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Red blood cell alloimmunization in pregnancy: A 10-year single-center study

Desetogodišnja studija jednog centra o aloimunizaciji eritrocitnim antigenima u trudnoći

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Abstract

Background/Aim. Pregnancy-induced red blood cell (RBC) alloimmunization is important not only because of the possible negative effects on subsequent pregnancy outcomes in case the fetus carries the antigen but also because of the optimal transfusion management in cases of obstetric hemorrhage. Timely detection of RBC antibodies is part of testing, prevention, and treatment strategy, aimed at achieving better outcomes for alloimmunized mothers with an affected fetus. The aim of the study was to determine the frequency and specificity of alloantibodies among pregnant women from the South Bačka District, Serbia, with special attention to the incidence of anti-D alloantibodies. **Methods.** A retrospective study was conducted in the Blood Transfusion Institute of Vojvodina and covered the period from January 1, 2010, to December 31, 2019. Screening and antibody identification were performed by an indirect antiglobulin test in gel-microcards (ID-Card Liss/Coombs) with two test RBC (ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland) on an automated system (IH-500, Bio-Rad). **Results.** Among 25,694 tested pregnant women, 1.38% were actively immunized, while 1.12% of women acquired antibodies in the current pregnancy. Among 3,622 (14.09%) RhD-negative women, 1.77% produced anti-D antibodies during the ongoing pregnancy. Distribution of antibody specificity was: anti-D 23.34%, anti-M 11.85%, anti-E 9.41%, anti-K 9.41%, anti-C 5.92%, anti-Fy^b 5.92%, anti-c 3.13%, anti-S 3.13%, anti-Le^a 3.13%, anti-Le^b 3.13%, anti-C^w 1.75%, anti-Jk^a 1.40%, anti-P 1.05%, anti-Lu^b 0.70%, anti-Fy^a 0.35%, autoantibody of undetermined specificity 0.70%, and irregular antibodies of undetermined specificity 15.68%. **Conclusion.** Immunoglobulin prophylaxis has led to a significant reduction in the frequency of D-alloimmunization among pregnant women in the South Bačka District over the last ten years. However, the incidence of anti-D antibodies is still significantly higher than in published data for developed countries. We also identified the other, less commonly present, clinically significant antibodies. There is a need to introduce uniform recommendations for immunohematological testing in pregnancy on the territory of the Republic of Serbia in accordance with modern requirements.

Key words:
blood group antigens; blood group incompatibility; erythrocytes; immunity, humoral; prenatal diagnosis; rh-hr blood – group system.

Apstrakt

Uvod/Cilj. Aloimunizacija eritrocitnim antigenima izazvana trudnoćom nije važna samo zbog mogućih negativnih efekata na ishode sledećih trudnoća, ukoliko fetus nosi određeni antigen, već i zbog optimalnog upravljanja transfuzijom u slučajevima akušerskog krvarenja. Pravovremeno otkrivanje antieritrocitnih antitela deo je strategije ispitivanja, prevencije i lečenja, kako bi se postigao bolji ishod za aloimunizovane majke sa ugroženim fetusom. Cilj rada bio je da se utvrdi učestalost i specifičnost aloantitela među trudnicama južnobačkog okruga, Srbija, sa posebnom pažnjom na učestalost anti-D aloantitela. **Metode.** Retrospektivnom studijom, sprovedenom u Zavodu za transfuziju krvi Vojvodine, obuhvaćen je period od 1. januara 2010. do 31. decembra 2019. godine Skrining i identifikacija antitela kod trudnica vršeni su indirektnim antiglobulinskim testom u gel-mikrokarticama (*ID-Card Liss/Coombs*), dvoćelijskim test eritrocitima (*ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland*), automatizovanim sistemom (IH-500, Bio-Rad). **Rezultati.** Među 25 694 testiranih trudnica ustanovljeno je da je 1,38% aktivno imunizovanih, dok su kod njih 1,12% ova antitela nastala u tekućoj trudnoći. Među 3 622 (14,09%) RhD-negativnih žena, njih 1,77% je produkovalo anti-D antitelima tokom tekuće trudnoće. Raspodela antitela prema specifičnosti bila je: anti-D 23,34%, anti-M 11,85%, anti-E 9,41%, anti-K 9,41%, anti-C 5,92%, anti-Fy^b 5,92%, anti-c 3,13%, anti-S 3,13%, anti-Le^a 3,13%, anti-Le^b 3,13%, anti-C^w 1,75%, anti-Jk^a 1,40%, anti-P 1,05%, anti-Lu^b 0,70%, anti-Fy^a 0,35%,

