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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Neonatal hyperbilirubinemia caused by anti-Jr^a antibodies – The first case report in Serbia

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SUMMARY

Introduction Jr^a is a high-frequency antigen belonging to the JR blood group system. Population studies have established that the Jr (a-) phenotype is rare. The clinical significance of anti-Jr^a antibodies is controversial. This case report describes a newborn with prolonged jaundice due to alloimmunization against Jr^a antigen.

Case Outline A female Roma infant, 27 days of age, was admitted to hospital due to prolonged jaundice and failure to thrive. Immunohematological testing determined a blood group type A, D+ C+ E+ c+ e+, K-, and the presence of an antibody direct against a high-prevalence red blood cell antigens. On admission, total bilirubin was 199.6 μmol/l, direct bilirubin 10.3 μmol/l, hemoglobin concentration 132 g/l, hematocrit 41.1%, reticulocytes 1.08%. The newborn was the third child from a third routinely monitored pregnancy. Maternal sensitization to Jr^a antigen was detected during the second pregnancy. The titer of anti-Jr^a reached the highest value of 1,024 at the 28th week of gestation.

Conclusion This is the first description of neonatal hyperbilirubinemia caused by anti-Jr^a antibody in the Republic of Serbia. This case report provides new data about the clinical significance of anti-Jr^a in pregnancy and the newborn.

Keywords: Jr^a alloimmunization; neonatal jaundice; rare blood

INTRODUCTION

More than 340 types of red blood cell antigens have been described so far.

The antigen Jr^a was first reported in 1970 by Stroup and MacIlroy, who determined that five individuals had an antibody to the same antigen. The new blood group antigen, one of the first five probands, was named Jr after Rose Jakobs [1]. Since 1990, the Jr^a has been placed in the 901 series of high incidence antigens with an incidence of greater than 90% in most of the explored population [1].

In 2012, based on the published reports, the gene responsible for Jr^a expression was identified. The International Society for Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Terminology ratified establishment of a new blood group system, JR (ISBT 032) [2–4].

The JR blood group system consists of one antigen Jr^a, and an ISBT number 032001 has been assigned to it. It is a membrane glycoprotein which is encoded by the *ABCG2* gene on chromosome 4q22.1. Fourteen unique *ABCG2* null alleles define Jr (a-) blood group phenotype [2, 3].

Population studies worldwide have established that the Jr (a-) blood type is very rare. Most cases have been reported among Japanese and other Asian populations, European Romani population, but also among Bedouins. In Japan, the incidence of the Jr (a-) phenotype ranges from a high of one in 60 in the Niigata area, to a low of one in 3,800 in the Tokyo area [2–4].

Anti-Jr^a may be stimulated by transfusion or by pregnancy. A review of literature indicates that anti-Jr^a may be clinically significant because they can cause hemolytic disease of the fetus and newborn (HDFN) and acute or delayed hemolytic transfusion reactions [5–7].

In this article, the authors describe a newborn with prolonged jaundice due to maternal alloimmunization against Jr^a antigen.

CASE REPORT

A female Roma newborn, 27 days of age, was admitted to hospital in the Institute for Mother and Child Health Care "Dr Vukan Čupić" because of yellow skin and failure to thrive. Weight at admission was 3,390 g, body length was 49 cm, the child was afebrile. The skin and the sclera were yellow. The rest of physical examination was normal.

