

KELL Null PHENOTYPE IN A PREGNANT WOMAN - IMMUNOHEMATOLOGICAL CHARACTERISTICS, MOLECULAR ANALYSIS AND TRANSFUSION MANAGEMENT

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SUMMARY

In Laboratory for antenatal testing NBTI, a pregnant woman with Kell null phenotype was discovered few years ago. The findings were serologically confirmed in Blood Group Reference Laboratory in Bristol, and Immunohematological Laboratory in Frankfurt. The molecular analysis of this KEL gene was performed by Sohee Lee, New York Blood Center. It showed a homozygous T366QA mutation (T to A mutation at nt 366 of Kell c DNA) in exon 4. The mutations changed a cysteine codon (TGT) to a stop codon (TGA) resulting in a premature stop codon encoding residue 83. All mutations occurred preceding the zinc binding enzyme active site. Molecular analysis of the remaining 7 Kell null phenotypes from all over the world were also performed. The blood group of the investigated person was O, RhD negative, ccddee. Since every transfusion of red blood cells could be a potential risk to the alloimmunization to this woman, our colleague from the Institute of transfusion Medicine Of MMA succeeded to collect and froze two units of autologous red blood cells for her possible needs in the future.

Key words: molecular analysis, Kell null phenotype, autologous frozen red blood cells

Introduction

Kell blood group system is one of 29 so far discovered systems. Within this system, 27 red blood cell antigens were identified (1). First among them, K antigen (Kell 1), was discovered in 1946, thanks to an antibody which caused hemolytic disease of the newborn in a pregnant woman, certain Ms. Kell. K gene is present in around 9% of the Caucasian population and in 2% of the black race population. Antithetic k allele which determines the presence of k antigen (KEL 2) is present in 99% of the human population (2-5). After the discovery of the above stated antigens, other antigens, also belonging to the Kell blood group system, were discovered later on.

Expression of corresponding Kell antigens on red blood cells is dependent on two proteins, Kell and XK, linked by a single disulfide bond. Autosomal KEL gene is located on chromosome 7 at the position q33-35 distally from the cystic fibrosis locus (6-9). Besides,

expression of Kell blood group antigen expression on red blood cells is controlled by another protein - Kx. This protein is coordinated by XK gene found on the short arm of the chromosome X on Xp21, between loci of Duchenne's muscle dystrophy and chronic granulomatosis disease (10, 11). Kell protein is a membrane glycoprotein type II and its structure is equal to the zinc neutral endopeptidase. So far, protein Kell function on the red cell membrane is still unknown, although it has been noted that there are structural similarities with the family of neutral endopeptidases binding with zinc. Kx protein has 10 transmembrane domains and it is a membrane transporter (12-25).

As in other blood group systems, there are rare deficient phenotypes in the Kell system as well. One of them is K₀ (Kell null). Based on DNA sequences persons with so far discovered Kell₀ phenotype have mutations in exons 3, 4, 6, 9, 10 and 18. Characteristic of the Kell₀ phenotype is the lack of all Kell antigens on red blood cells and a strong expression of Kx antigen (5, 16).

Analyses performed on the molecular level, proved Kell₀ phenotype in 8 persons only, one of them is the woman from our country. The study presents results of the immunohematological and molecular investigations performed in the NBTI and in several competent international reference laboratories.

Besides, a short insight into the possibilities of the transfusion treatment using one of the autologous blood transfusion protocols.

Investigated Subject and Methods

In the course of the routine prenatal blood group testing and red blood cell antibody screening, we tested a blood sample of a pregnant woman, age 27, on the 27th gestation week, who delivered a healthy male child five years earlier. In the meantime, there were no other pregnancies or blood transfusions. Blood group was determined using tube method, while red blood cell antibody screening, antibody identification and red blood cell antigen typing were performed using both tube and gel method (DiaMed ID Micro Typing System). Direct and indirect anti globulin tests and enzyme treated red blood cells techniques were used for immunohematological investigations. Commercial test reagents were used for red blood cell antigen typing (Ortho Clinical Diagnostics, Johnson & Johnson Co; DiaMed, Switzerland, Biorad), and for red blood cell antibody screening and identification we used commercial red cell panels (DiaMed, Switzerland).