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autoantitela čija specifičnost se nije mogla odrediti 0,70%, i iregularna antitela čija specifičnost se nije mogla utvrditi 15,68%. **Zaključak.** Profilaksa imunoglobulinom značajno je smanjila učestalost D-aloimunizacije među trudnicama južnobačkog okruga tokom poslednjih deset godina, ali je učestalost anti-D antitela i dalje znatno viša od objavljenih podataka za razvijene zemlje. Takođe, identifikovali smo prisustvo i drugih, ređe prisutnih, ali klinički značajnih antitela. Potrebno je uvesti jedinstvene preporuke za imunohematološka ispitivanja u trudnoći na teritoriji Republike Srbije, u skladu sa savremenim zahtevima.

Ključne reči:
krvne grupe, antigeni; krvne grupe; nepodudaranje; eritrociti; imunitet, humoralni; dijagnoza, prenatalna; krvne grupe, rh-hr sistem.

Introduction

The blood group antigens recognized by the Working Party on Red Cell Immunogenetics and Blood Group Terminology (ISBT) are formally registered within 38 blood group systems [1]. The blood group systems consist of one or more antigens with varying importance to the transfusion care of patients and to the outcome of pregnancies in which the child inherits from the father an antigen that the mother does not possess [2–3].

Pregnancy-induced red blood cell (RBC) alloimmunization is important not only because of the possible negative effects on subsequent pregnancy outcomes if the fetus carries the antigen but also because of the optimal transfusion management in cases of obstetric hemorrhage. Timely detection of RBC antibodies is part of testing, prevention, and treatment strategy in order to achieve better outcomes for alloimmunized mothers with an affected fetus. The testing protocols are designed to provide recommendations for blood grouping and RBC antibody testing in pregnancy in order to protect pregnant women and their children [4].

The antigenic difference between the pregnant woman and her fetus, the patient's immune status, and the immunogenicity of the RBC antigens are some of the factors that influence the formation of antibodies. The Rhesus D antigen (RhD) has the greatest importance in pregnancy even though, in the case of maternal and infant RhD incompatibilities, there is the possibility of preventing immunization with human anti-D immunoglobulin. In the general population of Vojvodina (north part of Serbia), approximately 84% of the population is RhD-positive, while 16% is RhD-negative [5].

Once an antibody to RBC has been detected during immunohematological testing of pregnant women, their specificity must be determined in order to predict the possibility of occurrence of hemolytic disease in the fetus and newborn (HDFN) [6]. Pregnant women with antibodies (anti-D, anti-c, and anti-K) known for having the potential to cause clinically significant HDFN are monitored more frequently, according to established algorithms which define the frequency of testing, measurement of antibodies concentration, referrals to specialist examinations, monitoring after childbirth, etc. [6–8]. In the case of immunization, the child's biological father is also being tested (ABO blood group, Rh phenotype, the presence of the specific antigen). Defining the RhD fetal genotype from fetal cells and cell-free fetal DNA (cffDNA) in maternal plasma has opened up opportunities for improved noninvasive prenatal testing and allowed invasive procedures, such as amniocentesis and chorionic villus sampling, to be abandoned [9]. Cordocentesis can also be done after week 18 of pregnancy in order to perform the basic immunohematological analysis and to detect and treat fetal anemia in the pregnancies at risk (when pregnant women have clinically significant antibodies in a concentration above the critical titer). Collecting fetal blood by cordocentesis also became rare due to a high risk of complications to the child [4]. Monitoring of alloimmunized pregnancies with a risk of fetal anemia is possible using the fetal middle cerebral artery Doppler [4, 7].

