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Research Article

ANTICOAGULANT INDUCED PSEUDOTHROMBOCYTOPENIA - A CASE REPORT

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ABSTRACT
Pseudothrombocytopenia is a false low platelet count observed when blood samples are collected with Ethylene diamine tetra acetic acid, which is used as an anticoagulant. It is commonly identified by peripheral smear examination and can manifest with platelet clumping or rosette formation. We present two cases with pseudothrombocytopenia which was EDTA induced,the first case with platelet clump formation and the second with rosette (platelet satellitism) formation.

***Keywords*:** *Anticoagulant, Rosette, Platelet Clumping, Antiplatelet Antibodies, Platelet Satellitism*

INTRODUCTION
The normal platelet count ranges from 1,50,000 to 4,50,000 cells/mm3.Thrombocytopenia is defined as a drop in the platelet count below the lower limit of the normal range[<1,50,000 cells/mm3]. Pseudothrombocytopenia is a falsely low platelet count which can happen due to many factors. The incidence of Ethylene diamine tetraacetic acid [EDTA]induced thrombocytopenia is 1.9% in patients admitted in hospital and 0.15% in out patients (Suarez *et al.*, 1991). It may happen if the anticoagulant is not properly mixed with serum or if the quantity of anticoagulant is insufficient. It may also happen when the patient's blood sample is collected in a container where EDTA is used as anticoagulant which may induce platelet clumps or rosette(also called platelet satellitism) formation. We are presenting two case reports of patients, one who had platelet clumping with EDTA and the other with rosette formation with EDTA.

CASES
Case 1:
A 47 year old male with no significant past medical history was admitted with complaints of decreased appetite for one week duration,along with passage of two episodes of loose stools per day for two days.There was no history of fever or other localising symptoms.There was no significant past history of bleeding tendencies. General examination of the patient revealed no purpura, pethechiae or any other bleeding manifestations. Rest of the physical examination was normal.

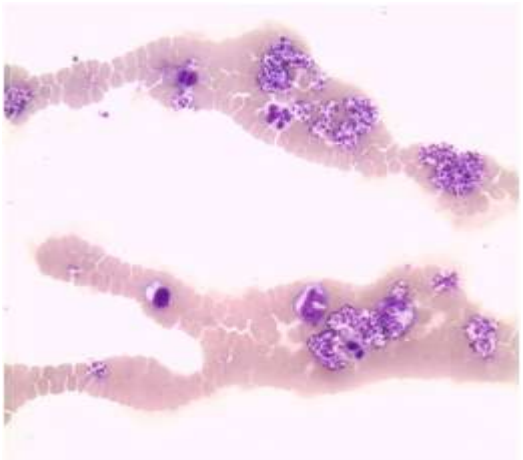

Figure 1: Multiple platelet clumps seen with EDTA blood sample

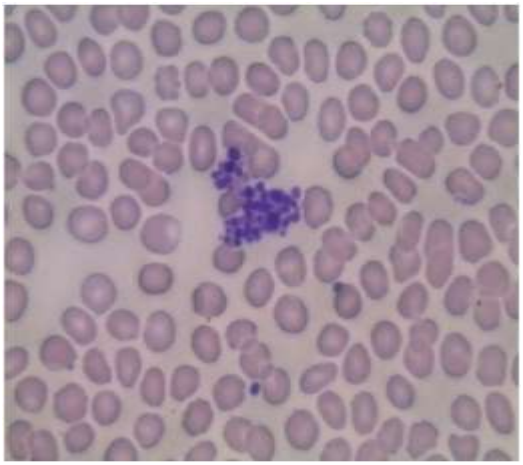

Figure 2: A giant platelet aggregate from the EDTA blood sample

The patient was investigated in an outside hospital for similar complaints and was found to have low platelet counts (27,000 cells/mm3) and was referred here for further management.The initial coulter counter platelet values was very low (7000 cells / mm3) and hence proceeded to peripheral smear examination which showed multiple platelet clumps on initial two samples collected (Figure 1 & 2). The sample was obtained in EDTA vacutainers. Repeat samples were obtained from the citrated vacutainer tube, which showed platelet clumping again (Figure 3).

