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CASE REPORT

EDTA-DEPENDENT PSEUDOTHROMBOCYTOPENIA: CASE REPORT

EDTA'YA BAĞLI PSÖDOTROMBOSİTOPENİ: OLGU SUNUMU

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ABSTRACT

EDTA-dependent pseudothrombocytopenia is the mismeasurement of the thrombocyte count in EDTA anticoagulated blood, due to aggregation of these cells. The situation may be mistaken with true thrombocytopenia and may lead to incorrect diagnosis and treatment, and to loss of power and time. The aim of this case report was to draw attention to EDTA-dependent pseudotyhrombocytopenia, which is one of the possible causes of thrombocytopenia. Case; isolated thrombocytopenia was detected in the EDTA blood sample sent to our laboratory, which had been obtained from a patient hospitalized with the diagnosis of epilepsy in the red area of the emergency medicine unit of our hospital. Another blood sample was collected into Na-citrate, in which the thrombocyte count was observed to be within the normal ranges. Furthermore, thrombocyte aggregations were observed in the peripheral blood smears prepared from the sample with EDTA, whereas no aggregation was observed from the sample with citrate.

Keywords: *EDTA, peripheral blood smear, pseudothrombocytopenia, thrombocytopenia*

ÖZ

EDTA'ya bağlı psödotrombositopeni; EDTA ile antikoagüle edilmiş kanda trombositlerin kümeleşmesi sonucu, normalden düşük olarak saptanması durumudur. Bu durum gerçek trombositopeni ile karışabilmekte, yanlış tanı ve tedavi uygulanmasına, gereksiz işgücü ve zaman kaybına neden olabilmektedir. Bu olgu trombositopeninin olası nedenlerinden biri olan EDTA'ya bağlı psödotrombositopeniye dikkat çekmek amacıyla sunulmuştur. Olgu; hastanemiz acil tıp kırmızı alanında epilepsi tanısı ile yatan hastadan alınıp acil gözetimli hizmet laboratuvarımıza gönderilen EDTA'lı kan numunesinde izole trombosit düşüklüğü tesbit edilmiştir. Hastadan yeniden alınan Na-sitratlı numunede trombosit sayısının referans değerler arasında olduğu tesbit edilmiştir. Ayrıca hem EDTA'lı hem de Na-sitratlı numuneden yapılan periferik yayma örneklerinde EDTA'lı numunede trombosit kümeleri gözlenirken, Sitratlı numunede gözlenmemiştir.

Anahtar sözcükler: *EDTA, periferik yayma, psödotrombositopeni, trombositopeni*

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Introduction

Thrombocytopenia is a common condition. Its causes include increased thrombocyte (PLT) destruction (immune thrombocytopenia, hemolytic uremic syndrome, disseminated intravascular coagulation), hemodilution, increased sequestration (hypersplenism), reduced PLT production (leukemia, HIV, and reduced thrombopoietin production) and drug intake (valproic acid, methotrexate, pantoprazole)[1-4].

Pseudothrombocytopenia (PTP) on the other hand, is a clinically unimportant condition, which is the mismeasured thrombocyte count. PTP arises against anticoagulants such as citrate, or oxalate; however, the most common cause of pseudothrombocytopenia is the ethylenediaminetetraaceticacid-related type (EDTA-PTP)[5].

It has been asserted that EDTA interacts with the glycoprotein IIb-IIIa molecule located on the thrombocyte membrane during calcium ion binding, and the glycoprotein IIb epitope is released[6,7], and thrombocyte aggregation is observed in individuals with autoantibodies against this epitope[8,9]. Due to aggregated platelets large structures, thrombocytes may be recognized as other cells in automatized counting instruments, and thus, lower levels of these cells may be reported[10].

Laboratory specialists are generally aware of the clinical situations of the patients, and clinicians should contact them in case of thrombocyte counts incompatible with the clinical condition. This case report was presented in order to attract the attention of the clinicians to EDTA-PTP count incompatible with the clinical condition of the patient.

Case Report

A 50-year-old male patient presented the emergency unit of our hospital due to a seizure and previous diagnosis of cerebrovascular event and epilepsy. The history of the patient included right middle cerebral artery infarction two and a half years ago and started to have seizures 6 months later. He had been prescribed acetylsalicylic acid (300mg 2x1), levetiracetam (1000mg 2x1), and sodium valproate (500mg CR 2x1). The patient had a generalized tonic-clonic seizure for approximately ten minutes and it was learned that he had not recieved his medication in the previous two days. The seizure did not continue. A blood sample of the patient was collected into a K2-EDTA tube and sent to the emergency unit laboratory. The sample was studied in Sysmex XE2100 (Sysmex Corporation, Kobe, Japan) instrument which revealed a platelet count of $33x10^3$/µL, a hemoglobin level of 14.9 gr/dl, and a WBC count of $8.20x10^3$/µL. The remaining biochemical parameters were within normal ranges. In order to evaluate whether that was an actual thrombocytopenia or a PTP, a blood sample was requested to be collected into a tube with Na citrate. In the blood sample collected into the Na citrate tube, the thrombocyte count was found to be $156x10^3$/µL. Additionally, peripheral blood smears were carried out from the blood samples collected both into EDTA and Na citrate tubes. In the peripheral blood smear obtained from the sample with EDTA, thrombocyte aggeragations were observed in many areas, which was significant in peripheral areas (Image-1).

