Case Report

Anticoagulant Dependent Pseudothrombocytopenia: A Case Report

Aryal B, Adhikari S, Aryal N, Pant V
Department of Clinical Biochemistry, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University
Correspondence author: Mr. Binod Aryal
E-mail: aryalbinod17@gmail.com

Abstract

Pseudothrombocytopenia is a laboratory finding caused by in vitro autoaggregation of platelets (an irreversible change) resulting due to the presence of EDTA in containers. In contrast to serious and potential life-threatening causes of thrombocytopenia, EDTA-pseudothrombocytopenia (EDTA-PTCP) is solely an in vitro effect without any clinical relevance. We report a 24 year old patient from MCTVC hospital who presented with common flu. On blood examination there was low platelets count in EDTA anticoagulated sample but he was clinically otherwise normal and there were no symptoms of thrombocytopenia. On smear preparation, in EDTA and Trisodium citrate sample his all blood count parameters were within normal range. This case highlights the importance of slide preparation.

Introduction

Anticoagulant most commonly used in today’s world in the field of laboratory science for whole blood analysis is Ethylene Diamine tetra acetic acid (EDTA). Calcium is one of the clotting factors required for blood coagulation and EDTA chelates this calcium thus inhibiting blood coagulation.\(^1\)\(^2\) Thrombocytopenia is due to increase hemorrhage and increased blood transfusion requirements after surgery. Thrombocytopenia is undesirable and may result in the need for platelet transfusion.

Pseudothrombocytopenia is a finding caused by in vitro autoaggregation of platelets (an irreversible change) resulting due to the presence of EDTA in containers. In contrast to serious and potential life-threatening causes of thrombocytopenia, EDTA-pseudothrombocytopenia (EDTA-PTCP)\(^3\) is solely an in vitro effect without any clinical relevance. There is an absence of exact data but (EDTA-PTCP) is often observed in our laboratory. EDTA-PTCP is often overlooked because all blood smears are not evaluated visually in routine laboratory practice. Histograms as well as warning flags of hematology analyzers are not interpreted correctly which results in misleading in low platelets counts.\(^4\)

Possible Mechanism of Platelet Aggregation

Cation chelation by EDTA leads to a conformational change of the platelet membrane cytoadhesive receptors GPIIb-IIIa complex unmasking a cryptic epitope that becomes accessible for autoantibodies. The autoantibodies are of IgG, IgM and IgA class and they stimulate the expression of activation antigens, trigger activation of tyrosine kinase, platelet agglutination and clumping in vitro, which finally lead to a spuriously decreased platelet count in vitro (some even at 37°c).\(^5\)\(^6\) Hematology analyzers count the resulting platelet clumps as single giant platelets or as small lymphocytes in the white blood cell gate and indicate thrombocytopenia.

Criteria for identification of EDTA-PTCP\(^7\).

The reliable and timely identification of this artifact is essential, since there is a high chance that it may be confused with other life-threatening platelet disorders, or otherwise lead to inappropriate clinical and therapeutic decision-making. Five basic criteria should be fulfilled to raise the clinical suspicion of EDTA-dependent pseudothrombocytopenia.

i. Abnormal platelet count, typically below 1,00,000/ l

ii. Occurrence of thrombocytopenia in EDTA-anticoagulated samples but to a much
lesser extent in samples collected with other anticoagulants

iii. Time-dependent fall of platelet count in the EDTA specimen

iv. Evidence of platelet aggregates and clumps in EDTA-anticoagulated samples with either automated cell counting or microscopic analysis

v. Lack of signs or symptoms of platelet disorders, either thrombotic or hemorrhagic

Visual evaluation of blood smears is regarded as gold standard for detection of EDTA-PTCP, but only a limited number of smears will be performed in routine laboratories. A simpler approach for detection of EDTA-PTCP is to inspect the histograms and flags of hematology analyzers. EDTA-PTCP is expected to be diagnosed correctly in most cases by this approach.

**Case Presentation**

A 24 year old patient from our hospital had common flu and was identified for showing the artefact EDTA-PTCP. Four months later when he was apparently healthy, once again his platelets count was performed with automated cell counter to see if the artefact had subsided or if it was only a result of the infection. Blood sample was collected in both EDTA and Tri Sodium Citrate and platelet count was performed with both samples at different time and at different temperatures. Within one minute of sampling while the blood was still warm, platelet count of EDTA sample was 159,000/µl. It was immediately followed by Citrated sample which showed a count of 200,000/ µl. After five minutes of the sample standing at room temperature, the platelet count with EDTA and Citrate was 7,000/ µl and 177,000/ µl respectively and at 10 minutes it was 5,000/ µl and 170,000/ µl respectively. Both samples were incubated for 10 minutes at 37°C and counts thereafter with EDTA and Citrate were 4,800/ µl and 170,000/ µl respectively. The Romanowsky stained slides of both EDTA (Figure 1) and histogram representation also showed variable curve in platelet zone (Figure 2) and Citrated samples at 30 min of withdrawal showed significant platelets clumps.

![Platelets from Direct sample](image1)

![Aggregated platelets : EDTA](image2)

*Figure 1: Smear examination through Direct sample and in EDTA sample*

![Platelet Histogram of a Normal Sample](image3)

![Platelet histogram from EDTA sample](image4)

*Figure 2: Platelet histogram representation in Normal sample and auto agglutinated EDTA sample*
Conclusion
Not only EDTA but citrate and heparin is also responsible for the in vitro platelet artifact EDTA-PTCP. This case also suggest that maintaining a temperature of 37°Celsius until platelet count is performed is not much relevant as it may not stop in vitro agglutination of platelets.

Recommendation
This study shows that not only EDTA but also Citrate induces in vitro thrombocytopenia but is less significant with citrated sample. Thus, for every case of isolated thrombocytopenia showing considerable variation in past platelet count and the absence of any clinical history, EDTA-PTCP should be suspected and sample for platelet count should be sent to laboratory in citrate vial. Along with this, smear from EDTA sample should also be evaluated for the presence of platelet clumps. To know the ‘near correct’ platelet count of people showing EDTA-PTCP, platelet count from citrated sample should be performed immediately after collection. Also, smear prepared from venous sample immediately after collection (without anticoagulation) should be evaluated for adequacy of platelets. Because even citrate shows the artifact, blood banks should also make it mandatory to screen for pseudothrombocytopenia among donors if a ‘Platelet Rich Plasma’ is to be prepared.

Conflict of interest: None declared.

References
7. Clinical Chemistry and Laboratory Medicine, ISSN (Online) 1437-4331, ISSN (Print) 1434-6621,