

ABO and Rh Blood Group Antigens and Natural Anti-A and ANTI-B Antibodies in the Neonates

SHORENA GABAIDZE¹, MARINA NAGERVADZE^{1,2}, LEILA AKHVLEDIANI^{1,2},
NANA NAKASHIDZE², ALISSAR ALFILO², IRINE TSINTSADZE², NATO GORGADZE²,
RUSUDAN KHUKHUNAISHVILI¹, MARINA KORIDZE¹, TEA KOIAVA¹, KETEVAN DOLIDZE¹,
TAMAR BAKHTADZE¹

¹Faculty of Natural Sciences and Health Care,
Batumi Shota Rustaveli State University,
St.35 Ninoshvili, Batumi, Adjara,
GEORGIA

²School of Medicine and Health Sciences,
BAU International University, Batumi,
St.237 Fridon Khalvashi, Batumi, Adjara,
GEORGIA

Abstract: - ABO blood group is determined by the presence or absence of A and B antigens on the surface of RBC and of anti-A and anti-B antibodies in the serum. The relatively weak expression of A and B antigens in newborns due to their developing immune systems poses challenges in accurately detecting naturally occurring IgM antibodies against these antigens. This difficulty in immunoserological methods contributes to the potential for errors in determining the blood groups of newborns. Despite this, the Rh antigen expression in newborns remains comparable to that in adults. Nonetheless, various factors contribute to diverse blood typing results in newborns, including the utilization of alternative testing methods. The complexity of blood typing is magnified when using samples from the umbilical vein. Furthermore, compared to adults, the exploration of ABO antigen expression in newborns is limited, and the identification of specific subgroups such as A1 and A2 is even rarer. This underscores the need for standardized testing procedures and further research to enhance our understanding of antigen expression patterns in newborns. Based on the aforementioned details, the primary objective of our study was to delve into specific aspects related to blood group characterization in newborns. This encompassed exploring the expression of A, B, AB, and D antigens on the surface of red blood cells (RBCs) and detecting anti-A and anti-B antibodies in the plasma of newborns. These analyses were conducted using samples obtained from the heels of 208 newborns and were typed by forward and reverse blood typing methods with monoclonal antibodies and standard erythrocytes. The distribution of phenotypic groups within the ABO system among the newborns was not uniform. The r allele was identified with the highest frequency in the analyzed samples (0.6), while the prevalence of the p allele significantly lags at 0.3. The q allele has the lowest frequency (0.1). In our study, we propose that for the majority of cases (43.94±3.5%) among the studied newborns, there was an absence of naturally occurring anti-A and anti-B antibodies (n=87). In a specific scenario, within the O(I) blood group newborns, partial synthesis of these antibodies was detected in 14.14±2.4% (n=28). Meanwhile, 41.92±3.5% of the newborns in our study exhibited natural antibodies similar to those found in adults. We didn't find any difficulties in typing the Rh blood group antigens in the newborns. In conclusion, our study's findings indicate that newborns, in certain instances, exhibit strongly pronounced natural anti-A and anti-B antibodies within the ABO system. However, in the majority of cases, these antibodies are not evident. Majority of cases erythrocyte A and B antigens were weakly expressed and for detecting these images optic microscopes were used.

Key-Word: - anti-A antibodies, anti-B antibodies; A and B antigens; newborns; Blood group typing, Rh factor.

Received: June 22, 2022. Revised: September 11, 2023. Accepted: October 3, 2023. Published: October 11, 2023.

1 Introduction

Blood group antigens are considered to play a role in how well the biological species of *Homo sapiens* has adapted to its environment. This is demonstrated by the fact that different races or ethnic groups have different blood group distribution frequencies, which is regarded as an example of gene geographic adaptation in the history of biological evolution, [1], [2], [3].

Presently, the International Society of Blood Transfusion (ISBT) recognizes a total of 45 blood group systems encompassing 360 antigens for human red blood cells, [4].

The majority of these antigens are polysaccharides, though they can also be proteins or complexes of proteins, carbohydrates, and lipids. Blood group antigens are primarily involved in the trophic and regulatory functions of blood cells. Due to their presence within cell receptors, they play a crucial role within the blood circulatory system, facilitating the transport of hormones, vitamins, enzymes, and other biologically active proteins. Additionally, they frequently serve as fundamental structural elements in the adhesion of cell membranes, [5].

The first known human genetic markers were the antigens of the ABO system, which were also the first blood groups to be recognized, [6]. Among the nearly 45 blood group systems investigated thus far, the identification of these systems and the subsequent revelation of naturally occurring antibodies targeting antigens absent on certain cells explained the prior inconsistencies in blood transfusion and organ transplantation. This breakthrough paved the way for safer transfusion practices in situations of critical blood loss, ensuring a higher level of patient safety, [7].

ABO blood group is determined by the presence or absence of A and B antigens on the surface of RBC and of anti-A and anti-B antibodies in the serum, [8]. The ABO blood group antibodies are inherent and predominantly IgM class. These antibodies are generated without antigen stimulation, unlike those produced during the conventional adaptive immune response. The exact nature of their production remains a subject of debate. Anti-ABO IgM is usually not present in newborns but appears in the first year of life. The antibodies may be produced against food and environmental antigens (bacteria, viral, or plant antigens), which are similar in structure to A and B antigens, [9], [10].

