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Role of minor erythrocyte antigens on alloimmunization in neonatal indirect hyperbilirubinemia background

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Summary

Aim: ABO/Rh incompatibilities are common causes of blood group incompatibility in newborns. On rare occasions, alloimmunization due to minor erythrocyte antigens may cause severe hemolytic disease requiring exchange transfusion. Most common minor erythrocyte antigens include non-D Rh antigens (c, C, e, E), Kell, Duffy, Kidd and MNS. In this study, we aimed to investigate minor erythrocyte antigens and their possible effects in newborns who were hospitalized for indirect hyperbilirubinemia and did not have any other detectable cause for neonatal jaundice.

Material and Method: : Between July 1st 2009 and September 31st 2009, 107 newborns were enrolled to investigate the relationship between minor erythrocyte antigens and neonatal jaundice. Patients with common causes of hyperbilirubinemia such as ABO/Rh incompatibility, hypothyroidism, glucose-6-phosphate dehydrogenase deficiency, inborn errors of metabolism and sepsis were excluded. The study was approved by the ethics commite ((25.06.2006/35-2009). Minor erythrocyte antigens were studied by performing gel centrifugation method using human monoclonal antibodies. Antibodies were detected in mothers with positive antibody screening. Antigens which countered the antibodies present in the mother and which were found to be positive in the infant were considered as a cause of incompatibility. Kell, C, E,c, e antigens were investigated in all newborns regardless of their antibody screening results.

Results: Minor erythrocyte incompatibility was detected in 7 out of 107 newborns (6.5%). Assessment among 230 newborns hospitalized for indirect hyperbilirubinemia revealed a rate of 3% for minor erythrocyte antigen positivity. The most common incompatibility was related to “s” antigen which was detected in 4 patients. Other antigens detected included C, Jka, S, Lub and N. Only 1 patient was found to carry Kell antigen. However his mother displayed negative antibody screening. Direct coombs positivity or severe hemolysis could not be detected in any of the patients with minor erythrocyte antigen incompatibility. Although the clinical course was similar, jaundice was realized much later in these infants when compared to other infants with indirect hyperbilirubinemia.

Conclusions: Currently minor erythrocyte antigens are not being investigated routinely in neonatal jaundice. However, clinicians should keep in mind that minor erythrocyte antigens can cause indirect hyperbilirubinemia and sometimes severe hemolytic disease. Therefore they should remember to study these antigens in newborns with pathologic jaundice. *(Turk Arch Ped 2013; 48: 23-29)*

Key words: Alloimmunization, erytrocyte antigene, hemolysis, hyperbilirubinemia, minör, newborn, subgroup.

Introduction

Neonatal jaundice occurs with a frequency of approximately 60% in term infants and with a frequency of 80% in preterm infants. It continues to be one of the significant porblems in the neonatal period because it occurs very frequently and leads to severe outcomes. These outcomes may be prevented when early diagnosis is made and appropriate treatment is administered. The important point is to determine if jaundice is physiological or not and to make early differential diagnosis in pathological jaundice and to administer appropriate treatment (1,2,3).

Among pathological jaundices, especially blood group incompatibilities are the main causes of jaundice. In the historical process, indirect hyperbilirubinemias related primarily with Rh sensitivity have decreased with the use of anti-D

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gammaglobulin and the frequency of subgroup incompatibilities is increasing currently (2,3,4).

The rarity of minor blood group incompatibilities is primarily a result of low antigenity of erythrocyte antigens. Clinically significant antibodies have been shown in 0.24-1% of pregnant women with prenatal screening programs. Although minor blood group incompatibility occurs very rarely, severe hemolytic responses requiring exchange transfusion may be observed. Among these minor blood groups, the most common ones include non-D Rh antibodies (c, C, E), Kell, Duffy, Kidd and MNS (3,4).

In our study, the frequency, clinical findings and outcomes of minor blood groups which may cause to jaundice were investigated in patients who were internalized in the neanotology ward with a diagnosis of indirect hyperbilirubinemia excluding the ones whose etiologies were found including ABO/Rh incompatibility, hypothyroidism, glucose 6 phosphate dehydrogenase (G6PDH) deficiency, metabolic disease and sepsis.

Material and Method

A total of 230 patients who were internalized in the Neanatology Clinic of our hospital between 07.01.2009 and 09.31.2009 with a diagnosis of indirect hyperbilirubinemia or who were found to have jaundice during hospitalization in the Neanatology Clinic were evaluated.

