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EDTA-Dependent Pseudo Thrombocytopenia: A Case Series

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1. INTRODUCTION

Pseudo thrombocytopenia (PTCP) is an in vitro phenomenon of platelet aggregation that results in spurious reporting of a low platelet count by automatic cell counters, which are typically EDTA dependent.[1] In general, EDTA, a calcium chelator, is considered a safe and reliable anticoagulant for a complete blood count test because of its stability in blood cell counting and sizing. However, platelet clumping occurs occasionally. People with malignancy, chronic liver disease, infection, pregnancy, autoimmune diseases, and cardiovascular diseases have an increased risk of EDTA dependent PTCP. It has also been observed in disease-free patients.[2,3] Platelet clumping in the presence of EDTA is caused by an autoantibody against glycoprotein IIb/IIIa located on the cell membrane of platelets.[4,5] Although other anticoagulants such as heparin and sodium citrate rarely induce such a phenomena, it is possible.[5,6] Misdiagnosis of PTCP leads to unnecessary diagnostic tests and treatments, such as bone marrow biopsy, holding surgery, splenectomy, steroid therapy, and platelet transfusion.[7] Therefore, when a low platelet count is noted, PTCP must also be considered. In this study, we report 4 cases of EDTA dependent pseudo thrombocytopenia.
Case 1:

A 56 year old female came for total knee replacement surgery. On examination, she had bilateral osteoarthritis knee with right more than left. Clinician advised CBC and peripheral smear evaluation. Sample from the antecubital vein was collected in BD K2 EDTA vacutainer under all precaution. CBC counts were all with in normal limits except for platelet count. Hemoglobin 11.6 g/dl., total leucocyte count 9000/mm^3 and platelet count 51000/$mm^{3.}$ Peripheral smear was done and stained with Leishman stain on examination smear shows numerous clumps of platelets (Figure 1).

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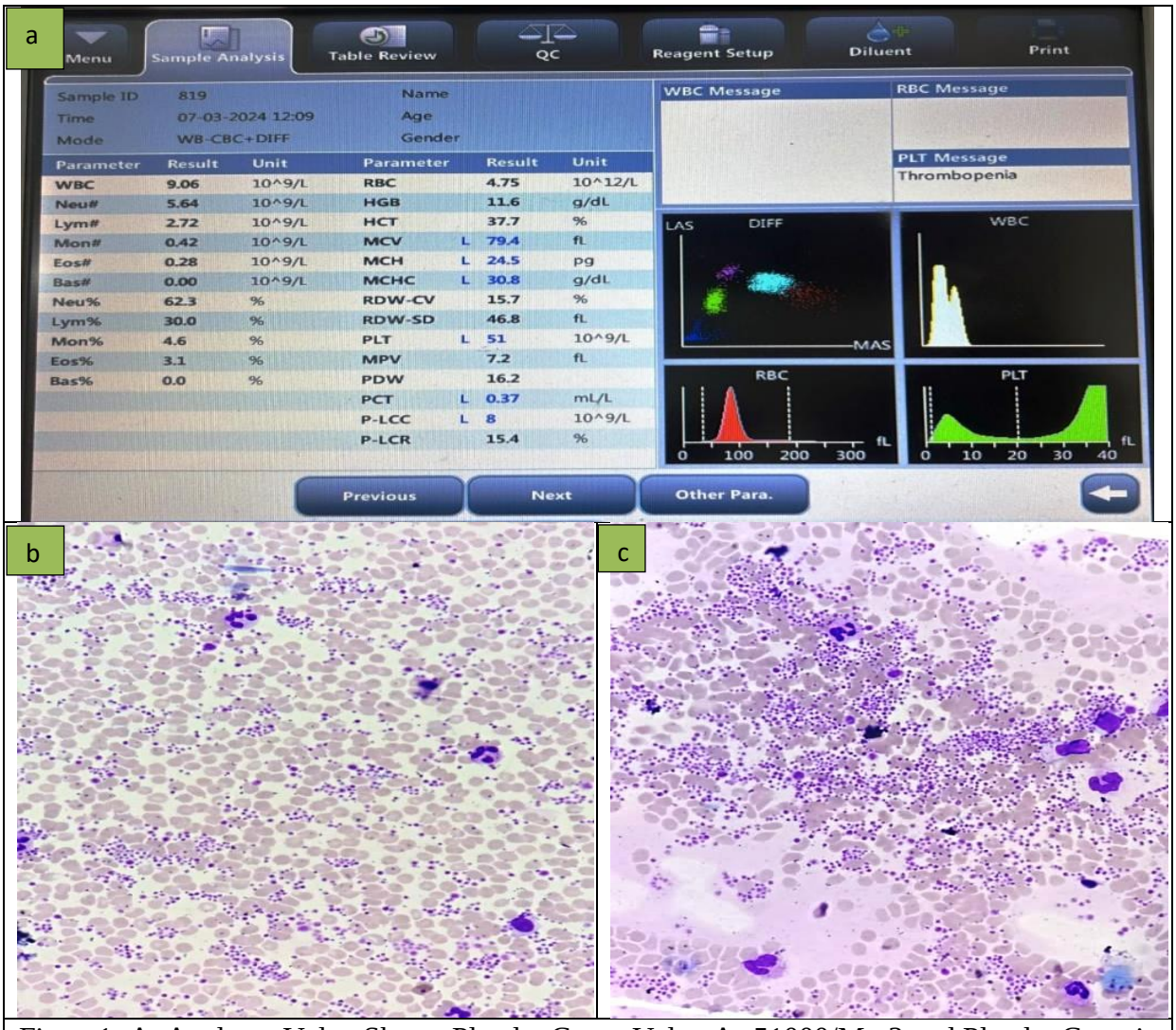