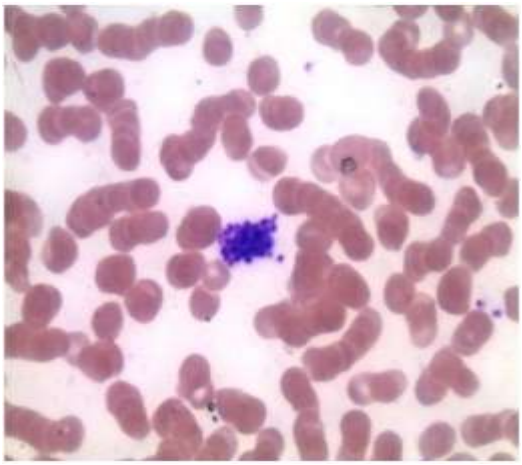

Figure 3: A giant platelet aggregate from the citrated blood sample

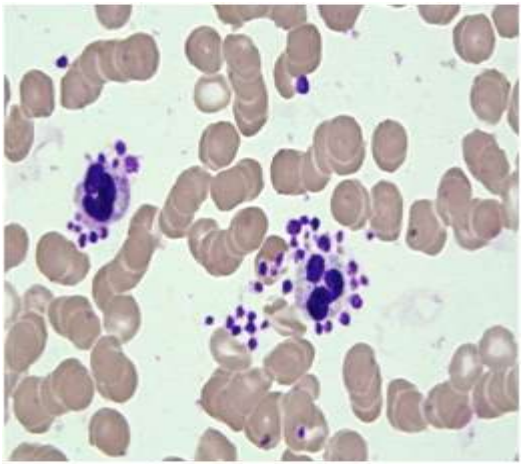

Figure 4: Platelet satellitism with neutrophil sorrounded by platelets

Hence the platelet count was repeated by direct sampling without any anticoagulant and was found to be 1,97,000cells/ mm3. The platelet count was repeated the next day and was found to be 1,88,000 cells/mm3(as direct sample). Hemoglobin was 13.8g/dl with hematocrit of 40.2% and mean corpuscular volume was normal. All other investigations including white blood cell count, serum creatinine, electrolytes, liver function tests, ultrasound abdomen, blood cultures were normal. Patient was treated symptomatically.The loose stools resolved within a day.

Case 2:

A 36 year old male patient with no significant past medical history presented with complaints of fever for 5 days. General and systemic examination showed presence of bilateral parotid swelling with no bleeding manifestations. There was no past history of bleeding tendencies.

Complete blood count showed presence of thrombocytopenia(30,000 cells /mm3). Peripheral smear showed presence of platelet satellitism (Figure 4) and repeat platelet count was normal (2,00,000 cells/mm3). White blood cell count was normal.Liver function tests showed mild derangement in aminotransferases. Renal function tests, blood sugars and serum electrolytes were normal. Blood culture was sterile. Patient was treated with antibiotics and symptomatically. Fever resolved within three days.

DISCUSSION

Whenever thrombocytopenia is detected on blood testing, pseudothrombocytopenia [PTCP] has to be excluded. Collection of blood samples with EDTA may sometimes lead to pseudothrombocytopenia due to formation of platelet clumps or platelet satellitism.This can be detected by peripheral smear examination and by collection of the blood sample with a different anticoagulant. PTCP is a benign condition and is not associated with hemorrhagic manifestations.

Field and MacLeod in 1963, upon investigating a 14 year old boy for a neurological disorder, found a new phenomenon of "Pseudothrombocytopenia" (Field *et al.*, 1963). From then on various case reports have been published and the reason for the platelet clumping was idenitified. Initially it was thought to be due

to some 'bridging substance' between the platelets and neutrophils and later was found to be due to the naturally occurring antibodies which gets attached to the altered glycoprotein platelet receptors.

Anticoagulants are routinely used in blood collection samples to prevent clotting of blood. EDTA is a commonly used anticoagulant for biochemical and hematological investigations. EDTA is a polyprotic acid with four carboxyclic acid groups (Banfi *et al.,* 2007) It also has two amine groups with lone pair electrons which play a role in calcium chelation. Calcium ions play an important role in the coagulation cascade and so calcium chelation by EDTA effectively blocks the coagulation cascade thereby preventing clotting of blood. The reason for using EDTA as an anticoagulant is because it helps in the preservation of cellular components and does not alter the cellular morphology (Banfi *et al.,* 2007). The other anticoagulants which are used include citrate and heparin. Most of the current blood investigations are carried out through the automated machines. The basic principle of automated analyzers is to assess the cells by it size.

Approximately 0.1 % of individuals have EDTA-dependent agglutinins.These agglutinins can induce platelet clumping (Vicari *et al.,* 1988). This is thought to happen because of a naturally occurring platelet autoantibody directed against an epitope which is concealed on platelet membrane glycoprotein [GP IIb/IIIa].This epitope becomes exposed by EDTA-induced dissociation of GP IIb/IIIa. The probable mechanism being, when the EDTA containing blood samples are processed in the automated processor under suitable temperature, the auto-antibodies (IgM,IgG and sometimes IgA) that are already present in the blood gets activated and attached to the glycoprotein receptor IIb/IIIa, thus producing the large clumps of giant platelets.Our first patient was a case of EDTA induced platelet clumping leading to PTCP. A clinical and epidemiological study done by Bizzaro showed that antiplatelet antibodies may persist for as long as a decade with no clinical significance other than PTCP (Bizarro 1995).This study showed that the same phenomenon could also occur when citrate was used as an anticoagulant, as was in our first case report.

Field and Macleod first described platelet satellitism in 1963 (Field *et al.,* 1963). Platelet satellitism is the phenomenon by which platelets adhere to and sorround neutrophils. It may occur when blood samples are collected with EDTA. Neutrophils are the most common cells involved in this phenomenon. Occasionally monocytes and lymphoma cells may also be involved (Cesca *et al.,* 2001) (Montague *et al.,* 2013). The mechanism of rosette formation is thought to be antibody induced, mainly of the IgG subtype(Bizarro 1995). In a study of patients with PTCP and rosette formation, analysis showed the presence of EDTA-dependent antibodies directed against both platelet membrane glycoproteins and neutrophils(Bizarro 1995). Anti-neutrophil activity was completely abolished when the sera were absorbed on normal platelets, which suggests that a single antibody is involved. Studies with monoclonal antibodies have shown that this IgG autoantibody was directed against the glycoprotein IIb/IIIa antigen of the platelet membrane, as well as the neutrophil Fc gamma receptor III. Our second patient was a case of EDTA induced rosette formation on peripheral smear leading to PTCP. Repeat peripheral smear with blood sample collected with citrate was normal.

Thus identification of PTCP is very essential as it may reduce the need for unnecessary investigations and interventions like bone marrow biopsy, steroid therapy, splenectomy and blood transfusions. These interventional measures may themselves be linked to side effects and complications and may be avoided if this condition is recognized early.

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