Among these, the patients in whom other known hyperbilirubinemia causes including ABO incompatibility, Rh incompatibility, congenital hypothyroidism, G6PDH deficiency, sepsis, cephalhematoma and metabolic disease could be found were excluded from the study and a total of 107 patients were included in the study.

The age, gender, birth weigth, gestational age, time when jaundice was recognized, delivery mode, maternal disease, maternal parity, consanguinity, history of jaundice in the siblings, time of hospitalization, physical examination findings, blood groups of the mother and the infant, direct Coombs, total bilirubin, direct bilirubin, TSH, G6PD, hemogram and reticulocyte count in the patients who were included in the study were recorded.

The gestational ages of the patients included in the study were determined according to the last menstrual period date. Total bilirubin values accepted for phototherapy and exchange transfusion were based on the recommendations of the American Academy of Pediatrics (AAP) and the Turkish Neanatology Association (5,6).

Tests performed in all newborns:

Determination of the blood group: Gel centrifugation method was used in determining ABO/RH in 1 cc blood sample with EDTA. Whole blood samples with EDTA were used for direct ABO grouping and in determining Rh, Rh subgroup and minor antigens. Serum samples were used for indirect ABO grouping and for antibody screening and antibody determination.

In newborns, Rh and weak D (D') determination together with ABO determination and direct Coomb test (DG Gel Neonatal, Diagnostic Grifolds, S.A, Spain) were performed. In all the newborns, Kell (K) antigen and Rh subgroup (C,c,E,e) antigens (DG Rh Subtyping, Diagnostic Grifolds, S.A, Spain) were also investigated.

Direct Coombs test: In determining the blood group of the newborns, presence of C3d and IgG was investigated in the subjects who were found to be positive with gel centrifugation method (DG Gel Neonatal, Diagnostic Grifolds, S.A, Spain).

Total bilirubin: Total bilirubin was measured with Jendrassik-Grof method (Bechman-coulter –DXC clinical systems USA) in the venous blood samples obtained.

Direct bilirubin: Direct bilirubin was measured with diazotised sulphonylic acid method (Bechman Coulter DXC Clinical Systems, USA) in the venous blood samples obtained. A direct bilirubin value above 1.5-2 mg/dL or above 10-15% of the total bilirubin was considered as direct hyperbilirubinemia.

TSH, FT_4 measurements; were done with electrochemiluminescnce method (Roche Elecsys) in the venous blood samples obtained. A FT4 level of 0.9-2.6 ng/dL and a TSH value below 20 µU/ml was considered as normal.

Glucose 6 phosphate dehydrogenase enzyme level measurement was done with manual spectrophotometric method in the blood placed in a tube with EDTA. Values between 6 and 14.5 U/gr Hb were considered as normal.

Hemogram and reticulocyte count were measured by placing a blood sample in a tube with EDTA using laser reading and cellular staining method (Horiba ABX, Diagno Group) and patients with anemia were determined (7).

In 107 patients in whom the etiology of jaundice could not be found, minor erithrocyte antibody was tested in the mother and minor erythrocyte antigen was tested in the infant in cases with a positive maternal antibody and the antigen corresponding the maternal antibody was accepted to be the cause of incompatibility.

Minor blood group systems and antigens tested included Kell blood group system (K,k, Kp^a, Kp^b), Duffy blood group system (Fy^a Fy^b), Kidd blood group system (Jk^a, Jk^b, Jk^aJk^b), Lutheran blood group system (Lu^a, Lu^b), MNS blood group system (M,N,S,s, U), Lewis blood group system (Le^a, Le^b), Rh subgroups (C,E,c,e).

Tests done in the mother: In a 2 cc blood sample with EDTA, direct and indirect ABO determination and Rh determination (DG Gel ABO/Rh, Diagnostic Grifolds, S.A, Spain) were done in addition to antibody screening test using four different cell series (Serascan Diana 4P, Diagnostic Grifolds, S.A, Spain). Minor erythrocyte antibody determination was done in mothers in whom antibody screening test was found to be positive using specific erythrocyte suspensions (Identisera Diana P, Diagnostic Grifolds, SA, Spain).

