Anticoagulant-Induced Pseudo-Thrombocytopenia: A Case Report

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ABSTRACT

We report a case of Pseudo-Thrombocytopenia among an eleven years old female patient who was referred as a case of Isolated Thrombocytopenia. Complete blood count revealed thrombocytopenia; a blood film was prepared and showed numerous platelet aggregations. Complete Blood Count was repeated in different tubes with different anticoagulants and also showed thrombocytopenia.
Cases of thrombocytopenia should be carefully examined; the peripheral blood morphology should be one of the first tests to be ordered to exclude Pseudo-Thrombocytopenia as well as other hematological disorders.

Key words: Thrombocytopenia, Pseudo-Thrombocytopenia, Peripheral blood.

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Introduction

Ethylenediaminetetraacetic acid (EDTA) is a well-knownanticoagulant in test tubes used to run most hematological 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 it has certain advantages over other anticoagulants.[1] EDTA most importantadvantage is that it does not distort blood cells, making it ideal for the most hematological tests.

Associated with this anticoagulant is a phenomenon that is very well known to cause erroneous results of platelet counts by automated hematological analyzersyielding low platelets counts.[2] This relatively rare phenomenon has been found in the normal population as well as being associated with some disease entities.[3] It occurs with an incidence of approximately 0.1% of the normal population,[4] and in such cases it has never been reported to be associated with bleeding tendency or dysfunction of platelets.

The false result of a platelet count may lead to surgical procedures being cancelled,[5] or rarely invasive measures such as unnecessary splenectomies, where the importance of diagnosing this rare phenomenon becomes a necessity.

We present a case of an eleven years old female patient who was presented as a case of Isolated Thrombocytopenia and found to have EDTA induced Pseudo-Thrombocytopenia with no associated pathologies.

Case Report

An 11 year-old-female patient was referred to the Prince Hamzah Hospital (PHH) as a case of newly diagnosed thrombocytopenia. She presented with fever and Upper Respiratory Tract Infection (URTI). Full Complete Blood Count (CBC) was performed as part of basic investigative tests and came up with normal parameters except for the platelet count which was extremely low; 4 x10^9/L. A blood film (Fig. 1) was ordered and it revealed platelet aggregations. Other laboratory investigations came back as normal kidney function tests, liver function tests as well as brain CT scan and abdominal US.

Despite the blood film finding, the case was still referred as thrombocytopenia to the hematology

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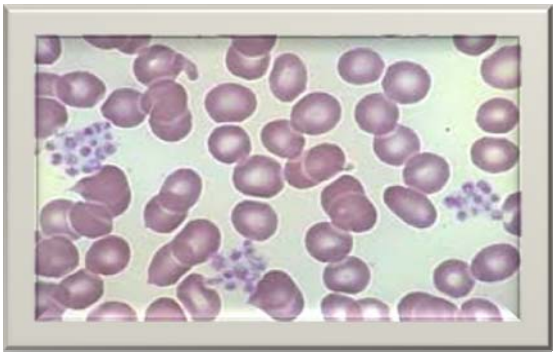

Fig. 1: Peripheral blood film showing platelets aggregations

Table I: Mechanism of thrombocytopenia

Decreased production
Decreased proliferation
Marrow infiltration – tumor, fibrosis, granulomas
Marrow hypoplasia – toxins, drugs, radiation, infection
Congenital disease – fanconi's pancytopenia, cyclic thrombocytopenia, congenital rubella
Ineffective thrombopoiesis
Megaloblastic - preleukemia, Di Guglielmo's Syndrome
Hereditary – Wiskott-Aldrich syndrome, May-Hegglin anomaly
Increased Destruction
Immunologically mediated
Drug induced thrombocytopenia heparin, quinidine, sulfa drugs
Idiopathic thrombocytopenia purpura
Post-transfusion purpura
Post-infections thrombocytopenic
Non-Immunologically mediated
Disseminated intravascular coagulation
Hemolytic-uremic syndrome
Thrombotic thrombocytopenic purpura
Cavernous hemangioma
Dilutional thrombocytopenia
Sequestration (hypersplenism)

clinic at PHH. In the clinic, the hematologist took a history which was unremarkable apart from a recent URTI. Physical examination was unremarkable. No purpura, no bleeding, no organomegaly or lymphadenopathy. The CBC revealed normal parameters apart from a low platelet count; 9 x10^9/L and another blood film revealed clearly aggregated platelets. Additionally, the physician asked for coomb's test and tests for autoimmune disease and all came up to be negative.

The lab asked for CBC to be drawn in a citrate tube as well as a Lithium Heparin tube to diagnose if this could be an EDTA induced platelet clumping and if that is the sole cause. The CBC data and the blood film results were the same in both tubes and similar to the EDTA tube results except that the count was higher with the Heparin tube; 45 x10^9/L.

The case was diagnosed as EDTA induced Pseudo-Thrombocytopenia and the patient was discharged in healthy condition.

