Paper Accepted

Case Report / Приказ болесника

Zorica Radonjić1,†, Snežana Jovanović-Srzentić2, Ivana Pešić-Stevanović3, Olivera Šerbić-Nonković1, Marija Popović1

Neonatal Hyperbilirubinemia Caused by Anti-Jrα Antibodies – The First Case Report in Serbia
Неонатална хипербилирубинемия узрокована анти-Јrα антителом – први случај у Србији

1Institute for Mother and Child Health Care "Dr Vukan Čupić", Belgrade, Serbia; 2Institute for Blood Transfusion of Serbia, Belgrade, Serbia; 3Gynecology and Obstetrics Clinic "Narodni front", Belgrade, Serbia

Received: February 9, 2016
Revised: July 11, 2016
Accepted: July 12, 2016
Online First: February 10, 2017
DOI: 10.2298/SARH160209016R

* Accepted papers are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the Serbian Archives of Medicine. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author’s last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

† Correspondence to:
Zorica RADONJIĆ
Institute for Mother and Child Health Care "Dr Vukan Čupić"
Radoja Dakica 6–8, 11070 Belgrade, Serbia
zorar@sezampro.rs
Neonatal Hyperbilirubinemia Caused by Anti-Jr\(^{a}\) Antibodies – The First Case Report in Serbia

Neонатална хипербилирубинемија узрокована анти-Jr\(^{a}\) антителом – први случај у Србији

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 Female Roma infant, 27 days of age, was admitted to hospital due to prolonged jaundice and failure to thrive. The 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, the hemoglobin concentration 132 g/l, hematocrit 41.1%, reticulocytes 1.08%. The newborn is 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 1024 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 antibody to the same antigen. A new blood group antigen named Jr after Rose Jakobs, one of the first five probands [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 population, 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 3800 in the Tokyo area [2-4].

Anti-Jr\textsuperscript{a} may be stimulated by transfusion or by pregnancy. A review of the literature indicates that anti-Jr\textsuperscript{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\textsuperscript{a} antigen.

CASE REPORT

A female Roma newborn, 27 days of age, was admitted to the hospital in the Institute for Mother and Child Health Care "Dr Vukan Ćupić" because of yellow skin and failure to thrive. Weight at admission was 3390 g, body length 49 cm, afebrile. The skin and the sclera were yellow. The rest of physical examination was normal.

On admission, total bilirubin concentration was 199.6 \(\mu\)mol/l, with direct value of 10.3 \(\mu\)mol/l, and C reactive protein (CRP) was 0.1 mg/l. The serum levels of bilirubin were such that phototherapy was not indicated. The concentration of hemoglobin (Hb) was 132 g/l, hematocrit (Hct) 41.1 %, 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 have been identified in the newborn’s plasma. Autocontrol was 1+. For the determination of the newborn’s blood group and DAT we used the gel method Bio Rad-IDMicro Typing System, ID Card "ABO/Rh for Newborns". Mother’s blood group was determined by gel method BioRad-IDMicro Typing System, ID Card "ABO/D + reverse grouping" and Rh phenotype was determined in the cards BioRad-IDMicro Typing System, ID Card "Dia Clon Rh Subgroups + K "(BioRad GmbH, Cressier FR, Switzerland).

Antibody screening and antibody identification were performed in the gel card (BioRad-IDMicro Typing System, ID Card 'LISS/Coombs') using screening test erythrocytes (ID-DIACELL A1 and B and ID-DIACELL I-I-III) and for antibody identification – ID Dia Panel (BioRad, Cressiers / Morat, Switzerland).

<table>
<thead>
<tr>
<th>Results</th>
<th>Blood group type</th>
<th>DAT</th>
<th>IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn on the 27th day after birth</td>
<td>A D+ C+ c+ E+ e+ K -</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Mother</td>
<td>A D+ C+ c+ E+ e+ K -</td>
<td>-</td>
<td>2+</td>
</tr>
<tr>
<td>DAT – direct antiglobulin test; IAT – indirect antiglobulin test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Results of immunohematological testing of the mother and the newborn on admission.
The newborn is the third child from a third pregnancy which was routinely monitored. Pregnancy ended at the 39th week of gestation (WG) in vaginal birth at the Gynecology and Obstetrics Clinic "Narodni front" (GOC NF) in Belgrade. At the time of birth, the neonate's weight was 2850 g, height 48.0 cm, a head circumference 33.0 cm. The neonate had Apgar scores of 9 points at 1 and 10 points at 5 minutes after birth. Serial bilirubin levels were followed with a total bilirubin concentration 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 Jrα antigen was detected in Gothenburg, Sweden. On that occasion, the titer of anti-Jrα was evaluated at 36th and 38th WG. The antibody titer was 32 and 128 respectively. Before the second delivery in Gothenburg, two red blood cells (RBCs) units of Jr (α-) 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 170 g/l. The phototherapy was not required.

