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# ETHYLENEDIAMINETETRAACETIC ACID-DEPENDENT PSEUDOTHROMBOCYTOPENIA IN PATIENT PREVIOUSLY TREATED AS DENGUE FEVER

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***Abstract: One of the most widely used anticoagulants for a complete blood count is ethylenediaminetetraacetic acid (EDTA).*** *Pseudothrombocytopenia (PTCP) may be caused by EDTA, this condition may lead to inappropriate diagnosis and treatment. We report a 25-year-old female with unspecific headache and joint pain with very low platelet count since 1 month before hospital admission. She was diagnosed with Dengue fever infection and got some platelet transfusion from the previous secondary hospital. She was carried out for a blood test with another anticoagulant (sodium citrate) and bone marrow aspiration. The results showed that she had normal platelet count and bone marrow cellularity. When a patient was identified with thrombocytopenia without any bleeding manifestation, hematology disease, and family history, PTCP should be taken into consideration to prevent unnecessary intervention.*

***Keywords:*** *platelet, pseudothrombocytopenia, ethylenediaminetetraacetic acid, Dengue fever*

## INTRODUCTION

Complete blood count (CBC) is one of the most frequently performed tests for the patient in the laboratory. Ethylenediaminetetraacetic acid (EDTA) is the most suitable anticoagulant for cell count and leukocyte differentiation and has become a standard anti-coagulant for an automatic cell counter. The advantages of EDTA are the irreversible calcium-complexing feature to prevent blood clotting, fluid component stabilization of the blood sample, and stable staining features for white blood cells. Those features are important for electronic counting.However, EDTA has some limitations such as time-dependent cell swelling and the risk of negligible pseudo-thrombocytopenia (PTCP). This PTCP was first reported by Gowland in 1969. False low platelet count results are associated with anticoagulants from in vitro spontaneous aggregate formation. This phenomenon is not a clinical diagnosis, but a laboratory diagnosis of EDTA incompatibility.[1,2]

PTCP has been reported in patients with numerous diseases, including viral infection, cancer, sepsis, autoimmune diseases, and normal healthy person.[3–5] The prevalence of PTCP affected by EDTA depends on the subject population. In the outpatient setting, PTCP ranges between 0.07 to 0.1% and up to 2% in outpatients. The prevalence of men and women are comparable.[1,2] In the thrombocytopenic patient population, a higher prevalence was found ranges between 1-15%. PTCP rate is relatively high in these thrombocytopenic patients show the need for a primary diagnosis to detect platelet aggregation in vitro depends on anticoagulants.[1,6]

## CASE REPORT

A 25-year-old female with unspecific headache and joint pain with very low platelet count since 1 month before hospital admission. She had no history of bleeding tendency, rash, bruise, or red spots on her body. There was no family history related to her condition. She was diagnosed with Dengue fever infection and got some platelet transfusion from the previous secondary hospital. She was referred to a tertiary hospital with a low platelet condition (6,000/$\mu$l) and immune thrombocytopenia suspect.

She did not have any significant complaints in the ward of the tertiary hospital. Her vital signs were within normal limits. There was no splenomegaly nor liver enlargement. At first admission, she had been tested for routine CBC with EDTA. The results showed that hemoglobin 12.5 g/dl, leukocyte 8,250/$\mu$l, and platelet 2,000/$\mu$l. The blood smear from the clinical pathologist revealed that there were some platelet clumpings with EDTA anticoagulation (Figure 1). She was carried out for a blood test with another anticoagulant (sodium citrate) and bone marrow aspiration (Figure 2). The results showed that she had a normal platelet count (394,000/$\mu$l) and normal bone marrow cellularity.

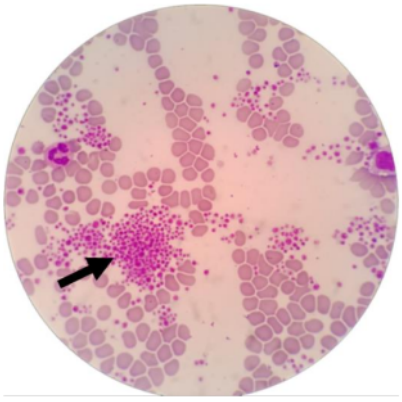

Figure 1. Platelet clumping with EDTA (2,000/$\mu$l)

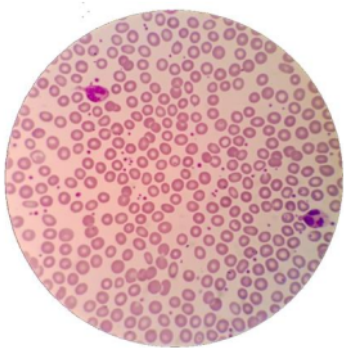

Figure 2. Normal platelet count with sodium citrate (355,000/$\mu$l)

## DISCUSSIONS

The accuracy and reliability of platelet count measurement are essential for diagnosis and management.[7] PTCP may be recognized as a pre-analysis problem, the suspected low platelet count reported by the laboratory provides important findings in the patient management, leading to incorrect diagnosis and clinical decision. Several cases of misdiagnosis or mistreatment of patients due to low platelet counts have been reported; most had a little clinical impact, but others were significant. This patient had inappropriate platelet transfusion caused by misdiagnosis.[8] Several effects of this diagnosis and therapy mistake are as follows:[1,9]

- Patients with infectious diseases such as dengue fever, mononucleosis, COVID-19, or HIV may have PTCP. This condition should be distinguished from true thrombocytopenia due to immunological phenomena of infection, splenic sequestration, or antiretroviral drugs from PTCP because it may affect the diagnosis, treatment, and prognosis of patients.
- Diagnosis of thrombocytopenia without an obvious cause may lead to unnecessary platelet transfusions, delay in invasive cardiology therapy, or delay in antiplatelet or anticoagulant treatment in patients with cerebrovascular and cardiovascular disease.
- Suspected thrombocytopenia due to drugs can lead to discontinuation of the drug. In cases of suspected heparin-induced thrombocytopenia, heparin therapy will be discontinued. In cases of false thrombocytopenia, heparin is irreplaceable for the patient.
- Thrombocytopenia cases due to viral infection may cause misdiagnosis and suboptimal therapy.
- In cases of suspected immune thrombocytopenia, misdiagnosis can occur and make steroid therapy disturbance. For patients with definite immune thrombocytopenia, PTCP may be considered resistant so that splenectomy is required. This is potentially harmful to the patient.

