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Original Article

# Evaluation of EDTA induced pseudothrombocytopenia and the effect of alternative anticoagulants

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**Keywords:**

Pseudothrombocytopenia;
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Heparin.

## ABSTRACT

**Background:** Artifactual Thrombocytopenia is a condition in which there is falsely lowered platelet in patients who have thrombocytopenia but the absence of petechiae or echymoses. Pseudothrombocytopenia is also an artifactual thrombocytopenia caused by anticoagulant dependent agglutinins. The aim of this study was to compare the platelet count in pseudothrombocytopenia in EDTA anticoagulated samples and other alternative anticoagulants.

**Materials and methods:** This study was performed in the department of hemotology hematology, Institute of medicine. All cases during study period were evaluated by EDTA-anticoagulated whole blood samples but criteria for selecting pseudothrombocytopenia patients was unexpectedly low platelet counts with clumping/aggregate on peripheral blood smear. Additional samples were collected in sodium citrate and heparin for examined.

**Results:** A total of 50 patients aged between 18 to 90 years were found to have pseudothrombocytopenia. Platelet counts in samples anticoagulated with EDTA ranged from 20x109/l to 149x109/l and samples from same patients anticoagulated with citrate ranged from 41x109 /l to 312x109 /l and heparin showed platelet count ranging from 29x10 9 /l to 210x109 /l. The mean platelet count in EDTA- anticoagulated blood of individuals with pseudothrombocytopenia was 104x109/l whereas the mean platelet count in citrate and heparin-anticoagulated samples was 151x109/land123x109/l respectively. Platelet counts decreased dramatically in the EDTA samples in contrast to the samples anticoagulated with citrate or heparin post four hours of collection.

**Conclusion:** Peripheral blood smears should be examined for platelet clumping/aggregates in cases with low platelet count not correlating with clinical presentation or in isolated thrombocytopenia flagged in hematology analyser. Alternative anticoagulants should be used for correct estimation of platelet count.

## INTRODUCTION

Thrombocytopenia is the most common cause of abnormal bleeding. Thrombocytopenia results from four process: artifactual thrombocytopenia, deficient platelet production, accelerated platelet destruction, and abnormal distribution or pooling within the body. Artifactual thrombocytopenia is falsely low platelets which should be considered in patients who have thrombocytopenia but the absence of petechiae or echymoses. In artifactual thrombocyctopenia, the cells are not counted accurately and the most common cause is giant platelets or platelet satellitism.1

Platelet clumping (pseudothrombocytopenia) is anartifactual

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thrombocytopenia caused by anticoagulant dependent agglutinins. These are immunoglobulins of IgG, IgA and IgM subtypes. Platelet clumping is most commonly seen when blood is collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant. However, other anticoagulants may also cause clumping. Platelet clumping is time dependent and varies with the type of instrumentation used for automatic counting.[1,2]

EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) is a common problem in laboratories. On evaluation for isolated thrombocytopenia, its prevalence is between 0.1-2% among hospitalized patients and 15-17% in outpatients. EDTA-PTCP is solely an in vitro effect without any clinical relevance.[3]

## MATERIALS AND METHODS

This study was performed in routine haematology department of Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University Teaching Hospital. All patients during study period were evaluated by EDTA-anticoagulated whole blood samples but the criteria for selecting pseudothrombocytopenia (PTCP) was unexpectedly low platelet counts of less than 150x109/lor positive flagging for platelet aggregates in Sysmex XE 5000 hematology analyser. Such cases were confirmed as PTCP after examination of peripheral blood film for platelet clumping or aggregates. Blood smears were prepared, Wright stained and examined.

After confirmation PTCP the patient was asked for additional blood samples using collecting tubes anticoagulated with sodium citrate and heparin and measured in parallel by automated routine haematological analyser (Sysmex XE 5000 system). To evaluate the time dependent influence, platelet counts were re-analysed after four hours of collection in all anticoagulants. Mathematical correction for dilution by sodium citrate was done by multiplying the obtained value by multiplication factor 1.1 (n x1.1). SPSS version 20 and word excel were used for statistical analysis.

## RESULTS

A total of 50 patients aged between 18 to 90 years were found to have pseudothrombocytopenia during the study period. Males accounted for 32% and females 68% with a M:F of 1:2.1.

Platelet counts in samples anticoagulated with EDTA ranged from 20x109/l to 149x109/l and samples from same patients anticoagulated with citrate ranged from 41x109 /l to 312x109 /l and heparin showed platelet count ranging from 29x10 9 /l to 210x109 /l (fig. 1).

The mean platelet count in EDTA- anticoagulated blood of individuals with PTCP was 104x109/l whereas the mean platelet count in citrate-anticoagulated samples was $151x10^9$/land in heparin-anticoagulated samples $123x10^9$/l.A higher mean platelet count was seen in citrate and heparin anticoagulated samples as compared to EDTA anticoagulated samples (Table 1). Citrate anticogulated samples show higher value as compared to heparin.

