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RESEARCH ARTICLE

EDTA INDUCED PLATELET AGGLUTINATION (EIPA) – A CURIOUS CASE REPORT AND REVIEW

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ABSTRACT

EDTA-Induced Platelet Agglutination (EIPA) is an in vitro phenomenon whereby EDTA causes agglutination of platelets in blood samples, leading to an artificially low platelet count on automated analyzers. A misdiagnosis of Thrombocytopenia may lead to unnecessary transfusion of platelets, thereby increasing the risks of associated morbidity. EIPA is thought to be the most common cause of pseudo-thrombocytopenia (PTCP) encountered in clinical laboratories. We report a case of pseudo-thrombocytopenia in a forty six year-old-female patient who was referred as a case of Isolated Thrombocytopenia and was found to have EDTA-Induced Platelet Agglutination (EIPA) with no associated pathologies.

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INTRODUCTION

Ethylene-diamine-tetra-acetic acid (EDTA) is a well-known anticoagulant in test tubes used to collect blood samples and run most haematological tests. It inhibits clotting by removing or chelating calcium from the blood. This anticoagulant has been used to inhibit clotting in blood specimens since the early 1950s and has certain advantages over other anticoagulants (Asim *et al.* 2012, Rod S Hangerman, 2000, Narasimhan *et al*, 2014, Izhar *et al*, 2014). The most important advantage is that it does not distort blood cells, making it ideal for various haematological tests and smears. It is known to sometimes lead to erroneous results in platelet c *et al. c*ounts by automated haematological analysers, causing low platelets counts (Mues *et al,* 2002, Nagata, 1992). This relatively rare phenomenon has been identified in the normal population and also specifically with some other pathologies (Izhar *et al.*, 2014). Bartels and colleagues have demonstrated a prevalence of 0.1% in the general hospital population (Asim, *et al*, 2012, Wilkes, *et al.*, 2000). Silvestri and co-workers discovered that 15% of patients referred to a specialist unit with diagnosis of isolated thrombocytopenia were, in fact, cases of EDTA-induced pseudo-thrombocytopenia (Wilkes *et al*, 2000). EIPA has never been reported to be associated with bleeding tendencies or platelet dysfunction. Far from being a harmless curiosity, unidentified PTCP is a dangerous finding that may lead to unwarranted investigations, platelet transfusions, treatment with gluco-corticoids, and delay or cancellation of surgical procedures. We conclude in our case report that collection of the blood sample in Sodium citrate instead of EDTA prevents platelet agglutination (Wilkes *et al*, 2000). A repeat Platelet count with the blood sample collected in Sodium citrate should be recommended as the first step in follow-up of all cases of thrombocytopenia that are not associated with any other pathology.

Case Report

A 46-year-old female patient was referred to our hospital as a case of newly diagnosed thrombocytopenia. She had presented to the clinician with fever for two weeks and no other significant past medical history. Her physical and systemic examination was unremarkable.

Laboratory Findings

CBC: WBC – 4990/µL, RBC – $3.58x10^6$/µL, HGB – 11.9g/dL, HCT – 33.3%, PLT – $7x10^9$/L, MCV – 93.1 fl, MCH – 33.3 PG, MCHC – 35.8 g/dl, RDW – 14.6%. Therefore, the CBC in our lab revealed normal parameters apart from a low platelet count of 7 $x10^9$/L, which was caused by platelet clumping in EDTA that affected the automated platelet count. Other laboratory investigations and an abdominal ultrasound were also normal. A blood film was prepared that revealed platelet clumps and aggregations. A blood sample, drawn in a citrate tube, was requested to diagnose if this could be a case of EDTA-induced platelet agglutination. The platelet count in the citrate tube sample was reported as 306 $x10^9$/L, and the blood film was likewise quite different in a as compared to the EDTA sample. The case was diagnosed as EDTA-Induced Platelet Agglutination (EIPA) and the patient was discharged in healthy condition.

DISCUSSION

Although the differential diagnosis of thrombocytopenia encompasses a diverse list of aetiologies, most cases fall within two major categories: impaired platelet production or accelerated platelet destruction. However, when a patient reports for evaluation with an abnormally low platelet count in the absence of a history consistent with thrombocytopenia, pseudo-thrombocytopenia (PTCP) should be suspected (Rod S Hangerman, 2000). The term is used to define a finding of a falsely low platelet count as reported by automated

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haematology analyzers due to platelet clumping. Collection of blood samples with EDTA may occasionally cause pseudo-thrombocytopenia due to formation of platelet clumps or platelet satellitism. PTCP is a benign condition and is not associated with hemorrhagic manifestations (Narasimhan, *et al,* 2014).