Additional immunohematological investigations were performed in the International Red Blood Cell Reference Laboratory, Bristol and Immunohematological Laboratory in Frankfurt, while Kell gene molecular analysis was performed in NYBC (DNA preparation, PCR, DNA sequencing, etc.) (15).

For providing compatible red blood cells for transfusion, two units of autologous blood were pre collected from the investigated subject. Separated red blood cells were frozen using glycerol, as a cryoprotector. Cryopreserved red blood cells were stored in the mechanical freezer on $-90\pm 5^{\circ}\text{C}$ to be used for autologous transfusion, if necessary.

Results and Comment

Pregnant woman's blood group was 0, Rh(D)-negative (ccddee). Her serum screening to the presence of red blood cell antibodies was positive regarding red blood cell panel, using both tube and gel method. Indirect anti globulin test, however, expressed positivity in gel method only. Antibody identification using enzyme treated red blood cell panel in gel method demonstrated the presence of anti-Kell antibody. Kell

antibody was demonstrated both by red blood cell homozygous for Kell antigen (KK) and heterozygous for Kell antigen (Kk). In the indirect anti globulin test, using gel method, antibody reacted with test-red blood cells KK phenotype only.

In our laboratory, phenotyping of the pregnant woman using anti-K, anti-k, anti-Kp^a and anti-Kp^b test reagents showed the following finding: K-, k-, Kp(a-b-). Reference laboratories in Bristol and Frankfurt confirmed that the pregnant woman's phenotype was Kell₀ with the following characteristics: K-, k-, Kp(a-b-), Js(a-b-), K11, Ku-. Results of the molecular analysis of Kell gene also confirmed Kell₀ phenotype.

In rare cases, red blood cells lack either XK or Kell protein. Red blood cells that lack XK have the McLeod phenotype and red blood cells that lack Kell protein and Kell antigens, have the Kell₀ phenotype. McLeod red blood cells have a greatly reduced amount of Kell protein and all of its antigens (16). Red blood cells show acanthocytosis and have shorter *in vivo* survival. Besides, persons with McLeod phenotype show the late onset forms of muscular and neurological disorders (5). This rare phenotype results from either deletion of the part of the X chromosome, or it is inherited by the defect allele at the locus XK. McLeod phenotype is often associated with the X-linked chronic granulomatous disease, Duchenne's muscular dystrophy or pigmentous retinitis.

Unlike McLeod phenotype red blood cells, Kell₀ red blood cells have normal shape. Kell₀ red blood cells have reduced amount of XK protein, but they have enhanced Kx antigen activity. This phenotype is obviously inherited through the silent allele on the Kell locus. When Kell phenotype persons become immunized by pregnancy or blood transfusion, they produce antibody (anti-KEL5) reacting with all Kell antigens (19).

In the pregnant woman, subject of this study, Kell null phenotype was determined after a thorough phenotyping of her Kell red blood cell antigen status. However, due to the lack of some rare test reagents, we succeeded to demonstrate the absence of the following antigens only: K, k, Kp^a and Kp^b on the woman's red blood cells, which pointed to a rare or Kell deficient phenotype. The same investigation was repeated in her parents, other siblings and children, demonstrating the same, K-, k+, Kp(a-b+) phenotype in all of them. It should be mentioned that the woman had a normal delivery without blood transfusion, despite the Hb level of 70 g/l and Hct of around 0,24. During pregnancy, she was administered iron substitution therapy only.

As already stated, the blood sample was forwarded to reference laboratories in Bristol and Frankfurt. Further testing confirmed our findings and, additionally, it was demonstrated that her red blood cells were Js(a-b-), K11, Ku-, showing the rare Kell null phenotype.

