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Quick Response Code:

Website: www.ajts.org

DOI: 10.4103/ajts.AJTS_76_16

Autoimmune hemolytic anemia caused by anti "e": A challenge: A case report with review of literature

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Abstract:

Autoimmune hemolytic anemia (AIHA) is featured by short red cell survival due to autoantibodies. AIHA caused by anti 'e' is a tough clinical situation as antigen 'e' is a highly prevalent antigen. The present case highlights the same and different issues related to it.

Keywords:

Anemia, anti-e, autoimmune, hemolytic

Introduction

Autoimmune hemolytic anemia (AIHA) is characterized by shortening of red cell survival due to autoantibodies. AIHAs are classified as warm, cold, mixed-type, and paroxysmal cold hemoglobinuria. Majority of warm-reactive autoantibodies are panagglutinin in nature with eluate showing reaction with all cells tested. Here, we report a case of AIHA with anti "e" specificity.

Case Report

A 2½-year-old boy presented to pediatric emergency with the complaints of intermittent fever with vomiting and yellowness of eyes for 7 days. The patient was lethargic and had a history of the altered sleep cycle and bilateral swelling of the lower limbs and abdomen for 4 days. There was no history of any previous blood transfusion or drug intake. On examination, he was irritable, icteric, and severely pale. The heart rate and blood pressure were 128/min and 104/50 mmHg, respectively. The peripheral pulses were hyperdynamic. Abdominal examination showed a hepatomegaly of 4 cm below right costal margin and splenomegaly of 1 cm below left costal margin. Rest of the systemic examination was within normal limits.

The investigations showed severe anemia with Hb being 3.6 g/dl. The corrected total leukocyte count and platelet count were 42,666/mm^3 and 5.1 lac/mm^3, respectively. The peripheral blood smear showed red cells demonstrating autoagglutination and presence of normocytic normochromic red cells, few macrocytes, and polychromatophils. The differential leukocyte count was N_{62} L_{34} E_{00} M_{04}. The corrected reticulocyte count was 4.5%. The serum bilirubin was elevated, being 21.3 mg/dl (direct: 19 mg/dl and indirect: 2.3 mg/dl). The liver enzymes were elevated (serum glutamic oxaloacetic transaminase: 652 IU/L, serum glutamic pyruvic transaminase: 1020 IU/L). The renal function tests were normal. The serology for antinuclear antibody, HIV, hepatitis B and C were negative. However, the serological tests for hepatitis E and A were not done. The rapid malaria antigen test was negative. The coagulation profile revealed markedly deranged partial thromboplastin time with

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Submission: 11-06-2016
Accepted: 20-03-2017

How to cite this article: Pahuja S, Verma D. Autoimmune hemolytic anemia caused by anti "e": A challenge: A case report with review of literature. Asian J Transfus Sci 2017;11:195-8.

kaolin (>180 s) and a normal prothrombin time. Based on the above findings, a provisional clinical diagnosis of acute hepatitis probably viral in etiology with hepatic encephalopathy Grade 1 with impending congestive heart failure and severe anemia was kept.

A requisition for packed red cells was received from pediatric emergency in the blood bank at night. The sample sent showed autoagglutination. The red cells were washed 10 times with warm saline to disperse autoagglutinates before any further work up. A discrepancy was noted between forward and reverse grouping at incubation at room temperature. Forward group was AB positive, whereas reverse group showed agglutination in A cells, B cells, and O cells. The details at various temperatures are shown in Table 1. The direct antiglobulin test (DAT) with polyspecific anti-human globulin was 4+ positive. On further profiling of DCT with monospecific antisera on gel card technique, positivity was noticed for both IgG and C3d. The 3 cell panel (ID-DiaCell I-II-III Asia, Bio-Rad) showed reactivity with I and III cell but was negative with II cell (R2R2) as shown in Figure 1. The antibody identification was done using 11 cell panel (ID-DiaPanel, Bio-Rad). The extended forward and reverse blood grouping was performed on washed cells at 4°C, 22°C and 37°C, which helped in resolving the discrepancy [Table 1].

