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Case Series

Ethylenediaminetetraacetic acid-induced pseudothrombocytopenia: The story of platelet clumps and report of three cases

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ABSTRACT

Ethylenediamine tetra-acetic acid (EDTA) induced pseudo-thrombocytopenia is an infrequent phenomenon occurring when samples are processed in an automated analyzer using EDTA as the anticoagulant. We report three cases of Pseudothrombocytopenia wherein peripheral smear evaluation and rerun in separate Haematology analyzers helped us in reaching a conclusion. The widespread use of automated instruments has led to the identification of this phenomenon. However, confirmation of PTCP can only be done under microscopic examination of the slide.

Keywords: Ethylenediaminetetraacetic acid, Pseudothrombocytopenia, Citrate, Platelets, Peripheral smear

INTRODUCTION

Complete blood counts (CBCs) are the most commonly ordered test for routine hematology analysis of patients. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant of choice for hematology testing in automated analyzers.[1,2] EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) is an *in vitro* phenomenon of false low platelet counts due to autoantibodies causing platelet agglutination in the anticoagulated blood.[3-6] The reported incidence of PTCP is approximately 0.1–2% in hospitalized patients and 15–17% in outpatients evaluated for thrombocytopenia.[7]

CASE SERIES

Case 1

A 45-year-old female presented with generalized weakness. On examination, she did not have any significant findings. The clinician had advised CBC and peripheral smear evaluation. Sample from the antecubital vein was collected and collected in a BD K2 EDTA vacutainer under all precautions. CBC counts were all within normal limits with a hemoglobin value of 11.9 g/dL, total leukocyte count of 6,730 mm^3, absolute eosinophil count of 590 mm^3 (mild eosinophilia), and platelet count of 39,000/mm^3. A peripheral smear was done and stained with Leishman stain,

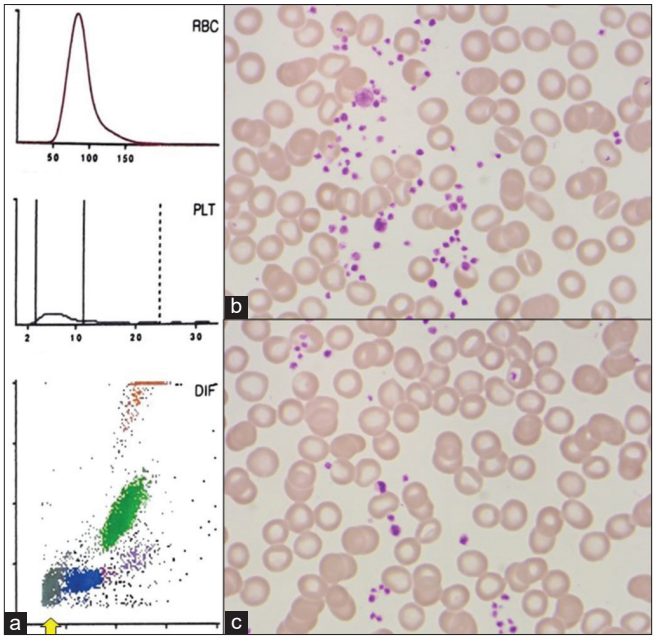

Figure 1: (a) Complete blood count curves of red blood cell (RBC), platelet, and white blood cell counts from K2 ethylenediaminetetraacetic acid vacutainer sample showing the graphs with platelet region showing an almost flat curve. (b and c) Peripheral smears show normochromic normocytic RBCs with numerous platelet clumps and occasional giant platelets (Leishman, ×100).

revealing the presence of numerous clumps of platelets along with a few giant platelets [Figure 1].

Case 2

A 60-year-old male patient presented with no personal or family history of bleeding to the outpatient department (OPD) with complaints of fatigue and was advised routine investigations. He had no history of any medications. BD K2 EDTA vacutainer was used for blood collection from the antecubital vein and done under all precautions. CBC of the patient showed hemoglobin of 9.4 g/dL with a microcytic hypochromic blood picture. The total leukocyte count was 9,810/mm^3 with mild neutrophilia (7,080 mm^3). The platelet count was 80,000/mm^3 on the analyzer. A look at the graph from the machine showed a spike in the platelet area beyond the 20 µ size range. Peripheral smear revealed a microcytic hypochromic blood picture with the presence of numerous platelet clumps. The manual platelet count on the smear was approximately 1,50,000/mm^3 [Figure 2].

