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EDTA INDUCED PSEUDOTHROMBOCYTOPENIA(PTCP): A PROSPECTIVE STUDY AT TERTIARY CARE HOSPITAL

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ABSTRACT:

Background -Spurious thrombocytopenia or pseudothrombocytopenia (PTCP) is a well known in vitro phenomenon that occurs when the anticoagulant used while testing the blood samples causes clumping of platelets which results in spuriously low platelet count with automated haematology analyser. It occurs most often in EDTA anticoagulated blood, however other anticoagulants though to a lesser extent have also been implicated in several reports. Clinical consequences include unnecessary platelet transfusions, bone marrow aspiration and inappropriate treatment like administration of steroids.

Material and methods –This is a hospital based prospective study in department of clinical pathology for one year duration from January 2021 to December 2021.

Results – A total of 82 were found to have EDTA induced pseudothrombocytopenia in the study period. The total case load during the study period was 2581 cases. The incidence of PTCP in our study being, 3.1%. Males accounted for 45% and females accounted for 55 % with male to female ratio of 2.5: 3.

Mean platelet count in EDTA anticoagulated samples with PTCP was 39,333/mm^3 mean platelet count in sodium citrate sample was 1,78,666/mm^3, and mean platelet count manually was 2,10,552/mm^3. Citrate anticoagulated samples showed higher values as compared to EDTA.

Conclusion -EDTA-PTCP is a common pre analytical error encountered in routine clinical laboratory practise. EDTA-PTCP should be suspected in all cases with a low platelet count but without any clinical bleeding manifestations. Platelet counts should be reviewed in all such cases to prevent unnecessary clinical interventions and treatment. Simple, inexpensive and diagnostic method of peripheral smear examination remains gold standard.

Keywords: EDTA-PTCP, STEROIDS, Anticoagulant, PSEUDOTHROMBOCYTOPENIA

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INTRODUCTION:

Pseudothrombocytopenia (PTCP) or spurious thrombocytopenia is awellknown in vitro phenomenon that occurs when the anticoagulant used while testing the blood samples causes clumping of platelets which results in spuriously low platelet count with automated haematology analyser.[1]Its prevalence is reported to vary between 0.1-2% among hospitalised patients and 15%-17% in patients evaluated for isolated thrombocytopenia.[4] It occurs most often in EDTA anticoagulated blood, however other anticoagulants though to a lesser extent have also been implicated in several reports.[2]Cation chelation by EDTA leads to a conformational change of the platelet membrane GPIIb-IIIa complex unmasking a cryptic epitope that becomes accessible for autoantibodies.[2]Hematology analysers count the resulting platelet clumps as single giant platelets or as small lymphocytes in the white blood cell gate and indicate thrombocytopenia.[3] Clinical consequences include unnecessary platelet transfusions, bone marrow aspiration and inappropriate treatment like administration of steroids.

AIMS AND OBJECTIVES:

This study aims –

1.To determine the incidence of spurious thrombocytopenia in EDTA anticoagulated blood samples.

2.To compare the platelet count in cases of EDTA induced PTCP with other alternate anticoagulants and manual platelet count.

3.To analyse abnormal platelet histograms of automated haematology analyser in cases of PTCP.

MATERIAL AND METHODS:

This prospective study was an observational study conducted in clinical pathology section of Department of Pathology, Guntur medical college. Duration of study is of one year from January 2021 to December 2021. Inclusion criteria was all cases of spurious thrombocytopenia identified by screening of peripheral smear and analysis of abnormal platelet histograms in Sysmex XE 5000 haematologyanalyser. All cases with normal platelet counts and thrombocytopenia in which haematology analyser counts correlated with peripheral smear were excluded from the study.

Suspected cases of PTCP were confirmed after examination of peripheral blood films for platelet clumping or aggregates. Peripheral smears were prepared, Leishman stained and examined. After confirmation of PTCP patients were asked for additional blood samples in sodium citrate vials and were run in automated analyser. Platelet count in EDTA vials, sodium citrate vials and manual platelet count are measured, analysed and compared in all these cases. Also, the abnormal patterns of platelet histograms in all these cases are analysed.

