

First Case Report of the Association of HbE Trait and Cold Agglutinin Disease

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Citation

V Chandrashekar, M Soni. *First Case Report of the Association of HbE Trait and Cold Agglutinin Disease*. The Internet Journal of Hematology. 2010 Volume 7 Number 2.

Abstract

This is the first report of the association of cold agglutinin disease with HbE trait. The patient was 58 year old male from Assam, India. Thalassemia, sickle cell disease and sickle-HbC have been found to be associated with cold agglutinin disease. Cold agglutinin disease has also been detected in patients with polycythemia vera. Erythrocytes in these disorders are likely to be immature and express more big "I" and little "I" antigens, the target antigens for cold agglutinins. Increased expression of big "I" and little "I" has been demonstrated in sickle cells. Conceivably, the increased expression of "I" and "I" in sickle cell disorders, thalassemia and HbE and polycythemia vera might render the erythrocytes in these disorders more vulnerable to cold agglutinins and hemolysis.

INTRODUCTION

Cold agglutinin disease or syndrome is a relatively uncommon autoimmune hemolytic anemia presenting in the middle aged or elderly (1). Overt hemolysis is often evident, and patients can have hemoglobinuria and acrocyanosis. The physical findings in a typical patient are pallor and jaundice. A minority of patients have splenomegaly (2). Cold agglutinin disease may be idiopathic or may be transient due to infections such as *Mycoplasma pneumoniae*, infectious mononucleosis and HIV. Cold agglutinin disease can also be associated with lymphoproliferative disorders and multiple myeloma. We would like to report the first case of cold agglutinin disease associated with HbE trait and to consider possibly pathophysiological consequences of the association

CASE REPORT

A 58 year old male hailing from Assam (a Northeastern state in India), presented to our medical outpatient department with complaints of exertional breathlessness and fever for the past few days. On examination, he was noticeably pale and icteric but not cyanotic. His spleen was palpable just below the left costal margin. He was a known diabetic who has been treated with antihypoglycemic drugs, Glibenclamide and Metformin, for the past four years. There was no significant family history. There was no prior history of blood transfusion.

A complete blood count in unwarmed blood revealed a

hemoglobin of 11 g/dl, a hematocrit of 29% and a RBC count was 3.1 million per cubic mm. The MCV was 93.5 fl, MCH was 35.4 pg and MCHC was 37.9g/dL. The leucocyte count was normal with a differential of 65% neutrophils, 30% lymphocytes and 5% monocytes. His platelet count was slightly reduced. A peripheral smear showed marked RBC agglutination (Fig.1) with polychromasia. The reticulocyte count was 12%.

After incubation at 37°C, the hematocrit was 34% the RBC count was 5 million per cubic mm. RBC agglutination was not seen (Fig.2). After incubation and the smear revealed a microcytic hypochromic picture with polychromasia and target cells (Fig.3). The post incubation RBC indices were consistent with microcytosis and hypochromia: MCV-68 fl, MCH-23 pg and MCHC-33.8 g/dL.

A hemoglobinopathy work up was done due to the microcytic hypochromic indices. On High performance liquid chromatography, HbA was 65%, HbF was 1.5%, and HbA₂ + HbE was 30%. The retention time for HbA₂ + HbE was 2.7 minutes. Biochemical investigations revealed hyperbilirubinemia with a total bilirubin level of 2mg/dL., and the serum lactate dehydrogenase levels were 545 IU/L. The direct Coombs test was positive for c3d but negative for IgG. His cold agglutinin titer were 1: 512. A bone marrow examination was not done since the patient refused the procedure. Serological tests for *Mycoplasma*, infectious mononucleosis and other infectious agents were not

available.

Figure 1

Figure 1: Agglutination of RBC at room temperature (40×).

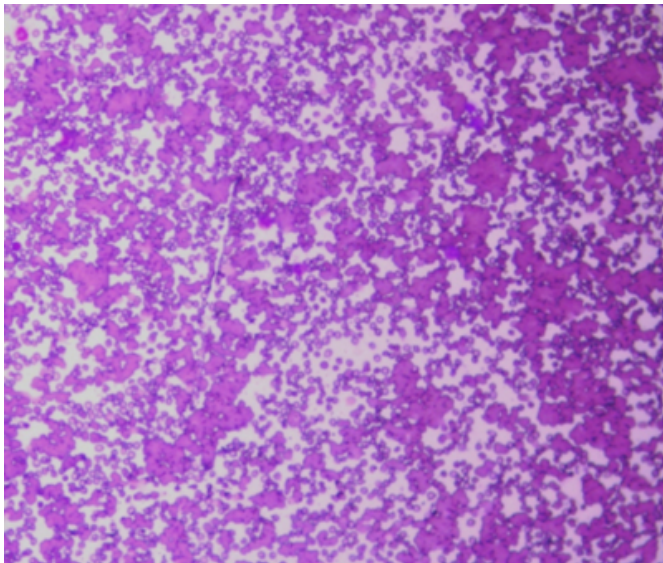


Figure 2

Figure 2: Absence of agglutination at 37°C (40×).