The antibody screening with 3-cell panel was positive as shown in Figure 1. The antibody identification with 11-cell identification panel showed a gradation of positive reactions between 1+ and 3+ grades and a negative reaction with 3rd phenotype (R_2R_2) as shown in Figure 2. Thereby presence of anti "e" antibody was established on antigrams. The elution was done using commercially available "Diacidal elution kit (Biorad)." It also confirmed anti-e nature of antibody. The Rh profiling of the patient's red cells revealed the presence of D, C, c, and e antigens [Table 2]. Hence, the patient was homozygous for antigen "e." Thus, autoimmune hemolysis by autoantibody "e" was established. A cold antibody was found at 4°C which was reacting with nonspecific A, B, and O cells. The titers of cold antibody were done at 4°C in saline phase and were 1:16. Hence, diagnosis of warm AIHA caused by anti "e" along with nonpathogenic cold antibody was given.

The cross matches performed with all the blood bags were found to be showing 3+ incompatibility. Pending complete immune-hematological investigations, on the clinician's demand, single unit of least incompatible AB positive unit was released to the patient at night in emergency.

The patient was started on parenteral steroids and antibiotics and intravenous fluids. Repeat IAT was performed after 2 days which showed a reduction in strength of reaction (between 1+ and 2+). The patient on further follow-up showed a good improvement with the restoration of hemoglobin to 11.8 g/dl and negativity of IAT with 3-cell panel.

Discussion

AIHA is an uncommon condition with overall reported incidence being 1 in 800,001[1] to 1 in 100,000 of a given population/year in the Caucasians.[2] It is characterized by shortened red cell survival because of the presence of autoimmune antibodies. The diagnosis of immune hemolytic anemia is a corroboration of both the clinical findings as well as laboratory investigations. Merely, the presence of DAT positivity does not label the diagnosis of immune hemolytic anemia.

An autoantibody is one that reacts with self-antigen on the red cells. Autoantibodies reacting optimally at 37°C are present in the serum of about 80% of patients with warm

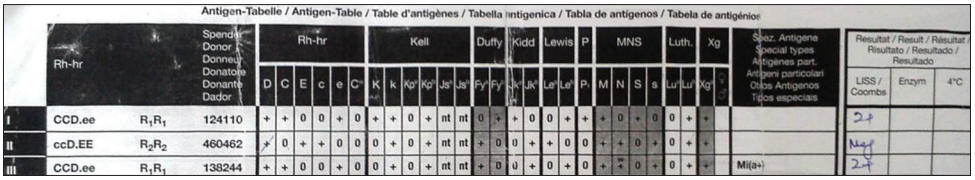

Figure 1: The antibody screening with 3-cell panel (ID-DiaCell I-II-III Asia, Bio-Rad)

Table 1: Results of forward and reverse grouping at 4°C, 22°C and 37°C

	Forward grouping				Reverse grouping			
	Anti A	Anti B	Anti D	Normal saline	A-cells	B-cells	O-cells	Auto control
4°C	4+	4+	3+	Negative	1+	1+	2+	2+positive
22°C	4+	4+	3+	Negative	Weak reaction microscopically#	Weak reaction microscopically#	Weak reaction microscopically#	Weak reaction microscopically#
37°C	4+	4+	2+	Negative	Negative	Negative/weak reaction microscopically#	Negative/weak reaction microscopically#	Negative

#Weak reaction microscopically: Presence of unevenly distributed 2-3 cells sticking together per low power field

Figure 2: The antibody identification with 11-cell identification panel (ID-DiaPanel, Bio-Rad)

Table 2: Results of Rh profiling

	D	C	c	E	E	K	Control
Reaction	4+	4+	4+	Negative	4+	Negative	Negative

AIHA.[3] In many cases of warm AIHA, no autoantibody specificity is apparent. The patient's serum reacts with all red cell samples tested (i.e. panagglutinin in nature). Apparent specificity for simple Rh antigens (D, C, E, c, e) is occasionally seen, especially in saline or low ionic strength saline in DAT.[4] However, specificity is rarely clear cut.

