DNA-based genotyping to assign extended blood group in thalassemic patients

Nada O. Abdelhameed[a], Maha Atwa[a], Eman Nasreldin[a], Khalid I. Elsayh[b], Sahar A. Elgammal[a]

Departments of [a]Clinical Pathology, [b]Pediatric Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Nada O. Abdelhameed, MSc, Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt. Tel: +20 106 693 7438; fax: 0882333327; e-mail: drnadaosman911@gmail.com

Received: 26 July 2020
Revised: 16 August 2020
Accepted: 1 November 2020
Published: xx xx 2020

Journal of The Arab Society for Medical Research 2020, 15:48–55

Background/aim
In multitransfused thalassemic patients, it is mandatory to assign a correct antigen profile for those patients. Unfortunately, hemagglutination fails to phenotype their blood group antigens owing to the presence of donor-derived red blood cells (RBCs). Genotyping can overcome this limitation to determine the correct antigen profile of these patients. The aim of this study is to compare the results of serological phenotyping and DNA-based red cell antigens genotyping in thalassemic patients.
Patients and methods
This study was conducted on 210 thalassemic patients who were divided into two groups, viz., the newly diagnosed patient group (n=20) and another 190 patients who were previously diagnosed with a history of blood transfusion, where 25 patients of them were selected with a negative screening test for antibodies. Two standard methods, the serological phenotyping and the DNA-based red cell genotyping, of 16 blood group antigens were performed.
Results
The present study indicated that there were no significant differences between serological phenotyping and DNA-based red cell genotyping results in the newly diagnosed patients with thalassemia (group 1) who had no history of blood transfusion. However, DNA-based genotyping was found to be significantly superior over the serological phenotyping for detection of some significant antigens in previously diagnosed transfused patients.
Conclusion
Hemagglutination is regarded as the gold standard method in blood group typing, but the results may be inaccurate in certain situations, particularly in the patients with transfused RBCs present in circulation. Hence, genotyping for RBC antigens could be more helpful in determining the blood group antigen profile in multiply transfused patients. Therefore, the integration between the serological phenotyping and DNA-based red cell genotyping will help in the correct interpretation of the results to increase transfusion safety.

Keywords:
Blood group antigens, DNA-based genotyping, serological phenotyping

J Arab Soc Med Res 15:48–55

1687-4293

Introduction

β-thalassemias are a group of inherited hemoglobin disorders that constitute major challenges to health care systems across the world [1]. Despite improvements in the management of β-thalassemia, there are still many challenges to control the disease [2]. Undoubtedly, regular red blood cell (RBC) transfusion and effective iron chelation therapy are milestone in the management of patients with β-thalassemia. However, because of the recognition that RBC transfusion could also cause major complications, the practice of donation, processing, testing, storage, and infusion has gone through major changes in the past decades. These improvements have resulted in much safer practice [3].

Alloimmunization is one of the most common complications that increase with repeated transfusions [4], and chronically transfused patients are at higher risk of alloimmunization, for whom the development of multiple antibodies may lead to death [5].

The risk depends on recipient's exposure to foreign antigens, their immunogenicity, number, frequency of transfusions, and genetic disparity between patient's and donor's antigen profile [6].

Most investigators agree that the extended RBC typing (beyond the routine ABO/D typing) has a beneficial effect on the incidence of alloimmunization in patients

with thalassemia major [7]. Serological testing (hemagglutination) is the conventional method used to determine extended RBC typing; this method relies on the use of monoclonal or polyclonal antibodies designed to detect specific epitopes of the antigens on the red cell surface [8].

Unfortunately, in multitransfused thalassemic patients, hemagglutination fails to phenotype the patient's blood group antigens owing to the presence of donor-derived erythrocytes. DNA-based methods can overcome the limitations of hemagglutination and can be used to determine the correct antigen profile of these patients [9].

Routine use of DNA-based extended phenotyping to provide optimally matched donations for patients with preexisting antibodies or patients with a known predisposition to alloimmunization, such as those with thalassemia, could limit post-transfusion complications by understanding antigenic differences between donors and patients [10]. Moreover, those patients could benefit from receiving extended genomic typing that can improve survival of transfused units of RBC and lead to reduced need for blood transfusion, less iron overload, and reduced risk of alloimmunization [10].

Therefore, the aim of this study is to compare the results of serological phenotyping and DNA-based red cell antigen genotyping in thalassemic patients to identify which cases should proceed into molecular testing.

