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Ethylenediaminetetraacetic acid (EDTA)-Induced pseudothrombocytopenia-A rare phenomenon

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Abstract
Pseudothrombocytopenia (PTCP) is a rare phenomenon of falsely low platelet count on automated analyzers due to in-vitro platelet agglutination in blood samples having calcium chelating anticoagulants, especially ethylenediaminetetraacetic acid (EDTA). The presence of large platelet aggregates on peripheral smear examination and normalization of platelet counts in citrated blood sample explains this phenomenon. We present here a case of a 31 years old male who came to our laboratory for regular master health check up. The automated analyzer showed a very low platelet count and on peripheral smear examination of EDTA blood sample, platelet clumping was evident. A repeat platelet count of citrated blood sample run in automated hematology analyzer revealed a normal platelet count with the absence of platelet clumps on peripheral smear examination made from the latter sample. This confirmed EDTA- induced pseudothrombocytopenia. Therefore, it is very important to recognize this entity, although rare, in order to avoid the unwarranted treatments and platelet transfusions. Examination of peripheral smears in all the cases of thrombocytopenia helps in identifying the platelet clumps and early diagnosis of EDTA-induced pseudothrombocytopenia, thereby, alleviating the unnecessary patient anxiety and interventions.

Keywords: ethylenediaminetetraacetic acid, pseudothrombocytopesia, platelet clumping, peripheral smear, platelet aggregates

Introduction

Pseudothrombocytopenia (PTCP) is a relatively rare phenomenon of falsely low platelet counts on automated analyzers found in the normal population as well as being associated with some disease entities [1]. PTCP is due to in-vitro platelet agglutination caused by autoantibodies directed against platelet membrane glycoproteins or anionic phospholipids that are modified by the anticoagulants and cold temperature, especially calcium chelating agents like ethylenediaminetetraacetic acid (EDTA) [2]. The prevalence of EDTA- induced PTCP in hospitalised patients is reported as 0.1- 2.0% and 17% in outpatient clinic [3, 4].

EDTA is the most recommended anticoagulant of choice for haematological testing because of its stability in cell counting and sizing [5]. EDTA- induced PTCP can be prevented by the use of other anticoagulants such as Sodium Citrate or Heparin. Misdiagnosed EDTA-induced PTCP cases lead to unnecessary patient anxiety, platelet transfusions, increased duration of hospitalisation, and inappropriate treatment [6].

Case report

A 31 years old male came to our laboratory for regular master health checkup. He had an unremarkable history, physical and systemic examination. The blood investigations were sought and his blood was collected in the EDTA vacutainer. The blood sample was run in the automated analyzer and it showed severe thrombocytopenia of $41x10^9$/L. A peripheral blood smear was made and on examination, it revealed many platelet clumps (FIG1A and 1B). A provisional diagnosis of pseudothrombocytopenia was made. Repeat blood sample was collected in 3.2% sodium citrate vacutainer which showed a normal platelet count of $329x10^9$/L. This was in concordance with the peripheral smear examination and it showed no platelet clumps. This confirmed the diagnosis of EDTA-induced pseudothrombocytopenia due to which the first sample collected in EDTA vacutainer showed low platelet count on automated hematology analyzer.

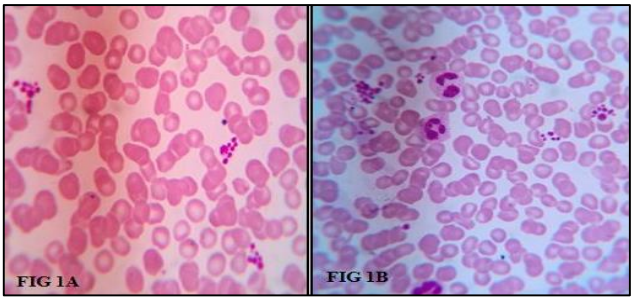

Fig 1A and 1B: Peripheral smear showing platelet clumps. (Leishman's stain, 1000X)

Discussion

EDTA -induced PTCP was first reported by Gowland *et al*. in 1969 [7]. It was referred as "laboratory disease" by Gschwandtner *et al*. in 1997 [8]. This entity is attributed to EDTA induced alteration of platelet surface glycoproteins and anionic phospholipids, which enables binding of antiplatelet antibodies, thereby, causing agglutination. These antibodies recognize Group IIb–IIIa receptors on platelet and trigger activation of tyrosine kinase which in turn causes platelet agglutination leading to spurious low platelet counts [9].

Pseudothrombocytopenia should be suspected, whenever, a patient presents with an abnormally low platelet count without any bleeding manifestations or family history of haematological disease. EDTA-PTCP can be confirmed by peripheral smear examination for presence of platelet aggregates.

Clumping of platelets in patients with EDTA- induced PTCP can be prevented by the use of other anticoagulants such as Sodium Citrate or Heparin. Citrate anticoagulant has also been implicated in causing spuriously low platelet counts but

with a lower frequency as compared to EDTA. In a study by Bizzaro, around 20% of cases with EDTA induced PTCP showed similar clumping in citrate anticoagulant as well [10]. Oxalate and heparin have also been implicated in PTCP. However, in our case, a repeat blood sample collected in 3.2% sodium citrate vacutainer showed a normal platelet count of $329x10^9$/L. This was also in concordance with the peripheral smear examination and it showed no platelet clumps.

In a study by Ohnuma O *et al*., the incidence and causes of thrombocytopenia were evaluated in 20,761 routine clinical blood samples. The study revealed that the incidence of thrombocytopenia was 0.15% and 72% of cases had EDTA induced platelet clumping. The rest 28% of cases had spuriously low platelet counts due to large platelets [11].

High dependence on automated analyzers with minimum slide reviews can cause delay in diagnosis and unnecessary interventions. It is, therefore, very essential to review the peripheral smears carefully whenever a patient presents with unexpectedly low platelet counts along with clinicopathological correlation.

Conclusion

This case highlights the importance of recognizing EDTA - induced PTCP which is spurious low platelet count caused by in-vitro platelet agglutination. Pathologists should be aware of this rare phenomenon as early recognition avoids misdiagnosis and unnecessary patient anxiety, unwarranted platelet transfusions and prolonged hospital stay. Examination of a well-stained peripheral blood smear is mandatory for all the cases of thrombocytopenia to rule out platelet clumping. The simultaneous collection of blood into EDTA and citrate containing anticoagulant tubes may provide a simple and rapid means for definitive diagnosis of EDTA-induced pseudothrombocytopenia.

Acknowledgement

Nil

Conflict of Interest

Nil

Source of Financial Support

Nil

Abbreviations

EDTA-Ethylenediaminetetraacetic acid, PTCP-Pseudothrombocytopenia

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