

# Pseudothrombocytopenia Case Report

## Psödötrombositopeni Olgu Sunumu

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### ABSTRACT

Pseudothrombocytopenia is a condition in which platelets are seen in sufficient numbers and aggregated in the peripheral smear although the platelet count is low in the complete blood count analysis of the patient. One of the causes of pseudothrombocytopenia is the Ethylenediamine Tetraacetic acid (EDTA) anticoagulant used for routine complete blood count samples. EDTA has a calcium chelator effect and changes the structure of the GPIIb/IIIa integrin complex found in platelets. The hidden GPIIb epitope is revealed as a result of the change in the structure of the GPIIb/IIIa integrin complex, and antibodies are formed against this epitope. These antibodies cause platelet aggregation. Due to aggregation of platelets, complete blood count analysis may give erroneous results and may be confused with true thrombocytopenia. Pseudothrombocytopenia is not a bleeding diathesis, but patients may be subjected to unnecessary diagnostic procedures and treatments. This case report was prepared to emphasize the clinical picture of EDTA-induced pseudothrombocytopenia. In our case, thrombocytopenia was observed as a result of the analysis of the EDTA whole blood count sample taken from the patient who was admitted to the internal medicine outpatient clinic for routine controls. Complete blood count analysis over the past three years also observed thrombocytopenia. It was deemed

### ÖZET

Psödötrombositopeni, hastanın yapılan tam kan sayımı analizinde trombosit sayısının düşük olmasına rağmen periferik yaymada trombositlerin yeterli sayıda görülmesi ve kümeleşmesi durumudur. Psödötrombositopeni nedenlerinden biri, rutin tam kan sayımı örnekleri için kullanılan Etilendiamin Tetraasetik asit (EDTA) antikoagülanıdır. EDTA kalsiyum şelatör etkiye sahiptir ve bu etkiyle trombositlerde bulunan GPIIb/IIIa integrin kompleksinin yapısını değiştirir. GPIIb/IIIa integrin kompleksinin yapısının değişmesi sonucunda, gizlenmiş olan GPIIb epitopu ortaya çıkar ve bu epitopa karşı antikorlar oluşur. Bu antikorlar nedeniyle trombosit kümeleşmesi gözlenir. Trombositlerin kümeleşmesi nedeniyle tam kan sayımı analizi hatalı sonuç verebilmekte ve gerçek trombositopeni ile karışabilmektedir. Psödötrombositopeni kanama diyatezi oluşturan bir durum değildir ancak bundan dolayı hastalar gereksiz tanı yöntemlerine ve tedavilere maruz kalabilmektedir. Bu olgu EDTA'ya bağlı psödötrombositopeni kliniğine vurgu yapmak için sunulmaktadır. Olgumuzda, dahiliye polikliniğine rutin kontrolleri için başvurmuş olan hastadan alınan EDTA'lı tam kan sayımı numunesinin analizi sonucunda trombositopeni görülmüştür. Geçmiş üç sene boyunca yapılmış tam kan sayımı analizlerinde de trombositopeni olduğu

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appropriate to send a complete blood count sample from the patient in a citrate tube. In the analysis of the citrated complete blood count sample obtained from the patient, it was observed that the platelet count was normal. A simultaneous peripheral smear was reported as pseudothrombocytopenia.

**Anahtar Kelimeler:** Thrombocytopenia, pseudothrombocytopenia, EDTA, complete blood count

görülmüştür. Hastadan, sitratlı tüpe alınan tam kan sayımı numunesinin gönderilmesi uygun görülmüştür. Hastadan alınan sitratlı tam kan sayımı numunesinin analizinde, trombosit sayısının normal olduğu görülmüştür. Eş zamanlı yapılan periferik yayma, psödötrombositopeni şeklinde rapor edilmiştir.

**Key Words:** Trombositopeni, psödötrombositopeni, EDTA, tam kan sayımı

## INTRODUCTION

Thrombocytopenia is a condition in which the platelet count is below 150,000/ $\mu$ L and is frequently encountered in daily practice. The grading of thrombocytopenia is as follows: mild (150,000/ $\mu$ L-100,000/ $\mu$ L), moderate (99,000/ $\mu$ L-50,000/ $\mu$ L), severe (<50,000/ $\mu$ L). However, this grading may change in some exceptional cases such as immune thrombocytopenia (1). Causes of thrombocytopenia include increased platelet destruction (disseminated intravascular coagulation, immune thrombocytopenia), sequestration (splenomegaly), decreased bone marrow production (leukemia, myelophytosis, aplastic anemia), dilutional conditions (excess fluid resuscitation). Thrombocytopenia may also occur due to infections affecting the bone marrow (HIV, Crimean Congo Hemorrhagic Fever, Hepatitis C, Malaria, EBV) and drugs (Furosemide, Penicillin, Acetaminophen, Methotrexate) (2).

Pseudothrombocytopenia is a condition in which platelets are seen in sufficient numbers and aggregated in the peripheral smear although the platelet count is low in the complete blood count analysis of the patient. Pseudothrombocytopenia is based on various etiologies including giant platelets, EDTA for the hemogram sample, platelet satellitism, and cold agglutinins (3). Giant platelets cannot be counted by the complete blood count analyzer due to their size and this can be observed in Bernard Soulier syndrome, gray platelet syndrome, May-Hegglin anomaly (MYH 9 gene mutation) which are the causes of congenital

thrombocytopenia (4). Platelet satellitism, platelets bind to leukocytes in the presence of EDTA and form rosettes. Therefore, complete blood count analyzers cannot count platelets. Platelet satellitism is mediated by IgG-type autoantibodies targeted to GpIIb/IIIa on the platelet membrane and Fc gamma receptor III on the neutrophil membrane (5). In thrombocytopenia due to cold agglutinins, platelet agglutination is independent of anticoagulants. It usually occurs at 4°C and is mediated by IgM autoantibodies directed to GpIIb/IIIa (3).

