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Research Article

Evaluation Of Laboratory Errors And Other Causes In Spurious Thrombocytopenia

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ABSTRACT

Background: Pseudothrombocytopenia (PTCP) is an in vitro phenomenon characterized by spuriously low platelet counts without associated clinical bleeding. It often results from preanalytical errors, particularly ethylenediaminetetraacetic acid (EDTA)-induced platelet clumping or platelet satellitism. Misdiagnosis may lead to unnecessary interventions, including platelet transfusions or inappropriate treatment for suspected thrombocytopenia. Aim and Objectives: The primary objective of the study was to identify and evaluate cases of PTCP and its etiological factors. The secondary objective was to assess laboratory errors contributing to PTCP and propose corrective strategies. **Methods:** This prospective observational study was conducted from June 2024 to May 2025 at Chamarajanagar Institute of Medical Sciences. Blood samples with low platelet counts flagged by automated hematology analyzers (Sysmex XL1000) were cross-verified with peripheral smear findings. Inclusion criteria comprised cases with discordance between analyzer readings and smear examination. A total of 1024 thrombocytopenia cases were evaluated, of which 186 were diagnosed as PTCP. **Results:** Among 186 PTCP cases, EDTA-induced pseudothrombocytopenia accounted for 176 (94.6%), platelet satellitism in 6 (3.2%), and heparin-related interference in 4 (2.2%). All PTCP cases showed platelet clumps or rosetting on peripheral smear, with no clinical bleeding. Most EDTA-dependent cases demonstrated platelet aggregation due to conformational changes in GPIIb/IIIa exposing cryptic antigens and forming immune complexes. **Conclusion:** EDTA-dependent PTCP is the most prevalent form of spurious thrombocytopenia. Peripheral smear correlation is essential to avoid misdiagnosis and overtreatment. Implementing alternate anticoagulants (e.g., citrate or heparin), timely smear analysis, and newer calcium-replacement strategies can reduce PTCP misclassification and enhance diagnostic accuracy.

Keywords: Pseudothrombocytopenia, EDTA-induced platelet clumping, laboratory error.

INTRODUCTION

Platelets are one of the cellular elements arising from megakaryocytic fragmentation in the circulating blood. Platelet count values between 150,000/µl and 400,000/µl which is considered normal[1]. Low platelet count or thrombocytopenia can be defined as platelet counts less than 150,000/µl.[2]

False reduction in number of platelets accounts to Spurious thrombocytopenia or Pseudothrombocytopenia (PTCP).[3] 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.Since then, this *in vitro* phenomenon has been identified in healthy subjects, various disorders, and the recent COVID-19.[4]

Causes of PTCP

Pseudothrombocytopenia (PTCP) is an *in vitro* phenomenon,which is defined as a false decrease in the number of platelets. The incidence ranges from 0.07% to 0.2% and in hospitalized patients it can be up to 0.1% to 2%. Various causes of PTCP are anticoagulant ethylene acid-diamino-tetraacetic acid (EDTA) induced ptcp, [5] platelet satellitism[1], active viral infections, capillary or central line blood collection. 6 Autoimmune conditions such as rheumatoid arthritis, and medications, such as the GPIIb/IIIa inhibitor abciximab used for the treatment of acute coronary syndrome when undergoing percutaneous coronary intervention are also other causes.[7] EDTA-dependent PTCP is the most common but two (EDTA and citrate), three (EDTA, citrate, and heparin), and even four (EDTA, citrate, heparin, and sodium fluoride) anticoagulant-dependent PTCPs were also described in the literature.[8]

There are different mechanisms which lead to ptcp.

EDTA-dependent mechanism of PTCP-Ethylenediamine tetraacetic acid (EDTA) is a polyprotic acid containing four carboxylic acid groups and two amine groups with lone-pair electrons that chelate calcium ions. As we know Calcium is necessary for a wide range of enzyme reactions of the coagulation cascade and its removal irreversibly prevents blood clotting within the collection tube. EDTA is a preferred anticoagulant of choice for hematological testing because it allows the best preservation of cellular components and morphology of blood cells. When in contact with EDTA, platelets undergo a time-dependent change from a discoidal to a spherical shape.An additional problem is the potential development of pseudothrombocytopenia in EDTA-anticoagulated specimens, which is typically characterized by a low platelet count due to in vitro platelet clumping or adhesion to white blood cells. Owing to the increased volume, these elements are not identified as platelets by most hematology analysers, but they may be counted as WBCs, producing spurious pseudothrombocytopenia and pseudoleukocytosis diagnoses.[9]