Compared to other causes, immune-mediated fetal and neonatal hemolysis are accompanied by a higher bilirubin level in the early stages after birth and a longer term of hyperbilirubinemia. In the most severe cases, phototherapy and immunoglobulin therapy are not sufficient, and hyperbilirubinemia must be treated with exchange transfusion [10, 11]. For all neonates at risk, cord blood should be taken immediately after the delivery in the following cases: if the mother's group was RhD-negative, if the mother's group was O, if antibody screening for the mother was not done or if the result was positive [11]. The routine testing includes ABO grouping and RhD typing, as well as a direct antiglobulin test (DAT). These tests provide information on indications for the use of anti-D immunoglobulin in RhD-negative women [12–14]. In the DAT-positive cases, the antibodies can be obtained by removing the bound antibodies of the neonatal RBC (elution). There are several different techniques for elution based on the process used to cause dissociation of the antigen/antibody complex. The choice of elution technique depends on the type of expected antibodies. Then, that eluate is tested against screening RBC [15].

Immunohematological testing of pregnant women is undertaken to determine the presence of RhD antigens, identify RhD-negative women who need anti-D immunoglobulin prophylaxis, and identify women with clinically significant RBC alloantibodies. This testing also serves as assistance in the diagnosis and treatment of HDFN [4].

The incidence of alloimmunization in pregnant women has rarely been reported in Serbia. The aim of the study was to determine the specificity and frequency of RBC alloantibodies in pregnant women of the South Bačka District of the Autonomous Province of Vojvodina (the northern part of Serbia). Special attention was paid to the

incidence of anti-D antibodies in order to compare the obtained results with data from other published studies and to evaluate anti-D immunoglobulin protection in the District.

Methods

Study design

The retrospective study was conducted to assess the frequency and specificity of RBC alloantibodies in pregnant women from January 1, 2010, to December 31, 2019. Testing was carried out at the Blood Transfusion Institute of Vojvodina (BTIV), Novi Sad, Serbia, the secondary/tertiary health care referral provider center. BTIV is the reference transfusion institution responsible for the antenatal RBC antibody screening and monitoring of alloimmunized women as well as neonatal testing. Data gathered from transfusion registers and information system of BTIV included: the age of the pregnant women, primary and other diagnoses, transfusion or pregnancy history, ABO blood groups, and RhD antigen typing of pregnant women, antibodies screen results, and identification results. The primary focus of this study was the presence of alloantibodies, antibody specificity, and its association with other patient characteristics.

The study was approved by the Ethics Committee Number 1/2020, on February 17, 2020.

Laboratory testing

At the first antenatal visit, basic demographic characteristics and a comprehensive history of all previous pregnancies were taken (miscarriages, ectopic pregnancy, known alloimmunizations, physical intervention with associated risks to the fetus, previous fetal-maternal hemorrhage and blood transfusion, previous anemic fetus or infant, etc.) in order to detect risk factors for the woman and her fetus.

Routine laboratory testing of maternal blood samples included ABO grouping and RhD typing, as well as screening for irregular RBC antibodies by indirect antiglobulin test (IAT). Further testing included: 1. Rh phenotyping – for RhD-negative women and the presumed father; 2. monitoring of RBC alloantibody development – for pregnant RhD-negative women; 3. antibody identification and monitoring of antibody titers – for pregnant women with positive antibody screening and clinically significant antibodies.

