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Original Article

Impact of Minor Blood Group Incompatibility Versus ABO and Rh Blood Group Incompatibility in Newborns with Indirect Hyperbilirubinaemia: A Single-Centre Clinical Experience

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Abstract

Introduction: Haemolytic disease of the fetus and newborn (HDFN) is caused by the destruction of red blood cells of the neonate or fetus by maternal immunoglobulin G (IgG) antibodies. Alloimmune HDFN primarily involves the major blood groups of Rhesus (Rh), A, B, AB, and O, although minor blood group incompatibilities (Kell, Duffy, MNS, P, and Diego systems) can also result in significant disease. ***Objective:*** The main objective was to provide insight about the impact of minor blood group incompatibility against ABO and Rh blood group incompatibility in newborns with hyperbilirubinaemia in terms of the demographic data, laboratory values, clinical course, and responses to treatment. ***Methods:*** Three hundred fifty-five infants hospitalised with the diagnosis of unconjugated hyperbilirubinaemia (UHB) had data collected retrospectively. The mother and the baby's blood group type as C, c, E, e, Kell, D, d, A, B, O was detected by gel centrifugation method. Patients were divided into 4 groups: group 1, ABO blood group incompatibility; group 2, Rh blood group incompatibility; group 3, ABO+Rh blood group incompatibility; and group 4, minor blood group incompatibility. A total of 355 patients of which 230 (64.7%) were in group 1, 68 (19.1%) were in group 2, 15 (4.2%) were in group 3, and 42 (12%) were in group 4 were evaluated, respectively. ***Results:*** Among those with minor blood group incompatibility, 12 (28%), 11 (26%), 9 (21%), 6 (14%), and 4 (9%) patients had "C," "c," "E+c," "E," and "Kell" incompatibilities, respectively. The mean age of diagnosing jaundice (8±7.5 days) was significantly higher (p=0.015), and the mean haemoglobin and the mean haematocrit levels at admission were lower (p=0.007, p=0.041) in group 4. The rebound elevation of serum bilirubin was remarkably high in group 2 and group 4 (p=0.025). The requirement of intravenous immunglobulin, exchange-erythrocyte transfusions, as well as rehospitalisation for phototherapy, after discharge was significantly higher in group 4. ***Conclusions:*** We should keep mind the minor blood group incompatibility in infants who have late hospital admission, prolonged jaundice, prominent anaemia, rebound elevation of serum bilirubin after treatment termination, requirement of exchange and/or erythrocyte transfusions, and increased rehospitalisation rate.

Key words ABO; Indirect hyperbilirubinaemia; Neonatal; Rh and minor blood group incompatibility

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Introduction

Neonatal jaundice is the most common cause of hospitalisation in the neonatal period. It is estimated that 60% of term newborns develop jaundice and 2% reach a severe hyperbilirubinaemia. In 2006, Sgro et al published an incidence of severe hyperbilirubinaemia of 1/2480 (not for haemolytic anaemia);[1] in 2007, it decreased to 1/8600 after the implementation of a Guideline of Clinical Practice aimed at improving the management of severe neonatal jaundice.[2,3] Risk factors for severe hyperbilirubinaemia include malnutrition, infection, history of jaundice in sibling(s), the presence of a large cephalhaematoma, premature labour, sequestration, polycythemia, isoimmunisation, erythrocyte enzymatic and structural defects, and disorder of conjugation.[1,4] The most common cause of severe early jaundice is fetal-maternal blood group incompatibility with resulting isoimmunisation. Maternal immunisation develops when erythrocytes leak from fetal to maternal circulation. Fetal erythrocytes carrying different antigens are recognised as foreign by the maternal immune system that forms antibodies against them (maternal sensitisation). These antibodies (IgG immunoglobulins) cross the placental barrier into the fetal circulation and bind to fetal erythrocytes.[5] This disease of the fetus caused by a maternal response to pregnancy is called the haemolytic disease of the fetus and newborn (HDFN). Although Rhesus (Rh) blood group incompatibility is well known in HDFN, the ABO and minor blood group incompatibility have become the increased frequent for HDFN in developed countries by virtue of the common using of anti-D immunoglobulin. In Rh incompatibility, sequestration and destruction of the antibody-coated erythrocytes take place in the reticuloendothelial system of the fetus. In ABO incompatibility, haemolysis is intravascular, complement-mediated, and usually not as severe as Rh disease. Significant haemolysis can also result from incompatibilities between minor blood group antigens (e.g., Kell).[6]

In this study, we aimed to compare the demographic data, laboratory values, clinical course, and responses for treatment between minor blood, Rh, and ABO blood group antigens incompatibility in newborns with HDFN, who were hospitalised due to jaundice.