Figure1: A. Analyser Value Shows Platelet Count Value As 51000/Mm3 and Platelet Curveis Depressed with a Spike after 20µ.B &C. Peripheral Smear with Numerous Platelet Clumps with Occasional Giant Platelets.

Case 2:

A 47year old female patient with extensor tendon injury right hand came for OPD, clinician asked CBC and peripheral smear. CBC value shows haemoglobin 11.5g/dl, total leucocyte count 7700/mm3 and platelet count 65000/mm3 on analyser. Platelet curve is depressed with double peak showing spike at 20µ peripheral smear shows normocytic normochromic anemia with few microcytes and numerous platelet clumps (Figure2).

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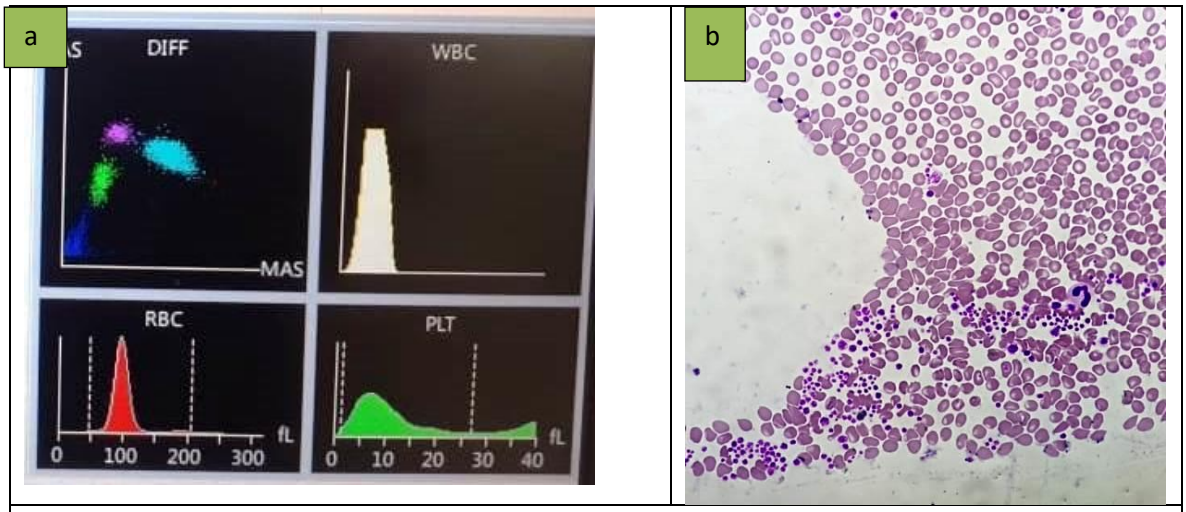
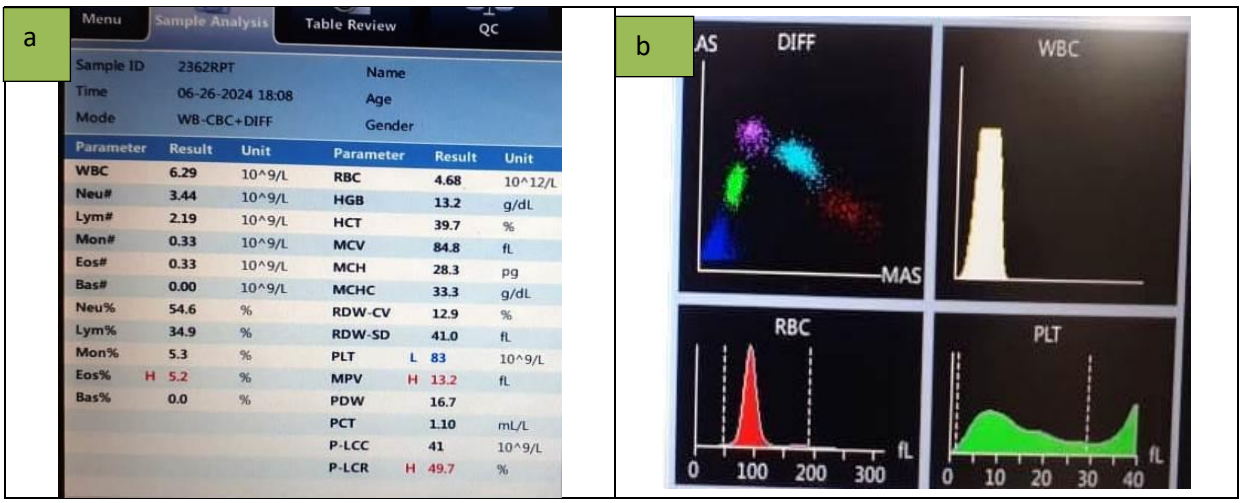


