Case Report

F. Sinem Hocaoglu-Emre* and Berna Yavuz Aksu

A pediatric case of EDTA-related pseudothrombocytopenia and implementation of the histograms by the automated cell counters

EDTA ile ilişkili Pediatrik Bir Psödotrombositopeni Olgusu ve Otomatik Hücre Sayıcı Histogramlarının Değerlendirilmesi

<https://doi.org/10.1515/tjb-2020-0584>Received November 21, 2020; accepted March 24, 2021;
published online September 6, 2021

Abstract

Objectives: Platelet aggregation in the presence of ethylenediaminetetraacetic acid (EDTA), is called EDTA-related pseudothrombocytopenia (EDTA-PTP), resulting in low platelet count by automatic cell counters.

Case presentation: Herein, we present a case of a 5-year-old female, who was referred to our laboratory due to persistent thrombocytopenia.

Conclusion: Our case report discusses the efficiency of the histograms and flag warnings of the cell counter, indicating the importance of these variables.

Keywords: EDTA-related pseudothrombocytopenia; EDTA-related thrombocytopenia; platelet aggregation; pre-analytical phase; pseudothrombocytopenia.

Öz

Amaç: Etilendiamintetraasetik asit (EDTA) varlığında trombosit agregasyonu, EDTA ile ilişkili psödotrombositopeni

(EDTA-PTP) olarak adlandırılır ve otomatik hücre sayıcılarda düşük trombosit sayısı sonuçları ile karakterizedir.

Olgu sunumu: Yazımızda persistan trombositopeni nedeniyle laboratuvarımıza sevk edilen 5 yaşında bir kız çocuğunu sunuyoruz. Farklı antikoagülan içeren tüpler ile yapılan trombosit sayımlarında trombositopeni gözlenmemiştir.

Sonuç: Olgu sunumumuz, hücre sayıcılar tarafından oluşturulan histogramlar ve flag uyarılarının dikkate alınmasının önemini ortaya koymaktadır.

Anahtar sözcükler: preanalitik evre; trombosit agregasyonu; EDTA ile ilişkili trombositopeni; EDTA ile ilişkili psödotrombositopeni; psödotrombositopeni.

Introduction

Platelet aggregation in the presence of ethylenediaminetetraacetic acid (EDTA), a widely used anticoagulant specifically for complete blood count (CBC) analysis is called EDTA-related pseudothrombocytopenia (EDTA-PTP). EDTA-PTP results in low platelet count by automatic cell counters, especially when the sample was analyzed a while after the phlebotomy procedure. Hematology analyzers might count the platelet clumps as lymphocytes, and increased white blood cell (WBC) count might occur occasionally [1].

Although EDTA-PTP is not a rare phenomenon with its 15–17% incidence in patients evaluated for thrombocytopenia, the diagnosis may still delay unless a peripheral blood smear examination was performed [2, 3]. Thus, EDTA-PTP should be considered when a low platelet count

*Corresponding author: F. Sinem Hocaoglu-Emre, Department of Clinical Chemistry, Duzen Laboratories Group, Istanbul, Turkey, Phone: +905065030680, E-mail: sinemhocaoglu@yahoo.com.
<https://orcid.org/0000-0003-2557-7378>

Berna Yavuz Aksu, Department of Clinical Chemistry, Duzen Laboratories Group, Istanbul, Turkey

without any other clinical symptoms of thrombocytopenia is present. In this report, we describe a pediatric case of EDTA-PTP.

Patients and methods

Case

A five-year-old female of Caucasian origin was referred to our laboratory by a pediatric hematologist because persistent thrombocytopenia was detected in the CBC results for her routine medical examination. She was asymptomatic, with no history of recent infection, a tendency to bleeding, petechia, purpura, drug use, or chronic disease. Previous CBCs demonstrated platelet count results varying between 60 and $110 \times 10^3/\text{mL}$ at three different outside hospitals within a week. Hemoglobin (Hb) and WBC were normal. Biochemistry tests and bleeding time with prothrombin time (PT) and activated partial thromboplastin time (aPTT) were within the reference intervals.

The patient was referred to our center for further testing for anti-streptolysin-o antibodies (ASO), rheumatoid factor (RF), antinuclear antibody (ANA), and direct Coombs analyses.

Results

Her parents decided to re-analyze her blood for CBC in our center, and the platelet count immediately performed after phlebotomy was $247 \times 10^3/\text{mL}$, and platelet clumping was present on the peripheral blood smear.