EDTA causes conformational change of cryptic epitopes i.e platelet surface glycoprotein IIb/IIIa (GPIIb/IIIa) which allows natural IgM or IgG auto-antibodies to bind to GPIIb/IIIa, leading to platelet agglutination. This phenomenon only occurs *in vitro* and has no known associated clinical significance.**7** The membrane glycoprotein IIb is the presumed antigen-bearing
protein of the EDTA-dependent antibody in EDTA-PTCP. Glycoprotein IIb exists as a Ca 2 + -dependent heterodimer complexed with glycoprotein IIIa. The dimer dissociates when the calcium concentration is lowered and re-associates when calcium is replaced in a reversible manner. The epitope of the antiplatelet antibody causing EDTA-PTCP is a cryptoantigen that is only revealed in the dissociated form of glycoprotein IIb. Therefore, it is the calcium chelating effect of EDTA, rather than EDTA itself, that induces the antigen antibody binding.This phenomenon may be observed with any EDTA formulation, and has also been described for molecules resembling EDTA such as ethylenetriamine tetraacetic acid. Most of times this conformational change is not observed with alternative anticoagulants such as citrate,although a recent report suggested that conformational changes could also appear with this latter anticoagulant or even with heparin.[1]

Platelet aggregation elicited by anticoagulant EDTA obviously begins immediately with the complexation of extra- and intracellular calcium. This explains the use of the term "pseudo-thrombocytopenia", even though the values are still above counts of 150×10^9/L.[10] The criteria for EDTA-PTCP include (a) Platelet count below <100 × 109/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.4

Platelet satellitism -One more mechanism leading to Spurious thrombocytopenia is platelet satellitism around white blood cells. It is observed as an in vitro phenomenon with platelet rosetting around the cytoplasm of neutrophils, it has been less frequently observed around lymphocytes.
The underlying mechanism thought to be IgG autoantibodies(antiplatelet
and antineutrophil) present in the patient sera targeting GpIIbIIIa complex of the platelet membrane would be involved in binding to neutrophil Fc Gamma receptor.Other non-immune hypotheses pinpoint that proteins from alpha granules or thrombospondin would be expressed on platelet membrane, thus leading to adhesion to neutrophils. This in vitro process would then evolve towards a more generalized agglutination of platelets and neutrophils in large aggregates containing over 100 cells. The latter phenomenon can be more rarely detected, and seems to be especially EDTA-dependent."[11]
Pseudothrombocytopenia (PTCP) has been increasingly described in patients suffering from various disorders like chronic inflammatory demyelinating polyneuropathy, giant platelet syndrome and exposure to certain drugs valproate, olanzapine, abciximab. Capillary collections are prone to clotting and formation of platelet clumps.12

Aims and Objectives of the Study
Primary- To detect the cases and various causes of Pseudothrombocytopenia
Secondary- To evaluate the laboratory errors causing Pseudothrombocytopenia and to make strategies to minimize them.

METHODOLOGY
(a) SUBJECTS
Inclusion criteria- All the blood samples with low Platelet count with further evaluation with peripheral blood smear examination
Exclusion criteria- Blood samples with low platelet count correlating with peripheral blood smear examination
SAMPLE SIZE is calculated using the formula
N(sample size)=$z\alpha^2$ p(1-p)/d^2
Z=1.96 at 5%level of significance
P=0.27%(prevalence taken from reference article [1])
d=% of margin error(07%)
N= 155
Trial Subject's AGE GROUP: Age ranges from 18 to 70 years.

METHODOLOGY
After obtaining approval and clearance from the institutional ethics committee, the patients fulfilling the inclusion criteria will be enrolled for the study after obtaining informed consent.
Samples are taken from the patients and the reports generated from the automated hematology analyzer and peripheral smear examination are correlated together.
The laboratory errors play a major role in causing pseudothrombocytopenia which forms the preanalytical errors. These are recognized and further strategies are formed to minimize them.
Research subjects undergo phlebotomy and the blood samples collected will be subjected for platelet count estimation using Sysmex XL1000 hematology analyzer and peripheral smear stained with Leishman stain is correlated.

RESULTS AND OBSERVATION
The present study was conducted at Chamarajanagar Institute of Medical Sciences. This prospective study was conducted from June 2024 to May 2025. Total number of all thrombocytopenia cases were

studied and among them cases of pseudothrombocytopenia was isolated and the laboratory errors contributing to ptcp were listed and also other causes leading to ptcp was studied. The studied samples were from patients aged between 18yr to 70 yrs. A total of 1024 thrombocytopenia cases were studied out of which about 186 cases were of ptcp cases.
Different causes leading to psuedothrombocytopenia-
EDTA(blood sample collected in edta vacutainers) induced ptcp -176
PTCP seen in patients on heparin treatment –4
PTCP due to Platelet satellitism-6
Majority of ptcp cases were due to EDTA induced(94%). Few percentage of cases were because of platelet satellitism and heparin induced. All decreased platelet counts detected by haematology analysers were confirmed by peripheral blood smear. Rest of the cases were correlating with peripheral smears with truly decreased in platelet counts.