In addition, research into the enzymology and structural details of A and B transferases is essential for other branches of medicine. For instance, ABO has become a key player in modern genomic medicine. It has also been studied in neurobiology, in the development of universal/artificial blood, and even in the hoax of "blood type diets", [11]. The understanding of how ABO relates to diseases has also grown, [12], [13].

The most notable advancements include a better understanding of the relationship between ABO and various diseases, [14], which can be discovered through genome sequencing, which has also improved our understanding of the evolution of ABO and related genes by identifying orthologous and paralogous genes in various organisms, [11].

Low expression of antigens on the RBCs as well as frequent blood transfusion history, hematological disorders, especially blood cell tumors, solid tumors, and surgical history, are the factors that can affect the expression of ABO antigens on RBCs and cause ABO typing discrepancies, [15], [16].

There is also a description of a different instance of antigen expression changes. In a malignant environment, epigenetic changes in blood group antigens of the ABO system are particularly noted. Blood cancers showed comparable cases. A patient with this cancer will typically require several transfusions, as is common knowledge. Genotyping of the blood group locus should be used in people with so-called "hard-to-detect blood types."

Compared to adult red blood cells, newborn red blood cells have a much weaker expression of A and B antigens. A significant protective factor against maternal antibodies that have passed the placental barrier is this relatively weak reactivity, [7]. From this perspective, indeed, the fetus is somewhat protected from the mother's immune system's reactivity due to the weak expression of antigens in the fetus' blood. However, it also makes it more challenging to correct blood typing. Additionally, consider another possibility is that newborns' blood serum may lack the corresponding antibodies, which causes incorrect blood group determination using immunoserological methods, particularly reverse methods. Regarding this inaccuracy, the use of blood for transfusion and treatment in newborns can result in both post-transfusion complications and immune sensitization of the baby. This fact is crucial because it can be challenging to find a suitable donor in an emergency transfusion situation for newborns with severe anamnesis (including those with hemolytic

disease and prematurity). In the case of the Rh system, Newborns and adults both express the Rh antigen in the same manner.

Our studies and clinical experience have shown that newborns with complicated anamnesis frequently have trouble identifying their blood group, and cause ABO typing discrepancy. The AB (IV) group is typically determined first, followed by the A (II) or B (II) group a week later. In some circumstances, different blood types are identified using distinct alternative techniques in the same sample of newborns.

In addition to the challenges mentioned earlier, there exists an increased potential for errors when ascertaining the blood group from samples obtained through the umbilical vein in newborns, which is the customary method of collection. In these situations, the transfusion and the proper donor selection can be difficult and take some time, and a delay is frequently linked to a risk to the patient.

Drawing from the available literature, the examination of erythrocyte antigens in adults has received more extensive attention compared to the analysis conducted in newborns. Notably, information about to A1 and A2 subgroups is limited in its availability.

Given these considerations, the central aim of our study was to thoroughly investigate various facets of blood group characterization in newborns. This encompassed a comprehensive analysis, involving the identification of A1, A2, B, H, and D antigens on the surface of red blood cells (RBCs), alongside the detection of anti-A and anti-B antibodies within the plasma of newborns.

In conclusion, directing efforts toward advancing our comprehension of antigen expression patterns in neonates holds significant promise for improving and standardizing testing methods. This proactive approach is crucial for reducing the potential for errors and inaccuracies in blood group determination, ultimately leading to more reliable and precise results.

2 Research Materials and Methods

2.1 Research Materials

Newborns' blood samples were collected from the "M. Iashvili Batumi Maternal and Child Central Hospital" and "Iris Borchashvili Health Center Medina", in accordance with the approved ethical guidelines. The mentioned clinics are located in the

city of Batumi, which is part of West Georgia. Informed consent was obtained from the legal guardians of the newborns' parents prior to sample collection.

A total of 208 newborn blood samples were enrolled in the study. The only inclusion criteria followed was that subjects must be less than one month old. No exclusion criteria were determined. All procedures were approved by the Ethics Committee of BAU Batumi International University and met the requirements set by the Declaration of Helsinki for Medical Research Involving Human Subjects (World Medical Association 2013).

The current research was carried out in the laboratories of Immunogenetics and Biosafety of the Department of Biology of the Faculty of Natural Sciences and Health of Batumi Shota Rustaveli State University (BSU), which are equipped with all the necessary equipment for carrying out the current research. Some part of the research was done at the Immunology and Microbiology laboratory of BAU International University Batumi (BAU, Batumi). The current research was carried out within the two years of 2020-2022. The research is done based on Batumi Shota Rustaveli State University targeted the grant project "Evaluation of immunogenetic characteristics of erythrocyte blood group antigen-antibodies of newborns", 2020-2022.

2.2 Research Methods

Peripheral blood samples (2 ml) were obtained from the newborn's heel, and the samples were collected into special tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Vigorous handling of specimens was avoided. The collected newborn's blood samples were stored at a temperature of 4°C until further processing and analysis.

