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Case Report

Red Blood Cell Autoagglutination: A laboratory challenge

Mawda Abdelsalam Mohamed Hamad[1], Ahmed A. Agab Eldour[2]

[1]Master in hematology and blood transfusion, First specialist medical laboratory at Elobied International Hospital.
[2]Department of pathology, Faculty of Medicine and Health Sciences, University of Kordofan.

Correspondence to Mawda Abdelsalam Mohamed Hamad: mawdaabdelslam21@gmail.com

Introduction:

Red cell agglutination or autoagglutination is a phenomenon in which red blood cells clump together, forming aggregates. It is caused by the surface of the red cells being coated with antibodies. This often occurs in cold agglutinin disease, a type of autoimmune hemolytic anemia in which people produce autoantibodies (termed cold agglutinins) [1]. Sometime may develop cold agglutinins from lymphoproliferative disorders, from infection with Mycoplasma pneumoniae or Epstein–Barr virus, or idiopathically. Cold agglutinins (CA) are monoclonal or polyclonal antibodies activated at low temperatures and lead to autoagglutination of the RBC membrane [2]. Binding of CA causes agglutination of erythrocytes and the antigen-antibody complex induces complement-mediated hemolysis. Intravascular hemolysis occurs due to direct complement mediated lysis while extravascular hemolysis is as a result of C3b coated red cell destruction by macrophages in the liver [3]. Patients show hemolytic anemia of varying degrees of severity, as well as episodes of hemoglobinuria and acrocyanosis, which arise or worsen upon exposure to low temperatures. Blood cell aggregates are counted as single cells by the automated analyzers used to run complete blood count tests [4]. This leads to a markedly decreased red blood cell count and hematocrit and markedly elevated mean cell volume and mean cell hemoglobin concentration. Red cell agglutination also interferes with routine methods for blood typing and blood compatibility testing, which rely on agglutination reactions. [5,6]

Case presentation:

A 72-year-old female was brought to emergency unit of Al-Daman Hospital, presented with stroke. Tests were requested for her by the doctor on duty, including a complete blood count and ABO blood grouping. When the sample was brought to the laboratory, it was noted that the sample was in a state of red blood cell agglutination.

The sample was analyzed by Zybo Z3 hematological analyzer. Findings were as follows: RBC 2.02 million, hemoglobin (Hb) 7.2 g/dl, hematocrit (Hct) 15.4%, mean corpuscular volume (MCV) 76 fL, mean corpuscular hemoglobin (MCH) 35.5 pg, MCH concentration (MCHC) 46.6 g/dl, red cell distribution width (RDW-CV) 15.7%. White blood cell 10.7 × 10^9/L differential leukocyte count: Neutrophils 68.4%, lymphocytes 21%, mid (monocyte, basophil, eosinophil) 10.6%, platelets 605 × 10^9/L (figure1). Aggregation of RBCs, which occurs mainly at temperatures lower than 30°C, causes invalid findings when working with automated hematology analyzer. Sample had no evidence of hemolysis or lipemia. To rule out a problem, the technologist was instructed to redraw a new sample. Another CBC test was run, but the results remained unchanged. Peripheral smear examination was done which showed the presence of red cell clumps and rouleaux formation (figure2). Suspecting cold agglutinins, the sample warmed at 37°C for 30 minutes and after the second run, reversibility of agglutination was observed. Thus, agglutination was confirmed as cold agglutinin and the results become valid. The findings were compared. MCHC reduced to 36.4g/dl at 37°C and the MCV also reduced to 19.2 pg. The increasing of RBCs counts to 3.2 million and HCT to 21.3%. the WBCs and differential count remain unchanged (figure3). Peripheral smear prepared from warmed sample showed normocytic normochromic cells with nucleated RBCs and polychromasia (feature of hemolysis) and there was no RBCs clumping observed (figure4).

