6%) AB, and 8276 (44.6%) O. An order of O > A > B > AB is observed. The chi-square test for goodness of fit between the observed and expected phenotypes is not statistically significant ($\chi^2 = 2.37$; $df = 2$; $.50 > P > .20$). Using the maximum likelihood method, the frequencies of the I^O, I^A, and I^B alleles were calculated and tested according to the Hardy-Weinberg law of equilibrium. The calculated gene frequencies are 0.6678 for ABO*O, 0.1768 for ABO*A, and 0.1554 for ABO*B ($\chi^2 = 3.013$; $df = 2$; $.50 > P > .20$). As it can be seen, the gene *O* (r) records the highest value in Kuwaiti population, followed by *A* (p) and *B* (q).

3.2. Molecular Genotyping

According to Yamamoto et al [13,14], there are 4 consistent nucleotide substitutions of A transferase leading to amino acid changes (residues 176, 235, 266, and 268) to form B transferase, B allelic cDNA. Formation of O allelic cDNA is due to the deletion of nucleotide position 258, which causes the loss of transferase activity. In the present study, we applied the PCR-RFLP method for the analysis of nucleotide positions 258 and 700 of cDNA from A transferase in a Kuwaiti sample population to determine the ABO genotyping. Two regions of the ABO glycosyltransferase gene were amplified, each containing a diagnostic restriction enzyme site (Figure 1).

Primers 1 and 2 amplified a 199- or 200-bp DNA fragment containing the position of nucleotide 258 of cDNA from the ABO locus. If the 258th nucleotide does not exist, the PCR product should be an 199-bp *O* allele–specific fragment, which will create a *Kpn*I cleavable site on the *O* allele–specific sequence. But if the 258th nucleotide exists, the fragment should be 200 bp, and there would be no *Kpn*I site. Therefore, if this fragment was completely digested with *Kpn*I, 171- and 28-bp fragments were found and recognized as homozygote *OO*, and the 171-bp fragment was used as an *O* allele marker. If the fragment was half digested, 200-, 171-, and 28-bp fragments were found and recognized to be heterozygote *AO* or *BO*. If no digestion occurred, no *O* allele was confirmed and genotypes *AA, AB,* and *BB* were possible (Table 2).

Primers 3 and 4 amplified a 128-bp fragment containing the position of nucleotide 700 of cDNA from the ABO locus. If the 700th nucleotide is A, this fragment should be *B* allele specific, and there would be a *Alu*I cleavable site on the *B* allele–specific sequence. Therefore, if this fragment was completely digested with *Alu*I, 88- and 40-bp fragments were found and recognized as homozygote *BB,* as shown in Table 2, and the 88-bp fragment was used as a *B* allele marker. If the fragment was half digested, 128-, 88-, and 40-bp fragments were found to be heterozygote *AB* or *BO*. If there was no digestion with *Alu*I, no B allele was confirmed and genotypes *AA, AO,* and *OO* were possible. If neither 200- nor 128-bp fragments could be digested, homozygote *A* allele was determined. Figure 2 shows the electrophoresis patterns of digested DNA from individuals with different ABO genotypes. By examining the digested patterns of these 2 fragments, ABO genotypes were easily determined, as shown in Table 3.

The results of ABO molecular genotyping of the 101 blood samples from Kuwaiti blood donors are shown in

Table 4.
Distribution of ABO Genotypes and Allele Frequencies in the Kuwaiti Population*

	Observed		Expected		
Genotypes	n	%	n	%	*P*
AA	4	3.97	4.34	4.30	>.80
AO	25	24.75	24.65	24.41	>.90
BB	3	2.97	4.20	4.16	>.60
BO	20	19.80	24.26	24.02	>.40
AB	14	13.86	8.55	8.46	>.05
OO	35	34.65	35.00	34.65	>.99
Total	101	100.00	101	100.00	>.40

*Allele frequencies: I^A (*p*) = 0.207; I^B (*q*) = 0.204; I^O (*r*) = 0.589 ($\chi^2 = 4.589$, $df = 5$, $P > .40$).