Patients and methods

Patients

The present study was targeted on 210 thalassemic patients at Assiut University Hospital, Egypt, during the period from January 2019 to December 2019.

Inclusion criteria

The following were the inclusion criteria:

(1) Egyptian patients diagnosed as β-thalassemia major or intermediate.
(2) Patients with no history of transfusion (first group).
(3) Multitransfused patients for at least 1 year (second group).

Exclusion criteria

The following were the exclusion criteria:

(1) Patients have other hemoglobinopathies.
(2) Thalassemia trait.
(3) Patients with positive screening test (second group).

Study design and sampling

This study was conducted on 210 thalassemic patients who divided into two groups as follows:

First group: this included 20 newly diagnosed thalassemic cases (β-thalassemia major/intermedia) with no history of blood transfusion. The ages of patients ranged between 1 year and 29 years old. There were 10 (50%) males and 10 (50%) female children. There were 11 (55%) cases of thalassemia major and nine (45%) cases of thalassemia intermedia.
Second group: Included one hundred and ninety thalassemia patients; beta thalassemia major or intermediate who had received transfusion at the Pediatric Haematology Unit during the period of the study. Their ages ranged between 2 years to 19 years old, 110 (57.9%) males and 80 (42.1%) female children. Antibody screening test was performed for all patients all over the period of the study for each time they admitted to the Pediatric Haematology Unit for transfusion to exclude those with a positive screening test. Twenty-five patients only were selected of this group with negative screening test for study analysis, there was 19 cases (76%) thalassemia major and 6 (24%) thalassemia intermedia.

Phenotyping and genotyping of 16 blood group antigens (D, C, c, E, e, Jk^a, Jk^b, Fy^a, Fy^b, Kell, Kp^a, Kp^b, M, N, S, and s) were performed on newly diagnosed patients and negative screened previously diagnosed and transfused patients to analyze the difference between their results.

Ethical consideration

The study was conducted according to the principles expressed in the Declaration of Helsinki. The Medical Ethical Committee of Faculty of Medicine Assiut University, Egypt, approved the protocol of this study, under the number, 17200485. Written informed consent was obtained from parents of each participant before inclusion in the study.

Serologic phenotyping

Serological phenotyping was done using fresh samples drawn into EDTA anticoagulant using ID-card (Diamed, Cressier, Switzerland) according to the manufacturers' instructions (Fig. 1).

Figure 1

(A) (B)

(a) Six cases performed on ID-card DiaClon Anti-M showing positive results in cases no. 1, 2, 3, and 4 and negative results in cases no 5 and 6. (b) Six cases performed on ID-card DiaClon Anti-Fy^b showing positive (agglutination dispersed in the gel) results in case nos. 2 and 5, whereas cases no 1, 3, 4, and 6 showing negative results.

Table 1 Primer sets used in our study (Biosearch Technologies-LGC)

	Antigen	Sequence (5′-3′)	Product size (bp)
1(mix 1)	Fy1	CAGCTGCTTCCAGGTTGGgAC CTCATTAGTCCTTGGCTCTTAT	713
	Jk1	GTCTTTCAGCCCCATTTGcGG CCAAGGCCAAGTGTCAGTGC	528
	MNS4	CGATGGACAAGTTGTCCCG GGAGTAATGGCTCCATATGCC	397
	KEL1	ACTCATCAGAAGTCTCAGCA CTAGAGGGTGGGTCTTCTTCC	322
	MNS1	CAGCATCAAGTACCACTGGT TTCAGAGGCAAGAATTCCTCCA	259
	KEL4	CAATCTCCATCACTTCACG CTGCCCGCACAGGTGGC	623
2(mix 2)	Fy2	CAGCTGCTTCCAGGTTGGgAT TCATTAGTCCTTGGCTCTTAT	712
	Jk2	AGTCTTTCAGCCCCATTTGcGA CCAAGGCCAAGTGTCAGTGC	529
	MNS3	CGATGGACAAGTTGTCCCA GGAGTAATGGCTCCATATGCC	397
	MNS2	TCAGCATTAAGTACCACTGAG TTCAGAGGCAAGAATTCCTCCA	260
	KEL3	TGTCAATCTCCATCACTTCAT CTGCCCGCACAGGTGGC	625
3	D	TAAGCAAAAGCATCCAA ATGGTGAGATTCTCCT	186
4	C	CGCTGCCTGCCCCTCTGC TTGATAGGATGCCACGAGCC	118
5	c	CTTGGGCTTCCTCACCTCAAA AAGCCGTCCAGCAGGATTGC	107
6	E	TGGCCACGTGTCAACTCTC CATGCTGATCTTCCTTTGGG	143
7	e	TGGCCACGTGTCAACTCTG CATGCTGATCTTCCTTTGGG	143

