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To Test the Accuracy of Platelet Count, as Corrected by Citrate Anticoagulant in EDTA Induced Pseudo-Thrombocytopenia

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Abstract: ***Background: EDTA- induced pseudothrombocytopenia (EDTA-PTCP) is a common laboratory phenomenonwith a prevalence ranging from 0.1-2% in hospitalized patients to 15-17% in outpatients evaluated for isolated thrombocytopenia.(EDTA-PTCP) is falsely low platelet countdue to antibodies directed against glycoprotein (gp) IIb-IIIacomplex leading to agglutination.Misinterpretation of artificial low platelet count lead to unnecessary transfusion of platelets increasing the risk of associated morbidity.Methodology & Results-A Total of 100 Cases of EDTA induced Pseudothrombocytopeniaas corrected by Citrate anticoagulant is studied. The PLT Count by Abotts Cell DYN Ruby using EDTA as anticoagulant is (55 ± 25.9)& citrate anticoagulant is (217.8 ± 61.16)x10^3/mm. Manual platelet count = 222± 58.7 x10^3/mm. P value < 0.05 which is statistically significant.This results clearly shows manual platelet count very well correlated with citrated blood platelet. Conclusion: EDTA induced PTCP can be corrected byimmediate blood analysis in hematology analyzers with Citrate as anticoagulant.So it can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood.***

Keywords: platelet aggregation, pseudothrombocytopenia, anticoagulation, EDTA, citrate, agglutination.

1. Introduction

EDTA-dependent pseudo-thrombocytopenia (EDTA-PTCP)is a common laboratory phenomenon. Its prevalence isreported to vary between 0.1-2% among hospitalized patients and 15-17% in outpatients evaluated for isolated thrombocytopenia. [1](EDTA-PTCP) is the result of aspuriously low platelet count induced byagglutinating antibodies that recognize cytoadhesivereceptorson platelet gpllb-IIIa which causes platelet clumping.[2]

Platelet clumping also termed as pseudo-thrombocytopenia is very commonly encountered in clinical practice especially in Obstetrics & G ynaecology section. The pregnancy related thrombocytopenia should be distinguished from pseudo-thrombocytopenia. A very easy safe & reliable method is to use Citrated blood for platelet count. This study was undertaken to assess the correlation of corrected citrate utilized platelet count & manual platelet count which will have a great diagnostic implication.

2. Methodology &Results

This study includes a total of 100 cases of EDTA- PTCP.

The criteria for case selection:

- Clinically suspected Pseudo-thrombocytopenia
- when flagged as suspicious for platelet aggregates by Autoanalyzers

For such cases slides were stained with Giemsa stain &reviewed for platelet aggregates or clumping. The platelet clumps are studied for their number and size in detail. The blood is collected in Citrate-Anticoagulated tube and immediately run in AbottsCell Dyn Ruby. Precaution should be taken that there should be no time lapse between blood collection & blood testing in Ruby. The sensitivity & p value is calculated to test the correlation of corrected platelet count in Citrated plasma and by manual platelet count.

The manual platelet count is done by using 1% ammonium oxalate as diluent(for lysing RBC) with 1:100 Dilution. 20 microlitreof blood is taken in a pipette & 1.98 ml of diluent is added.platelets are counted in NeubeurHemacytometer. This manual platelet count is cross checked with the platelet count obtained by Giemsa stained smears.

Formula for Corrected PLT Count Obtained By Citrated Blood
In Cell Dyn Ruby = (N X 1.1)x10^3/mm
N = Citrated blood platelet count

3. Results

A Total of 100 CASES of EDTA induced Pseudothrombocytopeniaas corrected by Citrate anticoagulant is studied

