Blocking Phenomenon Observed In HDFN Due To Anti-K

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Background/Case Study

A G2P1 female with anti-K (titer 256) identified at an outside laboratory was referred for evaluation for an intrauterine transfusion (IUT) due to a fetal hematocrit of 24.6%. Anti-K is an IgG antibody implicated in acute and delayed hemolytic transfusion reactions, as well as severe hemolytic disease of the fetus and newborn (HDFN) due to destruction of fetal erythroid progenitor cells causing suppression of erythropoiesis.

Study Design/Methods

Antibody detection test and an identification panel were performed on the maternal sample using solid phase media. A fetal blood specimen was obtained by cordocentesis. The direct antiglobulin test (DAT) was performed on the fetal specimen using polyspecific AHG, monoclonal anti-IgG and monoclonal anti-C3b, C3d reagents evaluated at immediate spin and room temperature. Phenotyping was performed on the fetal sample using commercial monoclonal anti-K antisera. One volume of fetal cells was treated with four volumes of chloroquine diphosphate (CDP) for a 90-minute room temperature incubation. Removal of bound antibody by CDP treatment was selected over EDTA-glycine acid (EGA) treatment due to inactivation of Kell blood group antigens by EGA.

Results/Findings

Figure 1 – Serologic Results Pre-Chloroquine Treatment

(A)

Direct Coombs Battery: Method: Tube Testing				
Phase	PS	IgG	C3	Saline
IS	3+	3+	w	0
5' RT	2+	2+	2+	0

(B)

Phenotype of Fetus: K Negative					
	Anti-K	Positive Control	Negative Control	Results	
Source	Immucor	Immucor	Immucor	Pos Cont. Cell #1	3+
Lot #	924670	46528	46528	Neg Cont. Cell #2	0
Exp. Date	4/10/2022	1/22/2021	1/22/2021	Patient	0

Figure 2 – Maternal Antibody Titer

	Tube Number	1	2	3	4	5	6	7	8	9	10	11	12
Cells	Incubation Time/Temp	Undiluted Serum	2	4	8	16	32	64	128	256	512	1024	2048
Kk	60' at 37C	1+	1+	0	0	0	0	0	0	0	0	0	0
	AHG	4+	3+	3+	3+	3+	3+	3+	2+	2+	1+	0	0

Figure 3 – Serologic Results Post-Chloroquine Treatment

(A)

Direct Coombs Battery: Method: Tube Testing				
Phase	PS	IgG	C3	Saline
IS	3+	3+	w	0
5' RT	2+	2+	2+	0

(B)

Phenotype of Fetus: K Negative					
	Anti-K	Positive Control	Negative Control	Results	
Source	Immucor	Immucor	Immucor	Pos Cont. Cell #1	3+
Lot #	924670	46528	46528	Neg Cont. Cell #2	0
Exp. Date	4/10/2022	1/22/2021	1/22/2021	Patient	3+

Anti-K was confirmed in the maternal specimen. The DAT on the fetal sample was strongly positive with polyspecific AHG and monoclonal anti-IgG. The fetal phenotype was initially K-; however, due to the strongly positive DAT result, the K phenotyping result was suspected to be falsely negative. The fetal RBCs were treated with CDP in an attempt to remove bound maternal antibody to allow for phenotyping. The DAT post CDP treatment was unchanged, suggesting removal of the maternal antibody was incomplete; however, repeat phenotyping was attempted and yielded a K+ result. A review of the patient's outside laboratory records indicated genotyping performed on a fetal amniotic fluid sample predicted the fetus to be K+k+ which correlated with the phenotype obtained post CDP treatment.

Conclusion

Antigen saturation of the fetal RBCs by high titer maternal anti-K inhibited binding of the commercial anti-K antisera resulting in a negative antigen phenotype. The fetal DAT result combined with a low hematocrit indicated HDFN, and a need for an IUT. A total of four IUTs were performed during the pregnancy, ensuring a healthy birth at 37w4d. This case illustrates that fetal phenotype alone may be inaccurate in predicting antigen status. Utilization of both genotyping and phenotyping methods can facilitate the recognition of blocking phenomenon, and provide a more accurate determination of fetal phenotype. Although our case demonstrated the blocking phenomenon with anti-K, it can occur with other high titer antibodies and has been reported in the Rh system.