MNS1 ('M'), MNS2 ('N'), MNS3 ('S'), MNS4 ('s'), KEL1 ('K'), KEL3 ('Kpa'), and KEL4 ('Kpb'). Nucleotides written in small letters mark mismatches to the complementary genomic DNA sequence.

Molecular typing

Gene JET Whole Blood Genomic DNA Purification Mini Kits (catalog no. #K0781, #K0782; Thermo Scientific, Waltham, Massachusetts, United States) were used to extract DNA from the blood samples.

(1) DNA-based red cell genotyping studies were carried out by conventional qualitative PCR, some in multiplex reaction mixes, consisting of up to six different amplification targets per mix with PCR products lengths ranging from 259 to 713 bp, and others in a single reaction, using primers sets (Biosearch Technologies-LGC, Novato, California, United States), as shown in Table 1. The PCR products were analyzed using 2% agarose gel electrophoresis.

(2) PCR first mixture (six primer sets) contained MyTaq Red Mix (2×) (Bioline Reagents Ltd, Humber road, London, United Kingdom), 12.5 µl; forward primer (20 µmol), 3 µl (0.5 µl for each primer); reverse primer (20 µmol), 3 µl (0.5 µl for each primer); deionized water (RNase/DNase free), 3.5 µl; and DNA, 3 µl (with a total volume of 25 µl). PCR second mixture (five primer sets) contained MyTaq Red Mix (2×) (Bioline Reagents Ltd), 12.5 µl; forward primer

(20 µmol), 2.5 µl (0.5 µl for each primer); reverse primer (20 µmol), 2.5 µl (0.5 µl for each primer); deionized water (RNase/DNase free), 4.5 µl; and DNA, 3 µl (with a total volume of 25 µl). For others in a single reaction, their PCR mixture contained MyTaq Red Mix (2×) (Bioline Reagents Ltd), 12.5 µl; forward primer (20 µmol), 1 µl; reverse primer, (20 µmol) 1 µl; deionized water (RNase/DNase free), 7.5 µl; and DNA, 3 µl (with a total volume of 25 µl).

The amplification reaction was carried out in Veriti 96 well thermal cycler (Applied Biosystems, Foster city, California, USA) with different cycling conditions:

Multiplex reaction mixes: the temperature profile started with 5 min at 94°C followed by six cycles at 94°C for 30 s, 67°C for 40 s (touch down for 0.5°C per cycle), and 72°C for 50 s. Subsequently, 27 cycles at 94°C for 30 s, 64°C for 40 s, and 72°C for 50 s were added. The protocol ended with a final step at 72°C for 2 min [7].
D antigen: 95°C for 5 min; 35 cycles of 1 min at 95°C, 1.5 min at 49°C, and 2.5 min at 72°C, and finally, one cycle of 9 min at 72°C [11]. C, c, E, and e antigens: 94°C for 10 min, 30 cycles at 94°C for 30 s, 58°C for 45 s, and 72°C for 45 s, and finally, 5 min at 72°C [9].

PCR products were loaded into the wells together with a 100-bp molecular weight marker. Electrophoresis was allowed for 1 h at 110 V, and then the gel was inspected using an ultraviolet transilluminator (Fig. 2).