RESULTS:

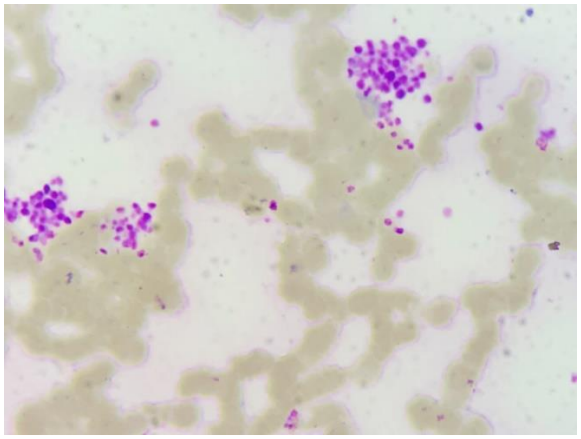
A total of 82 were found to have EDTA induced pseudothrombocytopenia in the study period. The total case load during the study period was 2581 cases. The incidence of PTCP in our study being,3.1%. Males accounted for 45% and females accounted for 55 % with male to female ratio of 2.5: 3.

Mean platelet count in EDTA anticoagulated samples with PTCP was 39,333/mm^3 mean platelet count in sodium citrate sample was1,78,666/mm^3, and mean platelet count manually was 2,10,552/mm^3. Citrate anticoagulated samples showed higher values as compared to EDTA.

DISCUSSION:

EDTA is the most commonly used anticoagulant for the estimation of blood cell counts.EDTA- PTCP is an in-vitro phenomenon due to antiplatelet antibodies that cause platelet clumping in blood that had been anticoagulated with EDTA[1].

In the present study EDTA-PTCP was diagnosed by examination of peripheral smear for platelet aggregates in patients with low platelet counts and abnormal platelet histograms on cell counter. EDTA-PTCP was diagnosed and confirmed by seeing platelet aggregates in smears. Since 1973, EDTA dependent PTCP has been reported in different literatures as well[4]



Peripheral smear showing plenty of platelet aggregates in a case of EDTA -PTCP.

A total of 82 cases of EDTA-PTCP were diagnosed during one year study period.Total number of hemograms in the study period was 2851.The incidence of EDTA-PTCP in the present study is 3%. The incidence in our study is among all the hemograms in outpatient as well as in hospitalised populations. This is slightly more than the incidence in studies by Cohen EM etal[7] who reported an incidence of 0.1 to 2%. The relatively high prevalence of PTCP in our study could be due to lack of microscopic examination of peripheral smear in the primary care laboratories.

A higher incidence of PTCP was seen in females in our study in concordance with study of Pullen et al[3] and Vicari A etal[13]. Male to female ratio in the present study is 2.5:3.

Blood samples were collected in citrate vacutainer in all 82 identified and diagnosed cases of EDTA-PTCP. Pseudothrombocytopenia was corrected in 69 of these cases but was still persistent in citrated samples in 13 cases accounting to 16% incidence of PTCP among EDTA-PTCP cases. Studies of Bizzaro etal[9] and Shresta etal[4] reported around 20% incidence in citrated samples, correlating with our study. Our study considers citrate as better anticoagulant to reduce PTCP than EDTA. Wu Wei etal[8] and Werner et al[6] also stated that citrate is superior to EDTA anticoagulant to reduce PTCP.

The mean platelet count in EDTA anticoagulant sample was 39,333 per mm ^{3} in PTCP cases. This is much lower when compared to citrated samples which showed mean platelet count of 1,70,000per mm^3 .This marked difference in mean platelet count was mainly because pseudothrombocytopenia was corrected in 84% cases of EDTA-PTCP upon use of sodium citrate anticoagulant. These values are in concordance with study of Werner etal[6].

Cell counter flagging and abnormal platelet histograms should be always be evaluated.Platelets are difficult to count because of their small size, marked variation in size, tendency to aggregation and overlapping size with microcytic red cells, cellular fragments and other debris.[14] In haematologyanalysers, this difficulty is addressed by mathematical analysis of platelet volume distribution so that it corresponds to log-normal distribution. Platelets are counted by electrical impedance method in the RBC aperture, and a histogram is generated with platelet volume on X-axis and relative cell frequency on Y-axis. Normal platelet histogram consists of a right skewed single peak.