DISCUSSION

As we know thrombocytopenia is caused by increased destruction of platelets (hemolytic uremic syndrome, immune thrombocytopenia,and disseminated intravascular coagulation),decreased production of platelets (leukemia, sepsis, human immunodeficiency virus, and decreased production of thrombopoietin), hemodilution, or the use of certain drugs (valproic acid, methotrexate, pantoprazole, and heparin).
Psuedothrombocytopenia which is caused by platelet aggregation in blood containing EDTA should also be considered when a low platelet count is noted. Since 1973, EDTA-dependent PTCP has been widely reported. The prevalence rate of EDTA-dependent PTCP is approximately 0.1 to 2% in hospitalized patients.[14] Platelet satellitism was first described by Field and Macleod in 1963, and since then, approximately only 100 cases have been described in the literature. It has been described exclusively in
EDTA mixed blood.[11] Immunological and nonimmunological mechanisms appear to play a role. As explained earlier the glycoprotein IIb/IIIa complex on the platelet and neutrophil FcgRIII receptor may play a role. These autoantibodies are present naturally in some individuals. It is also postulated that EDTA anticoagulant may expose some cryptic epitopes which are otherwise sequestered.[15]
In present study the number of EDTA induced psuedothrombocytopenia cases were more as compared to other causes. These cases were evaluated and were compared with peripheral smear examination after which corrected platelet count was given. Thereby preventing unnecessary platelet transfusions and critical care admissions.
Michael Nagler *et al.*, in conclusion says to study platelet and WBC histograms on automated hematology analyzers in EDTA-PTCP. Awareness of these patterns may alert to the presence of EDTA-PTCP in routine clinical practice. This may help physicians as well as laboratory personnel to be aware of EDTA-PTCP and to prevent unnecessary investigations as well as over-treatment.[10]
Lardinois, B, *et al.* in the year 2021 at Italy conducted a study in which it was concluded PTCP is a complex phenomenon, influenced by the method used for counting thrombocytes and also includes preanalytical issues. It seems that no particular disease may be specially associated with PTCP. This condition could still lead to misdiagnosis of thrombocytopenia,impairing diagnosis, management, and therapeutic decisions. Most cases of EDTA-dependent PTCP can be corrected by using different anticoagulants, whilst multiple anticoagulants PTCP is a less acknowledged laboratory phenomenon, resulting in more analytical challenges.[1]

CONCLUSION

Few Strategies to minimize PTCP In laboratory in investigation of platelet clumping the following steps may be included until a nonclumping smear is obtained, noting that Steps 3 and 4 are reserved for the rare instances, where Steps 1 and 2 do not resolve the platelet clumping.
Step 1. Verify method of blood draw (e.g., finger stick

versus venipuncture versus line draw) and exclude collection
method related clotting.
Step 2. Test a blood sample collected in sodium citrate.
If clumping persists, continue to Step 3.
Step 3. Test a sample collected in heparin. If Step 3 is not
possible, proceed to Step 4.
Step 4. Obtain a sample in ammonium oxalate, and count platelets utilizing a hemocytometer grid, if available, as per described methods16
Following Methods can be employed for distinguishing true thrombocytopenia from PTCP include using other anticoagulants (sodium citrate, oxalate, and heparin), elevating the temperature of a blood sample to 370C, examining a blood sample containing EDTA as soon as possible, examining a blood smear under a microscope. Misclassification of PTCP leads to unnecessary diagnostic tests and treatment.[14]
A novel method of calcium replacement while maintaining anticoagulation was designed to dissociate platelet clumps in EDTA-PTCP. This method consisted of calcium replacement with the addition of CaCl 2. Since calcium replacement would counteract the anticoagulating action of EDTA, heparin, whose anticoagulating mechanism is unrelated to calcium chelation, was added to maintain anticoagulation. This new method of calcium replacement while maintaining anticoagulation is based on the pathophysiological
mechanism of EDTA-PTCP and is an easily applicable, rapid, and efficient measure that dissociates platelet clumps in EDTA-PTCP.17
A high degree of suspicion is required. PTCP should be on the differential diagnosis for any patient presenting with moderate to severe thrombocytopenia on automated CBC but no clinical signs or symptoms of mucocutaneous bleeding, and a peripheral blood smear be obtained. Otherwise, additional costs and risks associated from further diagnostic testing and treatment for alternate causes of thrombocytopenia may occur. For example, a patient suspected of presenting with ITP may be treated with corticosteroids or even splenectomy, leading to potential iatrogenic injury. Therefore pseudothrombocytopenia cause has to be ruled out.

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