Discussion

Causes of thrombocytopenia fall into two major categories, either impaired platelet production or accelerated platelet destruction.[6] The mechanisms of thrombocytopenia are furtherly detailed is shown in Table I.[7] The term Pseudo-Thrombocytopenia is used to define a state with a falsely low platelet count reported by automated hematology analyzers due to platelet clumping or aggregation.[8]

This clumping is caused by a change in the surface glycoproteins of the platelets when they are incubated with a calcium chelating agent such as EDTA anticoagulant. These altered platelet antigens then react to anti-platelet immunoglobulins,[9] most of which have been described as IgG and react best at 37°C, butIgM and IgA antibodies have also been described,[10] to form large agglutinates.[9] The antibodies involved in EDTA-induced agglutination are most likely reactive with GP IIb/IIIa on the platelet surface.[10]

Some resources state that clumping of platelets in patients with EDTA- induced Pseudo-Thrombocytopenia can be prevented by the use of other anticoagulants such as Sodium Citrate or Heparin; other resources have found that even these agents can induce platelet clumping as well. This is comparable to the results in our study.

We drew blood for the patient in three tubes containing three different types of anticoagulants; EDTA, Sodium Citrate and Lithium Heparin. The three blood samples were run in Coulter Hmx fully automated Hematology analyzer which is based on laser technology. Immediately after processing, three slides were performed to inspect peripheral blood morphology. The platelet count with the EDTA tube was 4.0 x10^9/L, with the Sodium Citrate tube was 10.0 x10^9/L and with the lithium Heparin tube was 45 x10^9/L. The peripheral blood morphology for the three blood samples revealed platelets aggregations.

It has been stated that Pseudothrombocytopenia is associated with blood specimens collected in anticoagulants such as EDTA, sodium citrate, heparin, and sodium fluoride.[10] A prospective study of 20,761 routine clinical blood specimens was

conducted to evaluate the incidence and causes of thrombocytopenia.[12] It revealed that the incidence was 0.15% and 72% of which had EDTA induced platelet clumping sometimes associated to Sodium Citrate and 28% had spuriously low platelet counts due to large platelets.

One study demonstrated that Theophylline can be useful in the investigation of Pseudo-Thrombocytopenia when an automated cell counter was used. It states that when Theophylline was used as an anticoagulant in blood samples showing aggregation with EDTA, blood film examination revealed no platelet clumping.[12]

So, clumping can be seen with many types of anticoagulants. Such case have been reported and labeled as multianticoagulant dependent Pseudo-Thrombocytopenia.[13]

Conclusion

Unrecognized Pseudo-Thrombocytopenia may result in unnecessary laboratory testing and unwarranted interventions. Examination of a well prepared peripheral blood smear is mandatory for every case of thrombocytopenia to rule out platelet clumpings (Pseudo-Thrombocytopenia). For a definitive diagnosis of EDTA Induced Thrombocytopenia, the simultaneous collection of blood into EDTA- and citrate-containing or other anticoagulant containing tubes may provide a simple, rapid mean of identifying the presence of EDTA- Induced Pseudo-Thrombocytopenia .

References

1. **Gordan HG, Larson NL**. Use of sequestrene as an anticoagulant. *Am J Clin Pathol* 1955; 23:613-618.
2. **Mori M, Kudo H, Yoshitake S, *et al*.** Transient EDTA-Dependent Pseudothrombocytopenia in a patient with sepsis. *Intensive Care Medicine* 2000; 26, 218-220.
3. **Allerheiligen D, Houston R, Vermedahl B.** EDTA-Induced Pseudothrombocytopenia. *Jornal of the American Board of Family Practice* 1996; 9: 212-214.
4. **Undersea Dive RSHDO.** Ethylenediaminetetraacetic acid dependent Peudo-Thrombocytopenia, a case report of an incidental but important finding. Medicine on-line 2000; 12: 120-123.
5. **Matarazzo M, Conturso V, Di Martino M, *et al*.** EDTA-Dependent Pseudothrombocytopenia in a case of Liver Cirrhosis. *Panminerva Medica* 2000; 42: 155-157.
6. **Warkentin TE, Kelton JG.** Management of thrombocytopenia. In: Colman RW, Hirsh J, Marder VJ, Sazman EW, eds. Hemostasis and Thrombosis: Basic principles and clinical practice. Philadelphia: JB Lippincott 1994; 469-88.
7. **Dalamangas LC, Slaughter TF.** Ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia in a cardiac patient. AnesthAnalg 1998; 86: 1210-1211.
8. **Bartels P, Schoorl M, Lombarts A.** Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. *Scand J Clin Lab Invest* 1997; 57: 629-636.
9. **Sesok-Pizzini D, Bissell M.** Clinical abstracts, American College of Pathologists, March 2012
10. **Bizzaro N.** EDTA-dependent Pseudo-Thrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. *American Journal of Hematology* 1995; 50: 103-109.
11. **Gracia Suarez J, Merino JL, Rodrigeus M, *et al*.** Pseudo-Thrombocytopenia: Incidence, causes and methods of detection. *Sangre (Barc)* 1991; 36(3): 197-200.
12. **Ohnuma O, Shirata Y, Miyazawa K.** Use of theophylline in the investigation of Pseudo-Thrombocytopenia induced by EDTA. *J Clin Pathol* 1988; 41(8): 915–917.
13. **Zhou X, Wu X, Deng W, Li J, Luo W.** Amikacin can be added to blood to reduce the fall in platelet count. *Am J Clin Pathol* 2011; 136:646-52 .