Statistical analysis

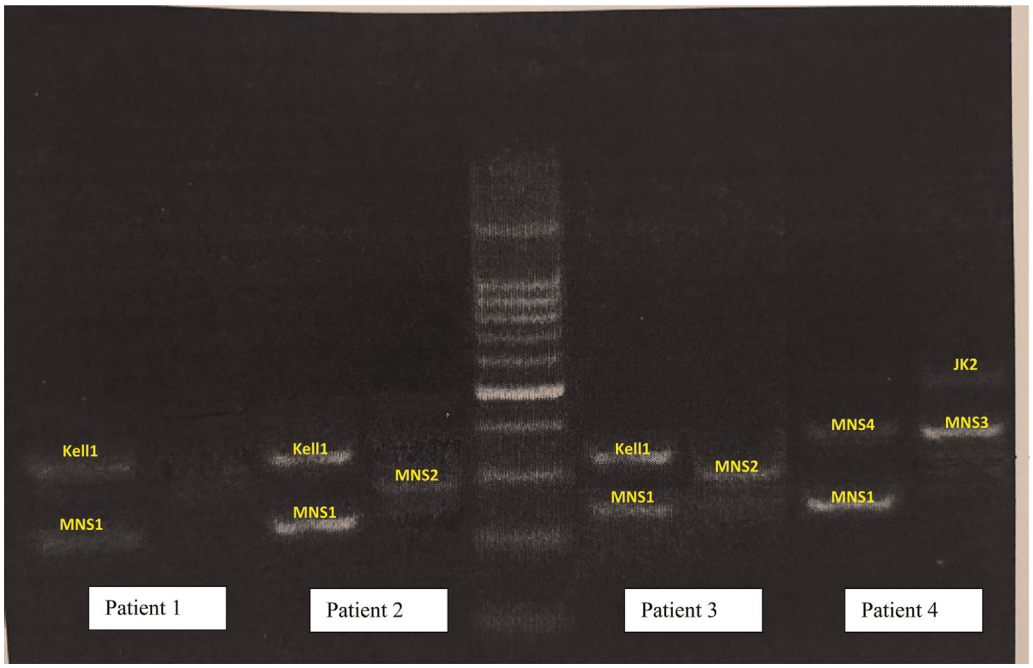
Data were analyzed using IBM SPSS, version 21 (SPSS Inc., Chicago, Illinois, USA). Quantitative variables were described as median and range. Qualitative data were expressed as frequency and percentage. χ^2 test was used to examine the relation between qualitative variables. *P* value of less than 0.05 was considered significant. The χ^2 test was used to compare results between two groups.

Results

In this study, comparison of the results of phenotyping and genotyping of different antigens for 16 blood group antigens in each group was performed. Regarding the results of the newly diagnosed thalassemic patients, there were insignificant differences between the results of phenotyping and genotyping of different antigens, except for Kp^b antigen, where its detection by phenotyping was significantly higher (20%) than genotyping (5%) at *P* value of 0.04, as shown in Table 2.

On the contrary, the results of previously diagnosed thalassemic patients (Table 3) indicated insignificant differences between phenotyping and genotyping of different antigens except for the following:

Figure 2



Multiplex PCR RBC antigens genotyping: ethidium bromide-stained agarose gel. Example for typing results of four patients (1–4), each in two lanes (first for mix. 1 and second for mix. 2), with a size ladder of 100 bp is running in the middle of the gel. RBC, red blood cell.

Table 2 Results of phenotyping and genotyping in the newly diagnosed group (first group)

Antigens	Phenotyping	Genotyping	*P* value
D	17 (85)	18 (90)	0.55
C	17 (85)	17 (85)	–
c	20 (100)	20 (100)	–
E	0	1 (5)	0.34
e	20 (100)	20 (100)	–
K	1 (5)	2 (10)	0.09
Kp^a	1 (5)	1 (5)	–
Kp^b	4 (20)	1 (5)	0.04
JK^a	11 (55)	13 (65)	0.23
JK^b	5 (25)	7 (35)	0.14
Fy^a	3 (15)	2 (10)	0.12
Fy^b	3 (15)	4 (20)	0.06
M	18 (90)	17 (85)	0.08
N	12 (60)	15 (75)	0.15
S	12 (60)	11 (55)	0.23
s	16 (80)	14 (70)	0.10

Data are expressed as frequency (%). *P* value was significant if less than 0.05.

Table 3 Results of genotyping and phenotyping results among the previously diagnosed group (second group) (*n*=25)

Antigens	Phenotyping	Genotyping	*P* valuePhenotypingGenotyping
D	21 (84)	23 (92)	0.45
C	19 (76)	20 (80)	0.56
c	16 (64)	24 (96)	<0.001
E	1 (4)	2 (8)	0.55
e	23 (92)	25 (100)	0.06
K	0	3 (12)	0.06
Kp^a	0	1 (4)	0.33
Kp^b	5 (20)	4 (16)	0.09
JK^a	8 (32)	16 (64)	<0.001
JK^b	1 (4)	5 (20)	0.04
Fy^a	2 (8)	4 (16)	0.35
Fy^b	5 (20)	8 (32)	0.07
M	19 (76)	24 (96)	<0.001
N	10 (40)	16 (64)	<0.001
S	12 (48)	9 (36)	0.06
s	17 (68)	16 (64)	0.09

Data are expressed as frequency (%). *P* value was significant if less than 0.05.