**Table: 1 Platelets value in different anticoagulants instant and after 4 hrs**

| Anticoagulants | Minimum value | Maximum value | Mean |
|---|---|---|---|
| EDTA | 20200.00 | 149000.00 | 104296.00 |
| Citrate | 41000.00 | 312000.00 | 151320.00 |
| Heparin | 29000.00 | 210000.00 | 123560.00 |
| EDTA after 4hrs | 4000.00 | 141000.00 | 53647.00 |
| Citrate after 4 hrs | 40000.00 | 232000.00 | 131060.00 |
| Heparin after 4 hrs | 29000.00 | 198000.00 | 106940.00 |

Platelet counts decreased dramatically in the EDTA samples in contrast to the samples anticoagulated with citrate or heparin post four hours of collection (fig. 2).

## DISCUSSION

Ethylenediaminetetraacetic acid (EDTA) is commonly used as an anticoagulant for estimation of blood cell counts. EDTA-PTCP is an in-vitro phenomenon due to antiplatelet antibodies that cause platelet clumping in blood that had been anticoagulated with EDTA.[1]

In this study EDTA-PTCP was diagnosed by examination of peripheral blood smear for microscopic aggregates or clumping of platelets in patients with low platelet count on Coulter counter. EDTA-PTCP was diagnosed and confirmed by seeing platelet aggregates in smears in different literatures as well.[4-7]

Pullen et al found that EDTA-PTCP was seen more commonly in females rather than in males with female: male ratio of 3:2.8 A higher incidence of PTCP was seen in females in this study as well.

In this study, PTCP diagnosed from EDTA anticoagulated samples showed lower platelet count than samples anticoagulated with heparin and citrate. Citrate anticoagulated samples show higher platelet value than heparin. Wu Wei et al also found citrate as better anticoagulant in management of PTCP.[7] Werner et al also found that citrate is superior to EDTA anticoagulant to reduce PTCP.[5]

The mean platelet count in EDTA- anticoagulated blood of individuals with PTCP was lower in comparison to citrate-anticoagulated and heparin-anticoagulated samples with PTCP. Literature shows that mean platelet count was increased in samples anticoagulated with magnesium sulphate than in EDTA samples.[5]

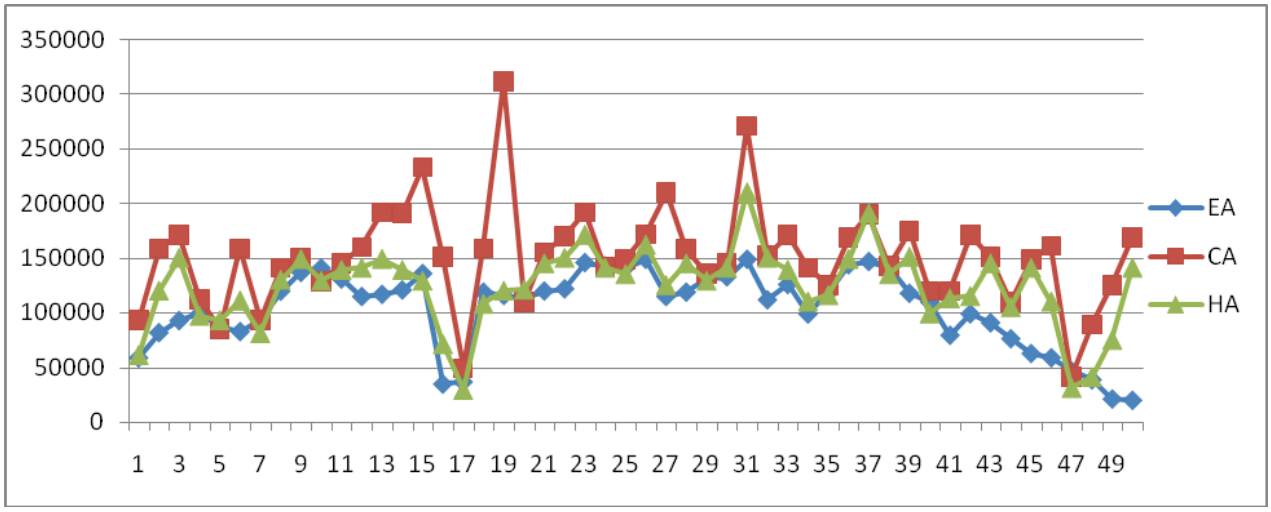


*Figure 1: Platelets count in different anticoagulants measured immediately after sample collection*