On admission, total bilirubin concentration was 199.6 μmol/l, with direct value of 10.3 μmol/l, and C-reactive protein was 0.1 mg/l. Serum levels of bilirubin were such that phototherapy was not indicated. The concentration of hemoglobin (Hb) was 132 g/l, of hematocrit

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Table 1. Results of immunohematological testing of the mother and the newborn on admission

Results of immunohematological testing of the mother and the newborn on admission	Blood group type	DAT	IAT
Newborn on the 27th day after birth	A D+ C+ c+ E+ e+ K-	1+	2+
Mother	A D+ C+ c+ E+ e+ K-	/	2+

DAT – direct antiglobulin test; IAT – indirect antiglobulin test

Table 2. Results of the newborn's laboratory tests

Laboratory tests	Hour 12 after birth	Day 4 after birth	Day 27 after birth
Hemoglobin (g/l)	194.0	184.0	132.0
Hematocrit (%)	59.0	53.0	41.1
Erythrocytes ($\times 10^{12}$/l)	/	5.1	4.4
MCV (fl)	110.2	/	94.4
MCH (pg)	36.3	/	30.4
MCHC (g/l)	329.0	/	322.0
Platelets ($\times 10^{9}$/l)	570.0	/	375.0
Leukocytes ($\times 10^{9}$/l)	22.3	11.8	7.9
Reticulocytes (%)	/	/	1.1
Total bilirubin (μmol/l)	45.0	234.0	199.6
Direct bilirubin (μmol/l)	/	102.0	10.3

41.1%, of reticulocytes 1.08%. The newborn's blood type was A, D+ C+ E+ c+ e+, K-, direct antiglobulin test (DAT) positive (1+), indirect antiglobulin test (IAT) positive (2+). The mother's blood group was A, D+ C+ E+ c+ e+, K-, DAT- , IAT 2+ (Table 1). Using the IAT, red cell antibodies with agglutination strength of 1+ and 2+ with all the test red blood cells were identified in the newborn's plasma. Autocontrol was 1+. For the determination of the newborn's blood group and DAT we used the ID-Micro Typing System, ID-Card "ABO/Rh for Newborns" (Bio-Rad GmbH, Cressier FR, Switzerland) gel method. The mother's blood group was determined by ID-Micro Typing System, ID-Card "ABO/D+ reverse grouping" (Bio-Rad GmbH) gel method, and Rh phenotype was determined by ID-Micro Typing System, ID-Card "DiaClon Rh Subgroups + K" cards (Bio-Rad GmbH).

Antibody screening and antibody identification were performed in a gel card (Bio-Rad ID-Micro Typing System, ID-Card "LISS/Coombs") using erythrocyte screening test (DIACELL A_1 and B and DIACELL I-II-III) and ID-DiaPanel (BioRad, Cressier sur Morat, Switzerland) for antibody identification.

The newborn was the third child from a third pregnancy, which was routinely monitored. The pregnancy ended at the 39th week of gestation (WG) in vaginal birth at the Gynecology and Obstetrics Clinic "Narodni front" (GOC NF) in Belgrade, Serbia. At the time of birth, the neonate's weight was 2,850 g, height 48.0 cm, head circumference 33 cm. The neonate had an Apgar scores of 9 points at one and 10 points at five minutes after birth. Serial bilirubin levels were followed with a total bilirubin concentration of 27 μmol/l at birth, gradually increasing, with a peak at 234 μmol/l on day 4. The neonate underwent phototherapy for 48 hours. Table 2 shows the main results of the newborn's laboratory tests at birth, on hour 12, day 4, and day 27.

During the mother's second pregnancy, alloimmunization to Jr^a antigen was detected in Gothenburg, Sweden. On that occasion, the titer of anti- Jr^a was evaluated at the 36th and 38th WG. The antibody titer was 32 and 128, respectively. Before the second delivery in Gothenburg, two units of red blood cells (RBCs) of Jr (a-) phenotype had been supplied from Barcelona, Spain. The newborn from the second pregnancy had the peak of total bilirubin concentration on day 4, 200 μmol/l, and Hb of 170 g/l. Phototherapy was not required.