An IAT with gel cards (ID-Card Liss/Coombs) with two RBC test cells (ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland) was used to detect antibodies in the serum of women during pregnancy. Testing was performed on an automatic immunoassay analyzer (IH-500, Bio-Rad).

In case of a positive antibody screen, antibody identification was performed on LISS/Coombs gel card using commercially available eleven RBC antibody identification panels (ID-DiaPanel, Bio-Rad, Cressier, Switzerland) typed for all clinically relevant antigens (D, C, E, c, e, C^w, K, k, Kp^a, Kp^b, Js^a, Js^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P, M, N, S, s, Lu^a and Lu^b). The antibody titer was determined by the test tube method [15]. In the case of anti-D antibody detection, the differentiation between prophylactic and alloimmune anti-D antibodies was done based on anamnestic data (preexisting anti-D antibodies, data of receiving routine antenatal anti-D prophylaxis, complications during pregnancy) and increase/decrease of anti-D antibody titer.

Antibody screening for all pregnant women was primarily performed in the first trimester of pregnancy. All pregnant women were advised to repeat the check of irregular RBC antibodies during the third trimester. RhD-negative and screening-positive pregnant women were controlled more often because of the risk of developing HDFN. The frequency of testing repetition (antibody screening and identification as well as antibody titration) depends on the specificity and strength of the antibody [4].

Statistical analysis

Data collected were analyzed using the statistical program Minitab 16. Descriptive statistics were conducted for all variables. Data are presented in tables and graphs. Statistical significance was set at $p < 0.05$.

Results

A total of 25,694 pregnant women were tested for ABO blood groups, RhD antigens, and antibody screening over the 10-year study period. Out of those tested, 3,622 (14.09%) were RhD-negative and 22,072 (85.91%) were RhD-positive.

A total of 761 (2.96%) antibodies were found, and out of that: 407/761 (53.48%) antibodies were passively introduced as anti-D antibodies as part of the prevention of RhD alloimmunization and 354/761 (46.52%) antibodies were actively produced. Passive anti-D antibodies were found in 128/407 (31.45%) first-time pregnant women, as well as in 279/407 (68.55%) women in second or subsequent pregnancies.

The alloimmunization rate of all pregnant women was 1.38% (354/25,694): 18.93% (67/354) of multiparous pregnant women with antibodies detected during previous pregnancies and 81.07% (287/354) of pregnant women with antibodies detected during the current pregnancy. The alloimmunization rate of pregnant women during the ongoing pregnancy was 1.12% (287/25,694). The Fisher test showed an extremely statistically significant difference between actively immunized RhD-negative pregnant women 4.53% (164/3,622) and RhD-positive pregnant women 0.56% (123/22,072) ($p < 0.0001$).

Anti-D alloantibodies were detected in 1.77% (67/3,622) of RhD-negative pregnant women during the ongoing pregnancy.

None of the pregnant women had a positive history of RBC transfusion.

The specificity of RBC antibodies detected during previous pregnancies is shown in Table 1. Only two pregnant

Table 1

Specificity of red blood cell (RBC) antibodies which are the result of previous pregnancies

Mothers' RhD status	RBC antibodies specificity					
	Anti-D	Anti-D + Anti-Fy[a]	Anti-E	Anti-K	Anti-M	Total
D-positive			9	10	8	27
D-negative	20	2	8		10	40
Total	20	2	17	10	18	67

women alloimmunized during previous pregnancies had multiple antibodies (D + Fy[a]).

The specificity of RBC antibodies detected during current pregnancies is shown in Table 2.

Trend analysis of the annual number of pregnant women with anti-D alloantibodies is given in Figure 1.

The study found 287 women with antibodies detected in current pregnancy: 1) in 141/25,694 (0.55%) women who were pregnant for the first time, antibodies were detected initially at a repeated screening during the third trimester; 2) in 146/25,694 (0.66%) women who reported previous pregnancies.