Minor erythrocyte antibodies were tested in infants of the mothers in whom antibody screening test was found to be positive: for minor erythrocyte antigens in newborns, presence

of Jk^a Jk^b, S, s was investigated with human specific monoclonal antibodies (Bioscot, Millipore Limited, UK), presence of Fy^a, Fy^b, Lu^a was investigated with human polyclonal antibodies (Alba Bioscience, UK), presence of M, N, Le^a, Le^b (Bioscot, Millipore Limited, UK) and presence of k ve Lu^b (Alba Bioscience, UK) was investigated with Mouse specific monoclonal antibodies on neutral gel cards (DG Gel Neutral, Diagnostic Grifolds, S.A, Spain) or gel cards with anti-human globulin (DG Gel Coombs, Diagnostic Grifolds, S.A., Spain) in accordance with the instructions of the manufacturer. Patients who had antigens corresponding to maternal antibodies were considered as minor erythrocyte antigen incompatibility.

Statistical Analysis

In statistical analysis of the data obtained in the study SPSS (Statistical Package for the Social Sciences Computer Software) for Windows 11.0 (SPSS Inc.Chicaco, IL) program was used. In statistical evaluation of the data, mean values, mean standard deviation, numerical values and percent values were determined. x^2 test was used for values determined by counting. For values determined by measurement students's t-test was used for paired comparisons and variance analysis was used for triple comparisons. A p value below 0.05 was considered as statistically significant.

Results

The etiological evaluation of all the patients is given in Table 1. After the patients in whom the cause of indirect hyperbilirubinemia could be determined were excluded from the study, maternal parities of 107 patients who were included in the study and followed up because of hyperbilirubinemia were evaluated; it was found that 61 mothers (57.0%) had a parity of one, 33 mothers (30.8%) had a parity of two and 13 mothers (12.2%) had a parity of three or more. When the maternal medical history was evaluated, four mothers (3.7%) had gestational diabetes, one mother (0.9%) had antepartum bleeding, one (0.9%) had hypothyroidism, three (2.8%) had hyperthyroidism. No pathology was defined in 98 of the mother (91.6%). Consanguineous marriage was found in 12 cases (11.2%). A history of jaundice in the siblings was found in 8 patients (7.5%), no history of jaundice in the siblings was found in 38 patients (35.5%). The remaining 61 patients (57.0%) were the first child of the family.

56 infants (52.3%) were born by normal spontaneous vaginal delivery and 51 infants (47.7%) were born by cesarean section. 64 of the patients (59,8%) were male and 43 (40.2%) were female. SGA was found in 14 patients. 105 (98.1%) of 107 patients were hospitalized with a diagnosis of indirect hyperbilirubinemia and 2 (1.9%) were hospitalized with a diagnosis of transient tachycardia of the newborn. In 35 patients (32.7%), mild dehydratation accompanied indirect hyperbilirubinemia. The general characteristics of 107 patients are given in Table 2 and their general laboratory values are given in Table 3.

When the blood groups of the patients were evaluated, the blood group was found to be O Rh positive in 44 patients (41.1%), A Rh positive in 43 (40.2%) patients and B Rh negative in 0.9% of the patients. AB Rh negative blood group was not found in any of the patients. Direct Coombs test was found to be positive in one patient. However, antibody screening was found to be negative in this patient.

When 230 patients with hyperbiluribinemia were evaluated, the ratio of minor erythrocyte antigens was found to be 3%. Minor erythrocyte incompatibility was found in 7 (6.5%) of 107 patients included in the study. The most common incompatibility in these 7 patients was related with "s" antigen from MSN group which was found in 4 patients (3.7%). Other antigenes of incompatibility included C, Jka, S, Lub and N'di. Kell antigene was observed in one patient. However, the maternal antibody screening test was found to be negative in this patient. The clinical and laboratory properties of 7 patients whose maternal antibody tests were found to be positive are shown in Table 4.