Materials and Methods

A total of 355 patients who have been hospitalised between January 1, 2013, and December 31, 2015, in neonatal service of Dr. Behçet Uz Children's Hospital with indirect hyperbilirubinaemia defined as >95p below according to nomogram based on the hour-specific serum bilirubin values recommended in the 2004 guideline of the American Academy of Pediatrics (AAP)[3] for neonates with a gestational age of 35 weeks or more and detected to be with Rh, ABO, or minor blood group incompatibility were included in the study. The study data were collected via a retrospective review of the respective patient files which was carried out with the approval of the local ethics committee (14.01.2016-01.06.2016). Patients with a gestational age of less than 37 weeks and with glucose 6-phosphate dehydrogenase (G6PDH) deficiency were excluded. Demographic data, such as sex, gestational week at delivery, mode of delivery, age at admission, birth weight, body weight at admission, maternal age, the presence of dehydration, and laboratory values, such as total and direct bilirubin levels, blood groups of mother and baby, direct Coombs test, haemolysis findings on peripheral blood smear, reticulocyte, haemoglobin, haematocrit, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin/albumin ratio, and responses to treatment and clinical courses such as level at discontinuation of phototherapy, decreased in total serum bilirubin (TSB) level per hour, phototherapy time, rebound bilirubin level after phototherapy, other treatments (intravenous immunoglobulin (IVIG), erythrocyte transfusion**,** exchange transfusion, intravenous fluid) and rehospitalisation need, were reviewed in the patient files. Dehydration (severe) was defined the loss of >10% in body weight. Rehospitalisation were hospitalised again within 30 days of newborn's index admission. Bilirubin rebound was described as post-phototherapy bilirubin level needing reinstitution of phototherapy. Phototherapy and/or exchange transfusion procedure were carried out in patients according to curves recommended in the 2004 guideline of the AAP for neonates with a gestational age of 35 weeks or more.[7] In line with the recommendations of the AAP, an IVIG treatment at a dose of 1 g/kg was administered in case of a continuing increase in total bilirubin level or the presence of a bilirubin level within the range of ± 2 to 3 mg/dl to exchange transfusion limit in HDFN despite intensive phototherapy. An intravenous fluid replacement was administered to lethargic patients reluctant to oral feeding with a weight loss of >10%. ABO and Rh blood group incompatibility, dehydration, sepsis, haematoma, the presence of a level above the exchange transfusion limit

without G6PDH deficiency at admission, the absence of the expected response to phototherapy or reticulocytosis, anaemia, and C, c, E, e, Kell, Duffy, Diego, Kidd, MNSs antigens in both mothers and babies in the presence of direct Coombs test positivity were studied in patients. The mother and the baby's blood group type as C, c, E, e, Kell, D, d, A, B, O was detected by gel centrifugation method. Patients as only ABO, only Rh, both ABO and Rh blood group incompatibilities, or minor blood group incompatibility were separated to group 1, group 2, group 3, or group 4, respectively. Demographic data, laboratory values, responses to treatment, and clinical courses were compared between groups. The statistical analysis was carried out by using SPSS for Windows, version 20.0. For comparing numerical variables between groups, ANOVA test (if the data fit a normal distribution) and Kruskal Wallis test for the nonparametric were used. For comparing ratios between groups, the chi square test was used. The statistical significance was accepted as $p<0.05$.

Results

A total of 355 patients, of which 230 (64.7%) were in group 1, 68 were (19.1%) in group 2, 15 (4.2%) were in group 3, and 42 (12%) were in group 4 were evaluated. Among those with minor blood group incompatibility, 12 (28%), 11 (26%), 9 (21%), 6 (14%), and 4 (9%) patients had "C," "c," "E+c," "E," and "Kell" incompatibilities, respectively. When groups were compared in regard to demographic data, no significant difference was detected with respect to sex, gestational week at delivery, birth order, and the presence of dehydration. Birth weight in group 4 was lower (3085±440 g) compared with other groups ($p=0.001$). Mean diagnosis age for all groups was 5.4±4.5 days, and postnatal age at admission in group 4 (8±7.5 days) was significantly higher compared with other groups ($p=0.015$). The detailed distribution of demographic data is shown in Table 1 both in general and separately.

