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Peculiarities of Blood Group Distribution among Infants Born to Mothers with Negative Rh-Factor (Findings of 2014)

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Abstract

Our works consider the investigation of possible manifestation of hyperbilirubinemia in infants against the ground of genetic incompatibilities of the fetus according to ABO system and Rh-factor (D) concerning the maternal organism. From this point of view we deal with jaundice of mixed genesis against erythroblastosis domination as a primary antenatal factor of pathological process formation.

The present study presents the results of distribution of the group and rhesus determinants (Rh D) of infants born to mothers with negative Rh-factor in 2014. Analytical review and comparison with the previous investigations in this direction have been made. New trends of further work with the elements of chronobiological characteristics concerning possible signs of hemolytic diseases of newborns (HDN), neonatal isoerythrolysis, in Bukovyna region are outlined. The values of umbilical bilirubin concentration are taken as a biochemical criterion of HDN development which is the main diagnostic sign of pathological jaundice of newborns. The signs of HDN of various degree were observed in 24 infants out of 333 neonates born to mothers with negative Rh-factor during the period of 2014.

Keywords: hemolytic diseases of newborns (HDN), negative Rh-factor (anti-D), jaundice form, bilirubin concentration, Bukovyna region.

Introduction

Neonatal hyperbilirubinemia for the last decade has been characterized by the tendency to increase its possible signs during the neonatal period which is first of all explained by the spread of intrauterine infections, indirect influence of numerous lesions of the uterine-placenta complex during the antenatal period. All these factors together with the action of many medical agents form hepatobiliary insufficiency of neonates characterized by lees or higher degree of manifestation [1, 2].

Our works consider the investigation of possible manifetsation of hyperbilirubinemia in infants against the ground of genetic incompatibilities of the fetus according to ABO system and Rh-factor (D) concerning the maternal organism [3-6].

From this point of view we deal with jaundice of mixed genesis against erythroblastosis domination as a primary antenatal factor of pathological process formation. Fetal erythroblastosis

by AB0 system is known to constitute ⅔ of all the cases and ⅓ of cases – is incompatibility by the system of Rh-factor. It is interesting to know that erythroblastosis develops not in all the cases of incompatibility of the mother-infant system [7].

The level of umbilical bilirubin within the limits of 51,3-68,4 micromole/L is indicative of possible development of jaundice with indirect hyperbilirubinemia (hemolytic diseases of newborns, polycythemia, acquired and congenital hemoglobinpathy and enzymopathy) which is one of the causes of pathologic hyperbilirubinemia. The value of umbilical bilirubin concentration within the limits of 85,5-153,9 micromole/L is indicative of the development of jaundice with direct hyperbilirubinemia, that is, with domination of bilirubin-diglucuronide in the blood serum [7].

It should be emphasized that in infants with pathologic jaundice, only by clinical-anamnestic findings without laboratory tests initiated, the correct diagnosis is made only in 15% of all the cases.

As a rule, fetal erythroblastosis on the second-third day of life of an infant is characterized by adjoining of metabolic hyperbilirubinemia due to initial adaptation to new conditions of the surroundings.

Pregnancy with possible transplacental transmission of antibody-producing cells from mother to fetus is an important way of alloimmunization of the population especially in the areas of gene penetration including Bukovyna as well. Sensitization index calculated by the ratio containing the number of individuals with antibodies against general number and expressed in percentage will be considerably high for maternity homes and essential for local application in the medical establishment as a certain prognostic criterion. It will not be usually a reliable criterion for the region on the whole, but it will enable to prognosticate possible signs in mother-infant system and will be important to diagnose its spread among the population [8, 9].

During several recent years on the base of the Maternity Home № 1 in Chernivtsi, Ukraine, the distribution of groups (by AB0 system) and Rh-factor has been examined in the blood of infants born to mothers with different group determinants according to AB0 system, the value of umbilical bilirubin concentration was used as a prognostic criterion of hemolytic disease of newborns.

The article presents the analysis of the findings concerning the distribution of the criteria chosen among infants born to mothers with negative Rh(-) factor irrespective of the blood group. The results obtained were compared with the findings of 2013 (from January including July) with the aim to compile possible chronobiological characteristics of group distribution with the peak (maximal and minimal) values of hyperbilirubinemia in future.

Materials and methods

Laboratory examinations of the umbilical and maternal (in case of necessity) blood were made in the laboratory at the Department of Anaesthesiology with beds for intensive care units at the Maternity Home № 1, the town of Chernivtsi.

Detection of the blood group and Rh-factor was conducted according to the Order №164, the Ministry of Public Health of Ukraine dated 05.07.1999 “On Approval of the Instructions Regulating the Work of Blood Banking Establishments in Ukraine”, and “The Instruction to Detect Blood Groups and Rhesus by AB0 Systems” in particular [10].