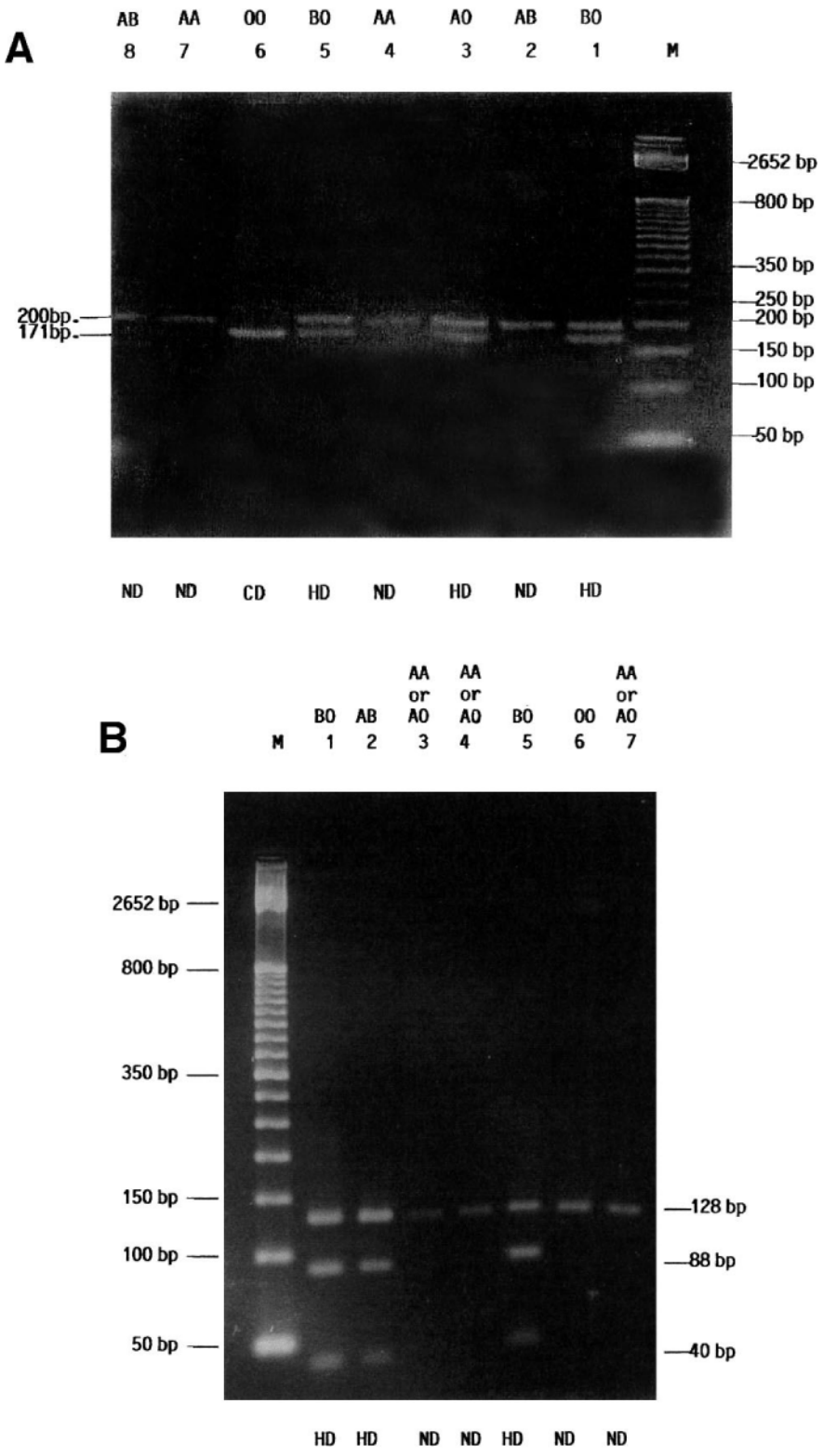

Figure 2. Restriction fragment patterns of the digested polymerase chain reaction (PCR) products obtained with the restriction enzymes as visualized on a 3% nusieve agarose (3:1) gel stained with ethidium bromide (10 mg/mL). The different lanes represent the different samples analyzed and their ABO genotypes. A, Restriction fragments obtained from the digestion of the ABO-1 and ABO-2 PCR products with *Kpn*I. B, Restriction fragments obtained from the digestion of the ABO-3 and ABO-4 PCR products with *Alu*I. (M is the lane with the 50-bp step DNA ladder.) CD indicates complete digestion; HD, half digestion; ND, no digestion.

Table 5.
ABO Allele Frequencies in the Kuwaiti Population and in Some Asian, African, and European Populations

Population	Allele Frequencies			Reference No.
	O	A	B	
Asia				
Kuwaiti	0.668	0.177	0.155	Present study
Saudi Arabian	0.708	0.137*	0.155	[3,4,29]
Iraqi	0.611*	0.212*	0.177	[3,4,29]
Iranian	0.525*	0.246†	0.229†	[3,4,29]
Indian	0.583†	0.149*	0.269†	[29,31]
Japanese	0.535†	0.288†	0.178	[3,4,29,32]
Chinese	0.599†	0.204†	0.197	[3,4,29]
Africa				
Egyptian	0.663	0.188	0.149	[3,4,29,30]
Sudanese	0.668	0.192	0.140	[3,4,29,30]
Europe				
Mean frequencies of European populations	0.632	0277†	0.090†	[29]

*$P < .05$.
†$P < .01$.

Table 4. The ABO phenotypes were determined serologically and examined by the present molecular method. Of 28 individuals with phenotype A, 4 (3.97%) were *AA* and 25 (24.65%) were *AO*. Of 23 individuals with phenotype B, 3 (2.97%) were *BB* and 20 (19.80%) were *BO*. Statistical analysis indicated that these data are in good agreement with the ratio calculated from the estimated gene frequencies of the ABO blood group system in the Kuwaiti population.