Figure2:A.Platelet Curve is Depressed with Double Peak Showing Spike at 20µ.B. Peripheral Smear Shows Numerous Platelet Clumps.

Case 3:

A 31 year male patient came to OPD with a clinical diagnosis of constriction band left leg, clinician asked CBC and peripheral smear. CBC analyser shows hemoglobin of 13.2g/dl, total leucocyte count 6200/mm3 and platelet count value as 83000/mm3, platelet curve is depressed with double peak showing spike at 20µ. Peripheral smear with numerous platelet clumps with occasional giant platelets (Figure 3).



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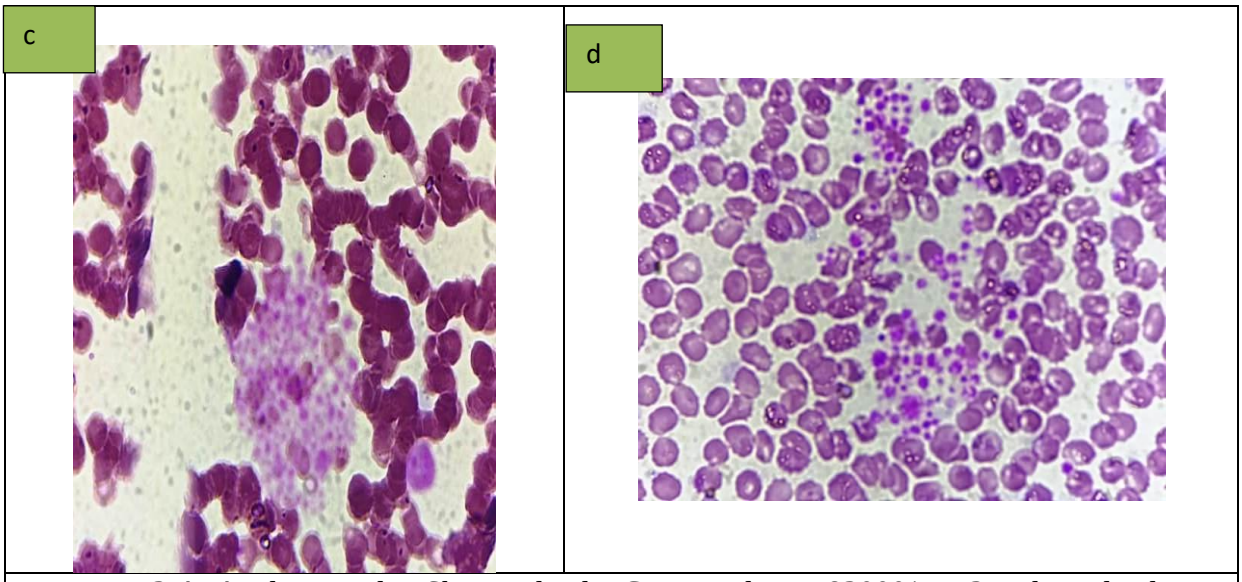
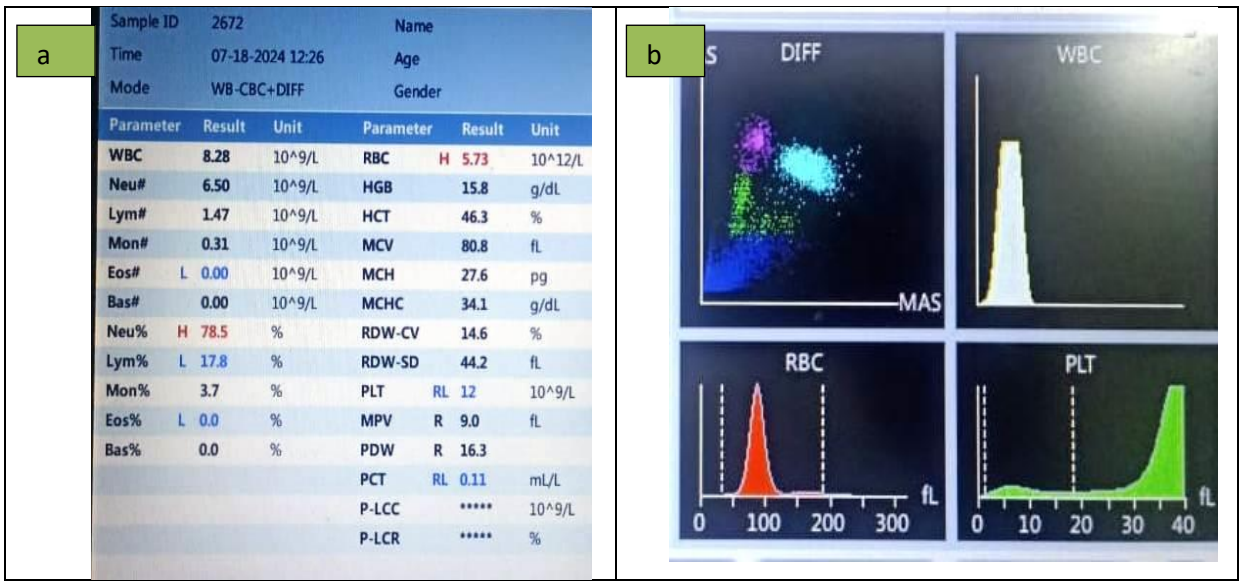