The test tubes containing blood samples were placed in the centrifuge. The tubes were positioned in a balanced manner to ensure an even distribution of forces during centrifugation. The samples were centrifuged at 4000 RPM for a duration of 1 to 2 minutes. Shortly after centrifugation, the uppermost layer constituting plasma was separated and stored in 0.5ml microcentrifuge test tubes for the anti-A and anti-B antibodies typing. The rest part of the centrifuged blood samples was used for typing of the erythrocyte A and B antigens.

To determine the ABO blood group of each newborn, blood typing was conducted using the slide and tube agglutination methods. There were used

Both forward and reverse blood typing procedures. We used monoclonal anti-A, anti-B, anti-AB, anti-D (Bio-Rad, cypress diagnostics) antibodies for typing of the ABO and Rh blood group antigens. Samples were tested on standardized blood typing plates. We allocated 4 phenotypes for the ABO system (A, O, B, and AB) and two types of Rh system (Rh⁺ and Rh⁻) based on this method. Agglutination processes were meticulously observed and recorded, following standardized protocols and guidelines. The standard erythrocytes with A and B blood groups and newborns' plasma were taken for reverse methods for ABO blood system natural antibodies (anti-A and anti-B) typing. Agglutination reaction was observed with the naked eye, but in some cases, especially in cases of so-called "weak" agglutination reaction, we used the optic microscope with different magnification lenses (10X4, 10X10).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software. P-values <0.05 were considered statistically significant. We also used the special calculator platform, [17].

3 Result

Determining the blood group of a newborn is an essential laboratory test conducted shortly after birth. It involves obtaining biological material from either the umbilical cord or the peripheral blood of the newborn. However, it is crucial to exercise caution and take special care when collecting blood from the umbilical cord to avoid potential contamination. Contamination can adversely impact the serological expression of antigens, leading to false agglutination or non-specific reactions that may result in the misinterpretation of test results.

The ABO system's phenotypic groups are unevenly distributed among the studied newborns. 43.75±3.4% of the studied newborns have O(I) blood group (n=91). A little low distribution characteristic has A (II) blood group. 41.35±3.4% of the studied newborns have A (II) blood group phenotypical characteristics (n=86). B(III) blood groups have 21 studied newborns (10.10±2.0%) and only 10 studied newborn's blood samples show both A and B antigens specifications and 4.8 ±1.4% of the studied newborns have AB (IV) blood group. There are four categories - O (I), A (II), B (III), AB (IV) and based on these degrees of freedom (df) is - 3. One variable Chi-square (χ^2) equals 89.65, which is 11.4 times

more than Critical Values (CV=7.815). This statistical characteristic shows a unique distribution of ABO blood groups in the studied newborns (Table 1).

Table 1. ABO blood group distribution in the studied newborns.

Blood group	Number (n)	Percent %	df*	CV*	χ^2 *
O (I)	91	43.75 ±3.4	3	7.815	89.65
A (II)	86	41.35 ±3.4			
B (III)	21	10.10 ±2.0			
AB (IV)	10	4.8 ±1.4			
Total	208	100			

*The P-Value is < .00001. The result is significant at $p < .05$.

*df – Degrees of freedom; * χ^2 - Chi-square; *CV- Critical values.

The ABO blood group system's gene distribution frequency in the newborns under study was also examined. The formula employed in the analysis of the three-allelic genetic system was utilized to determine their frequency. r allele was detected with the highest frequency in the studied samples and is equal to 0.6, while the prevalence of the p allele lags significantly behind and is 0.3, and the frequency of the q allele is the lowest at 0.1 (Table 2). r, p, q allele's frequency total equals 1 in our studied cohort.

Table 2. Frequency of distribution of the genes of the ABO system in the studied newborns

Three-allelic genetic system	Distribution
$r = \sqrt{O}$ *	0.6
$p = 1 - \sqrt{A+O}$ *	0.3
$q = 1 - \sqrt{B+O}$ *	0.1

*Where O, A, and B are the ratio of newborns carrying O, A, and B phenotypes to the total number of research subjects. Hardy-Weinberg equation where: $F = p + q + r = 1$.

In addition to screening newborns for group antigens, we were also interested in this target group's characteristics for identifying group-specific natural origin anti-A and anti-B antibodies. In adults, individuals with the O (I) blood group typically have both group-specific anti-A and anti-B antibodies in their blood plasma. However, it is important to note that the expression of the above-mentioned antibodies in newborns differs from that in adults.

38.46±5.0% of the studied newborns with the O (I) blood group carried both anti-A and anti-B antibodies (n=35) while none of the antibodies were detected in 30.77±4.8% (n=28) of cases, and 20.88±4.2% newborns carried only anti-A antibody (n=19) while 9.89±3.1% carry anti-B antibodies only (n=9). The degrees of freedom (df) is equal to 3 in this particular case. Chi-square (χ^2) equals 16.6, which is 2.12 times more than Critical Values (CV=7.815). The P-value is < .00001. The result is significant at $p < .05$ (Table 3).

Table 3. Anti-A and anti-B antibodies expression characteristics in the O (I) blood group newborns.

