Application Value of Blood Types Gene Detection in Blood Transfusion of Patients with ABO Blood Type Identification Difficulty

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The human red blood cell blood group system plays an extremely important role in blood transfusion medicine and the ABO blood group system is of great significance in clinical blood transfusion, organ transplantation, forensic medicine and genetics research. The aim of this study was to study the application of genotyping technology in difficult ABO blood types identification. A total of 12 patients were selected in our hospital from August 2019 to August 2020. Among them, 1 patient with peptic ulcer, 1 patient with chronic kidney disease stage V, 2 patients with diabetes, 1 patient with infertility, 3 patients with anemia, 1 patient with primary liver cancer, 2 patients with leukemia and 1 patient with systemic lupus erythematosus. Polymerase chain reaction-sequence specific primers genotyping method was used to detect these patients with difficult ABO blood type antigens and statistical method was used to analyze the correlation of ABO genotypes and difficult ABO blood types. The specific distribution of ABO genotypes in 12 patients were detected as follows: 6 cases of A/B, 3 cases of A/O and 3 cases of B/O. The genotype detected by polymerase chain reaction-sequence specific primers was consistent with serological phenotype. As an auxiliary method, ABO genotyping can be used to detect difficult ABO blood types.

Key words: Genotyping technique, ABO genotyping, polymerase chain reaction-sequence specific primers, blood group serology

The blood group system of human red blood cells plays an important role in blood transfusion medicine. At present, 36 human erythrocyte blood group systems have been detected, among which ABO blood group system is the earliest and most important one[1]. It is of great significance in clinical blood transfusion, organ transplantation, forensic medicine and genetics research[2,3]. ABO blood groups in addition to the most common A, B, O and AB types, there are also some subtypes, such as A_2, A_3, A_m, A_x, AEL, aAnt, B_3, B_m, B_x, CisAB, B (A), etc.[4]. The serological manifestations of these subtypes are often inconsistent, which brings great difficulties to clinical blood group identification and cross matching. Serological detection can fully understand the situation of ABO antigen and antibody, but the analysis of the causes of the positive and negative stereotypes in the detection is easily affected by a variety of factors, such as disease status, recent blood transfusion history, lack of pedigree, unable to analyze heredity, etc. However, gene detection can make up for the shortage of serological detection and provide a new detection method for the samples with difficult serological identification. In recent years, the continuous development of deoxyribonucleic acid (DNA) molecular detection technology provides a new possibility for the accurate identification of these difficult ABO blood group samples[5,6]. Therefore, the stable polymerase chain reaction-sequence specific primers (PCR-SSP) technology was used to determine the ABO genotypes of difficult ABO blood group samples in our hospital and the identification of 12 difficult ABO blood group samples was solved. From August 2019 to August 2020, 12 blood samples of clinical patients from various departments of our hospital were selected and all of them were anti-coagulated with dipotassium ethylenediamine tetraacetic acid (K2-EDTA). Serological typing of blood samples by routine serological techniques was

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carried out. The anti-A antibody, anti-O antibody, anti-B antibody, anti AB antibody and anti-H antibody used in all blood samples were prepared by the same company and the ABO reagent used for detection was prepared by our hospital. DNA extraction was carried out. The target gene was extracted from 5 ml whole blood of 12 patients anti-coagulated with K2-EDTA by guanidine hydrochloride/protease K cleavage extraction method and about 300 µl DNA was finally obtained. Specific primers were designed according to ABO nucleotide sequence and human growth hormone (HGH) was used as internal control gene. PCR amplification: 1 µl of extracted target gene, 1 µl of Taq polymerase (DNA polymerase) purchased by Promega company and 7 µl of human leukocyte antigen (HLA) genotyping primers produced by sigma company mixed with specific primers, deoxynucleotide triphosphates (dNTPs), buffer and internal control primers were used under hot start technology. The total volume of PCR reaction was 9 µl. The PCR reaction was carried out at 95° for 5 min. After preheat, denatured at 95° for 30 s, annealed at 60° for 30 s and extended at 72° for 1.5 min. The above operation process was cycled for 30 times, extended at 72° for 5 min and finally cooled to 4°. The sequence is shown in Table 1. All the primers were prepared in the kit of G & T Company. Gel electrophoresis of the final products obtained from PCR was carried out using 2 % agarose gel prepared from the same buffer. After ethidium bromide (EB) staining, 20 min electrophoresis was performed at 100 V voltage and the electrophoresis results were recorded under ultraviolet lamp. The inner control band appeared under ultraviolet (UV) transmission, indicating that PCR amplification was successful. The results of electrophoresis showed that those with specific amplification products were positive. The serological phenotypes of 12 clinical patients included 3 cases of AB, 2 cases of O, 3 cases of A and 4 cases of B. Analysis of serological phenotype in 12 clinical patients was shown in Table 2. The genotypes of 12 patients were A/B in 6, A/O in 3 and B/O in 3. The genotypes detected by PCR-SSP were consistent with serological phenotypes, as shown in Table 3. There are many kinds of antigens on the surface of human red blood cells, which are classified and combined by alloantigens, namely blood group system. There are more than 80 known blood group systems, including ABO, MNS, P, Rhesus (Rh), Lutheran, Kell, Lewis, Duffy, Kidd, Hh/Bombay, etc. These systems exist independently and the genes controlling the inheritance of all blood group systems are located on the same chromosome[7,8]. The most important blood group system in clinic is ABO system and Rh system. ABO system also has a variety of phenotypes and subtypes. ABO genetic gene is located in 9q34.1-9q34.2, with high homology, only a single base or several base substitutions. It is a highly conserved nucleotide sequence and its molecular basis is that highly homologous glycogen interacts with H under the action of galactosyltransferase. The precursor is formed by the linkage of galactose. Studies have shown that the identification of serological ABO subtypes is unreliable. Although there are many reports of ABO system subtypes in China, these reports often lack the experimental results of nucleotide mutation and the serological detection is affected by different monoclonal antibodies, different reagent specificity, antigen gene change, different laboratory detection conditions and even different operator operation[9,10]. We think that the blood samples with irregular reaction of antigen and antibody should be further genotyped and the experimental results should be used as a reliable basis for subtype diagnosis. For the units with poor experimental qualification, such blood samples should be sent to the regional or national reference laboratory for further confirmation, so as to prevent misdiagnosis. Since anti-A, anti-B and anti AB antibodies naturally exist in human serum, even if only a few milliliters of mismatched blood are injected, it can cause serious hemolytic reaction and even cause serious consequences of patient death[11-13]. Therefore, it is very important to determine ABO blood group in clinical practice. In this study, stable PCR-SSP method was used to identify ABO blood group subtypes accurately, which can exclude the errors caused by autoantibodies or irregular antibodies and also excludes the changes of ABO blood group subtypes caused by leukemia, myelodysplastic syndrome and other tumors. In addition, PCR-SSP method is not interfered by bacteria and hemoglobin disorder and has low requirements for samples. All nucleated cells can be used as experimental samples, including skin follicles, blood stains, amniotic fluid, tissue blocks, seminal spots, etc. qualified experimental results can be obtained by PCR amplification. At the same time, PCR-SSP technology is easy to obtain, preserve and transport samples and the results of experiments in different laboratories are comparable, which can make up for the lack of conventional serological detection. Different from conventional serological detection, which can only identify the phenotype of blood samples, PCR-SSP technology can accurately identify blood group subtypes, heterozygotes and homozygotes, which is of great significance for