Belonging to the blood group of patients was examined by means of agglutination reaction with the following reagents: standard serums and standard erythrocytes and monoclonal antibodies (coliclones anti-A, anti-B and anti-AB).

Standard serums and erythrocytes to detect blood groups were prepared in the laboratory at the Blood Banking Establishment of Chernivtsi Regional Center of Blood Banking (CRCBB). All the reagents were marked with the series and expiry date. Standard erythrocytes were prepared from the donor blood (according to the Instruction of taking and registration of blood received from donors in small doses to prepare standard erythrocytes). Monoclonal antibodies – erythrotest-coliclones produced in the Russian Federation, the company “Hematolog”, Moscow, certified on the territory of Ukraine were used in the study. Rh-factor was detected by anti-Rh_0(D) IgM monoclonal reagent produced by the company “Hematolog”.

The level of total bilirubin (TBR) and its fractions as one of the important biochemical prognostic criteria of HDN development was detected in the umbilical and infant blood.

Bilirubin and its fractions were detected according to the unified technique by Yendrashyk' method with the set of reagents produced by the company "Reagent" (Dnepropetrovsk, Ukraine). Photoelectrocolorimeter KFK-2 was used as a device to measure optic density of solutions.

The results were processed statistically with the detection of arithmetical mean value M and its error m ($\sigma\sqrt{n}$). Rank Kraskal-Wallis criterion was used for the comparative analysis of sampling.

Results and discussion

During 2014 on the base of the Municipal Clinical Maternity Home №1, Chernivtsi, 333 infants were born to 330 mothers with Rh(-) factor of blood by anti-D system through physiological labour of cesarean section. The majority of pregnancies were monocyesis, but twins were born: one to the mother with A(II) blood group and two to the mothers with AB(IV) blood group, mothers with B(III) blood group gave birth to stillborn twins, one was viable, that did not change general number of infants in this group concerning the number of women. It was interesting to know that the mothers with 0(I) blood group did not give birth to twins both in 2014 and in the first half of 2013. The majority of twins, six out from eight, inherited A(II) blood group as a dominant for our region. Out of general number of mothers with Rh(-) factor of blood the number of infants with A(II) blood group were 135: 75 from them were with positive Rh-factor. At the same time, during seven months of 2013 there were 83 newborns with A(II) blood group (64 with Rh(+) and 19 with Rh(-) factor) out of general number of newborns – 209 [11].

The distribution of group determinants of mothers and infants is presented in Table 1. The comparison of the distribution of blood groups both in 2014 and in the first part of 2013 revealed the priority of A(II) blood group both among mothers with negative Rh-factor and newborns, followed by 0(I), B(III) and AB(IV) respectively.

It should be noted that the majority of infants inherited maternal blood group. Only those born to mothers with AB(IV) blood group presented the distribution between A(II) and B(III) blood groups. In case to keep to the theory of development of AB(IV) blood group due to mixed marriages in the evolutional process but not under the influence of environmental factors, this kind of distribution appears to be quite natural and regular [12].

Mothers with AB(IV) Rh(-) blood group rarely give birth to babies with 0(I) blood group: one infant with 0(I) Rh(+) – during 2014 and none of the newborns with these characteristics during 2015. During the period of investigation in 2013 one newborn with 0(I) Rh(-) blood group born to the mother with AB(IV) Rh(-) blood group was characterized by a classical manifestation of HDN by AB0 system: the level of umbilical bilirubin was 158,7 micromole/L, a substitute blood transfusion was performed twice while staying in the Department of Neonatology of the Maternity Home. The concentration of umbilical bilirubin was 158,7 mciromole/L, and as a statistical value it was the highest for the manifestation of all the types of erythroblastosis during the last five years in our Maternity Home.

Table 1: Distribution of group and rhesus determinants in infants born to mothers with Rh(-) factor of blood group during 2014

Blood group (AB0) and Rh –factor	0(I) infant		A(II) infant		B(III) infant		AB(IV) infant		$\mathbf{N}_{infant}$
	Rh(–)	Rh(+)	Rh(–)	Rh(+)	Rh(–)	Rh(+)	Rh(–)	Rh(+)	
0(I) Rh(–) mother (n=97)	**27**	37	11	9	6	7	---	---	97
A(II) Rh(–) mother (n=130)	10	13	**33**	50	5	10	5	5	131
B(III) Rh(–)	6	5	5	5	**8**	19	3	10	61

$_{mother}$ (n=61)									
AB(IV)Rh(–) $_{mother}$ (n=42)	---	1	11	11	10	3	**1**	7	44
N$_{infant}$	43	56	60	75	29	39	9	22	333

Notes: the number of maternal and infant groups do not coincide due to the birth of twins; the numbers concerning identical reproduction of maternal signs according to the characteristics examined in infants are written in boldface font

According to the scientific data AB(IV) blood group does not possess immunity to the antigens A and B. The Ukrainians inherited AB(IV) blood group due to historical-geographical migration of the Pechenihies, Hungarians and Polish Jews, its spread is proved to coincide with the areas of population settlement of the Jews and Gipsy [12].