(1) c antigen, where its detection by genotyping was significantly higher (96%) than by phenotyping (64%) at *P* value less than 0.001.
(2) JK^a antigen, where its detection by genotyping was significantly higher (64%) than by phenotyping (32%) at *P* value less than 0.001.
(3) JK^bantigen, where its detection by genotyping was significantly higher (20%) than by phenotyping (4%) at *P* value of 0.04.
(4) M antigen, where its detection by genotyping was significantly higher (96%) than by phenotyping (76%) at *P* value less than 0.001.
(5) N antigen, where its detection by genotyping was significantly higher (64%) than by phenotyping (40%) at *P* value less than 0.001.

The present results indicated that on comparing the results of serological phenotyping and genotyping for 16 blood group antigens within both studied groups (*n*=45), it was noticed that there were insignificant differences between the results of both methods for the studied 16 blood group antigens among thalassemic patients, except for JKa antigen with *P* value = 0.01, as shown in Table 4.

Those significance differences mostly appear owing to the presence of mixed-field results, which were reported only with the second group (previously diagnosed group with blood transfusion history) for all blood group systems, with a total percent of 18.3, and with the highest frequency for Kidd system (32%),

Table 4 Results of genotyping and phenotyping results in the total studied patients (*n*=45)

Antigens	Phenotyping	Genotyping	*P* value
D	38/45 (84.4)	41/45 (91.1)	0.95
C	36/45 (80)	37/45(82.2)	0.22
c	36/45 (80)	44/45 (97.7)	0.05
E	1/45 (2.2)	3/45 (6.7)	0.11
e	43/45 (95.5)	45/45 (100)	0.10
K	1/45 (2.2)	5/45 (11.1)	0.22
Kp^a	1/45 (2.2)	2/45 (4.4)	0.20
Kp^b	9/45 (20)	5/45 (11.1)	0.06
JK^a	19/45 (42.2)	29/45 (64.4)	0.01
Jk^b	6/46 (13.3)	12/46 (26.6)	0.07
Fy^a	5/45 (11.1)	6/46 (13.2)	0.11
Fy^b	8/45 (17.7)	12/45 (26.6)	0.29
M	37/45 (82.2)	33/45 (73.3)	0.08
N	22/45 (48.8)	31/45 (68.8)	0.35
S	24/4 (55.3)	20/45 (44.4)	0.05
s	33/45 (73.3)	30/45 (66.6)	0.33

Data are expressed as frequency (%). *P* value was significant if less than 0.05.

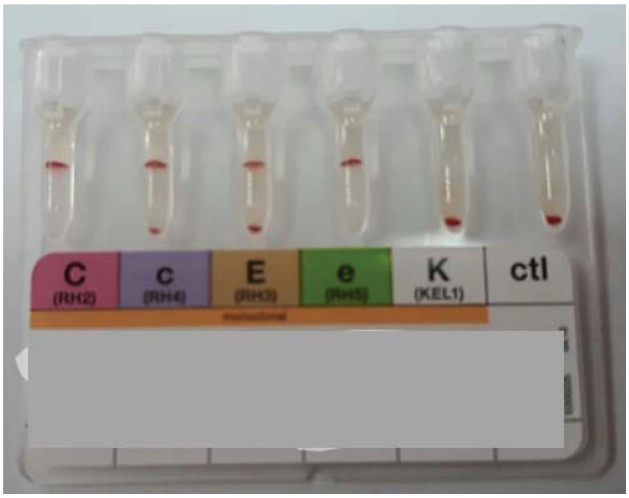
followed by Rh system, MNS system and Kell system (18.4, 16, and16%, respectively), as shown in Table 5 and Fig. 3.

Table 5 Frequency of mixed-field among previously diagnosed group (second group) for each blood group system

Antigens	Negative screening group (*n*=25)
Rh	23/125 (18.4)
MNS	16/100 (16)
Kidd	16/50 (32)
Duffy	6/50 (12)
Kell	12/75 (16)
Total	73/400 (18.3)

Data expressed as frequency (%).

