Unexpected Alloimmunization in a Young Sickle Cell Disease Patient: The Role of Duffy Antigen in Transfusion Management and Malaria Resistance

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Abstract

A nine-month-old Sudanese male with Sickle Cell Disease (SCD) developed alloimmunization after a single blood transfusion, an unusual event given his limited transfusion history. The patient also exhibited minimal symptoms during two malaria infections, suggesting a possible Duffy-negative phenotype, which has implications for both malaria resistance and transfusion compatibility. This case emphasizes the complexities of alloimmunization management in young patients with SCD and highlights the need for tailored transfusion strategies that consider genetic factors to improve patient outcomes and reduce the reliance on steroids and IVIG.

Keywords: Sickle Cell Disease, Alloimmunization, Duffy Antigen, Malaria, Plasmodium Vivax, Hemolytic Transfusion Reaction.

Introduction

Sickle Cell Disease (SCD) is one of the most common genetic disorder that affects millions of people worldwide. A common complication of SCD is alloimmunization, a phenomenon where the immune system produces antibodies in response to foreign antigens introduced through blood transfusions. While alloimmunization is not uncommon in SCD patients, affecting 5-75%,[1] its occurrence in young patients with minimal transfusion history is highly atypical and warrants further investigation.

This case report presents an intriguing instance of a young infant with SCD who developed alloimmunization after receiving only one unit of compatible blood. The rarity of this occurrence raises several questions: What could be the underlying reasons for the development of alloimmunization in this patient at such a young age and with a minimal history of transfusion? How can we prevent this from recurring in future transfusions, given the patient's SCD condition and the potential need for transfusions throughout his life?

Moreover, the patient's survival through two episodes of malaria infection with minimal symptoms adds another layer of complexity to the case. This observation led us to consider the role of the Duffy antigen, a protein found on the surface of Red Blood Cells (RBCs), with implications in both malaria infections and blood transfusions.

In the following discussion, we delve into these questions, exploring the genetic aspects, the role of the Duffy antigen, and the implications of the patient's heritage. We aim to shed light on this complex clinical scenario and propose strategies for managing similar cases in the future.

Case Report

A 9-month-old Sudanese male infant, known case of SCD, presented to the emergency department (ER) with non-bloody non-bilious vomiting. The mother was concerned because he was pale and noted reduced activity.

The patient was born at term, via urgent cesarean section due to potential placental insufficiency. His birth weight was >2 kg, and he had no antenatal or natal complications and no NICU admission. His parents are non-consanguineous, and he has two healthy siblings. Both the mother and her baby have O positive blood group. Extended phenotyping of the patient showed Rhesus Dec and Kell phenotype was k+.

He was diagnosed with SCD in our hospital at the age of 7 months. Initially he presented with acute splenic sequestration crisis (ASSC) with Hb 1.9 gm/dl. At that time, he received packed red blood cells (PRBCs) transfusion in two aliquots of 10 ml/kg from a single donor. He improved and was discharged on penicillin prophylaxis and folic acid.

Past medical history was positive for malaria twice: at the age of 2 months in his home country, where he did not require hospitalization, received short course of antimalarial drugs and the documentation for that incident is insufficient. Again, at the age of 7 months, Plasmodium Vivax (P. vivax) was detected by malaria parasite rapid test Immunochromatographic Diagnostic Test (ICT) which was positive, while blood smear was negative. Apart from fever, he was otherwise well, and he was treated with chloroquine.

On arrival at the ER, he was lethargic, dehydrated, extremely pale with scleral icterus, tachypneic and tachycardic with HR of 140/min. He looked in overt respiratory distress but maintaining O2 saturation at room air. Abdominal examination revealed splenomegaly of 10.5 cm and the liver was palpable 4 cm below the costal margin. Neither organ was tender to touch. There were no signs of focal neurological deficits.

laboratory investigations revealed severe anemia (Hb 3.1 gm/dL), reticulocytosis (36%) and thrombocytopenia, (platelet 80 $x10^3$/uL). Additional laboratory tests indicated increased bilirubin level (61.80 umol/L; normal: 3-17),

borderline AST (58 U/L, normal: 15-57), while other laboratory tests were within normal limits. A follow up of CBC parameters during the patient's hospital stay is presented in **Table 1**.

