

A RARE CASE OF NEONATAL ALLOIMMUNE THROMBOCYTOPENIA DUE TO ANTI-HPA-2b

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Despite the fact that neonatal alloimmune thrombocytopenia (NAIT) is a relatively rare disease (1/1500-1/5000 live births),¹ its importance stems from its potential to cause serious complications. NAIT accounts for 20% of cases of neonatal thrombocytopenia and is most commonly attributed to platelet-specific antigens. Diagnosis is usually confirmed by demonstrating that antiplatelet antibodies in the mother's serum are reactive with her baby's or husband's platelets. This test is, however, not always reliable.² Alternatively, platelets from the family could be serotyped, using specific antisera, but this is usually limited to reference laboratories. Recently, it has become possible to genotype platelet alloantigens, using various DNA-based techniques.^{3,4} This does not only simplify diagnosis but also allows early recognition and therapeutic intervention, such as intrauterine platelet transfusion and delivery by cesarean section. These therapeutic measures may prevent mortality or serious sequelae, such as permanent CNS damage. In this case report, we discuss the utilization of polymerase chain reaction–sequence specific primer (PCR-SSP) technology in a Saudi newborn with NAIT.

Case Report

A full-term, low birth weight (2.3 kg) baby boy was normally delivered in the Maternity and Children's Hospital, Dammam in 1997. Apgar score was normal and there were no dysmorphic features, petechiae or purpuric lesions. Routine biochemical investigations revealed low serum glucose (34 mg/dL). Hematological tests done on the first day showed normal Hb 21.1 g/dL, normal WBC ($8.5 \times 10^9/L$) and low platelet count ($100 \times 10^9/L$). The platelet count thereafter dropped to $66 \times 10^9/L$ on the 6th day of admission and became normal ($163 \times 10^9/L$) two weeks after delivery. Differential WBC count showed a

predominance of lymphocytes (54%), with 42% neutrophils, 2% monocytes and 2% eosinophils. Apart from a few nucleated RBC and physiological macrocytosis, RBC morphology was normal. A few platelets looked morphologically large. Coagulation studies excluded disseminated intravascular coagulopathy (DIC). Serological evaluation was negative for toxoplasma, but positive (IgG) for both rubella and cytomegalovirus (CMV), indicating maternally acquired antibodies. Blood culture did not grow any bacteria. The mother had three other healthy children. She aborted twice during her second and fourth pregnancies in the first and second trimesters for unknown etiology. She had a history of hypertension but was not on any medication. There was no history of a similarly affected sibling with thrombocytopenia or bleeding manifestations. The mother herself did not have any history of bleeding. Her platelet count was $216 \times 10^9/L$ on the day of the delivery.

Detection of Anti-Platelet and Anti-HLA Antibodies

Serum was collected from the baby and the mother, and 10 mL EDTA blood from the father provided platelets for the antibody test. Platelet indirect immunofluorescent test (PIFT) was performed by the recommended technique.⁵ The father's platelets were incubated in one tube with the maternal serum and in another tube with the infant's serum. A second layer of fluorescein isothiocyanate-labelled mouse anti-human IgG was added. Slides were examined under the epifluorescence microscope using FITC interference filter. A control normal serum was run with the father's platelets in parallel. This test revealed that maternal serum contained an alloantibody which reacted with the father's platelets.¹

TABLE 1. HPA 1-5 genotype of the family.

HPA system	HPA genotype		
	Father	Son	Mother
HPA-1	ab	aa	ab
HPA-2	ab	ab	aa
HPA-3	aa	aa	aa
HPA-4	aa	aa	aa
HPA-5	aa	aa	aa

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TABLE 2. Estimated risk of NAIT-complicated pregnancies in KSA: distribution according to HPA system.