Repeat analysis of CBC 1 h after the previous test revealed a $110 \times 10^3/\text{mL}$ platelet count. EDTA-PTP was suspected by the laboratory specialist, and the parents were informed about the possible condition. The parents asked for re-testing with simultaneous citrate and heparin containing blood collection tubes. In citrate blood, platelet counts were found to be $216 \times 10^3/\text{mL}$ whereas $246 \times 10^3/\text{mL}$ platelets were counted with the heparinized blood. There were no incidents of platelet aggregation on the blood smears prepared with citrate and heparin containing samples (Figure 1).

All samples were rerun again on automated analyzer after 2 and 8 h, and citrate and heparin containing samples did not demonstrate a decreasing trend of platelets over

time (Table 1). We did not detect in increased WBC count in the analyses, which has been reported as to be possible with analyzers falsely counting platelet clumps as lymphocytes. On a subsequent day, the results for ASO, RF and direct Coombs were negative, with a low positivity of the ANA. The patient's doctor was informed about the case and the experimental analyses, and a consensual diagnosis of EDTA-PTP was confirmed.

Discussion

EDTA is a commonly used anticoagulant for blood sample collection tubes, especially for the CBC analysis. In this report, EDTA-PTP was diagnosed and confirmed by the observation of platelet aggregates in peripheral smears prepared from EDTA-containing blood samples and normal platelet counts obtained from heparinized and citrated blood of the patient alongside the WBC-differential channel (WDF) and platelet scattergram and histograms on the Sysmex XN-1000 analyzer (Sysmex Corp, Kobe, Japan).

EDTA-PTP has been widely reported in hospitalized patients since its first description in 1969 in a broad range of conditions including recent-onset infectious mononucleosis cases with anti-EBV IgM and heterophilic antibodies, splenomegaly, and usage of medications, especially chemotherapeutic agents [4, 5].

The mechanism involves the Ca chelation by EDTA under low temperature expose an epitope site on the platelet surface glycoprotein IIb/IIIa complex which is recognized by platelet agglutinating autoantibodies [6]. However, a flow-cytometry based anti-platelet antibody testing revealed a negative result (Figure 2).

When EDTA-PTP is suspected, methods employed for distinguishing EDTA-PTP from other causes of thrombocytopenia include observing the time-dependent decrease in the platelet number with serial analyses, using other anticoagulant containing blood collection tubes, examining a blood smear under a microscope in terms of the presence of platelet aggregates and clumps in EDTA-anticoagulated blood samples [7]. In their *in vitro*

Figure 1: (A) Blood smear of an EDTA sample maintained at room temperature showing platelet clumps (black arrows). No platelet aggregation in blood smear of the citrate (B) and heparin (C) anticoagulated samples (Wright Stain, 100× oil lens).

Table 1: Platelet and white blood cell counts value in different anticoagulants on 0 and after 2 and 8 h of blood collection.

Time, h	Platelet count, $\times 10^3/\text{mL}$				White blood cell count, $\times 10^3/\text{mL}$			
	EDTA	Citrate	Citrate (corrected)	Heparin	EDTA	Citrate	Citrate (corrected)	Heparin
0	247	216	238	246	6.33	5.58	6.14	6.67
2	105	184	202	202	6.20	5.71	6.28	6.46
8	93	178	196	205	6.19	5.67	6.23	6.01

Figure 2: Flow cytometry analysis for total anti-platelet antibody detection. Fluorescein isothiocyanate (FITC)-conjugated total anti-human IgG, A, M antibodies on patient and control sample platelets yielded similar results. PLT: Platelet suggesting a negative pattern for anti-platelet antibodies.

experimental study, Casonato et al. showed that incubation of platelets with 5 mM EDTA at 37 °C diminished the expression of the epitope on glycoprotein IIb/IIIa complex, and prevented the development of EDTA-PTP [6]. Other studies suggest the addition of potassium azide, kanamycin, amikacin, and other aminoglycoside type antibiotics and anti-platelet agents such as acetylsalicylic acid, prostaglandin E_1 to the EDTA-containing samples in order to prevent EDTA-PTP or to provide dissociation of platelet clumps without producing any significant effect on blood cell morphology or counts [7].

In our case, we observed a “cell ghost” accumulation on the WBC-differential scattergram obtained from the WDF channel as an indicator of platelet clumps, which was not present compared to the other anticoagulant containing samples (Figure 3). Furthermore, there was a serrated pattern of platelet histogram in the EDTA samples, pointing out the platelet aggregates, which was initially described by Nagler et al. (Figure 4) [1]. Modern hematology analyzers adopt various “flags” indicating the abnormal distribution or pattern of blood cells. In our case, a flag message as “Platelet Abnormal Distribution”, indicating abnormal

Figure 3: White blood cell differential scattergrams. Ghost cell window attributed to platelet clumps (Left).

platelet distribution was present in the analyzer screen when the EDTA-containing samples were inspected. Taken together, all these findings highlight the essential implication of CBC scattergrams and histograms supplied by the hematology analyzers when interpreting the analysis results.