ABO blood group antibodies	Number (n)	Percent %	df*	CV*	χ^2 *
Both anti-A and anti-B antibodies	35	38.46±5.0	3	7.815	16.6
Only anti-A antibodies	19	20.88±4.2			
Only anti-B antibodies	9	9.89±3.1			
None of them	28	30.77±4.8			
Total	91	100			

* The P-value is < .00001. The result is significant at $p < .05$.

*df – Degrees of freedom; * χ^2 - Chi-square; *CV- Critical values

We have 86 newborns with A (II) blood group. Adult persons with A (II) blood group in the plasma have natural anti-B antibodies. We find that 40.7 ±3.4% case studied newborns have the anti- B antibodies in the plasma as an adult (n=35), but the majority of them 59.3±5.2% (n=51) did not show any agglutination reaction with standard erythrocyte mass with B blood group, which means that there weren't anti-B antibodies expression yet (Table 4). The degree of freedom (df) is 1 in this case because there are two categories. X^2 equals to 44.4, while the Critical Value (CV) is 3.841. The P-value is < .00001. The result is significant at $p < .05$.

Table 4. Anti-B antibodies expression in the A(II) blood group newborns.

Anti-B antibodies	(n)	Percent %	df*	CV*	χ^2 *
Present	35	40.7 ±3.4%	1	3.841	44.4
Don't present	51	59.3 ±5.2%			
Total	86	100			

*The P-value is < .00001. The result is significant at $p < .05$.

*df – Degrees of freedom * χ^2 - Chi-square *CV- Critical values

We also analyze the natural anti-antibody frequency in the studied newborns. We have 21 newborns with B (III) blood group. Adult persons with B (III) blood group in the plasma have natural anti-A antibodies. 61.9 ±3.4% (n=13) of our studied newborns have anti-A - A antibodies in the plasma, similar to an adult, 38.1 ±3.3 % (n=8) did not show any agglutination reaction with standard erythrocyte mass with A(II) blood group, which means that there weren't anti-A antibodies expression yet (Table 5). The degrees of freedom (df) is equal to 1 in this particular case. χ^2 equals 11, which is higher than Critical Values (CV=7.815). The P-value is .000911. The result is significant at $p < .05$.

Table 5. Anti-A antibodies expression in the B(III) blood group newborns.

Anti-A antibodies	Number (n)	Percent %	df*	CV*	χ^2 *
Present	13	61.9 ±3.4	1	3.841	11
Don't present	8	38.1 ±3.3			
Total	21	100			

*The P-value is .000911. The result is significant at $p < .05$.

*df – Degrees of freedom * χ^2 - Chi-square *CV- Critical values

The person with the AB (IV) blood group does not have anti-erythrocyte antibodies in the blood plasma. All our 10 blood samples with the AB blood group didn't show agglutination images with standard erythrocytes.

We also study the Rhesus system D antigen distribution in the studied newborns. It was typing the D antigens in the 148 samples. The majority of our samples (87.84 ±2.6%) have Rh+ phenotypical expression (n=130). The rest of them 12.16±2.6

belong to Rh⁻ group (n=18). We didn't find any difficulties in typing the Rh blood group in the newborns, because in all cases of the Rh⁺ samples D antigen was well agglutinated by using monoclonal anti-D antibodies (Table 6).

Table 6. Rh⁺ and Rh⁻ phenotypes in the studied newborns

Rh Phenotypes	Number (n)	Percent %	CV*	χ ² *
Rh ⁺	130	87.84 ±2.6	3.841	84.6
Rh ⁻	18	12.16±2.6		
Total	148	100		

*The P-Value is < .00001. The result is significant at p < .05.

*df - Degrees of freedom *χ² - Chi-square *CV- Critical values

4 Discussion

In our current study we found that unlike the adults, in some studied newborns, ABO blood group system A and B antigens are poorly expressed on the surface of the red blood cell. In the majority of cases, we used the optic microscope with low and/or high magnification lenses (10X4, 10X10, or 10X100) to detect the so-called "weak" agglutinated images (Figure 1).

We try to describe the reason for the poor expression of blood group antigens at the neonate stage. To answer the question of why they are so poorly expressed at the newborn stage we will follow the genetic mechanism of the expression of erythrocyte ABO antigens in the surface of the red blood cell. The main reason is that for the synthesis of the mentioned antigens, stepwise biochemical reactions are needed. Because the mentioned antigens are chemically carbohydrates and are not direct products of the specific genes, and accordingly, special transferases are formed first, which then change the structure of the precursor substance on the erythrocyte membrane, after which the final antigen-specific characteristic is formed at the embryonic life.

Features of the inheritance of antigens of the ABO blood group system have been well-studied at the current time. There are a lot of scientific papers regarding these issues, [6], [18], [19], [20]. The genetic inheritance of the ABO blood group system A and B antigens is due to multiple alleles and is one of the solid genetic traits. Usually, they do not change

throughout the ontogeny in a healthy person, but there are some cases of epigenetic changes of ABO blood group antigens in the cancer medium, [21], [22].

