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EDTA Induced Aggregation a Rare Phenomenon Causing Pseudo Thrombocytopenia in A Case of Dengue

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Case Report

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Abstract: EDTA-dependent pseudothrombocytopenia (PTCP) is the phenomenon of a low platelet count due to EDTA-induced aggregation of platelets. Since the failure to recognize EDTA-dependent PTCP may result in incorrect diagnosis and inappropriate treatment, the recognition of this phenomenon is very important in managing the patient and giving correct treatment. We here by discuss about a case of, A 21 year old male admitted for fever for evaluation showed severe thrombocytopenia by automated 5 part Mindray analyser. Manual count and peripheral smear done revealed higher count and small platelet aggregates. Following which citrated sample was collected and manual counting done both showed similar values, low count in singles without aggregation when compared to EDTA sample. EDTA induced platelet aggregation was thus conformed.
Keywords: EDTA, Thrombocytopenia, Automated cell counter.

INTRODUCTION

EDTA is commonly used as an anticoagulant for blood cell counts, but it may agglutinate the platelets in some patients. This EDTA-induced aggregation of platelets leads to pseudothrombocytopenia called EDTA-dependent PTCP [1]. EDTA-dependent PTCP can be recognized by the presence of platelet clumps in the peripheral smear of blood anticoagulated with EDTA. The aggregation of platelets in EDTA-dependent PTCP usually is prevented by other anticoagulants, such as sodium citrate or heparin [2]. The collection and examination of blood at 37°C also prevent the aggregation of platelets [3].

But in some cases platelet aggregation is not completely prevented by these methods for counting the platelets [3]. EDTA induced platelet aggregation is a rare phenomenon causes pseudothrombocytopenia due platelet aggregation in the sample which was fed in to the equipment which requires cells to pass through in singles. Direct smear study reveals no aggregation, where as smears from EDTA sample showed tiny aggregates of 4 to 6 cells. Patient was positive for Dengue serology and was suffering from falling platelet count. A timely recognition and appropriate correction helps in the management and helps inconcludinng the diagnosis for the treatment.

CASE REPORT

A 21 year old male was admitted on 1st august 2018 for 2 days with high grade fever associated with chills, rigor associated with vomiting. On admission T-103/F ,Pulse-90/mt, B.P-100/60 mmHg, CBC shows low platelet count , Urine routine was normal, smear for MP/MF -negative, RFT- normal, LFT-normal, Dengue serology - positive & USG Abdomen and Pelvis - normal. Platelet count was 1.3lakhs patient was started with supportive treatment.

On day 1 platelet was 1.30 lakhs in EDTA sample patient continued with fever clinically.

On day 3 platelet count was repeated which showed 86,000mm^3 falling count by automated method and peripheral smear showed many tiny clumps.

On 4th day platelet was 40,000mm^3 by automated chamber with many small clumps in peripheral smear. Hence direct smear was taken which showed severe thrombocytopenia and in singles. Hence advised to send the samples in the citrate

On 5th day platelet count was checked with both the citrate sample and EDTA sample which

showed decrease in the platelet count of patient in EDTA sample by automated method 30,000/mm^3, Manual method count was 38,000/mm^3 and smear shows tiny clumps of aggregates of platelets and citrate sample were done, platelet value was 37,000/mm^3 by automated method and direct smear count of platelet was 37,000/mm^3 , when both the values of citrate blood and direct smear sample showed equal values of platelets

Patient was put on high alert and multiple units of platelet transfusions were given and patient was followed up after transfusion .Platelet count improved to 81,000/mm^3 and 1,60,000/mm^3 subsequently within few days and the patients was discharged on 10th day of admission.