Normal platelet histogram begins with a sharp increase to a peak and tapers downwards as cell size increases(Image 1). This indicates that the majority of platelets are smaller in size with a fewerlargersized platelets. Mean platelet volume range is2 to 30 fl and platelet count is calculated by cell counter solely based on the volume. The reported platelet count is usually the maximum number of free platelets counted by analyser.In cases with true thrombocytopenia have a flattened curve. All abnormal platelet histograms should be evaluated by peripheral smear examination and also in all cases of low platelet count, platelet histograms need to be analysed. Multiple peaks in platelet histogram with saw tooth appearance indicate platelet clumping in cases of EDTA-PTCP[10,12]. Also in significant number of cases with pseudothrombocytopenia, there was a single peak but towards right of normal platelet peak.

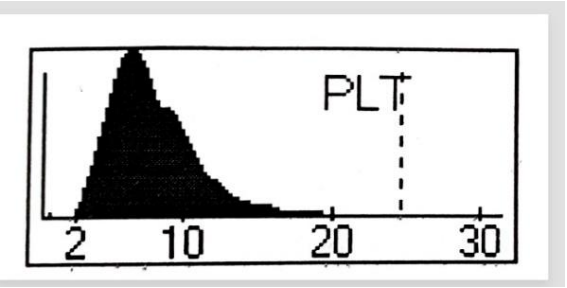


Image 1

Normal platelet histogram

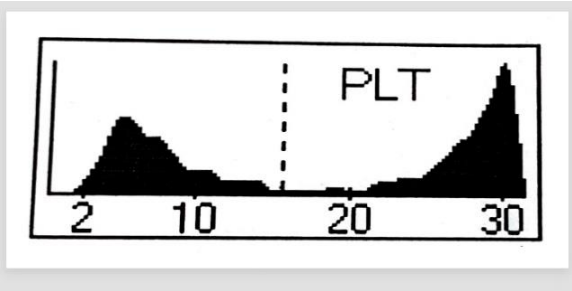


Image 2

PTCP - Abormal platelet histogram with two peaks

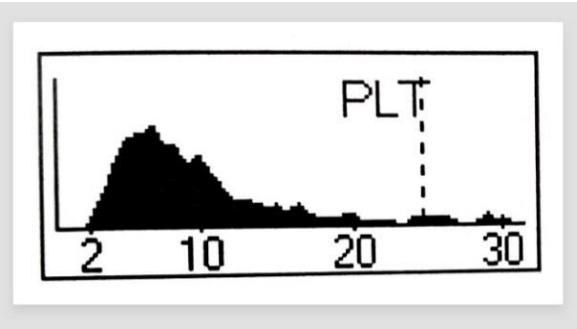


Image 3

PTCP- Abnormal platelet histogram with multiple peaks

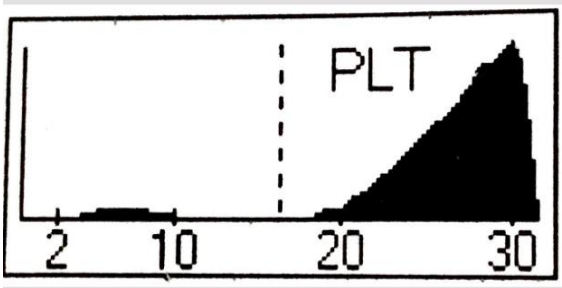


Image 4

Abnormal platelet histogram with right shift of peak

We also observed that there was further drop in platelet count when the EDTA sample was retested after 4 to 6 hours, signifying the importance of preventing delay in sample testing[6].
Out of the total 82 cases of our study, misdiagnosis and mismanagement was seen in the following cases-

- 4 cases were clinically diagnosed as ITP at primary health centres,
- 5 cases were referred to our tertiary hospital for platelet transfusion in view of idiopathic thrombocytopenia,
- 4 cases were referred to our centre for bone marrow examination,
- surgery was postponed in one case,
- one case of EDTA-PTCP was later diagnosed as Essential thrombocytosis,MPN with a platelet count of 12 lakhs.