Case 3

A 60-year-old female presented to our OPD with a clinical diagnosis of thrombocytopenia done by an outside clinician based on the CBC reports. However, the patient did not have any bleeding tendency or petechial spots in the body. There

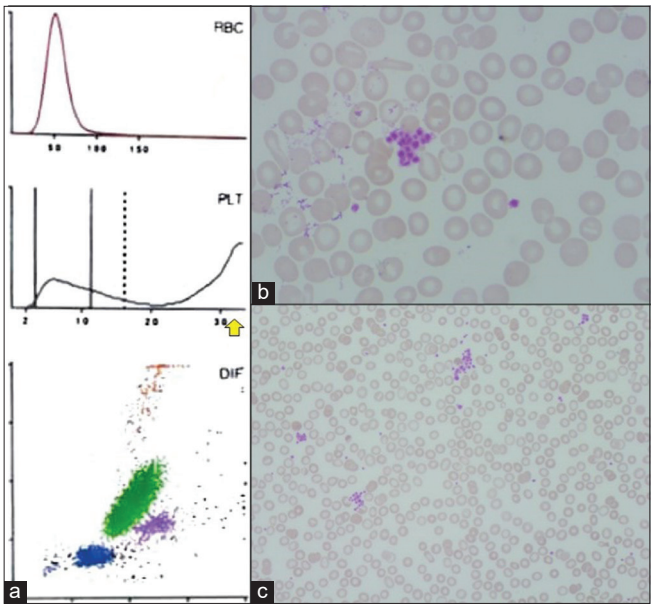

Figure 2: (a) Complete blood count curves of red blood cell (RBC), platelet, and white blood cell counts from K2 ethylenediaminetetraacetic acid vacutainer sample showing the graphs with platelet region showing a spike beyond the 20 µ. (b and c) Peripheral smears show microcytic hypochromic RBCs with numerous platelet clumps and occasional giant platelets. (Leishman, ×100).

was no previous history of similar conditions. Our clinician advised a repeat CBC from the pathology department. The sample was collected in BD K2 EDTA vacutainer from the antecubital vein under all precautions. The machine values revealed a hemoglobin value of 11.2 g/dL, a total leukocyte count of 5920/mm^3, and a platelet count of 10,000/mm^3. There was a flat curve in the platelet region, and the white blood cell graph showed areas of the ghost region/gray zone. This led us to do a peripheral smear, which revealed the normochromic normocytic blood picture with numerous clumps of platelets and occasional giant platelets in the tail region of the smear. We did a repeat collection of blood in sodium citrate BD vacutainer followed by a CBC run in a machine, hemoglobin value of 10.8 g/dL, total leukocyte counts of 5500/mm^3, and platelet count of 2,03,000/mm^3. The graph in the platelet region showed a curve in the 2–10 µ range [Figure 3].

DISCUSSION

Thrombocytopenia is defined as platelet counts lower than 140 × 10^9/L. Reduced platelet counts carry the risk of bleeding and are an indication of platelet transfusion.[8] PTCP is defined as spurious low platelet counts in the absence of clinical bleeding manifestations. PTCP was first identified in 1969 in a patient with non-Hodgkin's lymphoma.[9,10] Since then, this *in vitro* phenomenon has been identified in healthy subjects, various disorders, and the recent COVID-19.