All the investigations, subject of this presentation, were performed in the course of 1997 and 1998. Two years later, contacts were made with the research team from NYBC who performed molecular analysis of the investigated subject's Kell gene. The finding obtained in NYBC was compared with the findings of another 7 unrelated persons from all over the world in whom rare Kell null phenotype had also been demonstrated. (table 1)

Our subject had a single base mutation in exon 4, but in a different location (T366A). The mutation is homozygous and converts a TGT codon encoding cystein at position 83 to a TGA stop codon. Namely, all mutations occur on the loci preceding the enzyme binding site, where otherwise zinc is located.

Considering that any red blood or blood component transfusion is a risk of alloimmunization to red blood cell antigens for this woman, two units of autologous red blood cells were collected and cryopreserved at the Institute of transfusiology of the Military Medical Academy, in case of possible future demands. That precaution measure is particularly significant, since the woman has considerable menstrual bleeding episodes, associated with hypo chromic microcyte anemia. Besides, she is continuously on the supportive iron therapy, supervised by hematologist. Fortunately, her general condition and hematological status so far have not required autologous red cell transfusion.

Table 1. Described Kell null genotypes (NYBC, S Lee et al. Journal of Biological Chemistry 2001; 276:27:281-89)

Knull	Nucleotide substitution	Mutation	Zygosity
Michigan	C502T, exon 4	R128Stop	Homozygous
North Carolina	C502T, exon 4	R128Stop	Homozygous
Yugoslavia	T366A, exon 4	C83Stop	Homozygous
Portugal	C1162T,exon 9	Q348Stop	Homozygous
Israel	G2147A, exon 18	S676N	Homozygous
Reunion Island	G to A, 5' intron 3	Alternative splicing	Homozygous
Seattle	G1208A, exon 10	S363N	Heterozygous
	G to A, 5' intron 3	Alternative splicing	Heterozygous
New York	G1208A, exon 10	S363N	Heterozygous
	C694T, exon 6	R192Stop	heterozygous

Conclusion

The first extremely rare case of Kell null phenotype was demonstrated in our country. This case points to the necessity of founding a national rare blood group donor registry, as well as a national blood bank, along with the feasibility of certain blood cell types cryopreservation. Likewise, this case emphasizes the importance of regular contacts with the International Blood Bank in Amsterdam, so far the only possible source of assistance in cases when rare blood group individuals are vitally jeopardized. Thus, the reference immunohematological laboratory of the NBTI should be provided with all rare test reagents for the detection of rare red blood cell antigens, as well as different panels for antibody identification.

Summary

KELL NULL FENOTIP U TRUDNICE- SEROLOŠKE KARAKTERISTIKE, MOLEKULARNA ANALIZA I TRANSFUZIOLOŠKI TRETMAN

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Pre nekoliko godina, u Laboratoriji za prenatalnu dijagnostiku je otkriven fenotip Kell null u jedne trudnice tokom rutinskog određivanja krvne grupe i skrininga antieritocitnih antitela. On je serološki potvrđen u Laboratoriji za krvne grupe u Bristolu i Imunohematološkoj laboratoriji u Frankfurtu. Tokom 2000. godine izvršena je molekularna analiza gena Kell naše trudnice, u New York Blood Center-u, zajedno sa još 7 analiza osoba sa fenotipom Kell null iz različitih delova sveta. U naše trudnice, reč je o homozigotnoj mutaciji T366 (mutacija T u A na nt 366 Kell cDNA), na eksonu 4. Ona je izmenila kodon za cistein (TGT) u stop kodon (TGA) što je rezultovalo pojavom prevermenog stop kodona i otkriveno kodiranjem rezidua 83. Sve mutacije su se pojavile pre aktivnog mesta za koje se vezuje enzim, na

kome se inače nalazi cink. Krvna grupa pacijentkinje je O, RhD negativna, ccddee. Svaka transfuzija eritrocita ili ma koje komponente krvi koja sadrži eritrocite bila bi potencijalni rizik za aloimunizaciju jer naša pacijentkinja nema ni jedan od antigena Kell na membrani eritrocita. Zbog toga smo, zahvaljujući pomoći kolege sa VMA, uzeli i zamrznuti dve jedinice autolognih eritrocita za njene eventualne potrebe u budućnosti.