Table 1. The etiological distribution in all hyperbiluribinemia cases

Etiology	Number of patients (%)
ABO incompatibility	56 (24.3%)
Rh incompatibility	29 (12.6%)
Urinary tract infection	14 (6%)
Minor blood group incompatibility	7 (3%)
Hypothroidism	7 (3%)
Sepsis	6 (2.6%)
Cephal hematoma	6 (2.6%)
Omphalitis	3 (1.3%)
Metabolic disease	2 (0.86%)
Unknown	100 (43.47%)
Total	230 (100%)

Table 2. General properties of 107 patients

	Mean±SD	The least-the highest
Maternal parity	1.76±1.41	1-11
Gestational age at birth	38.3±2.11	31-42
Birth weight (g)	3084±546	1600-4400
Onset of jaundice (days)	3.85±1.89	1-10
Age at presentation (days)	6.87±4.75	1-23
Weight at presentation (g)	2905±558	1500-4270
Days of hospitalization	3.37±2.34	1-15

When the patients with positive antibody screening test were compared with the other patients in terms of gender and birth weight, no statistically significant difference was found; (p=0.589), (p=0.335), respectively.

When the patients with positive antibody screening test were compared with the other patients, no statisitcally significant difference was found in terms of age, gestational age, birth weight, body weight, hospitalization time, maternal parity, bilirubin level at hospitalization, bilirubin level at discharge, hemogram, reticulocyte count, G6PD and TSH values (Table 5). The day of onset of jaundice was found to be significantly later in the patients with a positive maternal antibody screening test (p=0.025).

Table 3. Laboratory values of 107 patients at presentation

	Mean±SD	The least-the highest
Bilirubin at hospitalization (mg/dL)	18.77±2.94	11.80-29.70
Bilirubin at discharge (mg/dL)	9.22±1.80	4.10-12.80
Hemogram (g/dL)	15.68±2.22	5.70-19.80
Reticulocyte (%)	1.41±0.93	0.50-6.00
G6PD (U/grHb)	11.55±2.42	6.60-18.00
TSH (µu/mL)	8.16±6.16	0.37-18.00

G6PD: glucose 6 phosphate dehydrogenase, TSH: Tyhroid stimulating hormon

Phototherapy was performed in treatment in all the patients. No complication except for skin eruption was observed during phototherapy. Total bilirubin level at presentation was found to be higher than the limit of exhange transfusion in a total of 21 patients (19.6%). In 2 of these patients, maternal antibody screening test was found to be positive. When compared with the other patients, no statistically significant difference was found in terms of bilirubin levels (p>0.05). Exchange transfusion was performed in 2 patients. Direct Coombs test and maternal antibody screening test were negative in both patients. In 2 patients, erythrocyte suspension and intravenous immunoglobulin (IVIG) were given in addition to phototherapy because of hemolysis findings and anemia. Direct Coombs test and antibody screening test were found to be negative in these patients.

Anemia was found in 25 (23.4%) of 107 patients. Minor erythrocyte antigene was found to be positive in two of them. In the other 23 patients, maternal antibody screening test was

Table 4. Antigens which were found to be responsible of minor antigen incompatibility and clinical findings and findings related with hemolysis

Patient number	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age at presentation(days)	4	4	14	9	7	7	7
Birth weight (g)	2900	4030	2600	2750	3050	2900	2900
Gestational week at birth	40	40	39	36	38	37	37
Mode of delivery	NSVD	C/S	NSVD	NSVD	NSVD	C/S	C/S
Gender	E	E	K	E	E	K	K
Body weight (g)	2720	3600	2870	2570	2650	2520	2500
Onset of jaundice (days)	4	4	5	5	4	5	6
Bilirubin at hospitalization (mg/dL)	19	20.3	22.2	17.7	25.5	15.6	17.2
Bilirubin at discharge (mg/dL)	9.8	9.8	10.6	11.8	7	9.3	9.5
Days of hospitalization	2	3	5	2	9	2	3
Hemogram (mg/dL)	13.8	18.2	15.6	14.6	13.7	19.8	15.5
Reticulocyte (%)	1.1	0.8	0.7	1	1.1	0.7	1
History of jaundice in a sibling	-	-	-	-	-	+	+
Maternal parity	1	1	2	1	1	2	2
Maternal disease	-	GDM	-	-	-	-	-
Blood group of the infant	A Rh^+	A Rh^+	A Rh^+	O Rh^+	O Rh^+	O Rh^+	O Rh^+
Maternal blood group	A Rh^+	A Rh^+	A Rh^+	O Rh^+	ARh^+	O Rh^+	O Rh^+
Antigens responsible of incompatibility	Lu^b, s	N,S,s	S	Jk^a	C	S	S

Table 5. Comparison of the general properties and laboratory findings between infants with positive maternal antibody screening test and other infants