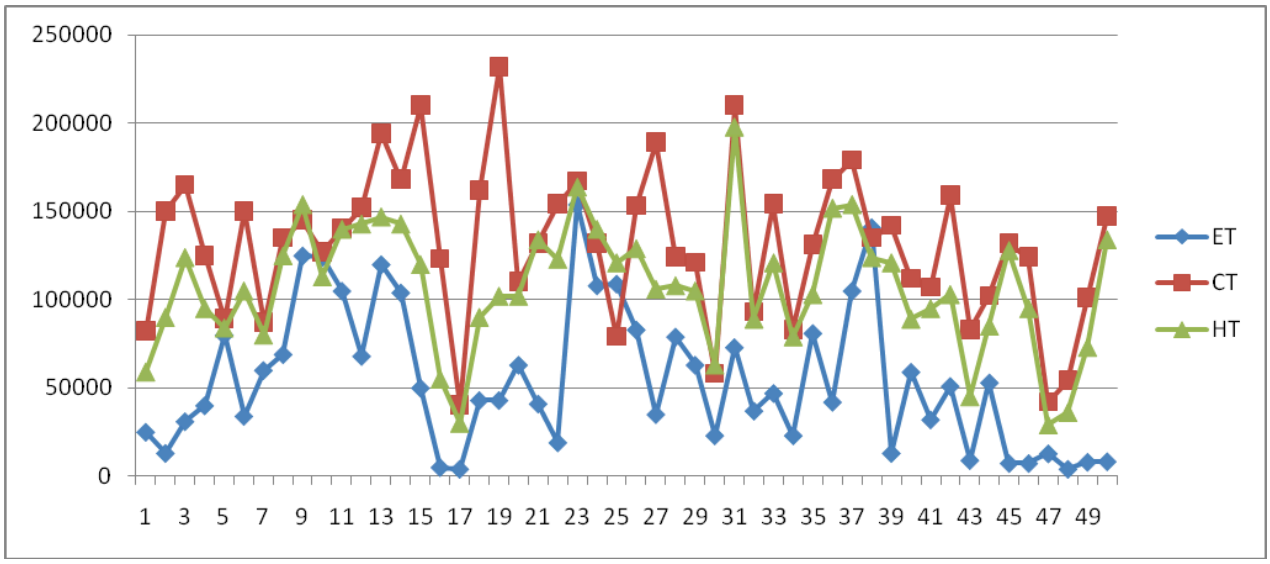


*Figure 2: Platelets count in different anticoagulants measured after 4 hrs of sample collection.*

After four hours of collection, platelet counts decreased dramatically in the EDTA sample, in contrast to the samples anticoagulated with citrate or heparin. Similar results were seen in the literature.[5] Fitzgerald et al postulated that cold-reactive antiplatelet antibodies directed against a hidden epitope becomes accessible due to the calcium complexing effect of EDTA leading to PTCP.[9]

Citrate and heparin anticogulated blood still showed platelet clumping in some of the cases in our study. A study by Bizzaro found that 20% of cases anticoagulated with citrate showed clumping.[10] To obviate this phenomenon, blood should be collected in ammonium oxalate and platelets counted in Burker chamber.[10] Other studies also show that anticoagulants such as citrate and heparin still show platelet clumping and aggregate resulting into PTCP but the incidence is lower as compared to EDTA anticoagulated samples.[5]

EDTA-PTCP may be diagnosed easily even by general practitioners without any experiences in blood film examinations as aggregated platelets lead to typical changes of platelet. However, blood smears are also not routinely evaluated by visual inspection and remains unnoticed because warning flags and histograms of hematology analyzers are not interpreted correctly. So, EDTA-PTCP if undiagnosed may lead to unwanted diagnostic testing, unnecessary transfusions and withhold of even emergency surgeries. This can lead to unnecessary cost and discomfort to the patient.[3]

It has been postulated that cation chelation by EDTA leads to a conformational change (changes in shape and size and

acquire more spheroid shape) of the platelet membrane GPIIb- IIIa complex and unmasking of cryptic epitope. This becomes accessible for autoantibodies and causes platelet clumps. Hematology analyzers count the resulting platelet clumps as single giant platelets or as small lymphocytes in the white blood cell gate and indicate thrombocytopenia. EDTA anticoagulation also leads to time dependent changes of mean platelet volume (MPV).[3,5,11,12]

Recently it has been proposed that EDTA-induced platelet clumps can be dissociated by a mixture of calcium chloride for re-association of glycoprotein (GP) IIb/IIIa complex and sodium heparin for maintaining anticoagulation to correctly estimate platelet counts.[5,13] The addition of an aminoglycoside antibiotic (e.g kanamycin) has similarly been used to count platelets in cases of PTCP.[5,14]

Schrezenmeir et al proposed that the phenomenon of in vitro-platelet aggregation should be collectively called anticoagulant induced PTCP and Gschwandtner et al referred it as a 'laboratory disease'.[15,16]

## CONCLUSION

Peripheral blood smears should be examined for platelet clumping/aggregates in cases with low platelet count not correlating with clinical presentation or in isolated thrombocytopenia flagged in hematology analyser. Alternative anticoagulants should be used for correct estimation of platelet count and to exclude EDTA induced PTCP in order to prevent unnecessary trouble and unwanted expenditure to the patient.

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