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Remarks:

Para.	Result		Unit
WBC	10.27	↑	10^9/L
Lym#	2.16		10^9/L
Mid#	1.09		10^9/L
Gran#	7.02	↑	10^9/L
Lym%	21.0		%
Mid%	10.6		%
Gran%	68.4		%
RBC	2.02	↓	10^12/L
HGB	7.2	↓	g/dL
HCT	15.4	↓	%
MCV	76.1	↓	fL
MCH	35.5	↑	pg
MCHC	46 6	↑	g/dL
RDW-CV	15.7		%
RDW-SD	36.2		fL

Figure 1: The CBC result before sample warming

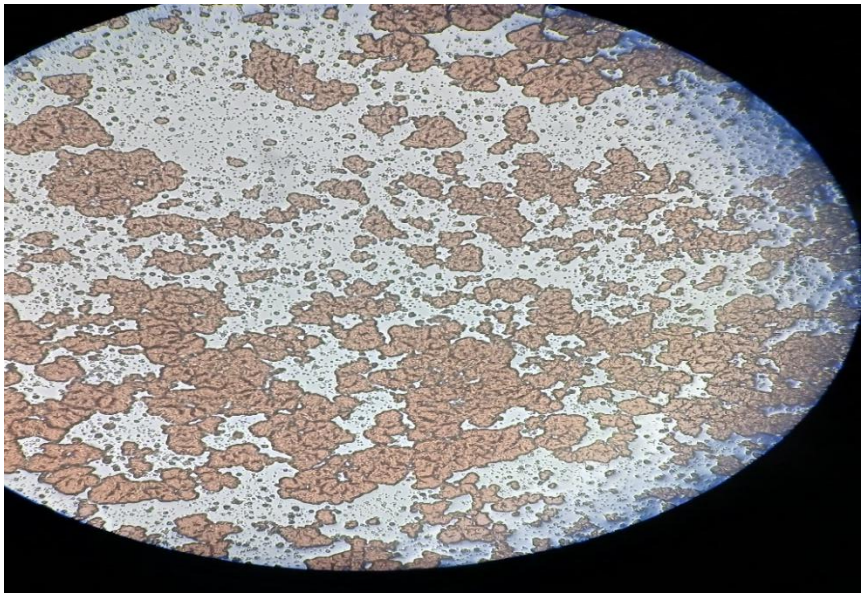

Figure 2: show RBCs agglutination in peripheral blood smear

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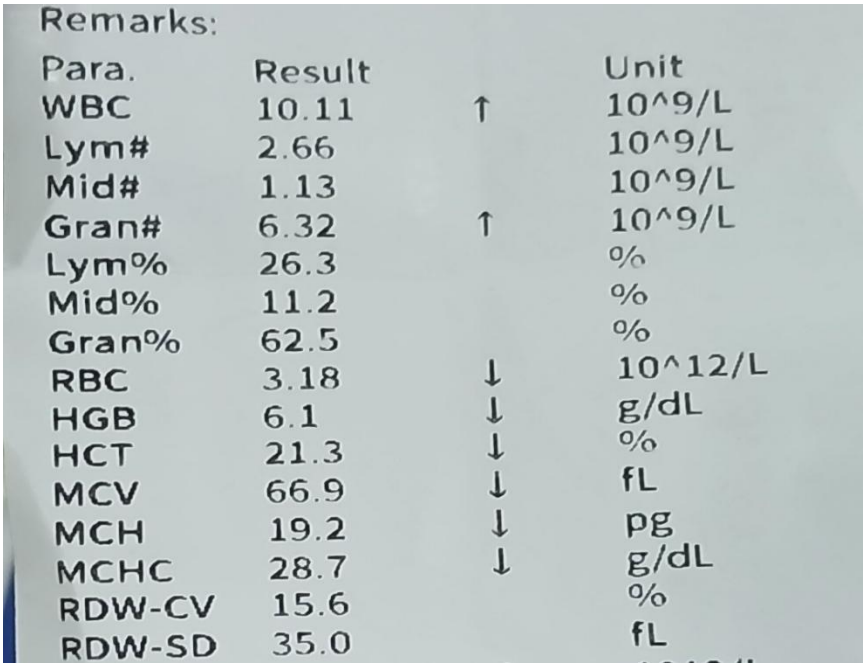

Remarks:

Para.	Result		Unit
WBC	10.11	↑	10^9/L
Lym#	2.66		10^9/L
Mid#	1.13		10^9/L
Gran#	6.32	↑	10^9/L
Lym%	26.3		%
Mid%	11.2		%
Gran%	62.5		%
RBC	3.18	↓	10^12/L
HGB	6.1	↓	g/dL
HCT	21.3	↓	%
MCV	66.9	↓	fL
MCH	19.2	↓	pg
MCHC	28.7	↓	g/dL
RDW-CV	15.6		%
RDW-SD	35.0		fL

Figure 3: CBC result after sample warming

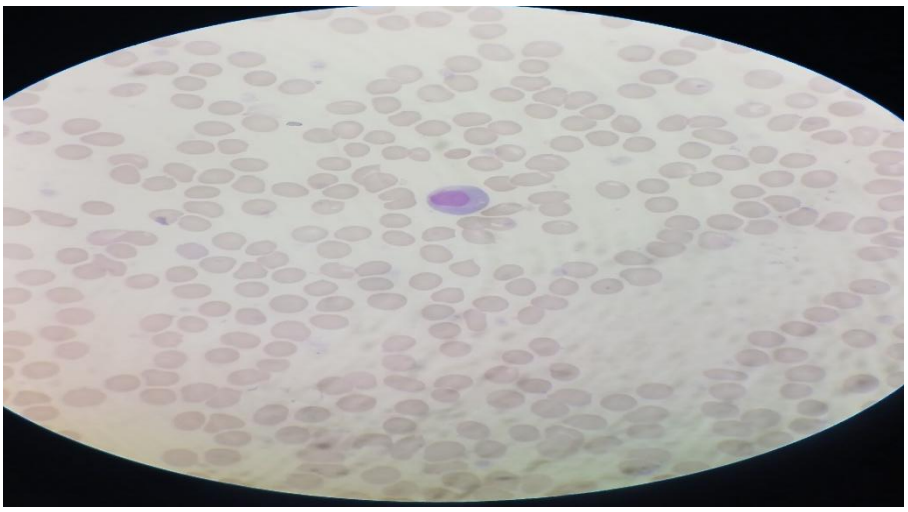

Figure 4: Peripheral blood picture after sample warming

In addition, the worked team faced difficulty to determine the blood group of the patient because the grouping depends on the visible RBCs agglutination and the sample was already in a state of agglutination even before the reagent added (figure5). To disband the problem, the sample was warmed at 37c for 30 min to ensure that antibodies were separated from its antigen. Then, the sample was washed by pr-warmed saline to remove the plasma and its antibodies. After that, the grouping was performed used that suspension and the result became more accepted (figure6). To confirm this red blood cell autoagglutination is associated with cold agglutinin antibodies the direct combs test was performed and result is positive.

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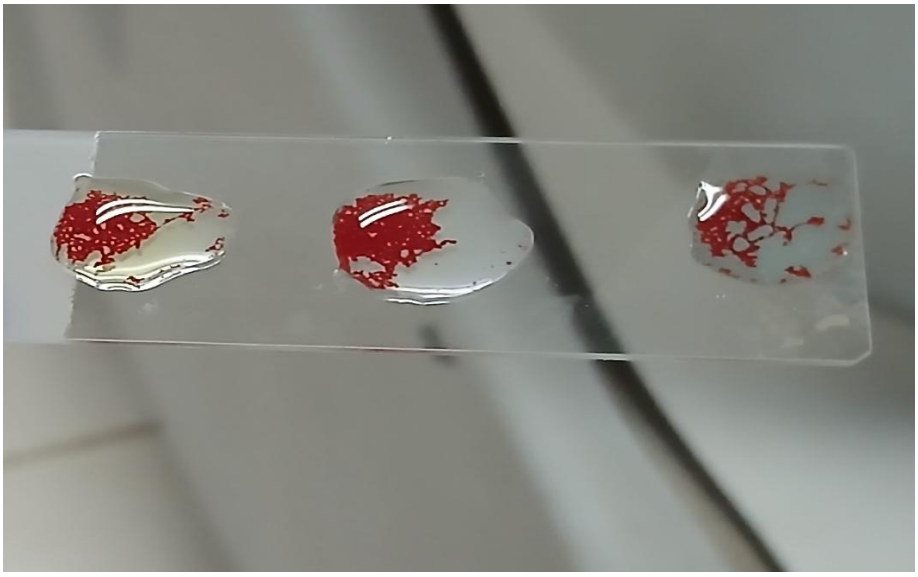