	Patients with maternal (+) AST	Patients with maternal (-) AST	p
Age (days)	7.42±3.40	6.84±4.84	0.351
Gestational week at birth	37.8±1.77	38.4±2.14	0.263
Birth weight (g)	3018±467	3088±553	0.416
Body weight (g)	2775±395	2914±568	0.514
Onset of jaundice (days)	4.71±0.75	3.79±1.94	0.025
Days of hospitalization	3.71±2.56	3.35±2.33	0.719
Parity	2.0±1.41	1.75±1.42	0.451
Bilirubin at hospitalization (mg/dL)	19.6±3.36	18.71±2.92	0.476
Bilirubin at discharge (mg/dL)	9.68±1.45	9.19±1.82	0.677
Hemogram (gr/dL)	15.52±2.67	15.70±2.20	0.579
Reticulocyte (%)	1.20±0.80	1.42±0.94	0.293
G6PD (U/grHb)	11.95±3.12	11.52±2.38	0.729
TSH (miu/mL)	7.10±6.30	8.24±6.17	0.478

AST: Antibody screeninbg testi, G6PD: glucose 6 phosphate dehydrogenase, TSH: thyroid stimulating hormon

found to be negative. No statistically significant relation was found between positivity in antibody screening test and presence of anemia (p=0.518). Antigenes responsible of minor antigene incompatibility and clinical findings and findings related with hemolysis are given in Table 4.

Discussion

The most common causes of pathological neonatal jaundice are hemolytic diseases due to blood incompatibilities including ABO, Rh and subgroup incompatibilities. These occur as a result of maternal antibodies produced against the antigenes in the erythrocytes of the newborn. This risk exists in 15% of live births, but disease symtomps occur in only 0.3-2.2% of the infants (13). Currently, the rates of Rh incompatibility in neonatal jaundice is gradually decreasing, since administration of anti-D immunoglobulin to women who have found to be Rh negative during pregnancy is a well-known practice (4).

Another cause of incompatibility is related with ABO system (11). In a study reported from Spain, ABO incompatibility was found with a rate of 13.6%, severe hyperbilirubinemia related to ABO incompatibility was found with a rate of 2.21% in healthy term infants, while severe hyperbilirubinemia was found with a rate of 16.27% in infants with ABO incompatibility (8). In publications reported from our country, ABO incompatibility was found with a rate of 32.9 by Kocabay et al. (9), with a rate of 38.9% by Satar et al. (10) and with a rate of 20% by Özkaya et al. (11) in patients followed up with hyperbilirubinemia, while Rh incompatibility was found with a rate of 15.2% by Kocabay et al., with a rate of 6% by Satar et al. and with a rate of 9.6% by Özkaya et al. Similiarly, ABO incompatibility was found with a rate of 24.35% and Rh incompatibility was found with ba rate of 12.6% in our study.

Currently, the frequency of subgroup incompatiblities found as a result of investigation of newborns with hyperbilirubinemia is increasing (4,5). The best known ones among these antigens which are responsible of 3% of the cases of neonatal hemolytic disease include Kell, Duffy, Diego, Kidd, MNS, C and E and they may lead to a hemolysis picture in the neonatal period similar to Rh disease. Hemolytic disease may be observed more frequently with Kell antigen and more rarely with Duffy, Lewis, Kidd, MNS and other minor blood groups. The antigens belonging to the Kell system have the strongest antigenic property after D antigen excluding A and B antigens. Kell antibodies are shown with Coombs test in vitro. They may cause to hemolytic transfusion reactions and neonatal hemolytic disease (5,15,12).

In hemolytic disease due to subgroup incompatibility, the disease spectrum may range from subclinical hemolysis to active hemolysis and hyperbilirubinemia requiring exchange transfusion. Its diagnosis and treatment is similar to Rh incompatibility (13). In a study performed to determine the frequency of erythrocyte alloimmunization causing hemolytic disease, the frequencies of antibodies which led to development of hemolytic disease causing mortality among 452 women with a positive indirect Coombs test were as follows: anti-D 18.4%; anti-E 14%; anti-c 5.8%; anti-C 4.7%; anti-Kell 22%; anti-MNS 4.7%; anti-Fya (Duffy) 5.4% and anti-Jka 1.5% (14). In a study performed in Poland, non-anti-D antibodies were found in 106 of 507 fetus and newborns with a positive maternal antibody test. Rh subgroups (C,c,E,e,G,Rh17) were found in 46 (43%) of these, K and k (only 1) were found in 35 (33%) and other antigens were found in the remaining 25 (24%) (15). In pregnant women, screenings are performed in different trimesters to determine non-anti-D erythrocyte alloimmunization and to take precautions against development of hemolytic disease. In these studies, the prevelance of clinically related non-anti-D alloantibody has been found to be 0.15-0.27% and the prevelance of related severe hemolytic disease hs been found to be 0.01-0.03% (16,17).