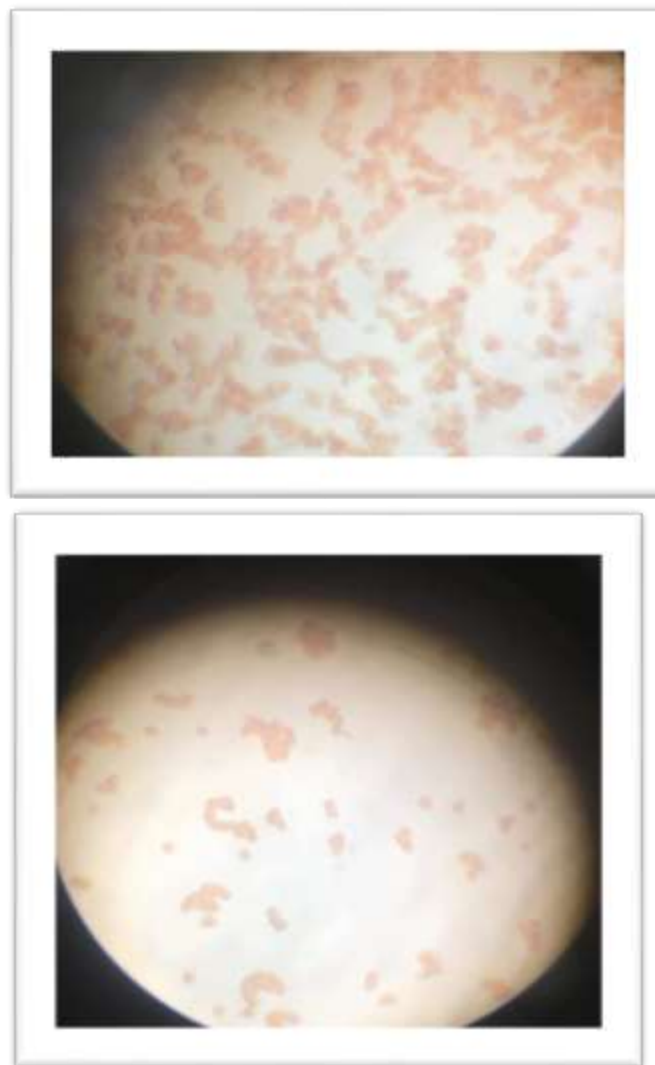


Fig. 1: Agglutination image in the newborn blood samples with 10X4 magnification lenses of the optic microscope.

The ABO system gene locus is located on the 9th pair of the autosomal chromosome, at position 9q34.1-q34.2. This region consists of 7 exons. Exons 6 and 7 encode the main catalytic domain of glycosyltransferases for the expression of erythrocyte A and B antigens. Expressions of the ABO system antigens are directly related to the H locus which is located in the long arm of the 19th pair of chromosome and occupies 19q13.3 position, [23]. As it turned out, the H locus is an antigenic system independent of the ABO system. It is well known

that A and B genes do not produce erythrocyte antigens; their direct products are glycosyltransferase enzymes. Gene A encodes 1,3-N-acetyl galactosamine transferase, gene B encodes 1,3-galactosyltransferase, and gene H encodes 1,2-fucosyltransferase which can transfer the fucose residue to the terminal chain of the galactose oligosaccharide. Transferases encoded by genes A and B can attach the corresponding immunodominant sugar residues to galactose, but only if the fucose residues are already attached (i.e., the middle chain has already been transformed to the H antigen). A and B antigen-determining carbohydrates reciprocally reduce the number of H-antigen molecules, [18].

The ABH antigen synthesis processes first appeared in embryos, which is correlated with the cellular differentiation and the histogenesis process of the embryo. ABH antigen occurs in tissues and biological fluids of more primitive mammalian species but appears in RBCs only among the great apes and human beings, [24]. Chimpanzees' blood typing showed A or O phenotypical characteristics. The gorillas were shown B phenotype specification. The expression of A and B antigens here is like humans, but lower primates do not have these characteristics. Interestingly, they express A and B antigens in respiratory or digestive epithelium. In some cases, it is also expressed in the biological secrets, especially in the saliva, [19].

Information about the synthesis of these antigens at an early stage of embryogenesis can be found in the literature. First of all, it is interesting to describe how and when erythrocytes are formed during embryonic life. In mammals as well as in human beings, the embryo first is going to have extraembryonic erythropoiesis, [25], [26]. The mentioned process first is detected in the umbilical vesicle (yolk sac) nearly 14 days after fertilization.

RBCs are vital for normal growth and development of the embryo and fetus. During embryonic and fetal life, erythropoiesis occurs in two different types. The first form consists of nucleated erythroblasts that form the formation process going into the yolk sac. The final form consists of anucleate erythrocytes that differentiate processes going in the embryonic liver and fetal bone marrow, [25], [27], [28], [29]. After is the formation of RBCs antigens, which is one most important components of cell membranes. But at the stage of the neonate, the rate of ABO gene expression is less compared to the early childhood period.

What about the ABO blood group's natural anti-A and anti-B antibodies? It's well known that the ABO system is the only one system where the natural antibodies are present in the serum. and the corresponding antibodies may be absent in the blood serum which leads to erroneous determination of the blood group of newborns using the immunoserological method, especially if reverse methods are used for newborn blood typing. In this regard, this inaccuracy in the use of blood for transfusion and treatment in newborns can lead to immune sensitization of the newborn as well as post-transfusion complications. This fact is of vital importance in newborns with severe anamnesis (with hemolytic disease and prematurity), when it is quite difficult to find a suitable donor in case of urgent transfusion.