Table: Comparison of platelet count in EDTA & Citrate as Anticoagulant

ANTICOAGULANT	EDTA – PTCP (PLT Count by Abotts Cell DYN Ruby)	CITRATE (PLT Count by Abotts Cell DYN Ruby)	MANUAL PLT COUNT	P
PLATELET COUNT = (mean ± standard deviation)	(55 ± 25.9) x10^3/mm	(198 ± 55.6)x10^3/mm	(222± 58.7) x10^3/mm	< 0.05
PLT COUNT RANGE	(29.1 – 80.9) x10^3/mm	(142.4 – 253.6) x10^3/mm)	(163.3 – 280.7) x10^3/mm)	

Corrected PLT Count Obtained By Citrated Blood IN CELL DYN RUBY = (198 X 1.1) = Average PLT Count = 217.8x10^3/mm

Corrected Standard Deviation (SD) = 55.6 x 1.1 = 61.16x10^3/mm
Range of PLT Count = (156.64 – 278.96) x10^3/mm
Manual platelet count = 222± 58.7 x10^3/mm and the range =(163.3 – 280.7) x10^3/mm)
P value < 0.05

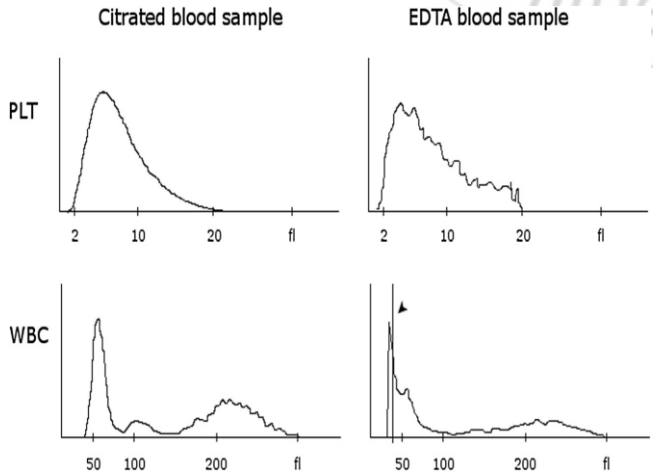
4. Discussion

EDTA-dependent pseudothrombocytopenia (EDTA-PTCP)is a common laboratory phenomenon. Its prevalence isreported to vary between 0.1-2% among hospitalized patients and 15-17% in outpatients evaluated for isolated thrombocytopenia. [1](EDTA-PTCP) is the result of aspuriously low platelet count induced byagglutinating antibodies that recognisecyto-adhesive receptorson platelet gpllb-IIIa which causes platelet clumping.[2]

InternationalCouncil for Standardization in Haematology (1993)[3]&the NCCLS has recommended EDTA (salts) as the anticoagulant of choice for CBC,principally for its cell preservation properties.EDTAchelates divalent cations including calcium, thus avoidingcoagulation and stabilizing the sample for subsequent haematologicalanalysis (Banfiet al, 2007).[4]

Visual evaluation of blood smears is regarded as goldstandard for detection of EDTA-PTCP, but only a limitedamount of smears will be performed in routine laboratories.

A simpler approach for detection of EDTA-PTCP isto inspect the histograms and flags of hematology analyzers& run the blood sample in hematology analyzers with Citrate as anticoagulant.[5]PTCP is a time- andtemperature-dependent phenomenon. Therefore with citrate direct samplingand immediate analysis results in higher platelet counts as compared to those obtained after delay. [5]



Aggregated platelets are plotted as a serrated (“saw-teeth”) curve in the PLT histogram.[6,7]

In this study a total of 100 Cases of EDTA induced Pseudothrombocytopeniaas corrected by Citrate anticoagulant were studied

The PLT Count by Abotts Cell DYN RubyusingEDTA as anticoagulant is (55 **± 25.9)** & citrate anticoagulant is (1**98 ± 55.6).**
Corrected PLT Count Obtained by Citrated Blood
IN CELL DYN RUBY = (198 X 1.1) = Average PLT Count = 217.8**x10^3/mm**
Corrected Standard Deviation (SD) = 55.6 x 1.1 = 61.16 **x10^3/mm**

