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Case report

Revealing Weak D: A Unique Exploration at a Hospital in Bangladesh

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Abstract

The weak D phenotype is a variant of the Rh blood group system, specifically related to the D antigen. Individuals with the weak D phenotype exhibit reduced expression of the D antigen on their red blood cells, making it challenging to detect using standard blood typing methods. This case report brings attention to an extraordinary instance involving a 45-year-old male who was serendipitously identified as possessing the weak D phenotype, a condition not only uncommon in Bangladesh but also exceptionally rare on a global scale.

Key word: Weak D, partial D, Rh type

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Introduction

The Rh blood group system is a critical component in transfusion medicine and clinical assessment that has gained increased attention due to its intricacies, especially the phenomenon of weak D variants. In 1946, the weakly reacting D antigen (DU antigen) was delineated by Stratton.[1] These cells possess the D antigen but have fewer D antigen sites per red cell than normal Rh-positive cells.[2] This distinct feature makes the weak D antigen less immunogenic, presenting difficulties in its identification.[3] Groundbreaking work in 1948 by Race et al. and in 1950 by Renton and Stratton discovered that DU red cells did not agglutinate directly with anti-Rh (D) serum. Instead, the presence of this antigen was revealed only after the subsequent addition of anti-globulin.[4] The prevalence of this weak D antigen varies between 0.2% and 1% among Caucasians,[5] while in India, the estimated occurrence of weak D ranges from 0.0075% to 0.2% within diverse geographic donor populations.6 A multicenter study reported that the prevalence of Weak D among the Bangladeshi population is 0.19%.[7] Despite its rarity, the significance of Weak D lies in its potential ramifications for blood transfusions, urging careful consideration in clinical practices.

Case Summary

Admitted to our hospital, Mr. Ahsan (pseudonym), a 45-year-old gentleman, expressed concerns about fever and generalized body aches persisting over a 4-5 day period. The fever manifested suddenly, characterized by a continuous pattern. His medical history notably revealed him as a documented case of type 2 diabetes mellitus being managed with medication (Tab. Metformin 500 mg once daily). He was free from symptoms like diarrhea, vomiting, rash, or any other complications. Moreover, he didn't have hypertension or bronchial asthma. During the general examination, the patient exhibited a heightened body temperature of 102°F. Furthermore, moderate anemia, mild

dehydration, joint tenderness, and subtle flushing of the face were noted. Systemic examination revealed no evident abnormalities.

His Dengue NS1 antigen tested positive. His platelet count registered at 202,000/uL, only to precipitously drop to a mere 23,000/uL after two days. Recognizing the urgency, we recommended a comprehensive blood grouping and Rh typing test.

In managing Mr. Ahsan, a patient of admirable cooperation, a discrepancy emerged in his blood grouping and Rh typing report, initially identified as B-negative, conflicting with his documented B-positive status on his Saudi Arabian driving license. Upon retesting and further consultation with the Department of Transfusion Medicine, an unexpected and rare weak D phenotype was unveiled.

ABO grouping of the patient's sample (slide method):

Cell grouping		Serum Grouping		Interpretation
Anti A	Anti B	A cell	B cell	
-	4+	3+	-	B

RhD grouping of the patient's sample (slide method):

Anti D (Tulip)	Anti D (Biorex)	Interpretation
-	-	RhD Negative

So initially, the patient's blood group was reported as B negative.

Due to the patient's historical RhD positivity, we conducted further investigations. Immediate centrifugation with one drop of anti-D (Tulip) mixed with a 5% suspension of the patient's red cells in a test tube resulted in a 'w+' reaction (barely visible agglutination, turbid background). Subsequently, the same test tube was incubated at 37°C for 15 minutes. After incubation, the red cells were washed three times with normal saline. Following the last wash, 2 drops of Anti-Human Globulin (Atlas) were added, mixed gently, and centrifuged at 1000 rpm for 30 seconds. Gentle resuspension of the cell button revealed 2+ agglutination. The ultimate interpretation regarding RhD status was B Positive (Weak D).

