Nonspecific red cell aggregation interferes in the interpretation of gel test results

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Introduction

Samples with abnormal serum protein concentration, altered serum protein ratio, or high-molecular weight volume expanders are known to induce rouleaux formation which can mimic agglutination. Serum factors inducing rouleaux and spontaneous red cell aggregation interfere in the interpretation of routine serological tests.

Observation

A 24-year-old lady with a provisional diagnosis of Immune hemolytic anemia, was referred to us for the direct and indirect antiglobulin test (DAT, IAT). The anticoagulated blood sample showed visible agglutinates along the sides of the test tube, and a wet preparation from the EDTA sample also showed similar findings [Figure 1a]. However, on addition of drops of normal saline onto the slide, the agglutination disappeared; thereby, raising the possibility of rouleaux formation.

Clinical details of the patient revealed that she had intermittent high-grade fever for past 5–6 months with a moderate splenomegaly. The patient had severe anemia (Hb 50 g/l), a total leukocyte count of 4.6×10^9/l with relative lymphocytosis, and platelets were just adequate. Bone marrow aspiration done showed a reactive marrow with excess of plasma cells (18%). A careful search revealed amastigote forms of Leishman donovani, both intracellular and extracellular forms, confirming a diagnosis of visceral leishmaniasis. Biochemistry tests revealed elevated total proteins (patient 78 g/l, normal 55–70 g/l) with reversal of albumin-globulin ratio (patient albumin 27 g/l, normal 35–55 g/l). With these findings, an impression of rouleaux formation due to altered albumin-globulin ratio was considered and an attempt to rule out any additional antibodies was made. An autocontrol was performed on the Diamed ID microtyping system. Results showed 2+ agglutination with 0.8% LISS suspended red cells, and 1+ agglutination with washed red cells in LISS. However, when warm saline washed red cells in LISS were used, the autocontrol was negative [Figure 1b]. The IAT and DAT were performed by the tube method. For the IAT, after routine incubation, saline replacement technique[1] was used and for the DAT, red cells were first washed thoroughly to remove serum factors. Both IAT and DAT were negative.

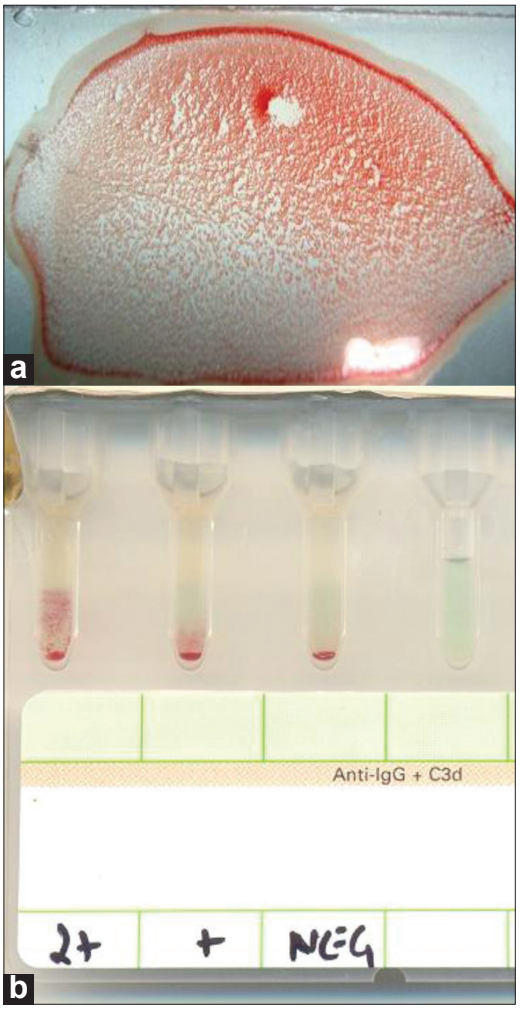

Figure 1: (a) Wet preparation of EDTA blood sample. (b) Autocontrol

Conclusion

Visceral leishmaniasis is common in coastal

states of eastern India and it may be associated with hypergammaglobulinemia.[2] In all such cases, the possibility of a false positive auto control exists. Abnormal concentrations of serum proteins are also known to interfere with blood grouping, by inducing rouleaux formation and spontaneous red cell aggregation.[3] This can be eliminated by thoroughly washing the red cells and retesting. For detecting antibodies in the presence of rouleaux, saline replacement or saline dilution method is used.[1] These techniques are also used to resolve ABO discrepancies due to rouleaux formation. The report emphasizes that nonspecific red cell aggregation interferes with the interpretation of gel test results. It highlights the need for warm saline washing of red cells before making the LISS suspension for the gel technique, in cases of hypergammaglobulinemia. In addition, reviewing the serological findings in the light of clinical and other laboratory data often helps resolve discrepant test results.

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