When laboratory data obtained from patients at admission were evaluated, it was found that haemoglobin and haematocrit values in group 4 were statistically significantly lower compared with the other groups ($p=0,007$ and $p=0.041$, respectively). There was no significant difference between groups regarding other laboratory data. However, TSB, direct bilirubin, level at discontinuation of phototherapy, reticulocyte, Haemolysis finding on peripheral blood smear were shown to be higher in group 4, although not statistically significant. The detailed distribution of laboratory data is given in Table 2 both in general and separately.

No significant difference was found between groups in regard to total phototherapy time applied to patients as from the first hospitalisation, decrease rate of bilirubin, and IVIG

Table 1 Distribution of demographic data according to patient groups

	General	Group 1	Group 2	Group 3	Group 4	p
	% (n)	% (n)	% (n)	% (n)	% (n)	
Sex						
Boy	53.2 (189)	53 (122)	54 (37)	53 (8)	52 (22)	
Girl	46.7 (166)	47 (108)	46 (31)	47 (7)	48 (20)	
Mode of delivery						
NSVD	36.9 (131)	32 (74)	35 (24)	60 (9)	57 (24)	
C/S	63.1 (224)	68 (156)	65 (44)	40 (6)	43 (18)	
Dehydration						
Yes	9.6 (34)	7 (18)	11.7 (8)	13 (2)	14 (6)	0.345
No	90.4 (321)	93 (212)	88.3 (60)	87 (13)	86 (36)	
*Gestational week at delivery	38.2±1.2	38.4±1.2	38.4±1.1	39±0.8	38.1±1.5	
*Birth weight (g)	3321±440	3324±409	3425±461	3403±556	3085±440	**0.001**
*Diagnosis age (days)	5.4±4.5	5.1±3.5	5±3.9	4.1±3.3	8±7.5	**0.015**
*Maternal age	28.2±5.2	27.9±5.1	29.9±5.3	26.2±5	27.8±5.7	**0.015**
*Pregnancy number	2.2±1.3	2.2±1.2	2.4±1.4	2.4±1.3	2±1.2	

NSVD=Normal spontaneous vaginal delivery; C/S=Cesarean section; *Data are shown as mean ± standard deviation.

need. However, the need for IVIG in group 4 was numerically higher compared with the other groups. Although exchange transfusion was administered to 1.6% (n=6) of all patients, the rate of exchange transfusion administration in the minor blood group incompatibility (group 4) was 11.9 % (n=5) of the total study patients. The need of exchange transfusion and transfusion with erythrocyte suspension in Group 4 was statistically significantly higher compared with the other groups (p=0.001 and p<0.001, respectively).

Rebound bilirubin levels after phototherapy were significantly higher in patients with RhD incompatibility and minor blood group incompatibility (p=0.025). Rehospitalisation due to unconjugated hyperbilirubinaemia (UHB) after discharge was significantly more common among patients with minor blood group incompatibility (p=0.005). Responses to treatment and rehospitalisation rates of patients are given in Table 3.

Discussion

The most common cause of Haemolytic disease of the newborn is Rh, ABO, and minor blood group incompatibilities, resulting from antibodies formed in the mother against neonatal erythrocyte antigens. Before the administration of anti-D immunoglobulin, the frequency of Rh alloimmunisation was about 16%, and it was the most frequently encountered cause of mortality- and morbidity-related Haemolytic disease in fetal and neonatal periods. By virtue of administration of anti-D immunoglobulin in the antepartum and postpartum period, the frequency of alloimmunisation has decreased to 0.17% to 0.28%, and the frequency of Haemolytic disease-related mortality has decreased from 46/100,000 to 1.6/100,000.[8,9] Anti-D is still regarded as one of the critical antibodies because of conditions, such as unmonitored pregnancies, suboptimal responses to immunoprophylaxis, and the like. In addition to RhD antigen, erythrocyte antigens related with Haemolytic diseases include non-D Rh antigens (C, c, E, e), ABO system antigens, Kell, and, as more rarely seen ones, Duffy, Kidd, and MNS antigens.[10] In our study, the most commonly reported causes were ABO blood group incompatibility with the ratio of 64.7%, Rh blood group incompatibility with the ratio of 19.1%, minor blood group incompatibility with the ratio of 12%, and ABO plus Rh blood group incompatibility with the ratio of 4.2%, respectively. In the study by Karagöl et al in which 106 patients with minor blood group incompatibility were evaluated, mean postnatal age at admission was reported as 6.1±5.2 days.[11] The mean diagnosis age of all patients was found as 5.4±4.5 days in our study. The mean diagnosis age in patients with minor