The newborn’s mother of Romani origin was a 21-year-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 pregnancy. Immunohematological testing was performed at the Institute for Blood Transfusion of Serbia in Belgrade, for the first time at 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-Jrα was regularly controlled using the father’s Jr (α+) erythrocyte (method in a liquid phase) [8]. The titer was 256 at 18th WG, 256 at 24th WG, and 1024 at 28th and at 32nd WG.

The mother was admitted for perinatal management to the Department of High Risk Pregnancies GOC NF at 36th WG. Initial laboratory tests showed a 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

**Table 2. Results of the newborn’s laboratory tests**

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>Hour 12 after birth</th>
<th>Day 4 after birth</th>
<th>Day 27 after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/l)</td>
<td>194.0</td>
<td>184.0</td>
<td>132.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>59.0</td>
<td>53.0</td>
<td>41.1</td>
</tr>
<tr>
<td>Erythrocytes (×10¹²/l)</td>
<td>/</td>
<td>5.1</td>
<td>4.4</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>110.2</td>
<td>/</td>
<td>94.4</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>36.3</td>
<td>/</td>
<td>30.4</td>
</tr>
<tr>
<td>MCHC (g/l)</td>
<td>329.0</td>
<td>/</td>
<td>322.0</td>
</tr>
<tr>
<td>Platelets (×10⁹/l)</td>
<td>570.0</td>
<td>/</td>
<td>375.0</td>
</tr>
<tr>
<td>Leukocytes (×10⁹/l)</td>
<td>22.3</td>
<td>11.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>/</td>
<td>/</td>
<td>1.1</td>
</tr>
<tr>
<td>Total bilirubin (µmol/l)</td>
<td>45.0</td>
<td>234.0</td>
<td>199.6</td>
</tr>
<tr>
<td>Direct bilirubin (µmol/l)</td>
<td>/</td>
<td>102.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>
the gestation. It was decided to treat anemia first with parenteral administration and then by oral application of hematonic (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 fluctuations type II b with no uterine activity [9].

Due to lack of Jr (a-) donors, 350 ml of maternal whole blood was obtained at 38th WG following correction of anemia and with the mother's agreement. Whole blood was deplasmatised, filtered and divided into three RBCs units of 50 ml. Because the newborn did not need transfusion, 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 the cases of Jr\(a\) incompatible transfusions of RBCs or in the ability of these antibodies to cause HDFN. Most of described examples in the literature refer to sensitization during pregnancy [9-12].

The Jr\(a\) antigen is located on ABCG2 transporter. It is a multipass membrane glycoprotein which is encoded by the \(ABCG2\) gene on chromosome 4q22.1. DNA sequences 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].

Antigen Jr\(a\) is fully developed at the birth. Anti-Jr\(a\) is generally IgG and may pass the placenta. So far all four subclasses of the IgG (IgG\(1\), IgG\(2\), IgG\(3\), IgG\(4\)) with large predominance of the IgG\(1\) have been found in 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 phototerapy. [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 H. et al. have presented the sensitization of nulliparous woman. A 33-year-old Korean woman at 32nd WG had a twin pregnancy after 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 patient’s serological testing determined anti-Jr\(a\) [13].

Ishihara and co-workers showed a severe hemolytic disease of the fetus in pregnant Japanese women. Fetal hydrops was diagnosed at 29th WG. Four intrauterine intravenous transfusions were successfully applied in the course of the treatment. The delivery was done at 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 [14].
Peyrard et al. 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 was evaluated at 1024 [15].

In our case report, the anti- Jr caused hyperbilirubinemia, which required treatment by phototherapy on the 4th day after birth for a period of 48 hours. The value of total bilirubin was 234 µmol/l. Jaundice and failure to thrive lasted till 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 Jr antigen which occurred in the first pregnancy did not significantly influence the development of the second pregnancy. Anti-Jr antibody detected in the second pregnancy had a maximum value titer 128 at 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 of 1024 was confirmed in the 28th WG and it was the same in 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 as well as spectrophotometric analysis of amniotic fluid were normal.

Exact explanation of pathogenetic mechanism of anti-Jr antibodies in HDFN is still unknown. It is assumed that a toxic effect of anti-Jr antibodies to the erythroid Jr of cell precursors (as described in the 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 is responsible for the regulation of the differentiation of precursor cells of red blood cells while on the other hand anti-Jr 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 9508 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 antibodies. Autologous blood collection or a collection of blood from Jr (a-) relatives is possible choice for elective treatment.

REFERENCES