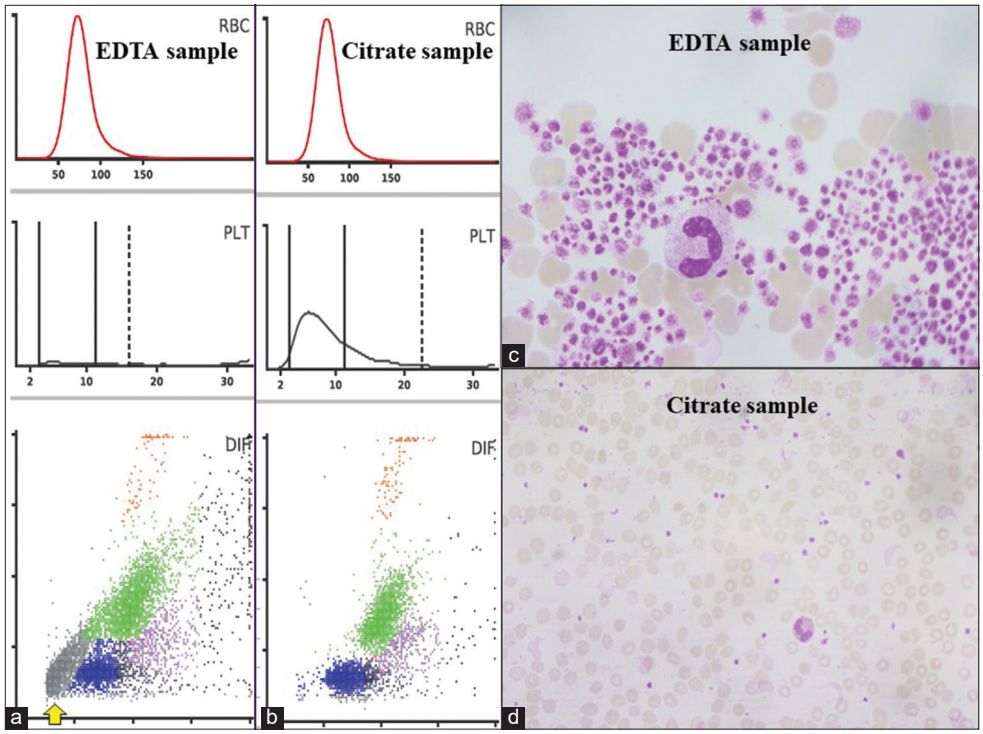

Figure 3: (a) Complete blood count (CBC) curves of red blood cell (RBC), platelet, and white blood cell (WBC) counts from K2 ethylenediaminetetraacetic acid (EDTA) sample showing the graphs with platelet region having near complete flat line. (b) CBC curves of RBC, platelet, and WBC scatters/graph from sodium citrate vacutainer sample showing curve in the 2–10 μ range of the platelet graph. (c) Peripheral smears from the K2-EDTA sample show normochromic normocytic RBCs with numerous platelet clumps and occasional giant platelets. (d) Peripheral smears from the sodium citrate sample show normochromic normocytic RBCs with platelets uniformly scattered in the background (Leishman, ×100).

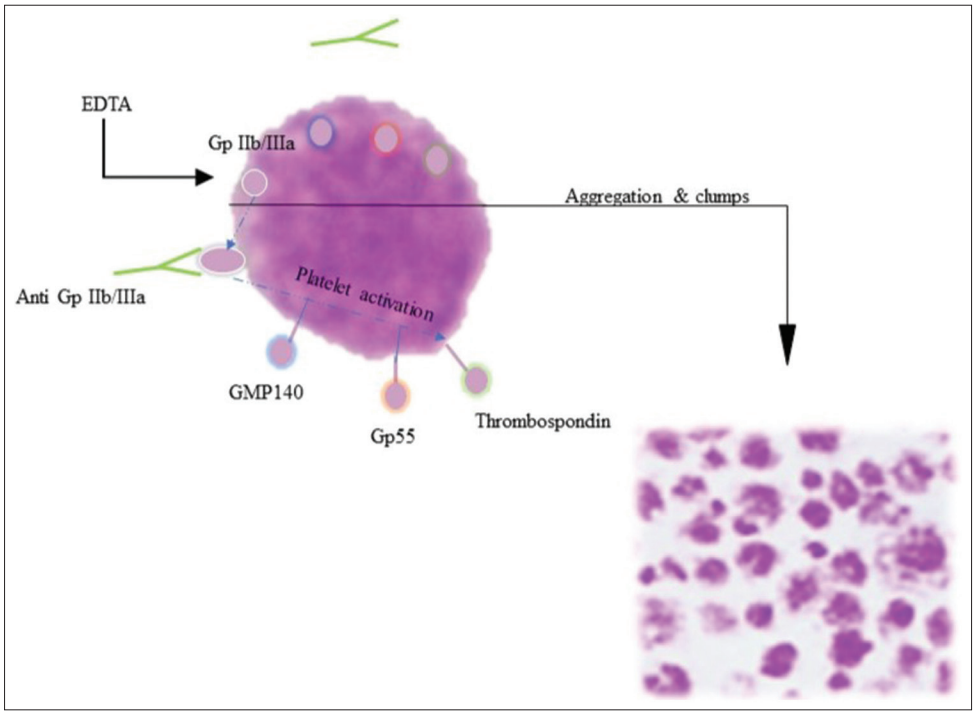

Figure 4: Ethylenediaminetetraacetic acid-induced thrombocytopenia pathogenesis.

EDTA has been indicated as the anticoagulant of choice for routine hematological analysis. It inhibits clotting by removal or chelation of calcium from the blood. They do not cause morphological alteration of RBC morphology. It is known to cause erroneous, false low platelet counts (EDTA-PTCP) in hematological analyzers. The reported incidence of this rare phenomenon is approximately 0.1% of the normal population with no history of bleeding tendency or dysfunction of platelets and approximately 2% in hospitalized patients.[11,12]

There is no age or sex predilection, rarely associated with burns, trauma, sepsis, human immunodeficiency virus, rubella, cytomegalovirus, malignancy, cardiac surgery, drugs, cardiovascular, and thrombotic disorders. Transplacental transmission of maternal serum to neonates and incidence in animals has also been reported.[13]

The mechanism by which EDTA can induce PTCP is by agglutinating antibodies (immunoglobulin G, immunoglobulin M, and rarely immunoglobulin A) that recognize cyto-adhesive receptors on platelet glycoprotein IIb-IIIa (α2β3) in the presence of EDTA. The chelating effect of EDTA on calcium ions and low temperature exposes the GpIIb epitope of platelets, inducing aggregation. The artifact can be triggered by any formulation of EDTA, including sodium (Na_2), potassium (K), calcium (Ca), and magnesium (Mg) salts [Figure 4].[2]