The Ukrainian population is characterized by the prevailing A(II) blood group by AB0 system.

The concentration of total bilirubin in the umbilical blood was characterized by a wide range of values: from 25,3 to 124,2 micromole/L infants from all the groups irrespective of the maternal blood group. HDN manifestation was found in 24 cases out of general number of newborns (333 infants). It is interesting to note that the value of umbilical bilirubin in case of HDN manifestation was also characterized by a wide range of indices: from quite safe 29,9 to maximally critical 124,2 micromole/L. The concentration of umbilical bilirubin 124,2 micromole/L was a record in 2014.

The greatest amount of HDN manifestation – 12 cases, that is, 50% out of general number, was found from mothers with 0(I) blood group. To the point, a record value of umbilical bilirubin was found in an infant with 0(I) Rh(+), who was born to mother with 0(I) Rh(-) blood group: a classical incompatibility by the Rhesus-factor system. All other cases of HDN in this group of infants were different combinations of the blood group and Rhesus-factor except B(III) Rh(-) blood group of infants who did not present erythroblastosis signs.

According to the literary data the majority of HDN cases is found by AB0 system than those found in Rh(D) system in the ratio 3:1 [7, 13].

Thus, HDN is characterized by extremely variable values of the umbilical bilirubin concentration which is a valuable initial criterion of the development of pathological jaundice but not the only one.

Especially interesting manifestation of HDN there was a case with a mother with A(II) Rh(-) blood group given birth to an infant with identical blood group by AB0 system but positive Rh-factor by Rh(D) system. The umbilical bilirubin level was 78,2 micromole/L, which was quite real in this case of erythroblastosis. Although the value of direct bilirubin concentration as a fraction of the total one was 48,3 micromole/L, being an alarming symptom and drawing the attention of neonatologists. As in the majority of cases HDN is characterized by the total bilirubin value at the expense of the content of indirect fraction. Thrombocytopenia was diagnosed in this newborn. Primary forms of thrombocytopenia of alloimmune or isoimmune character can occur due to the transmission of platelets from the fetus to mother (as it happens during Rhesus incompatibility) or blood group incompatibility of platelet antigens in the pregnant-embryo system [14].

An average content of total bilirubin in the umbilical blood of neonates irrespective of the maternal blood group was within the same rates: 36-38 micromole/L (Table 2).

Table 2: An average level of total bilirubin (micromole/L) in the umbilical blood depending on the maternal blood group with Rh(-) factor (2014)

	0(I)	A(II)	B(III)	AB(IV)
M ± m	37,95 ± 1,19	36,87 ± 0,78	37,85 ± 1,37	36,87 ± 1,37

Notes: M – arithmetic mean of bilirubin concentration and its error m=(σ√n).

The data presented in the table are indicative of the similar average content of total bilirubin in the umbilical blood irrespective of the maternal blood group, that is, the conversion of physiological jaundice into pathological one and the level of HDN manifestation are in reality individual characteristics of every newborn.

By means of Kraskal-Wallis criterion a cross comparison of the total bilirubin level in infants with the same blood group born to mothers with different blood group characteristics by AB0 system was conducted (for example, infants with A(II) Rh(+) born to mothers with 0(I), A(II), B(III), AB(IV) blood groups etc.). Thus, a leading criterion to compare and find a reliable difference in the total bilirubin level was the blood group inherited by an infant. Table 3 presents the average level of umbilical bilirubin in infants with B(III) Rh(+) blood group.

Table 3: An average level of total bilirubin (micromole/L) in the umbilical blood of newborns with B(III) Rh(+) blood group born to mothers with Rh(-) factor by D system (2014)

	$0(I)_{mother}$	$A(II)_{mother}$	$B(III)_{mother}$	$AB(IV)_{mother}$
M ± m	40,74 ± 4,42	37,26± 2,84	38,05 ± 1,66	36,03 ± 2,02

Notes: there is no reliable difference found between the groups by bilirubin level.

Analogical comparative analysis was made between newborns with different blood group characteristics concerning the value of umbilical bilirubin concentration. There were no reliable differences between the selected groups found by means of Kraskal-Wallis criterion, that is, inherited the same blood group and Rh-factor from mothers or different group determinants do not influence upon the average value of umbilical bilirubin. Therefore, there were no reliable differences found according to the blood group characteristics of infants concerning the maternal blood group determinant.