Corrected platelet count = mean± SD = (217.8 ± 61.16) x10^3/mm
Range of PLT Count = (156.64 – 278.96) x10^3/mm
Manual platelet count = 222± 58.7 x10^3/mm and the range = (163.3 – 280.7) x10^3/mm)
P value <0.05 .it is statistically significant

This results clearly shows manual platelet count very well correlated with citrated blood corrected platelet. It can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood by multiplying with diluent factor 1.1

Platelet aggregates were present in EDTA samples and their number and size were inversely relatedto the fall in platelet count. The most pronounced decrease in platelet count was associated with the presence of few and large aggregates composed of 10 to 20 platelets. These facts very well correlated with other studies. [8,9]

Possible Causes of Platelet Clumping

1) The most common cause of platelet clumping in an EDTAanticoagulatedspecimen is improper mixing of the tube.
2) Improper collection of the blood sample may cause thrombinrelease and a falsely low platelet count due to platelet aggregation. This may be due to an excessively traumatic venipuncture orin adequate anticoagulation.
3) Blood collection tubes should be filled to their stated draw volume. Overfilling an EDTAtube can result in an improper blood to additive ratio. Insufficient EDTA in the sample may contributeto platelet clumping and/or clotting of the blood.
4) Another cause of platelet clumping is EDTA-induced pseudothrombocytopenia. Thisphenomenon is believed to be caused by EDTA-dependant platelet agglutinins or antibodies present in the plasma. The clumping occurs due to an alteration of the platelet surface glycoproteins when they are incubated with a calcium chelator such as EDTA. These modified platelet antigens thenreact to anti-platelet autoantibodies (immunoglobulins of both IgG and IgM types to form the large agglutinates.
5) EDTA-induced pseudothrombocytopenia can be exhibited in normal, healthy individualsand those with diseases such as human immunodeficiency virus (HIV), rubella, cytomegalovirus(CMV), autoimmune disorders, thrombotic disorders and infectious mononucleosis.In infectiousmononucleosis, the patient may have increased

levels of cold agglutinins, which may contribute to platelet agglutination.[10, 11,12]

Managing Platelet Clumping in the Lab

When a platelet, white cell and/or red cell count is flagged on an electronic counter, there areseveral steps that can to be taken in order to get an accurate platelet count.

It isrecommended that the EDTA tube be inverted eight to 10 timesimmediately after the specimen is collectedTube with EDTA should be inverted 10times after collection, and mixed 10 times prior to testing.

Examine a stained blood smear microscopically to verify the platelet clumping.

Re-collect the patient's blood into a sodium citrate tube. Platelets will generally not exhibit clumping in sodium citrate. The sample in the citrate tube should be run immediately on the instrument, & then multiply theplatelet count by 1.1 to get the correct value.[11,12]

If the patient has infectious mononucleosis, or a cold agglutinin is suspected as the reason for platelet clumping, collect the blood into an EDTA tube that has been pre-warmed to37ºC in a water bath.[13]

The sample can be vortexed, which will cause the platelet clumps to break apart and they can then be counted more accurately. However, be aware that too vigorous mixing can, by itself, cause platelet activation.

Aminoglycosides (e.g. kanamycin) have been reported to be effective in dissociating platelet clumps in cases of EDTA-induced pseudothrombocytopenia.[14]

Once laboratory personnel understand the reasons and possible causes for platelet clumping in an EDTA-anticoagulated blood specimen, it can be much easier to deal with clumped platelets whenthey occur, and in many circumstances, prevent them from occurring in the first place.[11,12,15,16]

5. Conclusion

A simpler approach for detection of EDTA-PTCP isto inspect the histograms and flags of hematology analyzers& run the blood sample in hematology analyzers with Citrate as anticoagulant.PTCP is a time- andtemperature-dependent phenomenon. Therefore with citrate direct samplingand immediate analysis, results in higher platelet counts as compared to those obtained after delay.The results of this study clearly shows manual platelet count very well correlated with citrated blood corrected platelet

It can be confidently concluded that EDTA induced pseudothrombocytopenia can be effectively corrected by citrate anticoagulated blood

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