Figure 3:A. Analyser Value Shows Platelet Count Value as 83000/Mm3 and B. Platelet Curveis Depressed with Double Peak Showing Spike at 20µ .C, D... Peripheral Smear with Numerous Platelet Clumps with Occasional Giant Platelets.

Case 4:

A 31 year old male came to OPD and diagnosed as fourth metacarpal fracture, After examination clinician advised CBC and peripheral smear evaluation. Sample from the antecubital vein was collected in BD K2 EDTA vacutainer under all precaution. CBC counts were all with in normal limits except for platelet count. Hemoglobin 15.8 g/dl.,total leucocyte count 8200/mm^3 and platelet count 12000/mm^3.Peripheral smear was done and stained with Leishman stain on examination smear normocytic normochromic picture with numerous clumps of platelets and platelet curve shows almost flat curve (Figure 4).



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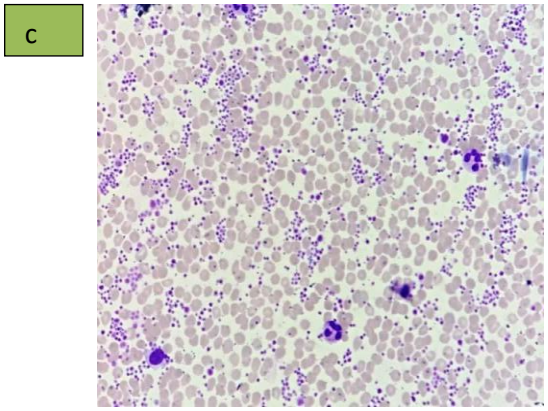

Figure 4:a. Analyser values shows platelet count as 12000/mm3 b.platelet curve shows almost flat curve. c. peripheral smear shows numerous platelet clumps.

2. DISCUSSION

EDTA-induced pseudo thrombocytopenia (EDTA-PTCP) is a laboratory phenomenon that is characterized by platelet clumping, which can be seen on a peripheral smear, and an abnormal platelet histogram with a typical saw tooth pattern. The incidence is about 0.1-0.2% in the general population,[8,9] which makes it an important differential for thrombocytopenia without any pathological causes. It is more commonly seen in women under the age of 50, and there is a slight male predominance in age groups over 50. Studies have shown that this phenomenon is not necessarily associated with any disease, and the patients do not show any clinical features of thrombocytopenia, but it can rarely be a serendipitous finding in Association with a disease or syndrome.[10] it is important for physicians to have a high degree of suspicion about this phenomenon, especially when dealing with asymptomatic patients showing thrombocytopenia on repeated tests.