One or 2 blood group systems give a far from complete picture of genetic variation within a population. However, because the ABO system has been so thoroughly investigated, the present results can be compared to those from other parts of the world. Table 5 compares the ABO allele frequencies of the Kuwaiti population with populations from some Asian, African, and European countries. There is a statistically significant difference in allele frequency distribution between the Kuwaiti population and the 3 neighboring countries, Saudi Arabia, Iraq, and Iran [3,4,29]. This difference may be due to the origin of Kuwaiti population. To obtain a clear picture of the Kuwaiti population, one must consider its background. The Kuwaiti population is a relatively young population that has roots originating from neighboring countries. The nucleus population of Kuwait State originated from small subpopulations that migrated mainly from Saudi Arabia, Iraq, and Iran, with some from India, Palestine, Syria, and Lebanon, forming a new heterogeneous population. This new population has increased rapidly over the last 4 decades, from 298,546 to 859,958 (according to the 2001 census), many of whom married into the Kuwaiti population, hence increasing the heterogeneity of the Kuwaiti population. This increased heterogeneity would contribute to a new ABO blood group distribution as observed in this study. Surprisingly, these figures from the Kuwaiti population show an ABO distribution similar to that of Egyptian and Sudanese populations from the Nile valley [3,4,30]. This similarity may due to the occurrence since the 1980s of more and more admixture, that is, marriages, between the members of the subpopulations and of other groups, especially from Egypt. However, in view of the population structure in Kuwait and its limited interaction with the Egyptian population, it is more likely that this similarity is accidental rather than an effect of intermarriages between the 2 populations.

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