Pseudoleukopenia due to ethylenediaminetetraacetate induced leukoagglutination in a case of hypovolemic shock

Sir,

A 45-year-old man presented to the emergency department with symptoms of acute gastroenteritis with severe dehydration leading to hypovolemic shock. On routine general physical examination, his face was flushed and the mouth and tongue were dry. He had cold clammy extremities. Pallor was noted. He was semiconscious, with a rapid feeble pulse of 96/min and a blood pressure of 62/44 mmHg. He had decreased urine output. There was no significant past medical or family history. Routine hematological investigations done on ethylenediaminetetraacetate (EDTA)-anticoagulated blood sample on an automated analyzer revealed the following values: hemoglobin, 10.2 g/dL; platelets, 156 x 10^3/μL; white blood cell (WBC) count, 3.6 x 10^3/μL; neutrophils, 1.5 x 10^3/μL; lymphocytes, 1.9 x 10^3/μL; and mixed (eosinophils, monocytes and basophils), 0.2 x 10^3/μL. However, when the peripheral blood smear was examined, the WBC count appeared to be elevated (15.5 x 10^3/μL), with numerous neutrophil aggregates comprising of 10–30 cells [Figure 1a]. A repeat blood sample in sodium citrate as anticoagulant was evaluated, which recorded a WBC count of 16.2 x 10^3/μL with neutrophils 12.1 x 10^3/μL on the automated analyzer. These findings were confirmed by peripheral blood smear examination [Figure 1b]. The routine biochemical investigations were within normal limits apart from mildly elevated serum creatinine and blood urea nitrogen. The patient was started on intravenous fluids and antibiotics. His recovery was unremarkable and he was discharged after 2 days.

Cell aggregation can be classified as neutrophil aggregation, lymphocyte aggregation, leukocyte aggregation and leukocyte–platelet aggregation. This is usually related to malignancies, infections, hepatic disorders or autoimmune diseases.[1] Pseudoneutropenia secondary to neutrophil agglutination is a relatively

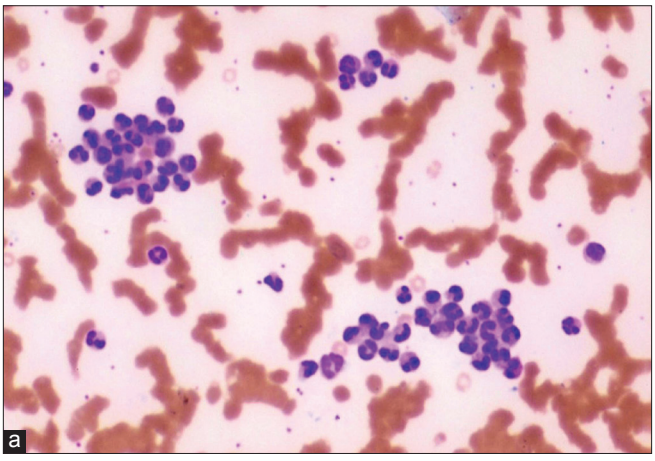

Figure 1a: Blood sample drawn in ethylenediaminetetraacetate showing leukoagglutination (Leishman stain, × 400)

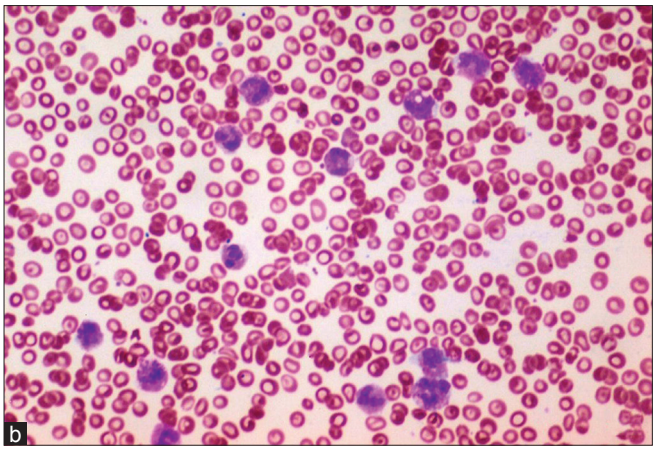

Figure 1b: Blood sample drawn in sodium citrate showing complete abolition of leukoagglutination with well-dispersed leukocytes (Leishman stain, × 400)

rare hematological phenomenon. While the exact mechanism is unknown, some authors attribute it to the presence of IgM antibodies directed against the membrane components of leukocytes, which act when EDTA is used as an anticoagulant for blood collection.[2] The formation of WBC aggregates is a time-dependent process, which starts gradually almost immediately after venipuncture and stabilizes after 60–90 min.[3] Leukocyte agglutination has been known to occur spontaneously and exuberantly at lower temperatures, which can be prevented by holding the blood sample at 37°C.[4] Leukocyte aggregation is a transient phenomenon varying from days to several months. WBC aggregates may comprise all major WBC classes or be limited to only one class, particularly granulocytes.[3] EDTA-dependent *in vitro* agglutination of neutrophils has been reported to resolve by the use of kanamycin, an agent previously shown to be effective in EDTA-dependent pseudothrombocytopenia.[5] Because the phenomenon of leukoagglutination is EDTA-dependent, blood samples should be drawn in containers with either citrate or heparin used as anticoagulant. This prevents the occurrence of leukoagglutination, and accurate leukocyte counts can be obtained. However, citrate can only be used as an auxiliary anticoagulant in parallel with EDTA. Citrate has an adverse effect on many other blood cell quantities, namely the erythrocyte quantities and the typical time-dependent granulocyte peak left shift that occurs earlier in citrate than in other anticoagulants. Measurements in both anticoagulants are necessary to calculate a sufficiently accurate dilution factor.[3]

To conclude, recognition of leukoagglutination is most important as the ensuing erroneous results may cause unnecessary additional diagnostic tests, false diagnoses and therapies.[3]

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