Figure 5: ABO blood grouping before sample warming

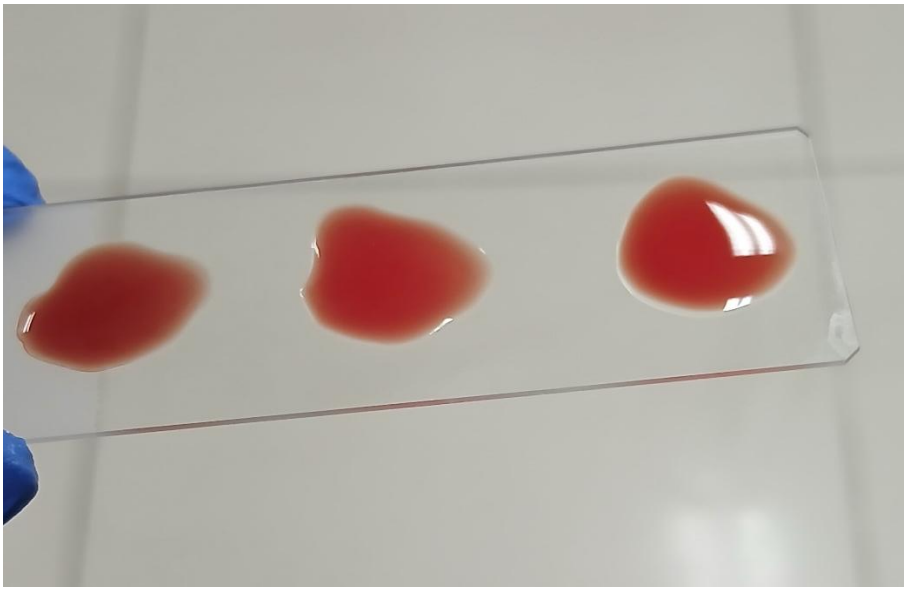

Figure 6: ABO blood grouping after sample warming

Discussion:

One of the challenges facing medical laboratory technicians is rare samples that require special treatment before starting their analysis to give better results. Sufficient awareness about these samples is very important. The case of red cell autoagglutination was considered one of these samples. Many case report studies had discussed this phenomenon and its redounding on the CBC and blood grouping test. This case report red cell agglutination due to unknown cold agglutinin. We met with the present case in winter. Therefore, the cold agglutinin forming might be the result of the fact that blood samples were exposed to cold weather during transfer from patient to our laboratory. In addition to that we suggest the antibody titer is high because the reaction is very strong and clearly visible. The invitro phenomenon of cold agglutination results in false increase in MCV and MCHC and a decrease in the RBC count

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and HCT given by automated hematology analyzer; however, different findings have been reported in some clinical case reports. Serif Ercan et al report 70-year-old female patient had a history of cerebrovascular diseases and rheumatoid arthritis. They found decreased HCT and elevated MCV values. Hemoglobin concentration was unaffected, thereby, calculated MCH and MCHC values were prominently elevated. cold agglutinin was evaluated as possible reasons for the mismatch between hematocrit and hemoglobin values. Leukocyte and platelet counts were also found to be unaffected by cold agglutinins. [7]

In a case report submitted by Lodi et al., the blood group of a 48-year-old male patient was not determined as a result of cold agglutinins and the patient died as a result of complications due to the emergency transfusion of universal RBCs (0 Rh-positive) [8]. Resolving blood grouping discrepancies in presence of cold agglutinins with warm washed (37°C saline) or 2 ME treated RBCs and reverse ABO tests at 37°C (control with group O RBCs) or with auto adsorbed or group O adsorbed serum-plasma seem to be a good suggestion in treatment [9].

Conclusion:

The present study showed that cold agglutinins may interfere with the analysis of RBCs and RBCs-related parameters (HCT, MCV, MCH and MCHC); in addition to blood grouping test and may cause ABO grouping discrepancy. The knowledge of this phenomenon can help prevent wasting too much time and make an early and accurate diagnosis.

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