Table 1. CBCs that were done throughout admission and after following up in the OPD.

Entity	Normal Ranges	Day 0 (Day of admission)	Day 1 *		Day 2	Day 3	1st Follow UP **	
			morning	evening			Before transfusion	After transfusion
WBC	16.00-6.00 x10(3)/mcL	14.31	8.45	7.40	7.77	11.72	18.08 H	18.24 H
RBC	5.10-3.90 x10(6)/mcL	1.13 L	2.19 L	2.46 L	2.80 L	3.13 L	1.64 L	3.49 L
Hgb	13.10-11.10 gm/dL	3.10 L	6.10 L	6.50 L	7.40 L	8.90 L	4.60 L	8.20 L
Hematocrit	38.00-30.00 %	%10.50 L	% 18.70 L	% 21.10 L	% 24.80 L	% 27.50 L	%14.50 L	% 26.20 L
MCV	84.00-72.00 fL	92.70 H	85.40 H	85.70 H	88.50 H	87.80 H	88.00 H	75.10
MCH	29.00-25.00 pg	27.80	27.70	26.60	26.40	28.40	28.00	23.40 L
MCHC	36.00-32.00 gm/dL	29.90 L	32.40	31.00 L	29.90 L	32.40	31.80 L	31.20 L
RDW	14.50-11.50	% 31.60 H	% 27.30 H	% 28.60 H	% 27.60 H	% 27.40 H	%28.80 H	% 30.90 H
Platelet	550.00-200.00 x10(3)/mcL	80.00 L	87.00 L	54.00 L	84.00 L	114.00 L	121.00 L	96.00x10 L
MPV	11.00-8.00 fL	10.80	11.50 H	7.80 L	13.20 H	11.90 H	7.80 L	8.20
Retic Count%	% 2.50-0.50	35.53 % H	-	-	-	26.41% H	31.18 % H	-
Retic Absolute	420.00-220.00 x10(3)/mcL	402.90	-	-	-	825.90 H	512.50 H	-

*A 10ml/kg blood was transfused to the patient before conducting the morning CBC.
** Second admission 3 weeks of the discharge, He received 15mg/dl PRBC.

The patient's clinical presentation and laboratory findings were consistent with ASSC. He was admitted to PICU for close monitoring. His blood type was O positive, and direct antiglobulin test (DAT) was negative, however, indirect antiglobulin test was positive and atypical antibodies were detected upon screening. Despite testing approximately 20 bags of donor blood, no compatible match was found, suggesting the possibility of alloimmunization. Given the critically low Hb level that dropped further to 2.1g/dl, and the potential for progression to anemic heart failure with generalized tissue hypoxia, we opted to transfuse him using the least incompatible available blood. In conjunction, iv methylprednisolone (5mg/kg) and intravenous immunoglobulin (IVIG at 1g/kg) were infused. A dose of 5 mg/kg rather than the classic higher pulse steroid doses was chosen considering that steroids might exacerbate vaso-occlusion in patients with SCD.[2] PRBCs transfusion at 10 ml/kg (least incompatible +1), was completed over 4 hours with no reactions.

Post transfusion Hb increased to 6.1 gm/dL, and treatment continued with IV then oral methylprednisolone at 2 mg/kg/12 hours, then tapered and discontinued over 1 week. Upon discharge, his spleen and liver regressed in size to 7.5 and 2cm below the costal margin respectively. His Hb levels further improved to 7.4 then 8.9 gm/dL eliminating the need for further transfusion, and he was discharged on folic acid and penicillin prophylaxis. The family was counseled that live attenuated vaccines should be postponed for 9 months from the date of the IVIG administration.

Three weeks later, he presented to the emergency again with another attack of ASSC, with splenomegaly 9.5 cm below the costal margin, and Hb level dropped to 4.6 gm/dL. Blood typing and antibody screening were conducted. Cross-matching was specifically done with a Duffy-negative donor, resulting in the identification of

compatible blood from the first bag tested. The patient received a transfusion of 15 ml/kg from a Duffy-negative bag. Post-transfusion, he was well with no distress. A repeated CBC after transfusion showed a hemoglobin level of 8.20 gm/dL. Other lab tests are shown in **Table 1**. Hydroxyurea at 15 mg/kg daily started to help in prevention of further vaso-occlusive crisis.