According to one hypothesis, group-specific antibodies are synthesized in two-three-month-old embryos, which is associated with the influence of intestinal micro-flora. This phenomenon is considered a result of bacterial immunization.

As it is mentioned in the introduction part the ABO blood group antibodies are inherent and predominantly IgM class. Anti-ABO IgM is usually not present in newborns but appears in the first year of life. The antibodies may be produced against food and environmental antigens (bacteria, viral, or plant antigens), which are similar in structure to A and B antigens, [9].

In our study, we suggest that 43.94±3.5% case of the studied newborns the natural origin anti-A and anti-B antibodies were not detected (n=87), in some particular cases (14.14±2.4%) of O(I) blood group individuals it was partially synthesis (n=28). 41.92±3.5% of our studied newborns expressed natural antibodies fully as an adult (Table 7).

Table 7. ABO system antibodies synthesis frequency in the studied newborns

ABO blood type	Normal expression	Partially expression	without expression	Total
O (I)	35	28	28	91
A(II)	35	-	51	86
B(III)	13	-	8	21
Total	83	28	87	198
%	41.92± 3.5	14.14±2.47	43.94±3.5	100

*The chi-square χ^2 statistic is 35.5725. The p-value is < 0.00001. The result is significant at $p < .05$.

Some studies show that the pneumococcal polysaccharide vaccine contaminated with blood group A-like substance stimulated long-lasting production of anti-A antibodies in subjects with O or B blood group, [30].

We didn't find a similar study of our research to compare our current study information with the available literature. In all studies, it is mentioned that newborns have specific ABO antigen and antibody expression, but it is not described in detail O, A, B blood group newborns antibodies prevalence and compared to adults. If this antibody synthesis process is related to food and environmental antigens we think that this current study will be different from other regions' newborns' study. All regions have their specific diet and environmental conditions.

Some scientists suggested using additional methods to clarify the ABO status in the newborn. The most important in this case is to determine ABO secretory status in the newborns. The secretory status of newborn infants can be determined from saliva samples collected from the newborns during the first few days of postnatal life. All errors can be detected by this method, [31].

The ABO secretory status also is detected in embryonic life by using the amniotic fluid, which is collected from the amniocentesis from 12 to 28 weeks of gestation. Scientific research has shown that

The secretory status of the amniotic fluid was correlated with the secretory status of the newborn, which is determined from the saliva (98% of cases), [32].

The best way to avoid errors in blood typing is through genotyping methods. There are a lot of alternative methods for genotyping the ABO blood group system status, [33], [34], [35], [36].

We didn't find any difficulties in typing the Rh blood group in the newborns, because in all cases of the Rh⁺ samples D antigen was well agglutinated with monoclonal antibodies. The reason is that the Rh antigen is the direct product of the RhD gene, [37], [38].

5 Conclusion

The ABO antigens and antibodies expression process starts prenatally and continues postnatally, especially during the first six months. We suggested that in some cases newborns have normal expression of ABO antibodies as adult, but they are not expressed in the majority of the studied newborn cases. The

erythrocyte A and B antigens were weakly expressed in the majority of the cases and for detecting the agglutination images the optic microscopes were used. The limitation of the study is less blood samples of the newborn. The next step of our research is the ABO system genotyping in the newborn. It is interesting also to study the quantitative characteristics (titer) of ABO system antigens and antibodies in the newborns and also the first six months of babies and compare it to the adults.

Acknowledgement:

Batumi Shota Rustaveli State University targeted the grant project "Evaluation of immunogenetic characteristics of erythrocyte blood group antigen-antibodies of newborns", 2020-2022.

BAU International University Batumi, supported by reagents too.

References:

- [1] F. Yamamoto, E. Cid, M. Yamamoto, N. Saitou, J. Bertranpetit, и A. Blancher, «An integrative evolution theory of histo-blood group ABO and related genes», *Sci. Rep.*, V. 4, Issue 1, p. 6601, octomber. 2014, doi: 10.1038/srep06601.
- [2] S. Xu *and et al.* «Structural basis of P[II] rotavirus evolution and host ranges under selection of histo-blood group antigens», *Proc. Natl. Acad. Sci.*, V. 118, Issue 36, p. e2107963118, september. 2021, doi: 10.1073/pnas.2107963118.
- [3] J. M. Moulds and J. J. Moulds, «Blood group associations with parasites, bacteria, and viruses», *Transfus. Med. Rev.*, V. 14, Issue 4, p. 302–311, Octomber, 2000, doi: 10.1053/tmrv.2000.16227.
- [4] ISBT, «Red Cell Immunogenetics and Blood Group Terminology | ISBT Working Party». <https://www.isbtweb.org/isbt-working-parties/rcibgt.html> (Accessed Date: 9th september, 2023).
- [5] G. Daniels, «Functional aspects of red cell antigens», *Blood Rev.*, V. 13, Issue 1, p. 14–35, march, 1999, doi: 10.1016/S0268-960X(99)90020-6.
- [6] E. Hosoi, «Biological and clinical aspects of ABO blood group system», *J. Med. Invest.*, V.