The specificity of the anti-RBC antibodies produced in the current pregnancy was as follows: anti-D 23.34% (67/287), anti-M 11.85% (34/287), anti-E 9.41% (27/287), anti-K 9.41% (27/287), anti-C 5.92% (17/287), anti-Fy[b] 5.92% (17/287), anti-c 3.13% (9/287), anti-S 3.13% (9/287), anti-Le[a] 3.13% (9/287), anti-Le[b] 3.13% (9/287), anti-C[w]

Table 2

Specificity of red blood cell (RBC) antibodies detected during current pregnancies

Antibody specificity	Number	Maternal characteristics			
		RhD status		The number of pregnancies	
		D-positive	D-negative	First	More than one
Anti-D	67		67	23	44
Anti-C	17		17	17	
Anti-c	9	9		9	
Anti-E	27	20	7	27	
Anti-C[w]	5	5			5
Anti-K	27	22	5	7	20
Anti-M	34	18	16	7	27
Anti-S	9	4	5	2	7
Anti- Fy[a]	1	1			1
Anti- Fy[b]	17		17	5	12
Anti-Le[a]	9	5	4	2	7
Anti-Le[b]	9	6	3	9	
Anti-Jk[a]	4	4		1	3
Anti-P	3	3			3
Anti-Lu[b]	2		2		2
Undetermined specificity	45	25	20	30	15
Autoantibody	2	1	1	2	
Total	287	123	164	141	146

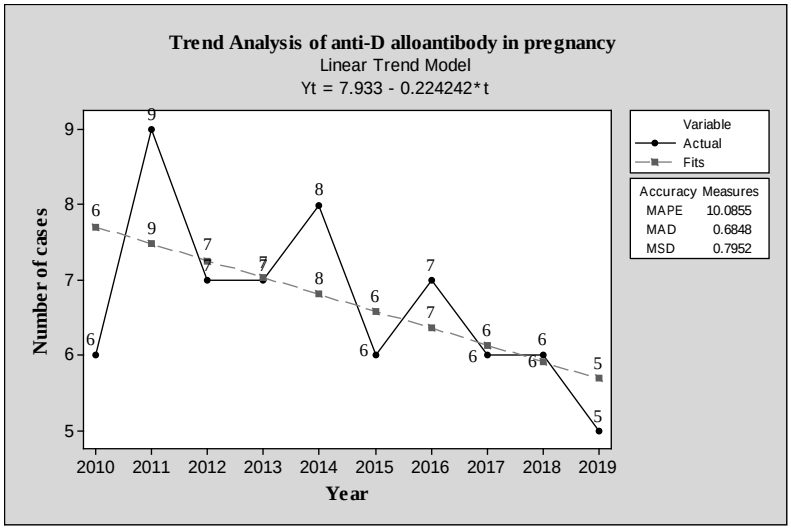

Fig. 1 – Pregnant women anti-D alloantibody, annually.

1.75% (5/287), anti-Jk^a 1.40% (4/287), anti-P 1.05% (3/287), anti-Lu^b 0.70% (2/287), anti-Fy^a 0.35% (1/287), autoantibody of undetermined specificity 0.70% (2/28,734), and irregular antibodies of undetermined specificity 15.68% (45/287).

All the pregnant women with antibodies were followed up, with an average of 3 (rank 1-6) controls for RhD-positive and 4 (rank 1-10) controls for RhD-negative pregnant women.

Discussion

The study revealed 1.12% of women who acquired anti-RBC antibodies by active immunization during an ongoing pregnancy. An extremely statistically significant difference was found between actively immunized RhD-positive pregnant women (0.56%) and RhD-negative pregnant women (4.53%). Anti-D antibodies showed the highest frequency (23.34%) among the detected antibodies. Anti-D alloantibodies were produced in 1.77% of RhD-negative pregnant women during the ongoing pregnancy.