Discussion

Alloimmunization in patients with SCD is not an uncommon phenomenon.[1] In a trial to minimize this risk the ASH 2020 guidelines,[3] the British Society for Haematology guidelines[4] and the National Institutes of Health Expert Panel[5] recommend prophylactic matching for Rh (C, E or C/c, E/e), and K in addition to ABO and D in patients with SCD. However, alloimmunization in the younger age group, especially in the absence of multiple transfusions history, is highly atypical. Interestingly, our patient was 9-months- old and had received only one compatible blood unit in his lifetime for an ASSC before developing alloimmunization. Given the current scenario where there is no compatible blood, we used IV methylprednisolone plus IVIG to minimize the risk for acute hemolytic transfusion reaction.[6] This approach poses significant challenges. Not only does it entail high costs, but it might also increase the patient's risk of vaso-occlusive crises because of the effects of steroids,[2] and the need to postpone live attenuated vaccines for a significant period post IVIg infusion.[7] Therefore, it is imperative to explore alternative approaches to manage this complex clinical scenario.

An intriguing observation in our patient was the survival through two episodes of malaria infection with minimal symptoms. While the heterozygous state of the sickle cell gene is known to provide a degree of protection against malaria, the homozygous state is associated with increased morbidity and mortality.[8] Remarkably, our patient, who is homozygous for the sickle cell gene, survived a malaria infection with minimal symptoms.

In view of this observation, the possibility that our patient might lack the Duffy antigen was considered. P. vivax requires the Duffy antigen to invade RBCs. Vivax binds to the N-terminal extracellular domain of the Duffy glycoprotein via one of its own proteins, the Duffy binding protein.[9] The absence of the Duffy antigen prevents the parasite from entering the RBC, resulting in a negative smear. However, the parasite can still be present in the blood and detected by a Malaria Parasite Rapid Test (ICT).[10] This explains the results of malaria testing in our patient.

The FY gene, which plays a crucial role in the production of Duffy antigens, exists in two major alleles, namely Fya and Fyb. These alleles are codominant, implying that both can be expressed simultaneously. In the absence of both alleles, an individual exhibits the Duffy null phenotype. If only one of the antigens is absent the patient will be labeled as heterozygous Duffy antigen[11] Exposure to that specific antigen through transfusion leads to sensitization and formation of antibodies that can be either anti-Fya and/or anti-Fyb antibodies. Subsequent exposure to the Duffy antigen might trigger a hemolytic reaction.

Considering the patient's Sudanese heritage, it further suggests the absence of the Duffy antigen. This is supported by epidemiological data indicating that the Duffy null phenotype, Fy(a-b-), is extremely rare in Caucasians but is found in approximately 68% of Blacks[11.]

In the Papua New Guinea population, carriers of the Fy(a-b+) or Fy(a+b-) express half the level of Duffy antigens on red blood cells compared to wild type homozygotes and exhibit reduced susceptibility to blood infection by P. vivax.[11] These observations suggest that total or partial restriction to access Duffy antigen reduces the ability of the parasite to invade new red blood cells and thus this might inhibit parasitemia by P. vivax[12,13]

Based on that, it was strongly suggested that our patient is either Duffy negative or heterozygous for the Duffy antigen. In addition, during the follow-up, upon crossmatching his samples with Duffy negative blood, the compatibility observed with the first tested bag lends credence to our hypothesis.

Unfortunately, due to resource constraints, we were unable to conduct a genetic test to determine the blood type. A phenotypic analysis was performed, focusing on the available testing of Fya and Fyb antigens, and revealed a Fy(a+b-) phenotype. To confirm this phenotype and assess for other clinically significant Duffy antigens, such as Fy3, which has been associated with alloimmunization among sickle SCD patients,[11] further genetic testing was indicated.

Conclusion

This case raises awareness regarding the role of the Duffy antigen in both malaria infections and blood transfusions, particularly in populations where the Duffy null phenotype is prevalent. It highlights the importance of considering genetic factors when managing patients with SCD and devising strategies for future transfusions to avoid alloimmunization and hemolytic transfusion reactions caused by anti -Duffy antibodies.

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