A group of patients with warm AIHA may also possess cold autoantibodies. These qualify the definition of mixed or combined warm and cold AIHA if these cold autoantibodies have a high thermal amplitude, that is, reacting at or above 30°C or having high titers. In the present case, cold autoantibodies detected were found to be interfering with blood grouping in saline phase at both 4°C and 22°C but showed only weak granularity with 2–3 cells agglutinating microscopically at 37°C. The titers at 4°C were 16. Furthermore on DAT, a 4+ reaction was found with polyspecific anti-human globulin reagent. Further reaction of warm saline washed red cells was seen with monospecific anti-IgG and anti C3d, thereby, establishing the existence of a warm antibody as well. The antibody identification performed by 11-cell panel showed an anti "e" specificity of the autoantibody. The Rh profiling of the patient's red cells revealed the presence of "e" antigen over red cells, thus, establishing the autoimmune character of the antibody. Moreover, there was the absence of any history of prior blood transfusion or drug intake in the present case. The on-going hemolysis in our case thus proved to be due to autoimmune antibody "e." Hence, the diagnosis of warm AIHA with nonpathogenic cold agglutinin was given.

Most of the warm antibodies in AIHAs are panagglutinins in nature with no apparent specificity.[4] Initially, it was thought that significant proportion of these antibodies were directed against antigens of Rh system (based on weak/negative reaction with the Rh null red cells); also it was observed that same red cell autoantibodies reacted preferentially with defined Rh antigen including e, E, or C.[5-8] This was proved wrong later as it was appreciated that absent or weak reactivity of autoantibodies with Rh null red cells does not indicate specificity to Rh complex as Rh null cells may be possessing other membrane abnormalities like absence of LW, Fy; marked decrease of U and to a lesser extent Ss and glycophorin B levels which are 30% of the normal.[9,10] Autoantibody having a clearly defined Rh specificity, for example, anti "e" was described by Race and Sanger in 1954.[11] Similar findings were also reported by several other authors.[12,13] However, after an extensive review of the literature, we did not come across many autoantibodies with specific anti "e" reactivity in India. Issitt *et al*.[14] reported on the specificity of the autoantibodies formed by 87 patients with warm antibody AIHA and found only four had formed anti-Rh antibodies (anti "e" or "c").

If the autoantibody has an apparent and relative specificity for a single antigen, for example, anti "e" and there is an evidence of hemolysis then blood lacking that antigen may be selected for transfusion. It was not that easier to find compatible blood in cases like ours where the antigen against which the antibody was directed, that is, "e" antigen is a highly prevalent antigen. Prevalence of "e" antigen in Indian population is very high and is present in 98% of population.[15] Procuring "e" negative unit is an uphill task and we need to revert to donor registries for finding "e" negative units, which may not always be possible if the patient is brought in emergency with urgent need for transfusion as in the present case. This explains the great difficulty that was faced by us in finding the

compatible unit for the patient. Among several blood units which were tested for compatibility, none could be found compatible. On clinician's demand, the least incompatible blood unit was released for transfusion on emergency basis. Das and Chaudhary[16] have opined that decision to transfuse in AIHA should be based on the clinical condition of the patient and no critical patient should be denied blood due to serological incompatibility.

According to Chaudhary and Das,[17] red blood cell transfusion is not a contraindication in AIHA; however, its use should be limited to cases of life-threatening anemia or a high risk of cardiac or cerebrovascular ischemic events. When transfusion is needed, then the least incompatible unit should be issued and the infusion should be slow and carefully monitored.

Another significant difficulty in transfusing such patients is that if the antigen profile of the patient is E-e+, then transfusing them with antigen "e" negative blood implies exposure to antigen E and hence further increasing the risk of alloimmunization. Hence, it must be ensured that red cells selected for transfusion in such cases do not possess an antigen which the patient lacks as it will lead to the production of alloantibodies. Moreover, it has been documented in the literature that knowledge of specificity of patient's autoantibody is not of much value, as the antibody of broader specificity outside Rh system were also present as well.[18]

The therapy directed for such patients aims at the first treatment of underlying disease if present. The general measures for cardiovascular support are very important for patients who are severely anemic. Transfusion should be avoided if possible on account of high risk for hemolysis. However, it should not be avoided in life-threatening anemia. Transfusion in such cases should be at clinicians' discretion.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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