Ključne reči: molekularna analiza, aloimunizacija, zamrznuti autologni eritrociti

Literature

- Daniels GL (chair) et al:** ISBT Committee on Terminology for Red Cell Surface Antigens. Vox Sang 2003.
- Mollison PL et al.** Blood transfusion in clinical medicine; 7th ed. oxford Blackwell, 1998, pp.
- Daniels GL.** Human blood Groups; Oxford Blackwell, 1995, pp.
- Issitt PD, Anstee DJ.** Applied blood group serology. 4th ed. Durham NC;Montgomery Scientific, 1988, pp.
- Schenkel-Brunner H.** Human Blood Groups - Chemical and Biochemical Basis of Antigen Specificity. 2nd completely revised edition. Springer Wien New York, 2000, pp 485-503.
- Lee S, Zambas ED, Marsh WL, Redman CM.** The human Kell blood group gene maps to chromosome 7q33 and its expression is restricted to erythroid cells. Blood 1993; 81: 2804-09.
- Murphy MT, Morrison N, Miles JS, Fraser RH, Spurr NK, Boyd E.** Regional chromosomal assignment of the Kell blood group locus (KEL) to chromosome 7q33-q35 by fluorescence in situ hybridization: evidence, for the polypeptide nature of antigenic variation. Hum Genet 1993, 91: 585-88.
- Purohit KR, Ewbur JL, Ward LJ, Keats BJB.** The Kell Blood group locus is close to the cystic fibrosis locus on chromosome 7. Hum genet 1992; 89: 457-58.
- Zelinski T, Coghlan G, Myal Y, White LJ, Philipps SE.** Assignment of the Kell blood group locus to chromosome 7q. Cytogenet cell genet 1991; 58:1927.
- Bertelson CJ, Pogo AO, Chaudhuri A, Marsh WL, Redman CM, Benerjee D et al.** Localisation of the McLeod locus (XK) within Xp21 by deletion analysis. Amer j hum genet 1988; 42: 703-11.
- Ho MF, Monaco AP, Blonden LAJ, Van Ommen GJB, Affara NA, Ferguson Smith MA, Lehrach H.** Fine mapping of the McLeod locus (XK) to a 150-380 kb region in Xp21. Amer J Hum Genet 1992; 50: 317-30.
- Garraty G, Dzik W, Issitt PD et al.** Terminology for blood group antigens and genes- historical origins and guidelines in the new millennium. Transfusion 2000; 40: 477-89.
- Lee S, Russo D, Redman CM.** The Kell blood group system: Kell and XK membrane proteins. Semin hematol 2000; 37: 113-21.
- Zelinski T, Coghlan G, Myal Y et al.** Genetic linkage between the Kell blood group system and prolactin-inducible protein loci: provisional assignment of KEL to chromosome 7. Ann Hum Genet 1991; 55:137-40.
- Lee S, Russo D, Reiner A et al.** Molecular defects underlying the Kellnull phenotype. Journal of Biological Chemistry 2001; 276: 27: 281-89.
- Vengelen-Tyler V,** editor. Technical Manual. 14th ed. Bethesda, MD: American Association of blood Banks; 2003.
- Allen FH, Krabbe SMR, Corcoran PA.** A new Phenotype (McLeod) in the Kell blood group system. Vox Sang 1961; 6: 555-60.
- Marsh WL, Redman CM.** The Kell blood group system: a review. Transfusion 1990; 30: 158-67.
- Redman CM, Marsh WL.** The Kell blood group system and the McLeod phenotype. Sem Hematol 1993; 30: 209-18.