Table 2 Comparison of patient groups for laboratory values

	General (mean±SD)	Group 1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)	Group 4 (mean±SD)	p
Total bilirubin (mg/dl)	19.5±4.9	19.6±4	18.1±4.8	20.7±6.3	21.1±7.6	0.106
Direct bilirubin (mg/dl)	0.6±0.5	0.6±0.2	0.5±0.16	0.5±0.14	1±1.5	0.168
Level at discontinuation of phototherapy (mg/dl)	10.9±5.5	11.3±6.2	10.3±1.9	9.5±1.4	11.5±5.9	0.468
Reticulocyte (%)	3.8±4.2	3.6±3.9	3.2±3	3.3±2.8	5.2±6.7	0.083
Haemoglobin (g/dL)	15.4±2.9	15.5±2.4	16.3±3.2	15.3±2.2	14.6±4.5	**0.007**
Haematocrit (%)	46.1±8.4	45.9±6.9	48.2±9.4	46.1±6.8	44.5±12.6	**0.041**
[a]AST (IU/L)	51±30.6	50±26.5	57±45	60.7±20.7	48.1±19.5	0.162
[b]ALT (IU/L)	18.6±12.7	18.5±113.7	19.8±12	17.6±5.6	17.8±10.4	0.837
Total bilirubin/albumin	5.5±1.4	5.5±1.2	5.1±1.3	5.8±1.8	5.8±2.5	0.067
*Direct Coombs positivity	12.9 (46)	10.4 (24)	16.1 (11)	20 (3)	19 (8)	0.378
*Haemolysis finding on peripheral blood smear	20.5 (73)	19.5 (45)	19.1 (13)	6.6 (1)	33.3 (14)	0.148

*Normal spontaneous vaginal delivery; [a] Cesarean section; [b] Data are shown as mean ± standard deviation.

blood group incompatibility was as 8±7.5 days, which was statistically significantly higher compared with the other study groups. The underlying reason for this significant difference may be the increased awareness both in families and health institutions as a result of routine scans for ABO and Rh blood group incompatibilities carried out within the scope of antepartum blood group analyses. In some countries, scans for non-anti-D erythrocyte alloimmunisation, with respect to minor blood group incompatibility, are carried out in pregnant women due to the risk of severe Haemolytic disease, even though the risk is very low.[8] However, there are no such routine scans in Turkey.

The AAP guideline on management of hyperbilirubinaemia highlights the importance of the presence of Haemolysis, with respect to hyperbilirubinaemia and neurotoxicity, and states that haemolytic disease should be primarily considered if jaundice is detected and the predischarge hour-specific TSB nomogram shows a TSB value within the high risk zone, especially in the first 24 hours.[12,13] Because haemolytic and nonhaemolytic factors are generally seen together in neonatal period, assessing TSB, reticulocyte, direct Coombs test, complete blood count, and erythrocyte morphology on peripheral blood smear may be insufficient to evaluate Haemolytic disease in these patients.[14] In another study carried out by Bolat et al on hyperbilirubinaemic neonates with ABO or RhD incompatibility, the researchers have determined that the rate of severe hyperbilirubinaemia in patients with ABO incompatibility was significantly higher.[15] In our study, no significant difference was found in laboratory findings between groups, except lower haemoglobin and haematocrit levels reported in patients with minor blood group incompatibility. These findings suggest that newborns with minor blood group incompatibility are exposed to Haemolysis for a longer time because this patient group applies to a hospital later compared with the other groups.

The most valid treatment method in UHB is phototherapy. In high TSB levels unresponsive to phototherapy, exchange transfusion should be urgently administered. Other medical treatment options apart from IVIG administration, such as phenobarbital and metalloporphyrins, are less frequently preferred. Particularly, in the presence of haemolytic disease of the newborn, IVIG is recommended if there is a TSB level within the limits of exchange transfusion or an increased TSB level despite phototherapy.[16] In the study by Bolat et al, the need for IVIG administration was more frequently reported among patients with Rh blood group incompatibility,[15] and in an another study by Nasseri et al, a shorter phototherapy time, a lower rate of exchange transfusion requirement, and a shorter hospitalisation period were reported for neonates receiving IVIG with a diagnosis of haemolytic disease of the newborn.[17] According to Beken et al, IVIG therapy did not decrease phototherapy nor hospitalisation duration in infants with Haemolytic disease-related ABO blood group incompatibilities. IVIG is not successful for decreasing haemolysis but IVIG prevents requiring exchange transfusion.[18] Likewise, in the Demirel et al's study, IVIG therapy, single or multiple, did not affect exchange transfusion, need of erythrocyte transfusion, and hospitalisation when used in combination with phototherapy.[19] In the study by Karagöl et al, in which the researchers have evaluated 106 patients with