Platelets are aggregated when activated under physiological conditions, such as by drawing blood after a vein or artery blockage. Proper anticoagulant tubes for blood sampling techniques and careful sample mixing are required to avoid in vitro platelet aggregation (clumping).[1,10]

This PTCP phenomenon happens within the presence of platelet aggregation due to anti-platelet antibodies with EDTA anticoagulation. EDTA may cause platelet aggregation in vitro by binding to GpIIB/IIIa receptors on the platelet surface. The EDTA-PTCP occurs due to platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa) conformational change.[4,11] This condition makes IgM or IgG autoantibodies bind to GPIIb/IIIa, leading to platelet aggregation. EDTA makes magnesium, calcium, and other divalent cations complex irreversible. Calcium is required to preserve the heterodimeric structure in Gp IIb/IIIa. It is considered that EDTA-related calcium cascade activation causes interaction between Ca and platelet antibodies by evacuating Ca

from GpIIb or GpIIIa official destinations. The calcium decrease by EDTA may induce some irreversible changes to the platelet membrane conformation. PTCP due to anticoagulation is activated by antibodies that cross-react with epitopes platelet receptor GPIIb/IIIa. This binding site is exposed by EDTA-related activation and platelet membrane changes; this phenomenon reveals the covered up platelet receptor epitopes of the fibrinogen GPIIb/IIIa receptor. These epitopes are considered as antigens that bind to non-pathogenic autoantibodies and make platelet aggregation.[1]

Bizzaro used the indirect immunofluorescence assay to show that in PTCP samples, the antibodies came from different immunoglobulin classes mostly IgG.[12] The origin of the nonspecific antibodies involved has not been able to explain the reasons for production and their sporadic occurrence, but it is suspected that there is a seasonal fluctuation of PTCP. The antibodies may react with phospholipids and suggest that they can bind to EDTA-modified antigens on the platelet membrane and responsible for the genesis of PTCP.[1]

The platelet aggregation due to anticoagulants in vitro is time-dependent. The decrease in the normal platelet count which was initially high becomes low in the first 2 hours after the blood draw. Anticoagulant-induced aggregation may occur after a 2-hour delay.[1] The platelet aggregation induced by EDTA begins with intracellular and extracellular calcium complex formation. The rate of platelet reduction depends on the time from which the sample was stored until measurement. This condition explains the term "pseudo-thrombocytopenia", although the value is still more than 150,000/$\mu$L. A spontaneous in vitro platelet aggregation can also be caused by other anticoagulants such as oxalate, citrate, and the thrombin inhibitors heparin so some authors write "PTCP-induced anticoagulant".[1,13]

Sodium citrate as an alternative anticoagulant can tie calcium ions reversibly, which is critical for the coagulation process. Citrate is important for the plasmatic coagulation test in laboratory diagnostics.[14] Some methods are developed to optimize the platelet counting in EDTA-anticoagulated blood. Some of them are Mindray SF-Cube technology and BC-6800 hematology analyzer with optical fluorescence platelet counting.[15,16]

Some preanalytical factors may affect platelet variabilities such as bad blood venous sampling, improper tourniquet application, and wrong patient posture. These factors should be avoided.[17] Patients with a low platelet count should be identified for any bleeding tendency, bruise, or red spots to rule out the diagnosis. History of hematology disease and family history should be checked. PTCP should also be considered if there are no signs and symptoms. If PTCP caused by EDTA is suspected, a blood sample of the patient with tubes containing other anticoagulants must be considered.[17,18]

The laboratory may sometimes ignore events of EDTA-induced PTCP so that the physician will get the wrong platelet count and potentially give the patient incorrect diagnosis and treatment. If there is no evidence that the patient has bleeding signs (e.g., bruise, bleeding gums, or prolonged bleeding), PTCP should be ruled out before assuming true thrombocytopenia. Regular evaluation of the platelet count may facilitate an accurate diagnosis of PTCP.[1,3] Some literature proposed diagnostic criteria for PTCP induced by EDTA:[2,19,20]

1. Platelet count <100,000/$\mu$l
2. Platelet aggregation in EDTA blood samples
3. Sample collection with EDTA tubes at room temperature

4. Time-dependent platelet count decrease in EDTA blood samples
5. There was no sign and symptom related to low platelet counts

One of the most common features of Dengue infection is thrombocytopenia. Misdiagnosis of this condition is potentially harmful to the patient. It may lead to unnecessary diagnosis and management. Some authors report the importance of peripheral blood smears especially in Dengue fever to confirm the thrombocytopenia condition.[21]

## CONCLUSIONS

PTCP doesn't have any clinical significance, but in Dengue fever cases, it may lead to overuse of diagnostic workup and treatment. When a patient was identified with thrombocytopenia without any bleeding manifestation, hematology disease, or family history, PTCP should be considered to prevent unnecessary intervention.

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