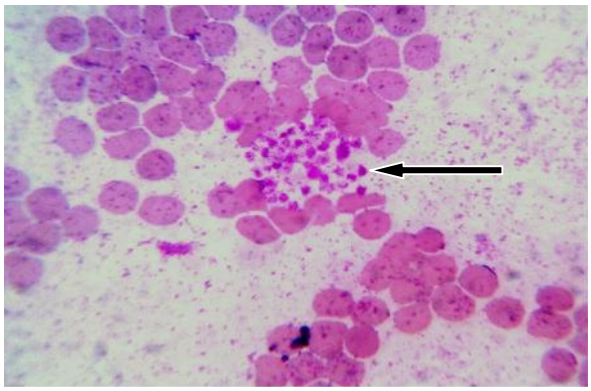

Figure 1 A giant platelet aggregate from the EDTA blood sample.

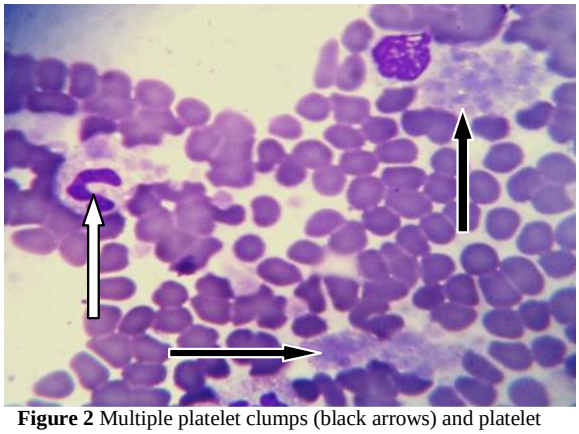

Figure 2 Multiple platelet clumps (black arrows) and platelet satellitism with a neutrophil surrounded by platelets (white arrow) from the EDTA blood sample.

'Pseudo-thrombocytopenia' was first identified and reported by Field and MacLeod in 1963, while investigating a 14 yr old boy for a neurological disorder. Platelet satellitism was also first identified and described by them in the study. Thereafter, various case reports have been published and the reason for the platelet clumping has been identified. Initially the clumping and satellitism was thought to be due to some 'bridging substance' between the platelets and neutrophils, but later it was identified to be due to the naturally occurring antibodies which get 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 haematological investigations. EDTA is a polyprotic acid with four carboxylic acid groups (Banfi *et al,* 2007). It 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). Other frequently used anticoagulants 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 a platelet membrane glycoprotein [GP IIb/IIIa]. This epitope becomes exposed by EDTA-induced dissociation of GP IIb/IIIa. Subsequently, 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 GP IIb/IIIa, thereby producing large clumps of giant platelets.

Platelet satellitism is the phenomenon by which platelets adhere to and surround neutrophils and other WBCs. Neutrophils are the most common cells involved in this phenomenon, but occasionally monocytes and lymphoma cells may also be involved (Cesca *et al,*2001, Montague *et al,* 2013). The mechanism of rosette formation is also thought to be antibody induced, mainly of the IgG subtype.

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 GP IIb/IIIa antigen of the platelet membrane, as well as the Neutrophil Fc Gamma Receptor III. The clinical and epidemiological study done by Bizzaro also showed that anti-platelet antibodies may persist for as long as a decade with no clinical significance other than PTCP. This study demonstrated that the same process could also occur when citrate was used as an anticoagulant, which was not observed in our case.

Our patient was a case of EDTA-induced platelet clumping and satellitism leading to PTCP. Repeat peripheral smear with blood sample collected with citrate was absolutely normal. The 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 (Narasimhan *et al,* 2014). Repeat platelet counts of a blood sample collected in citrate tubes may be helpful in early identification of the phenomenon.

CONCLUSION

Unrecognized pseudo-thrombocytopenia may result in unnecessary laboratory investigations and unwarranted interventions. Examination of a well prepared peripheral blood smear (platelet aggregations/ rosettes/ satellitism) is mandatory in every case of thrombocytopenia to rule out platelet clumping (pseudo-thrombocytopenia). For a definitive diagnosis of EIPA, the simultaneous collection of blood in EDTA and citrate (or other anticoagulant) containing tubes can provide a simple and rapid mean of identifying the presence of EDTA-Induced Platelet Agglutination (Asim *et al,* 2012). Given the widespread use of EDTA-containing vacutainers for blood collection, identification of PTCP requires a

high index of suspicion after the identification of thrombocytopenia in the absence of a suggestive medical history. Our patient is an example of an otherwise healthy individual who, in light of her current condition, was a suspicious candidate for EIPA. Nonetheless, an awareness of this particular condition is necessary, because its lack of recognition may lead to subjects with a normal platelet count to be considered, and at times treated, as if they were severely thrombocytopenic (Rod S Hangerman, 2000).

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