Table 3 Responses to treatment and clinical course in patient groups

	General	Group 1	Group 2	Group 3	Group 4	p
*Phototherapy time (hours)	37.1±20.1	37.6±21.5	35.1±16.6	34.6±19	38.8±17.8	0.729
*Decrease in total serum bilirubin level per hour	0.37±0.17	0.41±0.21	0.24±0.15	0.33±0.19	0.28±0.16	0.903
Rebound bilirubin	18 (64)	14.4 (33)	22 (15)	13.3 (2)	33.3 (14)	**0.025**
Intravenous fluid need	13.5 (48)	9.1 (21)	8.8 (6)	40 (6)	35 (15)	**0.000**
**IVIG need	16 (57)	14.3 (33)	14.7 (10)	6 (1)	30 (13)	0.067
Exchange transfusion need	1.6 (6)	0.4 (1)	–	–	11.9 (5)	**0.001**
Erythrocyte transfusion need	4.5 (16)	0.8 (2)	4.4 (3)	6.6 (1)	23.8 (10)	**<0.001**
Rehospitalisation	6.2 (22)	5.2 (12)	1.4 (1)	6.6 (1)	19 (8)	**0.005**

*Data are shown as mean ± SD and % (n); **Intravenous immunoglobulin

minor blood group incompatibility, 20.8% of all patients have been subjected to exchange transfusion.[11] In our study, the rate of exchange transfusion requirement was 1.6% for all groups, and the rate of exchange transfusion administration in the minor blood group incompatibility (group 4) was 11.9% (n=5) of the total study patients. In our study, there was no significant difference between groups with respect to the need for IVIG, whereas in patients with minor blood incompatibility, the need for exchange transfusion and erythrocyte transfusion was significantly higher compared with the other patients. In patients who have been subjected to IVIG treatment, no difference was found compared with the other patients regarding phototherapy time, exchange transfusion requirement, and rebound increase in TSB.

In our study, the rate of intravenous fluid need was higher in groups with minor blood group incompatibility and ABO+Rh blood group incompatibility compared with the other groups. This may be the result of the presence of a higher rate of dehydration in these two groups, although it was not statistically significant. Furthermore, for patients with minor blood group incompatibility, the higher rate of intravenous fluid use may be associated with the introduction of intravenous fluid administration with discontinuation of oral feeding which occurred because of the presence of a higher need for exchange transfusion and erythrocyte transfusion in the patient group.

Bilirubin levels may increase again after phototherapy, and a postdischarge rehospitalisation may be observed as a result thereof. The presence of haemolytic jaundice is a critical risk factor for rebound bilirubin levels. Other risk factors include premature labour, the onset time and severity of jaundice, and G6PDH deficiency and diet.[12,20] In a study by Bansal et al, the researchers have reported rebound bilirubin levels in 7.3% of patients with hyperbilirubinaemia.[21] In our study, the frequency value was found to be 18% for all patients, and patients with Rh or minor blood group incompatibility were more frequently associated with rebound bilirubin levels. The frequency of postdischarge rehospitalisation was reported as 13.3% in a study by Kaplan et al.[20] In our study, the frequency value was 6.2% in general and was significantly higher among patients with minor blood group incompatibility. This overall high rate reported in our study was thought to be dependent on the fact that unlike other studies, we have only included the patients with Haemolytic disease of the newborn in our study. Also, the patients with minor blood group incompatibility was associated with lower haemoglobin and haematocrit levels.

The authors have probably checked potential incompatibility at other blood group antigens when ABO or RhD incompatibility did not explain the Haemolytic disease of a newborn. A higher rate of incompatibility could be detected, if we have routinely examined suitable parameters in all newborns.

Conclusion

Consequently, it was found that minor blood group incompatibility constituted 12% of all blood group incompatibilities among patients included in this study. The older postnatal age at admission, more frequent co-occurrence of anaemia, rebound TSB levels, higher rates of intravenous fluid, exchange transfusion and erythrocyte transfusion needs, and higher rate of rehospitalisation were seen in the patients with minor blood group incompatibilities compared with the other patients. Therefore, when ABO and/or Rh blood group incompatibilities are not detected in neonates with jaundice requiring hospitalisation, minor blood group incompatibilities should always be taken into consideration.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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