Researches related with minor blood groups have generally been reported in newborns with hemolytic disease. In the study performed by Özkaya et al. (17), the rate of subgroup incompatibility was reported to be 10.4%. In our study, the rate of minor erythrocyte antigens in 230 patients with hyperbilirubinemia was found to be 3%. Minor erythrocyte incompatibility was found in 7 (6.5%) of 107 patients in whom

the etiology could not be found. The most common incompatibility in 7 patients was "s" antigen from the MNS group which was found in 4 (3.7%) patients. Other antigenes with incompatibility included C, Jka, S, Lub and N. Kell antigen was observed in one patient. However, maternal antibody screening test was found to be negative in this patient. Hemolysis findings were not observed in any patient with subgroup incompatibility in our study. In conditions related with subgroup nimncompatibility (for example, in conditions related with Kell antigene), anemia findings may be observed before hemolysis because erythroblast destruction occurs primarily (18). However, one of the questions considered is if the actual cause of jaundice was subgroup incompatibilty or another factor which could not be found with the investigations performed, since hemoglobin values in the group considered as subgroup incompatibility were not found to be different compared to the patients who had no incompatibility.

In minor blood group incompatibility, severe hemolytic reactions which require exchange transfusion may be observed, though very rarely. A patient with positive direct antiglobulin related with anti E who required exchange transfusion for two times was reported from our country (19). In the Chinese population in Taiwan, hemolytic disease related with maternal antibodies was found in 15 patients among 2615 children who developed hyperbilirubinemia in the neonatal period during a 10 year period and the frequency was reported to be 0.01%. Anti E was found in 6 patients, anti E+c was found in 3 patients, anti D was found in 3 patients, anti canti-Mur was found in one patient. Exchange transfusion was needed in 7 patients 3 of whom had anti D antibody (20). In our study, bilirubin level was found at the limit of exchange transfusion in only one patient in whom the cause of incompatibility was found to be C antigen, but exchange transfusion was not necessary after intensive phototherapy. In all patients, bilirubin values after phototherapy showed reduction in a similar time as in other patients.

Studies have found positive Coombs test with a rate of approximately 33% in patients with incompatibility. In patients with hemolytic anemia findings, direct Coomb test positivity may not alway accompany. However, a negative direct Coombs test is not an indicator that there will be no incompatibility. This is thought to arise from the weak antigenic properties of minor erythrocyte antigens (21). In our study, Coombs positivity was not found in any of the patients with minor erythrocyte antigen incompatibility.

In comparison of the patients with subgroup incompatibility with the other patients in our study, reported jaundice onset was observed to occur statistically significantly later. This might have arised from the difference in knowledge and attention of families related to jaundice. In addition, it may be associated with the fact that jaundice with a slower course was recognized by the families only in advanced days, since direct Coombs positivity and hemolysis findings were not found in any of the patients with subgroup incompatibility. Presence of mild dehydratation in 32% of 107 patients was interpreted as factors including insufficient nutrition and excessive water loss contributed to hospitalization in addition to breast milk jaundice in our study which was conducted in the summer period.

Although non-anti-D erythrocyte alloimmunization screenings in pregnant women in terms of subgroup incompatibilities are not recommended in some countries, since they occur rarely, they are evaluated to be feasable because of the low risk of severe hemolytic disease in other countries (20,21). In our country, such a screening is not at issue, since hemolytic diseases related to Rh alloimmunization can not be prevented completely.

Conclusively, minor erythrocyte antigen incompatibility was found with a rate of 6.5% in patients who were hospitalized becasue of indirect hyperbilirubinemia and in whom the etiology of jaundice could not be found in our study. Although hemolysis was not found in any patient with subgroup incompatibility in our study, it should be kept in mind that minor blood groups may lead to neonatal jaundice and minor blood groups should be tested in newborns with pathologic jaundice with unknown etiology and in newborns with hemolytic disease.

Conflict of interest: None declared.

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