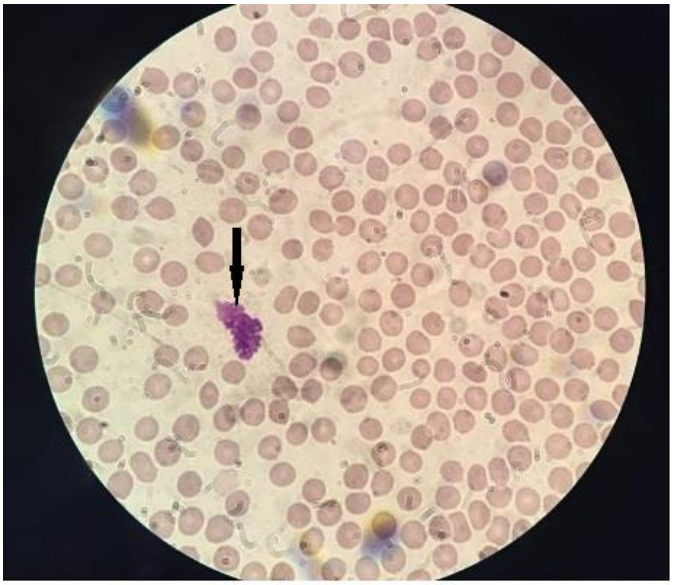

Image 1: Aggregated thrombocytes in EDTA sample, (Count: <100 at x100 magnification, light microscope).

No thrombocyte aggregation was observed in the smear obtained from the sample with Na citrate (Image-2). The situation was concluded to be EDTA-dependent PTP for the patient

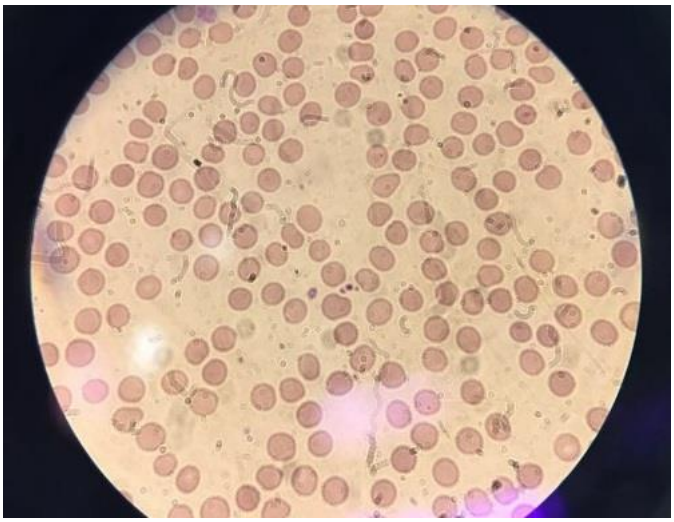

Image 2: Peripheral blood smear of Na-citrated sample, single thrombocyte (Count:<100 at x100 magnification, light microscope).

Discussion

The EDTA-PTP prevalence among hospitalized patients is estimated to be 0.1% or 0.2%[11]. EDTA-dependent pseudothrombocytopenia is an *in-vitro* phenomenon characterized by the false low count of platelets in EDTA anticoagulated blood measured by automatic analyzers, due to the anti-platelet auto-antibodies that lead to thrombocyte aggregation[12]. These anti-platelet auto-antibodies that are formed against glycoprotein IIb epitope localized in thrombocyte membrane, belong to the IgG, IgM or IgA class immunoglobulins. These antibodies are transient or permanent autoantibodies that are formed secondary to drug use or infections. Drug-related PTP cases have been reported in the literature[13,14]. In 2003, Yoshikawa reported a case of valproic acid-induced EDTA-PTP, who had used valproic acid for treatment of epilepsy[14]. In this case, the analysis of the blood sample collected into the EDTA tube revealed a platelet count of $5x10^3/\mu L$, which was $7x10^3/\mu L$, in the subsequent measurement, and the sample anticoagulated with heparin revealed a platelet count of $234x10^3/\mu L$. Valproic acid may lead to hematopoietic toxicity and frequently affects thrombocytes. Its effect on thrombocytes may be thrombocytopenia, thrombocyte dysfunction or bone marrow suppression. The authors of the same study have reported that autoimmune thrombocytolysis may be due to the molecular configuration of valproic acid that mimics the fatty acids on the thrombocyte membrane and to the presence of anti-platelet antibodies. The pathophysiology of valproic acid-induced EDTA-dependent PTP has not been clearly understood yet; however, it has been related to the structure of valproic acid[14]. History of medication with valproic acid is present in the case we presented as well. However, drug interaction is not the only cause of EDTA-PTP. Thus, other possibilities should be considered as well, and further studies should be conducted.

Different methods may detect PTP. These include use of anticoagulants other than EDTA such as Na citrate, heparin or oxalate, and examination of the sample anticoagulated with EDTA brought to 37°C, blood samples with added kanamycin and peripheral blood smears[15]. In our study, we used Na citrated samples in order to detect the presence of any EDTA-PTP. Furthermore, we investigated the peripheral blood smears of both EDTA and Sodium citrated samples. Different anticoagulants may be used instead of Na citrate. However, anticoagulants such as citrate, oxalate, acid-citrate dextrose and heparin may lead to PTP as well. The possible mechanism in the method of bringing the EDTA-anticoagulated sample to 37°C has been suggested as the inhibition of thrombocyte aggregation by unbinding of the glycoprotein IIb/IIIa complex at 37°C. However, aggregation may continue in some cases observed by this method as well, with unknown reasons[15].

In conclusion, although EDTA-PTP is not meaningful clinically, the misdiagnosis may lead to further examination requests, unnecessary loss of power and high cost. Therefore, we would like to emphasize that in all situations of incompatible clinical and laboratory findings, clinicians should contact the laboratory specialist, which would be beneficial for the patient, the clinician and the laboratory specialist.

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