Discussion

The Rhesus blood group system encompasses more than 50 antigens.[8] 'D' stands out as the most immunogenic antigen among these.[9] The presence of this antigen on red blood cells is denoted as 'Rh(D) positive' or simply 'Positive'. This antigen is mainly a transmembrane protein, containing 417 amino acids.[10] The entire RhD protein must be present for the D antigen to be expressed serologically, otherwise it is termed weak D.[11] This phenotype can manifest through distinct mechanisms. Through genetic inheritance, an individual acquires the RHD gene that codes for the weak expression, resulting in a mutation. This mutation impacts the amino acids within the transmembrane or intracellular regions of the RhD protein. So these changes in the protein occur only 'inside' the cell membrane rather than externally. Consequently, the number of expressed D antigens on the red blood cell surface is diminished, although the D antigen is usually complete. This genetic alteration gives rise to the Weak D phenotype.There is anothermechanism called "position effect" or "gene interaction effect"where the allele carrying RhD is in the 'trans' (opposite haplotype) position to the allele carrying C. (Such as in Dce/dCe haplotype). Despite the Rh antigen appearing normal, this arrangement impedes the proper expression of the D antigen on the cell membrane.[12,13] Partial D is another aspect. Typically, RhD encompasses more than 30 epitopes, and for an individual to be RhD-positive, all these epitopes must be expressed. The alteration or absence of some epitopes on the D antigen gives rise to the partial D phenotype.[14] Unlike weak D, these changes occur in extracellular regions.[15]

Observational studies from central Europe reported that individuals with weak D types 1, 2, 3, and 4.1 are not prone to developing anti-D and can be safely regarded as RhD-positive, making them suitable for transfusion with RhD-positive blood.[16,17] However, alloimmunization has been noted when RhD-positive units are transfused in specific weak D types, such as weak D types 4.2, 11, and 15 but it is rare.[17,18] Additionally, there have been several reports of RhD-positive individuals presumed to have a partial D phenotype forming anti-D antibodies.[19,20]

The preceding discussion highlights that when weak D results from a genetic mechanism or position effect, the alterations are confined to the interior of the red blood cell (RBC). This primarily impacts the quantity, not the quality, of RhD. Consequently, the likelihood of developing anti-D antibodies is low, allowing the individual to safely receive D-positive blood in most cases. Conversely, with partial D, the changes occur externally to the RBC, influencing the quality rather than the quantity.[21] This poses a risk of producing anti-D antibodies, potentially leading to complications such as Hemolytic Disease of the Fetus & and the Newborn or transfusion reactions.[12,13] Therefore, individuals with partial D shouldn't receive D-positive blood. Unfortunately, the challenge lies in the absence of a serological procedure to differentiate between weak D and partial D variants. This situation raises a critical question about the approach to blood transfusion for individuals with D variants, particularly when the distinction between weak D and partial D cannot be made. It is advisable to transfuse individuals with a D variant with RhD-negative blood for safety.[22] While these individuals have the 'D' antigen in their red blood cells, caution is recommended to prevent them from donating blood to RhD-negative individuals.[23] In the

laboratory, the 'D' antigen is identified using anti-D reagents. Typically, we expect a robust 3+ or 4+ reactions visible to the naked eye.[24] But in instances of weak D expression, the red blood cell agglutination is milder (≤2+) than anticipated for RhD typing or no reaction at all with potent anti-D reagents.[7] Approximate identification of the D antigen is achieved through moderate or strong agglutination observed in the indirect anti-globulin test (D^U test) after incubation with anti-D[25]. Despite the passage of numerous years since the discovery of the weak D antigen, its clinical significance, immunogenicity, and associated guidelines remain controversial.[26,27] RHD genotyping offers a more comprehensive characterization of these D variants.[28] As genotyping is unavailable in our center; we rely on the antihuman globulin test to label a sample as weak D. And to ensure patient safety, we still follow the policy of considering all weak D-positive donors as RhD-positive.

Conclusion

The discovery of a rare Weak D blood group emphasizes the imperative for accuracy in blood grouping tests, highlighting the risk of diagnostic oversights. Meticulous lab practices and rigorous protocols are essential for accurate clinical diagnoses. Specifically, taking extra caution in suspecting Weak D is crucial, as misdiagnosis is imminent without careful consideration.

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