The newborn's mother of Romani origin was 21 years old at the time of her third pregnancy. Her pregnancy was monitored and checked in Belgrade. The anamnesis did not contain information about previous transfusions and abortion. She had an obstetric history of two full-term pregnancies. Immunohematological testing was performed at the Blood Transfusion Institute of Serbia in Belgrade, for the first time at the 12th WG. On this occasion, initial laboratory investigation showed a blood group type A, D+ C+ E+ c+ e+. The DAT was negative and IAT 2+. The father's blood group type was determined as A, D+ C+ E – c – e+; DAT-. The mother's titer of anti-Jr^a was regularly controlled using the father's Jr (a+) erythrocyte (method in a liquid phase) [8]. The titer was 256 in the 18th WG, 256 in the 24th WG, and 1,024 in the 28th and 32nd WG.

The mother was admitted for perinatal management to the Department of High Risk Pregnancies of the GOC NF in the 36th WG. Initial laboratory tests showed low hemoglobin level (Hb 96 g/l, hematocrit 30.6%, serum iron 7.7 nmol/l). Other laboratory results were in the reference values for the gestation. It was decided to treat anemia first with parenteral administration and then by oral application of hematinic (iron supplements) and then to perform autologous blood collection.

Fetoplacental and uteroplacental circulation Doppler indices were normal. The peak systolic velocity of the fetal middle cerebral artery was 0.8 multiples of median. Amniocentesis was also carried out and the value was in the B1 zone of the Liley curve. Cardiotocographic record showed type II b fluctuations with no uterine activity [9].

Due to lack of Jr (a-) donors, 350 ml of maternal whole blood was obtained in the 38th WG following correction of anemia and with the mother's consent. Whole blood was deplasmatised, filtered, and divided into three RBC units of 50 ml. Because the newborn did not need a transfusion, the mother received autologous RBCs due to anemia [9].

DISCUSSION

Since the phenotype Jr (a-) is very rare, the clinical significance of anti-Jr^a antibodies is not well-established either in cases of Jr^a-incompatible transfusions of RBCs or in the ability of these antibodies to cause HDFN. Most of the described examples in literature refer to sensitization during pregnancy [9–12].

The Jr^a antigen is located on the *ABCG2* transporter. It is a multipass membrane glycoprotein which is encoded by the *ABCG2* gene on chromosome 4q22.1. DNA sequence analysis showed that the null allele *ABCG2* define the Jr (a-) phenotype. Under normal conditions, *ABCG2* is thought to have an important role in protecting the organism against various toxic substances by restricting absorption or facilitating elimination [2–4].

The Jr^a antigen is fully developed at birth. Anti-Jr^a is generally IgG and may pass the placenta. So far, all four subclasses of IgG (IgG_1, IgG_2, IgG_3, IgG_4), with large predominance of IgG_1, have been found in the described cases of HDFN [10, 11, 13–15]. When hemolytic disease of the newborn occurred, most reported cases were mild to moderate in intensity and often required no treatment beyond phototherapy [9–11].

In the published articles on HDFN caused by anti-Jr^a, pregnant women did not have history of blood transfusions, which indicates that the alloimmunization was the result of previous pregnancies.

Kim et al. [13] presented the sensitization of a nulliparous woman. A 33-year-old Korean woman in the 32nd WG had a twin pregnancy after an in vitro fertilization and embryo transfer. She had neither received a transfusion of RBCs nor had she been subjected to amniocentesis. On admission to the hospital, the patient's serological testing determined anti-Jr^a.

Ishihara et al. [14] showed a severe hemolytic disease of the fetus in pregnant Japanese women. Fetal hydrops was diagnosed in the 29th WG. Four intrauterine intravenous transfusions were successfully applied in the course of the treatment. The delivery was done in the 35th WG and the newborn's hemoglobin at birth was 72 g/l. The newborn received two transfusions of RBCs for the treatment of anemia.

Peyrard et al. [15] reported the first documented case of fatal HDFN. A 28-year-old woman of Romani origin from Spain had an obstetric history of four pregnancies and the last pregnancy with a severe HDFN. The neonate was hydropic with multiorgan system failure. Death occurred 30 hours after birth. The maternal serum titer of anti-Jr^a was evaluated at 1,024.