A study conducted 20 years ago in the Republic of Serbia showed that the incidence of potentially clinically significant antibodies was 2.4%. The majority belonged to the Rh system, followed by anti-M, -Fy^a, -S, -Jk^a, and -Jk^b [16].

The 20-year retrospective epidemiological study in the West Herzegovina region found positive IAT in 1.8% of pregnant women. This study found that even though there has been significant progress in the prevention of RhD immunization, monitoring of immunization is still necessary [17]. The 15-year observational study from a tertiary care university hospital in Spain found that 76.9% of all maternal antibodies were clinically significant, and the most frequent maternal antibodies were anti-D (53%) and anti-K (19%) [18]. A five-year study conducted in West Yorkshire confirmed that the prevalence of RBC antibodies in pregnancy varies with ethnicity and geographical location. The study found RBC antibody prevalence in Yorkshire is lower compared with reports from other Caucasian populations, 1 : 242 of pregnancies (0.41%). The anti-E and anti-M antibodies were the most common [19].

Exposure to non-self RBC antigens during pregnancy may lead to the production of antibodies against RBC antigens. Antibody screening enables the detection of maternal alloimmunization early in pregnancy and a timely start of treatment. In severe cases, clinically significant maternal antibodies may lead to fetal anemia with a risk of fetal death and to severe forms of neonatal hyperbilirubinemia. Most severe HDFN cases are caused by anti-D antibodies despite antenatal – and postnatal anti-D prophylaxis in the previous pregnancy.

Although all pregnant women are exposed to fetal RBC during pregnancy and delivery, most of them do not become alloimmunized after this exposure. Exposure to immunogenic RBC antigens, such as Rhesus, Kell, Duffy, and Kidd, results in alloimmunization and leads to clinically significant HDFN. ABO incompatibility between mother and fetus plays a protective role against RBC alloimmunization [2].

In 2015, a group of authors reported the prevalence of 0.73% of unexpected anti-RBC antibodies in pregnant women in Australia, with the highest frequency of anti-E-27.6%, anti-D-10.4%, and anti-K-9.5% antibodies. The data showed that most antibodies were found in RhD-positive women and that the most commonly detected, anti-E antibodies, were present in a small number of RhD-negative women. Anti-E antibodies had a much higher detection rate compared to other studies, but the method used in this study proved both classes (IgM and IgG) of antibodies. The study showed that anti-D antibodies remain a clinically significant problem but suggested that the other antibodies can also cause severe HDFN [20]. Our study has also shown a lower frequency rate of anti-E antibodies (9.41%) than observed by the Australian study.

The Michigan Immunohematology Laboratory reported an antibody prevalence of 0.74% in pregnant women. The most common clinically significant antibodies were: anti-E (38.2%), anti-K (20.6%), and anti-M (17.6%). The most common clinically significant alloantibodies were anti-E and anti-M in RhD-positive women, and anti-K, anti-D, and anti-c in RhD-negative pregnant women [21]. The prevalence of anti-K antibodies in pregnant women was 0.15%. These antibodies lead to the suppression of erythropoiesis as a contributing factor to fetal anemia, unlike the other antibodies that cause direct erythrocyte hemolysis. In the same study, IgM class anti-M antibodies were mainly identified (naturally occurring antibodies). Compared to the US authors, we found a higher prevalence of RBC antibodies in pregnant women (1.12%), as well as a lower prevalence of anti-M (11.85%) and anti-K (9.41%) antibodies. The most frequent antibodies among South Bačka pregnant women were anti-D (23.34%), followed by anti-M, while anti-E and anti-K ranked third and fourth, respectively.

HDFN can be caused by antibodies directed against RBC antigens other than D antigen. In a study conducted in India in 2019, gynecologists' education about the necessity of antenatal antibody screening in all pregnant women was evaluated. The positive effects of the transfusion activity led to an increase in the number of RhD-positive pregnant women who came to the prenatal antibody screening (18.2% in the first phase and 72.8% in the second phase after the intervention) [22]. In order to protect pregnant women and their babies, BTIV also recommended to gynecologists a testing algorithm that included blood grouping and RBC antibody testing in pregnancy.