- 55, Issue 3,4, p. 174–182, 2008, doi: 10.2152/jmi.55.174.
- [7] J. Storry and M. L. Olsson, «The ABO blood group system revisited: a review and update.», *Immunohematology*, V. 25, Issue. 2, p. 48–59, 2009.
- [8] J. Milland and M. S. Sandrin, «ABO blood group and related antigens, natural antibodies and transplantation», *Tissue Antigens*, V. 68, Issue 6, p. 459–466, December, 2006, doi: 10.1111/j.1399-0039.2006.00721.x.
- [9] D. R. Branch, «Anti-A and anti-B: what are they and where do they come from?: ABO ISOHEMAGGLUTININS», *Transfusion (Paris)*, V. 55, Issue S2, p. S74–S79, June. 2015, doi: 10.1111/trf.13087.
- [10] N. J. Wuttke, P. J. Macardle, и H. Zola, «Blood group antibodies are made by CD5⁺ and by CD5⁻ B cells», *Immunol. Cell Biol.*, V. 75, Issue 5, p. 478–483, October. 1997, doi: 10.1038/icb.1997.74.
- [11] F. Yamamoto, E. Cid, M. Yamamoto, and A. Blancher, «ABO Research in the Modern Era of Genomics», *Transfus. Med. Rev.*, V. 26, Issue 2, p. 103–118, April. 2012, doi: 10.1016/j.tmr.2011.08.002.
- [12] S. B. Abegaz, «Human ABO Blood Groups and Their Associations with Different Diseases», *BioMed Res. Int.*, V. 2021, p. 1–9. 2021, doi: 10.1155/2021/6629060.
- [13] H.-Y. Li and K. Guo, «Blood Group Testing», *Front. Med.*, V. 9, P. 827619, February. 2022, doi: 10.3389/fmed.2022.827619.
- [14] W. A. Flegel, «COVID-19: risk of infection is high, independently of ABO blood group», *Haematologica*, V. 105, Issue. 12, p. 2706–2708, December, 2020, doi: 10.3324/haematol.2020.266593.
- [15] S. Mu, C. Sha, S. Ren, and D. Xiang, «Clinical Characteristics and Influence Factor Analysis of ABO Typing Discrepancy Among Patients in a Tertiary Hospital», *Clin. Lab.*, V. 67, Issue 11/2021, 2021, doi: 10.7754/Clin.Lab.2021.210229.
- [16] S. Fathima and R. B. Killeen, «ABO Typing Discrepancies», в *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. [Online]. Available on: <http://www.ncbi.nlm.nih.gov/books/NBK585061/> (Asseced Date: 24 July 2023)
- [17] «Quick Statistics Calculators», *Social Science Statistics*. <https://www.socscistatistics.com/tests/> (Accessed Date: September 9, 2023).
- [18] G. A. Denomme, «Molecular basis of blood group expression», *Transfus. Apher. Sci.*, V. 44, Issue. 1, p. 53–63, February, 2011, doi: 10.1016/j.transci.2010.12.010.
- [19] F. Yamamoto, «Molecular genetics and genomics of the ABO blood group system», *Ann. Blood*, V. 6, p. 25–25, September. 2021, doi: 10.21037/aob-20-71.
- [20] S. P. Yip, «Sequence variation at the human ABO locus», *Ann. Hum. Genet.*, V. 66, Issue. 1, p. 1–27, January. 2002, doi: 10.1017/S0003480001008995.
- [21] M. Franchini, G. M. Liumbruno, G. Lippi, and F. Scatena, «The prognostic value of ABO blood group in cancer patients», *Blood Transfus.*, 2015, doi: 10.2450/2015.0164-15.
- [22] M. S. Waleed and W. Sadiq, «Multiple Myeloma and Change of ABO Blood Group Type: A Case Report», *Cureus*, September, 2020, doi: 10.7759/cureus.10654.
- [23] R. J. Kelly, L. K. Ernst, R. D. Larsen, J. G. Bryant, J. S. Robinson, and J. B. Lowe, «Molecular Basis for H Blood Group Deficiency in Bombay (Oh) and Para-Bombay Individuals», *Proc. Natl. Acad. Sci. U. S. A.*, V. 91, Issue 13, p. 5843–5847, 1994.
- [24] D. C. F. Aguiar, W. L. A. Pereira, G. D. C. B. De Matos, K. S. Marruaz Da Silva, R. D. S. P. De Loiola, and T. C. O. Corvelo, «Tissue expression of antigens of ABH blood groups in species of New World Monkeys (Aotus infulatus, Callithrix jacchus, Sapajus apella and Saimiri sciureus)», *PLOS ONE*, V. 15, Issue 11, p. e0241487, November, 2020, doi: 10.1371/journal.pone.0241487.
- [25] J. Palis and G. B. Segel, «Developmental biology of erythropoiesis», *Blood Rev.*, V. 12, Issue 2, p. 106–114, June, 1998, doi: 10.1016/S0268-960X(98)90022-4.
- [26] M. A. S. Moore and D. Metcalf, «Ontogeny of the Haemopoietic System: Yolk Sac Origin of In Vivo and In Vitro Colony Forming Cells in the Developing Mouse Embryo», *Br. J. Haematol.*, V. 18, Issue. 3, p. 279–296, March, 1970, doi: 10.1111/j.1365-2141.1970.tb01443.x.
- [27] «Red Blood Cell Indices: Implications for Practice», *Medscape*. <https://www.medscape.com/viewarticle/497032> (Accessed Date: 30 August 2023).