difficult to identify subtypes and prenatal fetal blood group diagnosis and its wide range of materials also enables it to play a role in forensic identification, genetic identification, etc.[14-16]. The results of this study showed that the serological phenotypes of 12 clinical patients included 3 cases of AB, 2 cases of O, 3 cases of A and 4 cases of B. The genotypes of 12 patients were detected by PCR-SSP, including 6 cases of A/B, 3 cases of A/O and 3 cases of B/O. The genotype detected by PCR-SSP was consistent with serological phenotype. Accurate identification of ABO blood group is the key to clinical safe blood transfusion[17]. If blood group identification is wrong, it may cause serious transfusion reaction and even lead to the death of patients. If the clinically difficult ABO blood group cannot be distinguished by serological means, it must be identified by molecular biological detection[18]. Molecular biology detection can significantly reduce the false detection rate of ABO blood group identification, which is of great significance for guiding clinical blood transfusion and more effective use of rare blood group resources and also provides the basis for individualized precision blood transfusion[19], further making clinical blood use accurate, reasonable and efficient.

TABLE 1: PRIMER SEQUENCES

Gene	F	R
HGH	5'-GGCCTTCCCAACCATTCCCTTA-3'	5'-CTCACGGATTTCTGTTGTGTTTC-3'
Mix-15i	5'-AGGAAGGATGTCCTCGTGGTA-3'	5'-GGCCCACGTGGTCGCGGAAC-3'
Mix-25i	5'-GGAAGGATGTGCTCGT-GGTGA-3'	5'-GCCCCGAAGAACCCCCCCAG-3'
Mix-35i	5'-GGAAGGATGTGCTCGTGGTGA- 3'	5'-GGCCCACGTGGTCGCGGATC-3'
Mix- 45i	5'-AGGAAGGATGTCCTCGTGGTA-3'	5'-GGCCCACGTGGTCGCGGATC-3'
Mix-55i	5'-GGAAGGATGTGCTCGTGGTGA-3'	5'-GCCCCGAAGAACGCCCCCAT-3'
Mix-65i	5'-GGAAGGATGTGCTCGTGGTGA-3'	5'-GTGCTTCCG-TAGAAGCTGGG-3'

TABLE 2: ANALYSIS OF SEROLOGICAL PHENOTYPE IN 12 CLINICAL PATIENTS

Number	Source of specimen	Serological phenotype
1	Peptic ulcer	AB
2	Chronic kidney disease stage 5	O
3	Diabetes	A
4	Infertility	B
5	Diabetes	AB
6	Anemia	B
7	Anemia	B
8	Primary liver cancer	O
9	Anemia	A
10	Leukemia	AB
11	Systemic lupus erythematosus	A
12	Leukemia	B

TABLE 3: ANALYSIS OF GENOTYPES AND PCR RESULTS IN 12 PATIENTS

Number	Source of specimen	Genotype	PCR primer			
			A	AB	B	O
1	Peptic ulcer	A/B	+	-	+	-
2	Chronic kidney disease Stage 5	A/B	+	-	+	-
3	Diabetes	A/O	+	-	-	+
4	Infertility	B/O	-	-	+	+
5	Diabetes	A/B	+	-	+	-
6	Anemia	B/O	-	-	+	+
7	Anemia	B/O	-	-	+	+

8	Primary liver cancer	A/B	+	-	+	-
9	Anemia	A/O	+	-	-	+
10	Leukemia	A/B	+	-	+	-
11	Systemic lupus erythematosus	A/O	+	-	-	+
12	Leukemia	A/B	+	-	+	-

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Conflicts of interest:

The authors report no conflicts of interest.

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