Platelet alloantigen	Estimated risk (%)	Total (%)
HPA-1a	0	
HPA-1b	24	24
HPA-2a	3.84	
HPA-2b	4.55	8.39
HPA-3a	4.72	
HPA-3b	15.39	20.11
HPA-4a	1.96	
HPA-4b	3.84	5.8
HPA-5a	2.97	
HPA-5b	20.96	23.13

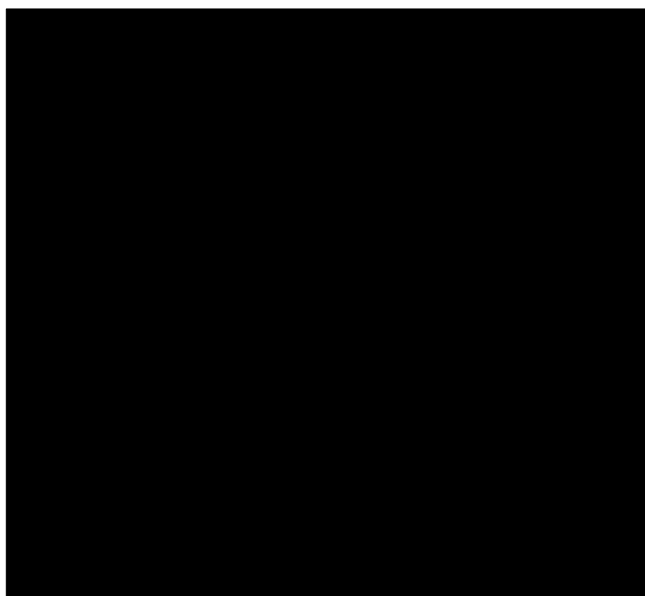


FIGURE 1. Agarose gel electrophoresis of PCR-amplified HPA-2 genes (a & b) from father (1 & 2), son (3 & 4) and mother (5 & 6). Wells 1, 3 & 5 contained HPA-2a specific primer. Wells 2, 4 & 6 contained HPA-2b specific primer. The fast band (258bp) represents the amplified HPA-2 gene. The slow band (429bp) represents the amplified positive control (HGH) gene. The father and son are heterozygous (i.e., have both HPA-2a & 2b). Mother is homozygous for HPA-2a.

The infant's platelets were not available for direct PIFT. Anti-HLA antibodies in the mother's serum were excluded by standard NIH lymphocyte cytotoxicity assay. Organ and non-organ-specific antibodies were tested by indirect immunofluorescence test using a composite block and showed no antibodies in maternal serum.

Platelet Genotyping

Five mL EDTA blood was obtained separately from the baby, the mother and the father, and DNA was isolated from lymphocytes by salting out and ethanol precipitation as previously described.⁶

Isolated DNA was amplified by the PCR-SSP technique described earlier,⁷ using a Perkin Elmer thermal cycler (Gene Amp PCR system 9600). The PCR reaction mix (10 μ L) contained 2 μ L (200 ng) genomic DNA, 1 μ L (20

pmol) of common and 1 μ L of specific primer (HPA-1a or HPA-1b, etc.), 2 μ L of positive-control (sense and anti-sense) primers for human growth hormone (HGH), 0.2 μ L (200 μ mol) of dNTP mix, 1 μ L of 10x reaction buffer, 0.09 μ L (0.04 U) of taq polymerase, and 2.72 μ L double-distilled water. Amplification was carried out for 34 cycles with three different set-ups of decreasing annealing temperature (i.e., 68°C, 61°C and 51°C).

Three primers were used for each HPA system: one common and two specific primers (for a or b). Each tube contained an allele-specific and a common primer. A 50 bp ladder was used as reference for the size of the amplicon. The amplified product was detected by electrophoresis on 2% agarose gel impregnated with ethidium bromide (140 v, 20 minutes).

Results of HPA1-5 genotyping of the family are shown in Table 1. Genotypes of HPA-3, HPA-4 and HPA-5 were the same in all three family members, but there were discrepancies in the distribution of HPA-1b and HPA-2b. It is obvious from Table 1 that the only HPA system that could have contributed to NAIT in this case was HPA-2b. This antigen was absent on the mother's platelets but expressed on both the father's and baby's platelets. Figure 1 shows the electrophoretic pattern of HPA-2 genotyping of the family.

Discussion

Isolated thrombocytopenia in our Saudi neonate was not initially suspected as NAITP, as there was no history of a previously affected sibling. Other etiologies of thrombocytopenia, including DIC, infection, maternal ITP, consumption hemangioma and drug intake were excluded. Diagnosis of NAIT was virtually confirmed by positive indirect PIFT and spontaneous recovery of the thrombocytopenia. However, identification of the specific platelet alloantigen linked to thrombocytopenia in this infant was confirmed by PCR. This was necessary for counselling the family regarding future pregnancies. It was also relevant for the management of future pregnancies, with or without the need for intrauterine treatment or cesarean delivery. Maternal platelets can be collected in advance for later transfusion to the newborn if NAIT is anticipated. Further genotyping becomes essential in those cases where PIFT or other serological tests are negative.

