

ORIGINAL ARTICLE

Pseudo thrombocytopenia: a true entity in automated hematological cell counter

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ABSTRACT

BACKGROUND: Thrombocytopenia is a condition in which platelet count is less than 150,000 / μ l. Pseudo thrombocytopenia is a spurious thrombocytopenia caused by anticoagulants dependent agglutinins. The aim of this study was to identify the cases in which spurious low platelet counts were given by the automated cell counter due to the anticoagulant EDTA. **MATERIALS AND METHODS:** This study was conducted in the Department of Pathology, Adesh Institute of Medical Sciences & Research (AIMSR), Bathinda, Punjab from 1st April 2014 to 30th September 2015. A total of 16830 patients were investigated for hematology parameters including platelet counts, out of which 300 patients were flagged for thrombocytopenia using the automated hematology analyzer. All the blood samples were collected in EDTA as anticoagulant. These 300 subjects were finally enrolled in the study. All these 300 subjects were further evaluated by peripheral smear examination for assessing platelet counts. **RESULTS:** 27 subjects out of 300 of our study showed pseudo thrombocytopenia by automated analyzer with samples collected in EDTA; Their platelet counts ranged from 22×10^9 μ l to 148×10^9 μ l. While their peripheral smear examination had platelet count ranged from 155×10^9 μ l to 200×10^9 μ l by three different observers. **CONCLUSION:** Thrombocytopenia flagged in hematology analyzer should be screened on peripheral smear for platelets. PTCP is also labelled as a laboratory thrombocytopenia which can be induced by use of EDTA anticoagulant. Falsely low platelet count may lead to unnecessary, expensive and even invasive diagnostic or therapeutic procedure on the patients.

Key words: pseudo thrombocytopenia, EDTA induced

INTRODUCTION

Thrombocytopenia is the most common cause of abnormal bleeding. Artificial Thrombocytopenia is falsely low Platelet count which should be considered in patients who have thrombocytopenia but in the absence of any underlying cause. In artificial thrombocytopenia, platelets are not counted accurately and most common cause is giant platelets or platelet satellitism¹.

Platelet clumping is an artificial thrombocytopenia caused by anticoagulant dependent agglutinins. This Platelet clumping is most commonly seen when blood is collected into EDTA anticoagulant. Platelet clumping is time dependent and varies with type of instrumentation used for automatic counting^{1, 2}. EDTA pseudo thrombocytopenia (EDTA –

PTCP) is not uncommon problem in Laboratories. It is solely an in vitro effect without any clinical relevance³

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, AIMS, Bathinda, and Punjab from 1st April 2014 to 30th September 2015. All the patients during the study period were evaluated by EDTA – anticoagulated whole blood samples but the criteria for selecting Pseudo thrombocytopenia (PTCP) was low platelet counts of $< 150 \times 10^9$ / μ l without any underlying cause of thrombocytopenia; positive flagging for Platelet aggregates in SYSMEX XS-800i- 5 part differential hematology analyzer with fluorescence technology. Such cases were further confirmed as PTCP after examination of peripheral blood film for platelet clumping or aggregates. At the time of collection of reports the subjects having thrombocytopenia flagged by auto analyzer and having normal peripheral smear and normal platelet count were again asked for additional blood sample but this time

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replacing EDTA anticoagulant by MgSO₄ as anticoagulant.

RESULTS

A total of 16830 patients were investigated for hematology parameters. Out of them 300 patients were having thrombocytopenia & evaluated clinically and proper history was taken. 27 subjects out of 300 aged between 17 to 90 years were found to have pseudo thrombocytopenia during the study period. Males accounted for 35% and females 65% with M: F ratio of 1:1.9. Platelet counts in samples anticoagulated with EDTA ranged from $22 \times 10^9/\mu\text{l}$ to $148 \times 10^9/\mu\text{l}$ while similar samples from peripheral smear examination ranged from $155 \times 10^9/\mu\text{l}$ to $200 \times 10^9/\mu\text{l}$.

Similar comparison was done in same patients by using MgSO₄ as anticoagulant and PTCP got corrected and ranged from $150 \times 10^9/\mu\text{l}$ to $200 \times 10^9/\mu\text{l}$.

The Mean Platelet count in EDTA anticoagulated blood of individuals with PTCP was 60,000 whereas the mean platelet count in Manual methods was 1, 75,000 and with MgSO₄ was 1,60,000/ μl .

DISCUSSION

EDTA is most suitable and commonly used as an anticoagulant for estimation of blood cell counts. EDTA – PTCP is an in vitro phenomenon due to anti platelet antibodies that cause platelet clumping in blood that had been anticoagulated with EDTA¹

In this study EDTA – PTCP was diagnosed by examination of peripheral blood smear for microscopic aggregates or clumping of platelets in patients with low platelet count on hematology analyzer. According to Momani A et al⁴, Yoneyama A et al⁶, Wu Wei et al⁷ EDTA – PTCP was diagnosed and confirmed by seeing platelet aggregates in smears.

In this study, PTCP diagnosed from EDTA anticoagulated samples showed lower platelet counts, and moreover mean platelet count in EDTA anticoagulated blood of individuals with PTCP was lower in comparison to MgSO₄. Literature also shows that mean Platelet count was increased in samples anticoagulated with MgSO₄ than in EDTA samples⁵

Fitzgerald et al postulated that cold reactive antiplatelet antibodies directed against a hidden

epitope becomes accessible due to calcium complexing effect of EDTA leading to PTCP⁸

It has been postulated that cation chelation by EDTA leads to conformational change of platelet membrane and unmask the cryptic epitope. This becomes accessible for autoantibodies and causes platelet clumps. Hematology analyzer counts the resulting platelet clumps as a single giant platelet or as small lymphocytes in WBC gate and indicate thrombocytopenia.^{3, 5, 9, 10}

Many a time's histograms and warning signs of hematology analyzer are not interpreted correctly by novice technicians and remains unnoticed. Hence these subjects will be subjected to unnecessary, costly and invasive procedures like bone marrow aspiration.

Schrezenmeir et al proposed that the phenomenon of in vitro platelet aggregation should be collectively called anticoagulant induced PTCP and gschwandther et al referred it as laboratory diseases.^{11, 12} (laboratory induced thrombocytopenia)

CONCLUSION

Peripheral blood smears should be examined for platelet clumping/ aggregates in cases with low platelet count not correlating with clinical presentation or in isolated thrombocytopenia flagged in hematology autoanalyzer. Therefore it is very essential for all laboratory personnel and clinicians to be familiar with this phenomenon of pseudo thrombocytopenia to avoid the unnecessary, expensive and even invasive diagnostic or therapeutic procedure.

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