Figure 3



A case performed on Rh phenotyping ID-card, showing positive results for C and e, mixed-field results for c and E antigens and negative results for KEL1 antigens with negative autocontrol (ctl).

Discussion

Accurate antigen typing by serology is difficult in case of multitransfused patients owing to the presence of donor RBCs in patient's circulation [9]. Accurate RBC typing of patients and blood donors is essential to prevent alloimmunization and hemolytic transfusion reactions. Beyond the basic level of ABO compatibility, numerous other antigens and alloantibodies are capable of causing both acute and delayed hemolytic transfusion reactions and may render it progressively more difficult to obtain compatible RBC units for a given patient [12].

Although the hemagglutination is regarded as the gold standard method in blood group typing, the results may be unreliable in certain situations, and molecular analysis can overcome these situations. DNA testing for red cell blood groups is not influenced by immunoglobulin coating of the RBCs, the presence of the recently transfused RBCs, or any form of polyagglutination, or by the limitations commonly found with the antisera, that is, no mixed-field reaction will occur, which leads to the undetermined result. DNA also can be easily extracted from any source such as whole blood, buffy coats, or buccal swab [8].

Our results indicate that there is no superiority for genotyping over phenotyping when performed to determine extended blood group typing for patients with no history of previous transfusion except for Kpb antigen. In agreement with our results, a previous study reported that among donors, the RH, KEL, FY, JK, and MNS genotypes were concordant with all the corresponding serological phenotypes [9], and in another one, there was a full agreement between phenotype and genotype of the 20 healthy volunteers [13].

On the contrary, the present results indicate that there is superiority for genotyping over phenotying when performed to determine extended blood group typing for patients with previous frequent transfusion in detecting some blood group antigens, little c, Jka and Jkb (Kidd system), and M and N antigens. In concordance with our results, Castilho *et al.* [14] showed that genotypes differed from the assumed phenotypes in nine of ten alloimmunized thalassemic patients. Moreover, in 2013, Bakanay *et al.* [15] showed that 19 out of 37 multitransfused patients had discrepancies between genotyping and phenotyping results in a total of 25 alleles.

The results indicate that there is superiority for genotyping over phenotying when performed to determine extended blood group typing for patients with previous frequent transfusion, in order to overcome mixed-field results and to avoid possibility of false results of phenotyping for those patients, which may cause hemolytic transfusion reaction if they have transfused with antigen mismatched RBC units [15].

Most of our patients were transfusion-dependent thalassemic patients who require a blood transfusion every 4 weeks or less. Based on the phenotype-genotype results, we have shown that there was mistyping when serological phenotyping was performed alone that could lead to false assignment of blood group systems especially when mixed-field reactions were recorded. Our study results are in agreement with the results of Osman *et al*. [13] and showed that mixed-field agglutination was observed in more than one antigen typing in all blood group systems, which makes difficulties in interpreting the patients' blood phenotype and selection of antigen-matched (antigen-negative) RBCs for regularly transfused patients, increasing the risk of alloimmunization. Genotyping can overcome mixed-field results and so avoid the possibility of false results of phenotyping for those patients [13].

It is well known that not all blood group antigens have the same significance in transfusion, and in our study, not all blood group antigens have significant difference between phenotyping and genotyping in their detection; only JKa, JKb, and little c antigens have a significant difference between the two methods in their detection and also have a great importance in transfusion. There were no previous studies that analyzed the difference between phenotyping and genotyping in detection of separate blood group antigens. However, previous studies showed the importance of these antigens in transfusion. The first study indicated that most transfusion reactions owing to JK incompatibility are delayed, although these also can be associated with rapid destruction of transfused red cells with accompanying oliguria, renal failure, and death [16]. However, another one showed that anti-c is clinically the most common Rh antibody after anti-D and is reported to cause hemolytic disease of newborn and delayed hemolytic transfusion reaction as a single or with anti-E antibody [17]. Reasons that explain the discrepancies were: recent transfusions, difficulties in differentiation of auto and alloantibodies, limitations of phenotyping due to poor or unavailable antisera, positive anti-globulin test (DAT), and weak and partial phenotypes [18].

The possibility of performing genotyping in conjunction with serological phenotyping will help in the correct interpretation of the results, thus increasing the safety of transfused patients [19].

Patients will benefit by receiving antigen-matched RBCs and show better in-vivo RBCs survival, show increased hemoglobin levels, and have diminished frequency of transfusion and by the way reduce its complications [20].