In our patient's family, the father was heterozygous for HPA-2b, whereas the mother was homozygous for HPA-2a allele. This means that there is a 50% chance of having HPA-2b-positive babies in future pregnancies. This may explain the previous three unaffected children, who were probably homozygous for HPA-2a. It is also remotely possible that the two abortions might well have been due to NAIT, as a result of the heterozygous state of the fetus. However, further pregnancies would be carefully monitored for possible recurrence of the same problem.

Among the different platelet alloantigens recently

classified by Von dem Borne,⁸ the most frequently implicated in Caucasians are HPA-1A (PI^{A1}), accounting for 78%, and HPA-5b (Br), accounting for 19% of cases of NAIT. Thus, HPA-2 (Ko), HPA-3 (Bak) and HPA-4 (Pen) account for only the remaining 3%.⁹ The contribution made by various HPA to NAIT appears to be racially determined; the Japanese, for example, have a higher incidence of anti-HPA-4-related NAIT.⁷ Anti-HPA-1 is the most commonly reported cause of NAIT. HPA-2 (Ko) system is a rare cause of NAIT. Since the discovery of this system in 1960, only a few cases have been reported. One rare case of prolonged neonatal alloimmune megakaryocytopenia was attributed to anti-HPA-2b. Molecular studies have shown that the two allelic forms of HPA-2 (i.e., 2a and 2b) are polymorphic at a single amino acid at position 145 from the N-terminal elastase-sensitive fragment of glycoprotein 1b chain.¹⁰

To our knowledge, this is the first reported case of NAIT due to anti-HPA-2b in Saudi Arabia. The HPA system responsible for the majority of NAIT in the Kingdom is not yet known. In our recent study on healthy Saudis, HPA distribution revealed a pattern peculiar to this population that was different from Western and Oriental populations.¹¹ The distribution of the different HPA alleles in Saudis was as follows: HPA-1a 80%, HPA-1b 20%, HPA-2a 80.5%, HPA-2b 19.5%, HPA-3a 88%, HPA-3b 12%, HPA-4a 97%, HPA-4b 3%, HPA-5a 84.5%, and HPA-5b 15.5%.

The estimated risk (ER) of NAIT in pregnancies can be predicted from the probability that an antigen-negative mother becomes pregnant with an antigen-positive baby. ER for NAIT = $aa \times (ab + bb) + bb \times (ab + aa)$, where a and b are codominant alleles of each HPA type. The ER for NAIT associated with anti-HPA antibodies is: HPA-1a=0%, HPA-1b=24%, HPA-2a=3.84%, HPA-2b=4.55%, HPA-3a=4.72%, HPA-3b=15.39%, HPA-4a=1.96%, HPA-4b=3.84%, HPA-5a=2.97% and HPA-5b=20.96%. It must be stated that not all pregnancies at risk progress to clinical disease. This may be partly due to variable immunogenicity of different HPA. It may also be related to the immune response in the mother (i.e., high or low responder). Some authors have described the influence of HLA-DR phenotype in the mother on her immune response to foreign HPA. Thus mothers with the HLA haplotypes B8, DR3, DRw52 are reportedly more likely to develop anti-HPA antibodies when stimulated.¹²

These estimated risk figures overestimate the actual

risk. NAIT is seen much less clinically than is suggested by these figures. This data, however, may be useful to predict the relative contribution of different HPA antigens and antibodies. As can be predicted from Table 2, approximately half of the NAIT in Saudis may be related to anti-HPA-1b and HPA-5b antibodies. This is more or less similar to Western experience. Here again anti-HPA-2b is predicted to play a less significant role (around 5%). Thus, our patient may represent one of those rare cases. The actual contribution of HPA system to NAIT in Saudis requires a larger study.

PCR-SSP is a useful method for the diagnosis of this rare condition, and we hope this method, as well as genetic counselling, will be introduced in other laboratories in the Kingdom.

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