So undiagnosed EDTA-PTCP may lead to unwanted diagnostic testing,unnecessary transfusions, inappropriate clinical interventions and withhold of even emergency surgeries[11].
There is a lot of unnecessary expenditure,psychological trauma and anxiety to the patient.
Peripheral smear microscopy can be regarded as gold standard for detection of EDTA-PTCP.
A simpler approach is to inspect the histograms and flags of haematologyanalyser in all cases

of thrombocytopenia. This is especially important in peripheral laboratories where there is limited availability of pathologist to screen the peripheral smear.

In our tertiary hospital we screen peripheral smears in all cases with new or unexpected thrombocytopenia below 75,000mm^3 and in cases with abnormal platelet histograms along with warning analyser flags. If platelet aggregates are found in these cases we confirmed as EDTA-PTCP and assessed the correct platelet count by obtaining a fresh sample in citrate vacutainer.

CONCLUSION:

EDTA-PTCP is a common pre analytical error encountered in routine clinical laboratory practise. Far from being a harmless curiosity, undiagnosed PTCP is a pitfall leading to unnecessary diagnostic tests and therapeutic interventions. EDTA-PTCP should be suspected in all cases with a low platelet count but without any clinical bleeding manifestations. Platelet counts should be reviewed in all such cases. Simple, inexpensive and diagnostic method of peripheral smear examination remains gold standard.

REFERENCES: –

1.Chun-Hui Fang, Yueh-Li Chein. EDTA Dependent pseudothrombocytopenia. Formosan Journal of Surgery. 2015; 48:107-109.

2.Casonato A, Bertomoro A, Pontara E. EDTA dependent pseudothrombocytopenia caused by antibodies against the cystadhesive receptor of platelet gpIIB-IIIA. Journal of Clinical Pathology.1994;47:625-630.

3.Pullen R, BriechlE. Pseudothrombocytopenia; Etiology, incidence and significance of a alaboratoryartifact. Medizinischeklinik. 1994;89:196-7.

4. Shresta A, Karki S. Evaluation of EDTA induced pseudothrombocytopenia and the effect of alternative anticoagulants. Journal of Pathology of Nepal.2014;4:626-629.

5.Shreiner DP,Bell WR. Pseudothrombocytopenia: manifestations of a few type of platelet agglutinin.Blood.1973;42:541-549.

6.Werner PS, Steiner M, Fenger S, Gross HJ. Effective estimation of platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. British Journal of Haematology. 2013; 16:684-92.

7.Cohen AM, Cycowitz Z, Mittleman M. The incidence of pseudothrombocytopenia in automated blood analyzers. Hematologia 2000; 30(2):117-21.

8.Wu W, Guo Y, Zhang L, Cui W, Li W, Zhang S. Clinical utility of automated platelet clump count in the screening for EDTA dependent pseudothrombocytopenia. Chin Med J (Engl). 2011;124:3353-3357.

9. Bizzaro N: EDTA- dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases with 10 year follow up. Am J Hematol. 1995; 50(2): 103-109.

10.Michael Nagler, Peter Keller. A case od EDTA-dependant pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon. BMC Clinical Pathology. 2014;14:19.

11.Payne BA, Pierre RV. Pseudothrombocytopenia: a laboratory artifact with potentially serious consequences. Mayo clinic proc.1984;59: 123-125.
12.Bartels PC, Schoorl M, LombartsAJ.Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. Scand J Clin Lab Invest 1997,57(7):629-636.
13.Vicari A, Babfi G, Bonini PA.EDTA-dependent pseudothrombocytopenia: a 12 month epidemiological study. Scand J Clin Lab Invest .1988;48(60):537-542.
14.Zandecki M, Genevieve F,Gerard J, Godon A. Spurious counts and spurious results on hematology analysers: a review. Part 1: Platelets.Int J Lab Hematology. 2007;29(1): 4-20.