Studies have also shown that additional antibody screening in the third trimester of pregnancy increases the number of detected late alloimmunizations in RhD-negative pregnant women, thus enabling early treatment of HDFN [13]. Blood transfusion, multiparity, and chorionic villus sampling/amniocentesis are known risk factors for alloimmunization. A much faster secondary immune response, due to the action of these factors before the current pregnancy, explains most late alloimmunizations [12].

Administration of routine antenatal anti-D immunoglobulin prophylaxis significantly reduces the incidence of RhD immunizations. Bollason et al. [23] examined

the prevalence of anti-D antibodies in Iceland, where postnatal anti-D immunoglobulin prophylaxis was introduced in 1969 and antibody screening in 1978. Before these measures, the prevalence of alloimmunized RhD-negative pregnant women was 1.9%. In the period 1996–2015, the incidence of anti-D antibodies was significantly reduced (1.04%). Compared to their results, pregnant women in our study had a higher prevalence not only of anti-D antibodies (1.77%) but also of total antibodies (1.12%). The most frequent antibodies among Iceland pregnant women were anti-M (19.4%), anti-E (19.0%), and anti-D (12.5%). The authors of this study highlight the unusually high prevalence of anti-M antibodies, whose cause is unclear, as well as the fact that anti-E plus anti-c were the most common antibodies combination in alloimmunized pregnancies [23].

Chatziantoniou et al. [24] from London, UK, demonstrated that morbidity and mortality caused by HDFN were minimal, and reported anti-D antibodies as the most commonly encountered, followed by anti-c and anti-E antibodies.

The frequency of alloimmunization in pregnant women as well as antibody specificity varies around the world. Gavrančić et al. [25] carried out a retrospective study similar to this one from 2003 to 2010 among pregnant women from the South Bačka District. The authors found anti-D antibody specificity in 74 alloimmunized pregnant women, which represented 44% of all detected alloantibodies. Almost one decade later, our study showed a downward trend of alloimmunized pregnant women with anti-D antibodies.

In 2015 Southern Pakistan authors reported 1.6% of non-anti-D incidence and 2.9% of anti-D incidence [26]. Prevalence of RBC alloantibodies among multiparous antenatal females in North India is found to be 2% (anti-D, anti-E, anti-C, and anti-K) with anti-D antibodies most commonly encountered [27]. Ngoma et al. [28] reported incidence of RBC alloantibodies among pregnant women in Africa, ranging from 1.1% to 12.1%. Anti-D antibodies were ranked as the most common, followed by anti-K and anti-E antibodies. Al-Dughaishi et al. [29, 30] have determined an alloimmunization rate of 10% in RhD-negative Omani pregnant women and an alloimmunization rate of 2.7% in RhD-positive Omani pregnant women [29, 30]. In the Hubei province of China, among the Han population, RBC alloantibodies prevalence was 0.50%, and the most frequently identified alloantibodies were anti-E, anti-D, and anti-M [31].

Conclusion

The study found that anti-D, anti-M, anti-E, anti-K, anti-C, and anti-Fy^b were the most frequent specificities, respectively. Antenatal and postnatal anti-D immunoglobulin prophylaxis has led to a significant reduction in the frequency of D-alloimmunization over the last ten years but did not eliminate D-alloimmunization. Unfortunately, the incidence of anti-D antibodies among alloimmunized pregnant women in the South Bačka District is still significantly higher than published data for the developed countries.

The introduction of mandatory perinatal immunohematological testing of pregnant women and fetuses/newborns in a uniform manner throughout the Republic of Serbia, in all private and state healthcare institutions, would certainly lead to improved health care provision in this area.

Conflict of interest

None to declare.

R E F E R E N C E S

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