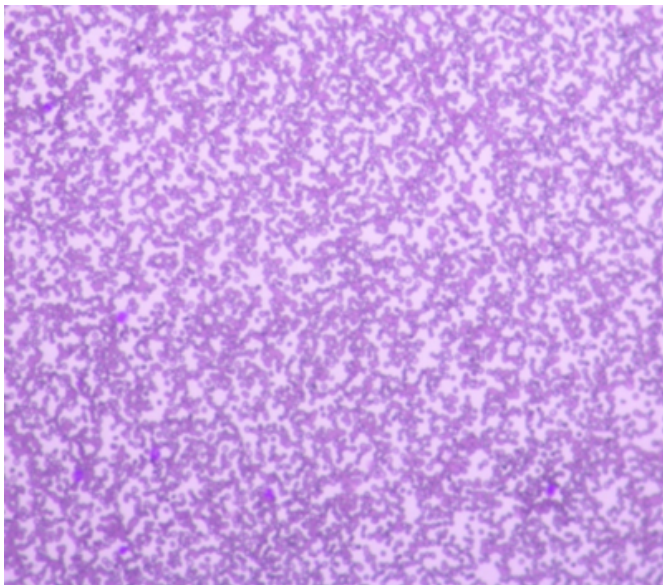
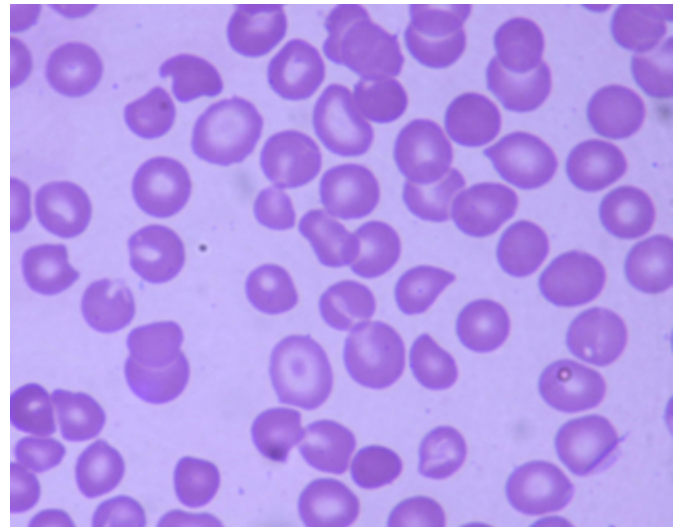


Figure 3

Figure 3: Microcytic hypochromic RBC with target cells at 37°C (400×).



DISCUSSION

The patient presented with evidence of hemolysis. Cold agglutinin disease was suggested by the agglutination of RBC in cold and the absence of clumping when blood was incubated at 37°. Rouleaux formation was unlikely since clumping did not occur when blood was warmed. Cold agglutinin disease was diagnosed by the moderate but significant increase in the cold agglutinin titer and a direct Coomb's test that was positive for c3d but negative for IgG.

The basis for cold agglutinin disease in the patient was not established. He had a fever and splenomegaly, suggesting an underlying infection or lymphoma. Both of these conditions are associated with cold agglutinin disease. However, we could not complete the work up since the patient refused a bone marrow and requested to be transferred to another medical institution.

In most cases of cold agglutinin disease, the MCV and MCH are falsely elevated when unwarmed blood is passed through the cell counter due to low level agglutination and platelet clumping. However, these RBC Indices will revert to normal if the blood has been prewarmed since clumping does not occur at 37°C. In our patient, the microcytic hypochromic RBC indices were masked and appeared to be normal when unwarmed blood was tested due to low level agglutination. When the patient's blood had been warmed, his true RBC indices became apparent and reflected a microcytic and hypochromic anemia. Therefore, a hematological workup was initiated.

The results of an evaluation revealed the presence of HbE trait based on the decreased HbA level of 65% with an increase in HbE. Even though HbE and HbA₂ comigrated on electrophoresis, the possibility of an increase in HbA₂ and beta thalassemia HbE disease was unlikely since the HbA level of 65%. Therefore, the patients appeared to have cold agglutinin disease associated with a HbE trait.

The prevalence of HbE carriers in India is 0-3.5% of which 50-80% are in Assam (3). Our case is the first to report the association of cold agglutinin and HbE trait. The question is whether there are pathophysiological consequences of this association.

In cold agglutinin disease, the targets of the agglutinin are big "I" antigen on the surface of adult erythrocytes and little "i" antigen on the surface of fetal erythrocytes. These interactions occur at sites in which the body temperature is less than 37°, the nose or extremities. The agglutinin-antigen interaction activates the complement pathway on erythrocyte membranes that is the basis for the hemolysis.

Low titers of cold agglutinin are present in normal people and do not cause hemolysis. Titers of 1/64 or greater at 4° are abnormal and can cause hemolysis. Hemolysis associated with *Mycoplasma pneumonia* is due to cold agglutinin-big "I" interactions. Hemolysis associated with infectious mononucleosis are usually due to little "i" interactions. Cold agglutinin disease also is associated with lymphoma and multiple myeloma.

Relevant to our patient, is that cold agglutinin disease has

been found to be associated with beta-thalassemia (4), sickle cell disease (5), SC disease (6) and Polycythemia Vera (7). There is evidence that more "I" and "i" antigens are expressed in sickle cells due to their immaturity (8) that may render sickle cell cells may be more sensitive to cold agglutinins. Conceivably, thalassemic, sickle cells, HbE and erythrocytes in polycythemia vera may also express big (I) and little (i) antigens due to their immaturity.. This may render erythrocytes in these disorders more vulnerable to cold agglutinins and hemolysis.

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