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Case Report

Blood grouping in cold agglutinin disease: A preventable medico-legal predicament

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ABSTRACT

Cold agglutinin disease (CAD) is a rare autoimmune condition characterized by presence of cold-reacting antibodies. This can lead to hemagglutination and complement-mediated hemolysis. Sometimes, it can interfere with serological tests as well. We report a case of CAD where there was a discrepancy in blood grouping initially. The patient presented with complaints of shortness of breath (oxygen saturation of 84% on admission), abdominal swelling , along with evidence of chronic liver disease with cirrhosis.
His total and unconjugated bilirubin were raised. Direct antiglobin test was positive. The blood sample showed auto-agglutination at collection, which was grossly visible in the vial. The discrepancy in blood grouping and cross-matching were subsequently resolved. This case points towards the fact that respiratory illness and liver failure can have an underlying association with CAD. A timely diagnosis of CAD and resolving the discrepancies in immuno-hematological tests can avoid many unwanted results and complications, thereby preventing medico-legal issues.

1. Introduction

Cold agglutination disease (CAD) is an uncommon form of auto-immune hemolytic anemia (AIHA).[1–3] AIHAs are characterized by production of auto-antibodies directed against surface antigens on red blood cells (RBCs). AIHAs are classified as: warm, cold or mixed type, depending on the optimum temperature at which autoantibodies bind surface antigens.[2–4] Both warm and cold autoantibodies can be either idiopathic (primary) or secondary due to an underlying condition. Cold AIHAs can be further classified as primary chronic cold agglutinin disease (CAD), paroxysmal cold hemoglobinuria, or secondary cold AIHAs associated with an underlying condition such as infection or malignancy.[2–5]

Here in we present a case of an elderly male who had respiratory illness and features of liver failure along with dernaged bilirubin levels. On investigations, a discrepancy was noted in blood grouping & cross matching of the patient. Proper screening was done with detailed serological evaluation and the issue was resolved, giving correct interpretation of blood group. The current case highlights, how important is to diagnose this entity timely so that any medico-legal issue can be averted.

2. Case Presentation

A 70-year-old male patient presented to emergency department with chief complaints of shortness of breath, nervousness, pain and swelling abdomen with severe acidity

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along with swelling over left knee. The patient gave history of chronic smoking for last 30 years. There was no history of hypertension, diabetes, or chronic disease or any surgical intervention. On admission the oxygen saturation was 84% at room air. The patient's saturation was stabilized at 99% on oxygen for an hour, following which his saturation came to normal levels at room air.

There was a history of fall 15 days back following which patient had swelling in the left knee. On physical examination, the patient was conscious with stable vitals but restless due to pain abdomen and difficulty in breathing. Bilateral air entry was normal. Per abdomen examination showed tense ascites. 2-D echo-cardiography showed normal left-ventricular function with no significant valvular pathology. However, on electro-cardiogram [ECG], an unintentional ventricular conduction delay was noted with sinus bradycardia.

Ultrasound (whole abdomen) was done and significant findings were noted. Chronic liver parenchymal disease with dilated portal vein and mild splenomegaly was noted with gross ascites, suggestive of Cirrhosis. There was minimal right sided pleural effusion. Bilateral grade I medical renal disease was also found.

The patient's routine investigations were sent to pathology department. Biochemical tests showed increased fasting blood sugar levels and deranged liver function tests. Total bilirubin, direct bilirubin & indirect bilirubin levels were increased, with values being 4.74 mg/dl, [Normal range- 0.2-2 mg/dl], 3.51 mg/dl [Normal range- 0.1-0.4 mg/dl] & 1.23 mg/dl [Normal range- 0.1-0.8 mg/dl]. Liver enzymes were elevated with serum SGOT levels of 594.6 U/L [Normal range, 0-35 U/L], SGPT levels of 387.6 U/L [Normal range, 0-45 U/L], Serum Alkaline phosphatase levels of 215 IU/L [Normal range, 53-141 IU/L], Gamma glutamyl transferase levels of 115.5 U/L [Normal range, 0-55 U/L], along with hypoalbuminemia showing decreased albumin [2.11 gm/dl, normal range- 3.5-5.2 gm/dl] and increased globulin levels [4.64%, normal range- 2.3-3.6 gm/dl]. These all deranged parameters confirmed the chronic liver disease.

Further, the blood sample was received in EDTA-vial for complete blood count [CBC]. CBC showed leukocytosis, total leukocyte count being 15,000/cumm. Visible agglutination was noted in the vial with blood sample (Figure 1 A). All tests were performed after incubation. Marked increase in MCV, MCH & MCHC levels were noted. Peripheral smear was made and stained with Leishman stain. Smear showed mild increase in leukocyte count with excessive rouleaux formation & agglutination. The CBC reading was repeated in the 5-part as well as 3-part differential hematology analyzer. Still the RBC indices were markedly elevated. A repeat sample was asked for and re-run. Further the sample was incubated at 37*C for 30 min & 1 hour, subsequently. In all samples there was excessive rouleaux formation even after incubation.

A detailed immuno-hematology work-up was done. On naked eye examination, EDTA & plain vials showed visible agglutinates. Blood grouping by conventional slide method and tube method showed discrepancy (Figure 1C). Cell grouping revealed AB positive blood group, while serum showed O positive. Initially gel card showed pan-agglutination with auto-control also showing agglutination(Figure 1B).