- [28] D. J. Kuruvilla, J. A. Widness, D. Nalbant, R. L. Schmidt, D. M. Mock, and P. Veng-Pedersen, «A Method to Evaluate Fetal Erythropoiesis from Postnatal Survival of Fetal RBCs», *AAPS J.*, V. 17, Issue 5, p. 1246–1254, September, 2015, doi: 10.1208/s12248-015-9784-y.
- [29] K. M. Moritz, G. B. Lim, and E. M. Wintour, «Developmental regulation of erythropoietin and erythropoiesis», *Am. J. Physiol.-Regul. Integr. Comp. Physiol.*, V. 273, Issue 6, p. R1829–R1844, December, 1997, doi: 10.1152/ajpregu.1997.273.6.R1829.
- [30] P. Koskela, T. Nurmi, and V.-M. Häivä, «IgA, IgG and IgM anti-blood group A antibodies induced by pneumococcal vaccine», *Vaccine*, V. 6, Issue 3, p. 221–222, June, 1988, doi: 10.1016/0264-410X(88)90214-9.
- [31] R. L. Kirk and G. H. Vos, «A Preliminary Check on the Accuracy of ABO Typing of Newborn Infants», *Vox Sang.*, V. 2, Issue 6, p. 422–427, 1957, doi: 10.1159/000478355.
- [32] S. L. M. Gibson and M. A. Ferguson-Smith, «The secretor status of the foetus», *Clin. Genet.*, V. 18, Issue 2, p. 97–102, April, 2008, doi: 10.1111/j.1399-0004.1980.tb01018.x.
- [33] D. S. O'Keefe and A. Dobrovic, «A rapid and reliable PCR method for genotyping the ABO blood group», *Hum. Mutat.*, V. 2, Issue. 1, p. 67–70, 1993, doi: 10.1002/humu.1380020112.
- [34] M. M. El-Zawahri and Y. A. Luqmani, «Molecular genotyping and frequencies of A 1, A 2, B, O 1 and O 2 alleles of the ABO blood group system in a Kuwaiti population», *Int. J. Hematol.*, V. 87, Issue. 3, p. 303–309, April, 2008, doi: 10.1007/s12185-008-0036-0.
- [35] H. Y. Lee, M. J. Park, N. Y. Kim, W. I. Yang, and K.-J. Shin, «Rapid Direct PCR for ABO Blood Typing*: A RAPID DIRECT ABO GENOTYPING», *J. Forensic Sci.*, V. 56, p. S179–S182, January. 2011, doi: 10.1111/j.1556-4029.2010.01591.x.
- [36] G. Ferri и S. Pelotti, «Multiplex ABO Genotyping by Minisequencing», в *DNA and RNA Profiling in Human Blood*, P. Bugert, Ред., в *Methods in Molecular Biology*, V. 496. Totowa, NJ: Humana Press, 2009, p. 51–58. doi: 10.1007/978-1-59745-553-4_5.
- [37] N. D. Avent and M. E. Reid, «The Rh blood group system: a review», *Blood*, V. 95, Issue 2, p. 375–387, January. 2000, doi: 10.1182/blood.V95.2.375.
- [38] J.-P. Cartron, «Defining the Rh blood group antigens», *Blood Rev.*, V. 8, Issue. 4, p. 199–212, December, 1994, doi: 10.1016/0268-960X(94)90108-2.

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

- SHORENA GABAIDZE was responsible for reparation of the published work, specifically writing the initial draft (including substantive translation).
- MARINA NAGERVADZE had oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
- LEILA AKHVLEDIANI was responsible for development and design of research methodology.
- NANA NAKASHIDZE was responsible for application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.
- ALISSAR ALFILO was conducted a research and investigation process, specifically performing the experiments and data/evidence collection
- IRINE TSINTSADZE has organized and executed the experiments of Section 2.
- NATO GORGADZE - was Provided of study materials from the "Iris Borchashvili Health Center Medina"
- RUSUDAN KHUKHUNAISHVILI was responsible for verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.
- MARINA KORIDZE was responsible for preparation of the published work, specifically visualization/data presentation.
- TEA KOIAVA was responsible for the Statistics.
- KETEVAN DOLIDZE was responsible for formulation of research goals and aims.
- TAMAR BAKHTADZE was Provided of study materials from the "M. Iashvili Batumi Maternal and Child Central Hospital".

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself

Batumi Shota Rustaveli State University targeted the grant project "Evaluation of immunogenetic characteristics of erythrocyte blood group antigen-antibodies of newborns", 2020-2022.

Conflict of Interest

The authors have no conflict of interest to declare.

Creative Commons Attribution License 4.0 (Attribution 4.0 International, CC BY 4.0)

This article is published under the terms of the Creative Commons Attribution License 4.0

https://creativecommons.org/licenses/by/4.0/deed.en_US