Conclusion

The results of this study concluded that the integration of serological and molecular tests in the immunohematology routine as well as the evaluation, resolution, and classification of the discrepancies found would help in the correct interpretation of the results found and, consequently, increase transfusion safety.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, *et al.* β-Thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis 2017; 9:17–22.

2 Russo G, De Franceschi L, Colombatti R, Rigano P, Perrotta S, Voi V, *et al.* Current challenges in the management of patients with sickle cell disease – a report of the Italian experience. Orphanet J Rare Dis 2019; 14:120–121.

3 Sarihi R, Amirizadeh N, Oodi A. Study of Kell blood group genotype in alloimmiunized thalassemia patients. Sci J Iran Blood Trasfus Organ 2019; 16:259–269.

4 Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. Br J Haematol 2012; 159:394–404.

5 Evers D, Zwaginga JJ, Tijmensen J, Middelburg RA, De Haas M, De Vooght KM, *et al.* Treatments for hematologic malignancies in contrast to those for solid cancers are associated with reduced red cell alloimmunization. Haematologica 2017; 102:52–59.

6 Gupta R, Singh DK, Singh B, Rusia U. Alloimmunization to red cells in thalassemics: emerging problem and future strategies. Transfus Apher Sci 2011; 45:167–170.

7 Makarovska-Bojadzieva T, Velkova E, Blagoevska M. The impact of extended typing on red blood cell alloimmunization in transfused patients. Open Access Maced J Med Sci 2017; 5:107–111.

8 Osman NH, Sathar J, Leong CF, Zulkifli NF, Sabudin RZAR, Othman A, Asnawi AWA. Importance of extended blood group genotyping in multiply transfused patients. Transfus Apher Sci 2017; 56:410–416.

9 Kulkarni S, Choudhary B, Gogri H, Patil S, Manglani M, Sharma R, Madkaikar M. Molecular genotyping of clinically important blood group antigens in patients with thalassemia. Indian J Med Res 2018; 148:713–719.

10 Belsito A, Magnussen K, Napoli C. Emerging strategies of blood group genotyping for patients with haemoglobinopathies. Transfus Apher Sci 2017; 56:206–213.

11 Jungbauer C, Hobel C, Schwartz D, Mayr W. High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors. Vox Sang 2012; 102:234–242.

12 Stendahl K, Tormey CA, Baine IL. Methods of RBC alloimmunization to ABO and non-ABO antigens, and test methodologies. Immunol Concepts Transfus Med 2020; 13:15–33.

13 Osman NF, Ragab SA, Soliman MA. Alloimmunization in Egyptian children with transfusion-dependent B-thalassemia: a major challenge. Egypt J Haematol 2017; 42:9–13.

14 Castilho L, Rios M, Bianco C, Pellegrino JrJ, Alberto FL, Saad ST, Costa FF. DNA-based typing of blood groupsfor the management of multiply-transfused sickle cell disease patients. Transfusion 2002; 42:232–238.

15 Bakanay SM, Ozturk A, Ileri T, Ince E, Yavasoglu S, Akar N, *et al.* Blood group genotyping in multi-transfused patients. Transfus Apher Sci 2013; 48:257–261.

16 Poisson J. Other Rh antibodies that can impact transfusion. Transfus Manage Obstetr Patient 2018; 8:107–111.

17 Sachan D, Jayakumar R, Varghese J, Rela M. An acute hemolytic transfusion reaction due to the “anti-c” rhesus antibody: a case report emphasizing the role of transfusion medicine. Asian J Transfus Sci 2015; 9:213–219.

18 Menegati SF, Santos TD, Macedo MD, Castilho L. Discrepancies between red cell phenotyping and genotyping in daily immunohematology laboratory practice. Transfus Apher Sci 2019; 8:102–110.

19 Quirino MG, Colli CM, Macedo LC, Sell AM, Visentainer JEL. Methods for blood group antigens detection: cost-effectiveness analysis of phenotyping and genotyping. Hematol Transfus Cell Ther 2019; 41:44–49.

20 Belsito A, Costa D, Signoriello S, Fiorito C, Tartaglione I, Casale M, *et al.* Clinical outcome of transfusions with extended red blood cell matching in β-thalassemia patients: a single-center experience. Transfus Apher Sci 2019; 58:65–71.