The criteria for EDTA-PTCP include (a) Platelet count below $<100 \times 10^9$/L; (b) occurrence in room temperature as compared to 37°C; (c) time-dependent fall in platelet counts; (d) evidence of platelet aggregates and clumps in peripheral smears; and (e) lack of bleeding signs/symptoms.[14]

The differential diagnosis of PTCP can encompass numerous etiologies most of which fall into two categories: Impaired platelet production or accelerated platelet destruction. Giant platelets and platelet satellitism can also be causes of PTCP. There are substitute anticoagulants for avoiding the PTCP, such as heparin and sodium citrate. However, these also can cause PTCP to a lesser extent. The solution for such PTCP without any clinical evidence of bleeding can be through examination of peripheral smear (gold standard) or collection of blood in a citrate vacutainer.[4,8]

The management of EDTA-PTCP can be divided into three stages: Identification, confirmation, and prevention. Identification includes rechecking the sample for visible clots, the gap between collection and processing, the method of sample collection (needle prick), and the temperature of the sample during processing [Figure 5]. Confirmation

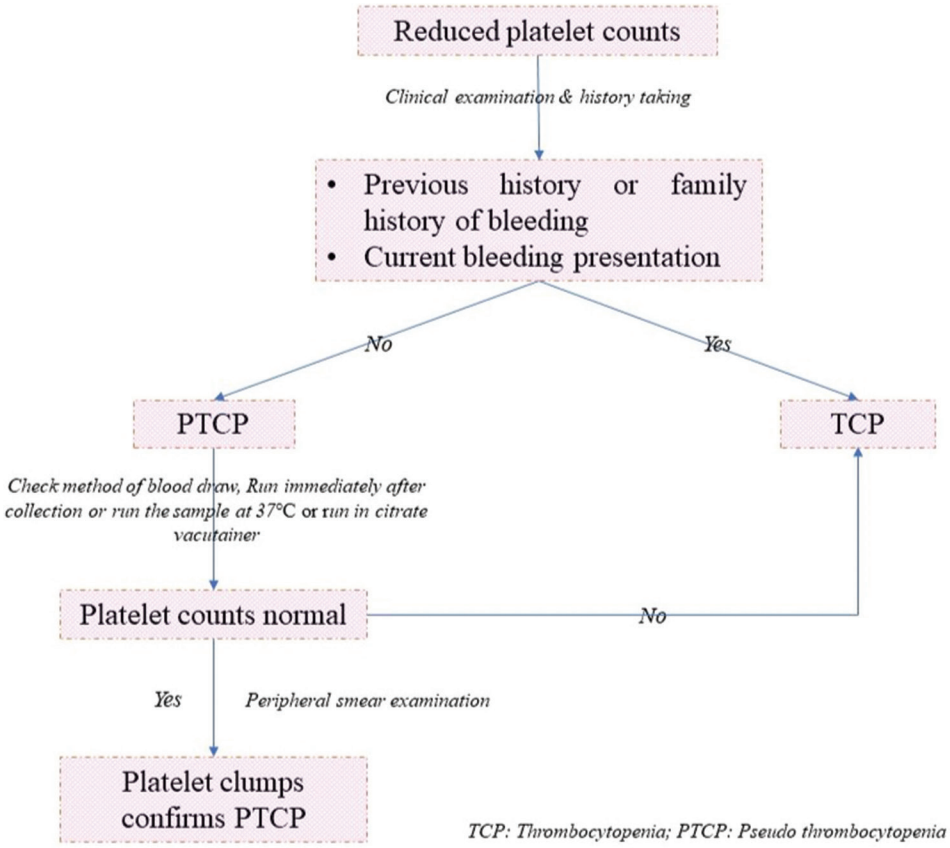

Figure 5: Approach to a case of pseudothrombocytopenia.

is the use of different anticoagulants (citrate or heparin), peripheral smear examination, ammonium oxalate, and Neubauer chamber for platelet counts. Prevention of PTCP can be by proper training to a phlebotomist, check of expiry of EDTA vacutainer, and alertness on automated analyzer flags.

CONCLUSION

Unrecognized PTCP may result in unnecessary laboratory investigations, undue stress to patients, unwanted caution of treating clinicians, and unwarranted interventions. Peripheral smear examination provides definitive evidence for the exclusion of EDTA-induced PTCP.

Availability of data and materials

All the data regarding the findings are available within the manuscript.

Authors' contributions

TS carried out concepts and design, literature search participated in clinical study, and manuscript preparation will stand as guarantor also. MKP carried out data acquisition, data analysis, and clinical study. All the authors have read and approved the final manuscript.

Ethical approval

The research/study complied with the Helsinki Declaration of 1964.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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