Another cause of pseudothrombocytopenia is the EDTA anticoagulant used for routine hemogram samples. Although aggregation is most commonly reported with EDTA, it can be caused by other anticoagulants such as citrate, oxalate, and heparin. Since platelet aggregates are large, the complete blood count device cannot count platelets. It has been proposed that EDTA-induced pseudothrombocytopenia is mediated by autoantibodies of IgG, IgM, and IgA subclasses formed against the epitope on glycoprotein IIb/ IIIa on the platelet membrane (3). This epitope is normally hidden in the GpIIb/IIIa membrane because ionized calcium maintains the heterodimeric structure of the GpIIb/IIIa complex. Through its calcium chelating effect, EDTA reveals the epitope hidden in the GpIIb/IIIa membrane (6).

## CASE

A 67-year-old woman with known hypertension, diabetes mellitus and coronary artery disease was

admitted to the internal medicine outpatient clinic of our hospital for routine follow-up. A blood sample was collected in a tube with EDTA for routine blood tests. The sample was analyzed with the Sysmex XN-1000 device in our hospital laboratory. The results of the complete blood count analysis of the patient are as follows: Wbc:  $8.08 \times 10^3 / \mu\text{L}$ , hemoglobin: 14.9 g/dl and platelets:  $15 \times 10^3 / \mu\text{L}$ . The patient's sample was repeated, and the platelet count was  $60 \times 10^3 / \mu\text{L}$ . On the 2nd repeat, the sample was incubated for 30 minutes, and the result was  $25 \times 10^3 / \mu\text{L}$ . Thrombocytopenia was observed in the patient's blood results since 2019. The result of the peripheral smear performed in 2019, which was reported as platelets were sufficient in number and aggregated, was evaluated. Based on this, it was reported that it would be appropriate to send the patient's sample with a tube with Na-citrate. The platelet count was  $195 \times 10^3 / \mu\text{L}$  in the Na-citrated tube sample of the patient. A peripheral smear sent simultaneously was reported as pseudothrombocytopenia.

The study was approved by the Ministry of Health Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Research Ethics Committee (Date: 12.09.2022 and Number: 146/20).

## DISCUSSION

It has been proposed that antibodies against the epitope of the GPIIb/IIIa integrin complex in platelets in the presence of EDTA cause EDTA-associated pseudothrombocytopenia. Bizzaro et al. discovered

that 81.8% of EDTA-related pseudothrombocytopenia patients had antiplatelet antibodies and 63.6% had anticardiolipin antibodies in a study on EDTA-related pseudothrombocytopenia patients (7). According to Balçık et al., 25% of patients with pseudothrombocytopenia had Heparin-Platelet Factor 4 antibody positivity (8).

EDTA-related pseudothrombocytopenia has been estimated to have a prevalence of 0.1%-2% in hospitalized patients (9). EDTA used as an anticoagulant in routine complete blood count samples has been associated with pseudothrombocytopenia in the foreground. It may be useful to use citrate, oxalate, and heparin as anticoagulants. Chaicharoen et al. showed that samples treated with kanamycin had higher platelet counts than samples processed with EDTA (10). Schuff-Werner et al. proved that magnesium sulfate could prevent aggregation in EDTA-associated pseudothrombocytopenia (11). Pseudothrombocytopenia was found to be less common in samples treated with amikacin versus EDTA, according to Engelmann et al (12). An alternative approach is to treat the complete blood count specimen with kanamycin, amikacin, or magnesium sulfate.

In conclusion, while the precise cause of EDTA-associated pseudothrombocytopenia is unknown, autoimmunity and the GPIIb/IIIa complex are thought to be important. In terms of autoimmunity, reported cases of pseudothrombocytopenia caused by Covid-19 vaccines are encouraging (13-14). The finding that pseudothrombocytopenia does not occur in Glanzmann thrombasthenia, a disorder characterized by quantitative or qualitative abnormalities of the GPIIb/

**Table 1.** Patient's complete blood count analysis results

Date	WBC $10^3 / \mu\text{L}$	RBC $10^6 / \mu\text{L}$	HGB g/dl	Anticoagulant	PLT $10^3 / \mu\text{L}$
29.07.2019	13.2	4.3	13.1	EDTA	49
01.10.2019	8.3	4.36	13.1	EDTA	57
24.12.2020	8.93	4.49	13.2	EDTA	52
26.06.2021	4.18	4.39	12.8	EDTA	41
31.03.2022	13.77	4.41	13.5	EDTA	7
09.08.2022	8.08	4.12	12.1	Na-citrate	195

IIIa complex, lends support to the GPIIb/IIIa complex's critical role (15). Pseudothrombocytopenia and true thrombocytopenia can be confused. As a result, patients may be subjected to unnecessary diagnostic

and treatment procedures. Patients with isolated thrombocytopenia without signs of thrombocytopenia such as bleeding, ecchymosis, or petechiae should be evaluated for pseudothrombocytopenia.

### ETHICS COMMITTEE APPROVAL

\* The study was approved by the Ministry of Health Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Research Ethics Committee (Date: 12.09.2022 and Number: 146/20).

### CONFLICT OF INTEREST

The author declares no conflict of interest.

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