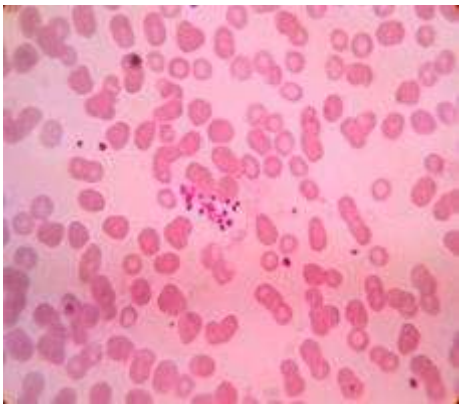

Fig-1: EDTA sample –smear shows platelet aggregation

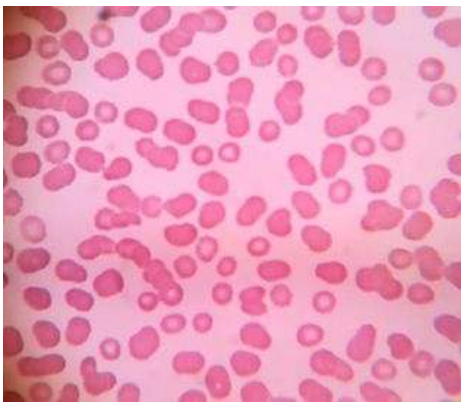

Fig-2: Citrate sample –smear shows thrombocytopenia

DISCUSSION

EDTA induced platelet aggregation is not a commonly observed phenomenon. It frequently impedes with automated counters cause pseudothrombocytopenia as small aggregates are not taken up for counting. EDTA induces alterations in surface glycoproteins and enables binding of antibodies, which in turn causes platelet aggregation. Antibodies are formed against Surface GpIIb and IIIa. They may be IgG or IgM type. Oxalates and Heparin can also cause similar changes.

In our case patient was already developing thrombocytopenia (Dengue) due to illness .The variations observed caused considerable trouble in interpretation; as the platelet values are taken up for Platelet transfusion besides clinical downhill course.

The discrepancy between counter values and presence of clumps was recognized hence a direct smear was done which did not show aggregation but severe thrombocytopenia. The reducing platelets counts were correctly deducted by changing to Citrated sample. This helped us to treat the patient with platelet transfusion and early recovery. Patients platelet count started improving from day 7 and was discharged on day 10 of admission.

EDTA-dependent PTCP is the low platelet count due to the appearance of antiplatelet autoantibodies that cause platelet clumping in blood anticoagulated with EDTA [5, 6]. These antiplatelet antibodies IgG or IgM, and IgA, recognize platelet antigens on the platelet membrane modified by EDTA [4, 5]. The platelet membrane glycoprotein complex IIb/IIIa might be involved in EDTA-dependent antibody reaction. The GP is normally hidden in GP IIb/IIIa complex, but becomes accessible to the cold antibody after dissociation of the glycoprotein complex due to the chelating effect of EDTA on calcium ions, which

are associated with alterations in protein conformation caused by low temperature [7, 8].

A low platelet count due to platelet aggregation in EDTA-dependent PTCP is of practical importance because the lack of recognition of this clinical entity may result in wrong diagnosis and wrong treatment of the patient [9, 8]. The first step in any case of newly diagnosed thrombocytopenia is to rule out falsely low platelet counts resulting from cold reactive, EDTA-dependent antiplatelet autoantibodies [10, 11]. The EDTA-dependent PTCP can be confirmed by microscopic evaluation of a peripheral blood smear for aggregation of platelets and difference in platelet counts between EDTA and other anticoagulants. Anticoagulants, such as heparin and sodium citrate, are usually used for obtaining accurate platelet counts in EDTA-dependent PTCP patients. In this case, platelet clumpings can be observed in a blood sample anticoagulated with sodium citrate and heparin also. Therefore, various methods for obtaining accurate platelet count in such PTCP patients have been suggested like direct smear study and manual method may provide proper evaluator technique for confirmation in EDTA dependent PTCP.

In our case patient was developing thrombocytopenia (Dengue) due to illness .The variations observed in the results and resulted in discrepancy in interpretation .The discrepancy between counter values and presence of clumps was recognized hence a direct smear was done which did not show aggregation and decreased platelet count. The reducing platelets counts were corrected by changing to Citrated sample. This helped us to treat the patient with platelet transfusion and early recovery. Patients platelet count started improving from day 7 and was discharged on day 10 of admission.

When our study was compared with the other study made by Sakurai et al. reported that the supplementation of aminoglycosides to EDTA-anticoagulated samples after blood withdrawal induced dissociation of aggregated platelets in blood samples from patients with EDTA-dependent PTCP. So, it has been suggested that the supplementation of aminoglycosides to samples from EDTA-dependent PTCP patients, either before or after blood withdrawal, is an easy and effective way to diagnose EDTA-dependent PTCP and accuratley evaluate the platelet count of PTCP patients. Another method for counting platelet in EDTA induced thrombocytopenia by direct peripheral smear method.

CONCLUSION

EDTA induced pseudothrombocytopenia is if suspected can be reduced by collecting blood samples in citrated tubes and manual method of counting of platelets as in our study.

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