In our case report, anti-Jr^a caused hyperbilirubinemia, which required treatment by phototherapy on the fourth day after birth for a period of 48 hours. The value of total bilirubin was 234 μmol/l. Jaundice and failure to thrive lasted until the end of the infant's first month of life.

Like in case reports of most other authors, hemolytic disease was mild in the presented newborn. Sensitization to the Jr^a antigen which occurred in the first pregnancy did not significantly influence the development of the second pregnancy. Anti-Jr^a antibody detected in the second pregnancy had a maximum value titer of 128 in the 38th WG. The second, also full term, pregnancy ended in the vaginal birth of a healthy child. Two units of typed Jr (a-) erythrocytes that had been supplied from Barcelona were not transfused. During the third pregnancy, the maximum value of the anti-Jr^a of 1,024 was confirmed in the 28th WG and it was the same in the 32rd WG, when it was checked the last time. Despite the antibody titer being significantly higher than in the second pregnancy, ultrasound measurements of fetal development and spectrophotometric analysis of amniotic fluid were normal.

Exact explanation of pathogenetic mechanism of anti-Jr^a antibodies in HDFN is still unknown. It is assumed that a toxic effect of anti-Jr^a antibodies to the erythroid Jr^a of cell precursors (as described in cases of anti-Kell and anti-Ge3 antibodies) is the direct cause of severe fetus/neonate anemia [16–18]. It is believed that the *ABCG2* gene is responsible for the regulation of the differentiation of precursor cells of RBCs, while on the other hand anti-Jr^a antibodies can be included in an uncontrolled differentiation [19].

In conclusion, we should emphasize a very important fact that the Jr (a-) phenotype is rare among Caucasians. For example, in the French National Registry of rare blood group phenotypes and genotypes in 2008, there were 9,508 people. Out of that number, 0.27% or 26 persons were phenotype Jr (a-), and most of them are Romani. Only nine were occasional blood donors [14].

It is very difficult to provide erythrocytes of corresponding phenotype for transfusion therapy of Jr (a-) immunized patients and newborns with anti-Jr^a antibodies. Autologous blood collection or a collection of blood from Jr (a-) relatives is possible choice for elective treatment.

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Неонатална хипербилирубинемија узрокована анти-*Jr^a* антителом – први случај у Србији

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САЖЕТАК

Увод Еритроцитни антиген велике учесталости *Jr^a* припада крвногрупном систему *JR*. Популационе студије су показале да је фенотип *Jr* (a-) врло редак. Клинички значај анти-*Jr^a* антитела је контроверзан. У овом раду аутори приказују новорођенче са продуженом жутицом због алоимунизације мајке на *Jr^a* антиген.

Приказ болесника Женско новорођенче узраста 27 дана, ромског порекла, хоспитализовано је због продужене жутице и ненапредовања. Имунохематолошким испитивањем новорођенчету је утврђена крвна група A, Д+ Ц+ E+ ц+ e+, K- и присуство антиеритроцитних антитела на високофреквентни еритроцитни антиген. Укупни билирубин на пријему био је 199,6 *μmol/l*, директни билирубин 10,3 *μmol/l*, концентрација хемоглобина 132 *g/l*, хематокрит 41,1%, ретикулоцити 1,08%. Новорођенче је треће дете из треће уредно контролисане трудноће. Сензибилизација мајке на антиген *Jr^a* откривена је током друге трудноће. Максимална вредност титара антитела од 1024 утврђена је у 28. недељи гестације треће трудноће.

Закључак Ово је први описани случај неонаталне хипербилирубинемије узроковане анти-*Jr^a* антителом у Србији, који даје нове податке о клиничком значају анти-*Jr^a* антитела у трудноћи и код новорођенчета.

Кључне речи: *Jr^a* алоимунизација; жутица новорођенчета; ретка крвна група