It should be noted that the most prominent manifestation of HDN was found among infants born to mothers with 0(I) Rh(-) blood group: 12 cases constituted 12,4% out of general number of neonates in this subgroup (in other subgroups this percentage varied from 4,6 to 6,5%) (Fig.). In addition, the analysis of distribution of group determinants from mothers with 0(I) Rh(+) blood group during 2014 demonstrated reliable differences of an average bilirubin level among infants with 0(I) Rh(+) blood group only in its comparison with diametrically opposite groups: with A(II) Rh(–) ($p<0,05$) and B(III) Rh(–) ($p<0,01$) [6].

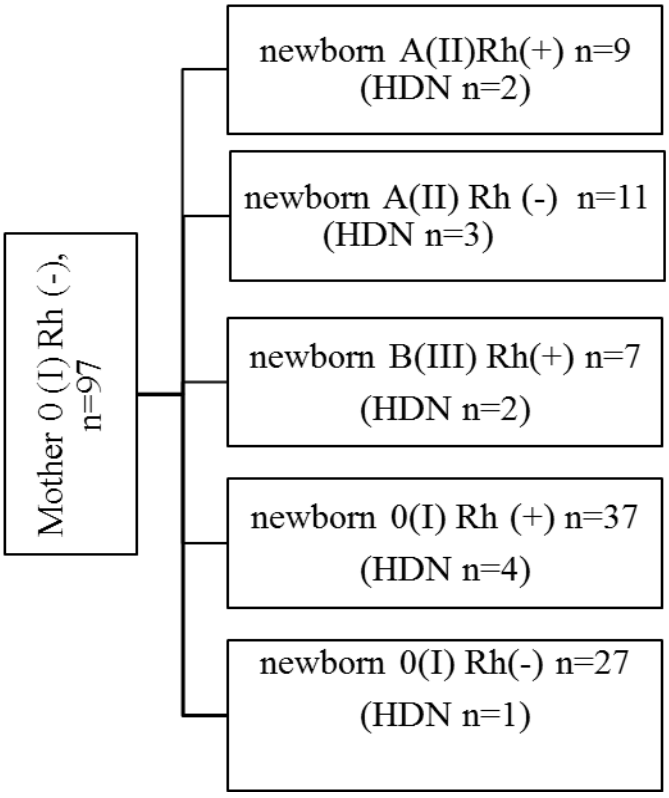

Figure 1. The number of HDN in infants born to mothers with 0(I) Rh(-) blood group by AB0 system during 2014

Hyperbilirubinemia is more often found in newborns with A(II) and B(III) blood groups born to mothers with 0(I) blood group, which is connected with anti-A and/or anti-B antibodies of IgG class present in their blood serum [15].

In case of double incompatibility both by the group and Rhesus-factor HDN caused by A- or B-antigens is likely to develop much easier than in case of isolated Rh-conflict. The birth of a baby with positive Rh-factor and incompatible blood group concerning the maternal one by AB0 system is likely to reduce the probability of immunization at the expense of competition for the antigen or "moderate" the course of jaundice

The family of Rh-proteins is known to be an important constituent of the erythrocyte cytoskeleton, they participate in the transportation of water and ammonium ions through the membrane. Rhesus-proteins Rh(D) are the molecules piercing the erythrocytic membrane 12 times in the direction from internal to external site, and again to the internal one; C- and N-ends of this protein are oriented into the cytoplasm site. Immunogenic characteristics of D antigen is not sufficiently studied [16, 17].

Conclusion

- umbilical bilirubin level is in fact an individual characteristics and not always an informative parameter to diagnose HDN;
- in early postnatal period it is difficult to diagnose HDN as it can develop in a latent form;
- bilirubinemia screening should be elaborated and introduced in the "risk group" of neonates (during the first 24-36 hours);
- Hb level is not always a prognostic criterion of HDN, first of all we deal with jaundice forms and transient polycythemia;
- umbilical bilirubin concentration within the limits of 50,0 micromole/L is the factor of a special attention for neonatologists (several years ago it was much higher – 65-70 micromole/L);
- possibility of fulminant development of HDN in a latent form and danger for further development of a newborn;
- increased frequency of HDN manifestation occurs with reduced light period (November-December-the first decade of January);
- various manifestations of HDN can be important modulators of the formation and further development of the immune system of infants.

These are the main issues for possible further directions of the investigation. In perspective a comparative analysis of the distribution of blood group characteristics in the mother-infant system will be made by the results of the studies conducted the previous year in our region including the characteristics of seasonal manifestation of HDN.

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