Following this, cell grouping was done with red cells washed with prewarmed saline in pre-warmed tubes with Anti-A, Anti-B and Anti-AB. A 5% red cell suspension of A, B, O cells were made and kept at 37*C. The patient's serum was separated and kept at 37*C for 45 minutes. The auto-adsorbed serum was used for cross-matching. A pre-warmed pipette was used for cell & serum grouping and cross-matching. This discrepancy was resolved & blood was confirmed to be O positive after pre-warming the serum and re-confirming with gel card test (Figure 2).

Direct Coomb's test was positive on gel card. The blood group was interpreted as O positive. There was no history of blood transfusions in the patient, so previous exposure to any blood products and presence of red cell allo-antibody was ruled out. Rh typing of patient cell was done using the pre-warming technique.

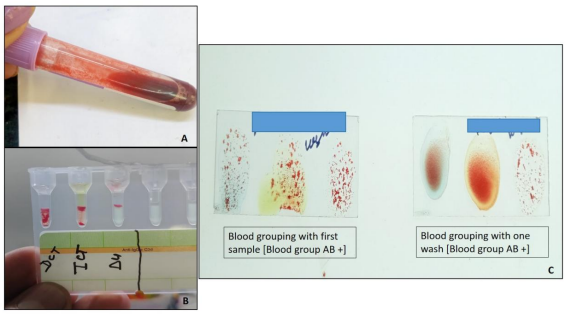

Figure 1: A) Showing visible agglutination in EDTA vial; **B)** Showing DCT, ICT and Du positivity; **C)** Showing blood group as AB positive which was initially done with pre-warm saline wash.

3. Discussion

Cold heam-agglutination disease (CHAD) is an auto-immune condition resulting from cold-reactive antibodies, leading to hemagglutination and complement-mediated hemolysis when body temperature drops.[1–3] Cold agglutinins or cold autoantibodies are found naturally in nearly all individuals.[2–4] These antibodies occur at low titres, less than 1:64 measured at 4*C and are not active at higher temperature.[1,2]

CHAD is rarely noted and benign cold agglutinin, which is a variant of CHAD is detectable on routine evaluation for some other ailments. The importance of cold agglutinins

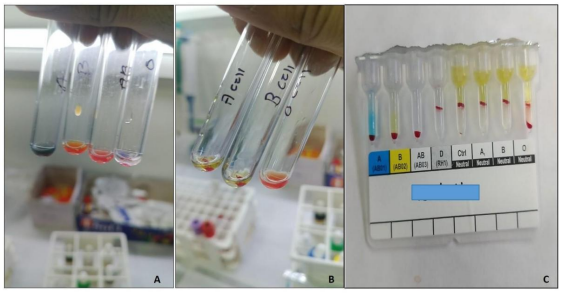

Figure 2: A-B) Showing blood group as O positive in forward **[A]** & reverse **[B]** grouping; **C)** showing gel card method as blood group O.

depends on two factors; plasma concentration and thermal temperature at which hemagglutination takes place.[1–3] In healthy individuals low levels of cold agglutinins are noted in the sear (1:16). Increased levels of agglutinins are see when there is activation due to an underlying cause [secondary to malignancy, infections, autoimmune diseases, liver disease, respiratory illness, etc.[3]

Our patient was admitted due to cirrhosis of liver and mild respiratory illness. Initially in work up of the patient, outside blood group report was noted to be AB positive. Blood transfusion was required in view of hemolysis and decreasing hemoglobin. A thorough blood examination was done and it was found that there was discrepancy in blood grouping due to excessive agglutination present in the EDTA sample. This was resolved with further detailed evaluation as mentioned in case history above. Few more authors have published similar findings.[2–4]

A study by Sharma et al. in 2023, regarding challenges in serological work up and management of the patient with CAD.[6] They reported 2 cases, first one was a 61-year-old female with symptoms of weakness, bone pain, difficulty breathing with high levels of cold agglutinin titres (1:1024). A detailed bone marrow examination confirmed the diagnosis of plasma cell dyscrasias. Initial blood grouping revealed B positive group, which was corrected to be O positive. In second case reported by them, the patient did not have any underlying disease, just mildly raised cold agglutinin titres of 1:64. In this case, the correct blood group was A positive which was initially wrongly interpreted as AB positive. Both cases were managed successfully. In both these cases, a thorough evaluation was needed which if not done could have lead to unwanted results.

Blood group discrepancy was noted in a study by Raghuwanshi B,[7] where the patient had presented with Covid infection, pneumonia along with anemia & thrombocytopenia. The patient's bilirubin levels were also deranged and direct antiglobin test was also positive. Blood sample in EDTA-vial showed auto-agglutination and discrepancy in reporting of blood group was resolved taking appropriate measures using pre-warmed saline wash technique.

Similarly, in a study by Shah et al. CAD was found in coexistence with left main coronary artey involvement and ventricular dysfunction[1]. They managed the patients timely with rituximab, bortezomib, and meticulous pre-operative protocol to reduce hypothermia. Our patient had cirrhosis liver with deranged bilirubin levels and mild respiratory illness. This case brings into picture the importance of diagnosis of blood group properly, to avoid any litigation at any stage.

4. Conclusion

There can be varied secondary causes leading to CAD. High levels of pathological cold autoantibodies may result in severe adverse outcomes in patients which should be duly taken care of. Benign cold agglutinins are detected incidentally and may be associated with underlying disease, as was seen in our case. Timely and correct interventions are important in managing these patients. These patients can be managed properly by counselling them to avoid exposure to cold, maintaining their hemoglobin levels and treatment of the underlying pathology. Any medico-legal issue arising from the discrepancy in serological tests can be avoided by a diligent approach.

5. Source of Funding

None.

6. Conflict of Interest

None.

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