A frequently asked question is the possibility of transmission of PTCP to the recipient after blood transfusion, plateletpheresis, or peripheral blood stem cell transplant. As per the available data and case reports, it has been repeatedly concluded that PTCP should not be a reason to withhold any of the aforementioned procedures and is usually not transferred to the recipient.[11] However, theoretically, there is a chance of developing PTCP in peripheral blood stem cell transplantation, especially after the development of the B-cell repertoire, which might take a significant amount of time and was observed in a case reported by Di Francesco et al.[12] Hence, it is also important to examine the patient's peripheral smear after a certain period following the stem cell transplant, which will allow mature B-cells to repopulate the recipient's bloodstream. Neonates can also display pseudo thrombocytopenia due to the transplacental transmission of maternal EDTA-dependent antibodies.[13]

There was a significant association noted between PTCP and viral infections, especially hepatitis A, followed by cytomegalovirus and H1N1 influenza (swine flu). Apart from the abovementioned scenarios, PTCP is also observed in patients with bladder cancer, autoimmune thrombocytopenia, SARS CoV2 infection, post-Oxford-AstraZeneca COVID-19 vaccine, and Graves' disease.[14]

The estimated prevalence is 0.1 to 2% in hospitalized patients and 15-17% in out-patients.[15] The antibodies described are primarily IgG, although there may be mixtures of IgA, IgM that precipitate at low temperatures.[16] Although considered an in vitro phenomenon without clinical relevance Fukuda Ohashi et al., reported that the presence of the phenomenon could

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be associated with increased mortality and malignancy.[17]It is presumed that the membrane glycoprotein IIB is the site for protein coupling of EDTA dependent antibody on the platelet membrane. Glycoprotein IIb exists alongside IIIa glycoprotein, as a heterodimer dependent of calcium. It is theorized that the dimer dissociates when the concentration of calcium decreases and it re-associates when the availability of the ion increases (Figure 3). The anti-platelet antibody epitope that causes EDTA pseudo thrombocytopenia is a crypto antigen that is only revealed when the IIb glycoprotein dissociates.[18]

Figure 5. Pathogenesis of thrombocytopenia induced by EDTA. Note the antibody interaction with the heterodimer and expression of GMP 140, gp55 and thrombospondin proteins, with subsequent aggregation and clumping. Adapted by Lippi et al. [19]

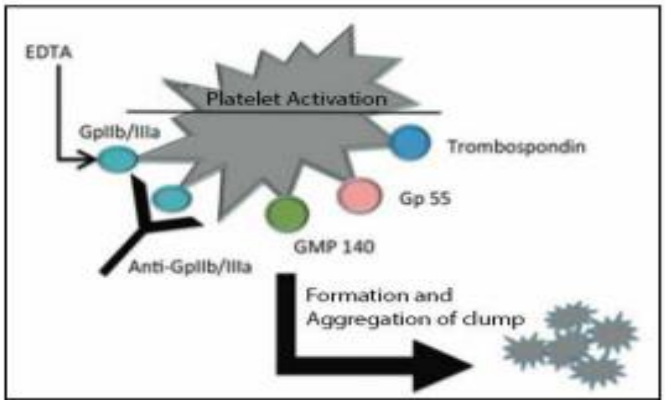

Figure 5. Pathogenesis of thrombocytopenia induced by EDTA.

By this mechanism it is assumed that EDTA, when calcium concentration decreases, it allows the exposure of the binding site of the antibody and the clumping of platelets. In fact, Hyojin et al., achieved to dissociate the platelets upon introduction of calcium chloride in the samples in vitro. 17 Recent research have clarified the type of reaction. Lippi et al. describe that autoantibodies react optimally between 0 and 4 degrees Celsius, and after binding with glycoprotein, the expression or activation of antigen the expression of several proteins is stimulated: CD62P, known as protein granulation membrane 140 or GMP140, CD63 known as lysosomal glycoprotein gp55 or type III, and thrombospondin. In the end, this triggers the tyrosine kinase, which brings together groups and platelets, reducing their count.[19]

In conclusion, the PT-EDTA should be recognized as an artifact and does not require other diagnostic studies or unnecessary transfusions. Its recognition can be assisted by a simple method, such as addition of amikacin to sample, to reverse platelet clumping. This case shows the importance of the clinical suspicion of the presence of this artifact and to discard true thrombocytopenia.

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