Some studies reported that citrate and heparin anticoagulated blood still showed platelet clumping in 20% of the cases, and blood collection into ammonium oxalate containing tubes are also suggested [8, 9]. The relatively lower number of WBC and platelet count in citrated samples was a result of the volumetric difference in anticoagulant, and the multiplication of results by 1.1 which is suggested for correction yielded similar platelet and WBC counts as with other anticoagulant containing samples [10].

The previous platelet counts of the patients were performed in large scale hospitals, where blood collection tubes are transferred to the clinical laboratory with a porter system taking them hourly. Thus, the persistent low platelet count might be a result of the delayed sample processing due to delivery systems that allow the samples rest for a while on the blood collection units.

If undiagnosed, EDTA-PTP may lead to unnecessary examinations, diagnostic tests, and treatment, leading to an incorrect diagnosis, complications, additional medical

cost, and patient anxiety. Preliminary workup of our patient included ultrasonography of abdomen, routine biochemistry analyses, coagulation studies, immunological tests for a broad spectrum of viral and autoimmune diseases, and finally, a bone marrow biopsy was scheduled, which was subsequently canceled following the EDTA-PTP diagnosis.

Conclusion

When a patient with a low platelet count is identified, EDTA-PTP should be considered, notably in patients without a family history, tendency to bleeding and recent viral infection. EDTA-PTP should be confirmed by the investigation of the EDTA-containing sample under the microscopy after staining. Platelet aggregates in the blood smear would contribute into the diagnosis. Sampling into blood collection tubes containing other anticoagulants, routine microscopic inspection of the blood smear, interpretation and utilization of warning flags and histograms of hematology analyzers might confirm the prompt diagnosis, preventing additional health system- and patient-related costs.

Figure 4: Platelet (PLT) histograms of the EDTA (left), citrate (middle), heparin (right) anticoagulated samples. Note the serrated curve in the PLT histogram of the EDTA sample indicated with an arrow showing platelet clumps, in contrast to normal histograms.

Competing interests: None declared.

Ethical approval: None.

References

1. Nagler M, Keller P, Siegrist D, Alberio L. A case of EDTA-dependent pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon. *BMC Clin Pathol* 2014;14:19.
2. Cohen AM, Cycowitz Z, Mittelman M, Lewinski UH, Gardyn J. The incidence of pseudothrombocytopenia in automatic blood analyzers. *Haematologia* 2000;30:117–21.
3. Silvestri F, Virgolini L, Savignano C, Zaja F, Velisig M, Baccarani M. Incidence and diagnosis of EDTA-dependent pseudothrombocytopenia in a consecutive outpatient population referred for isolated thrombocytopenia. *Vox Sang* 1995;68:35–9.
4. Shi X, Lin Z, He L, Li W, Mo L, Li Y, et al. Transient appearance of EDTA-dependent pseudothrombocytopenia in a postoperative patient with sepsis: a case report. *Medicine (Baltim)* 2017;96: e6330.
5. Hsieh AT, Chao TY, Chen YC. Pseudothrombocytopenia associated with infectious mononucleosis. *Arch Pathol Lab Med* 2003;127: e17–8.
6. Casonato A, Bertomoro A, Pontara E, Dannhauser D, Lazzaro AR, Girolami A. EDTA dependent pseudothrombocytopenia caused by antibodies against the cytoadhesive receptor of platelet gpIIb-IIIa. *J Clin Pathol* 1994;47:625–30.
7. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. *Clin Chem Lab Med* 2012;50: 1281–5.
8. Ahn HL, Jo Y, Choi YS. EDTA-dependent pseudothrombocytopenia confirmed by supplementation of kanamycin; a case report. *Korean J Intern Med* 2002;17:65–8.
9. Pitkin F. Ethylenediaminetetraacetic acid (EDTA)-induced thrombocytopenia: a case report. *J Tradit Med Clin Natur* 2017; 6:1.
10. Perrotta G, Roberts L, Glazier J, Schumacher HR. Use of sodium citrate anticoagulant for routine hematology analysis on the CELL-DYN® 4000: an opportunity to enhance efficiency in the clinical laboratory. *Lab Hematol* 1998;4:156–62.