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EVALUATION OF EDTA DEPENDENT PSEUDOTHROMBOCYTOPENIA AND THE EFFECT OF ALTERNATIVE ANTICOAGULANT- A TERTIARY CARE CENTRE EXPERIENCE

Pathology

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ABSTRACT

Background: - Pseudothrombocytopenia is a phenomenon of spurious low platelet count. EDTA- dependent pseudothrombocytopenia (EDTA-PTCP) is due to presence of agglutinating anti-platelet antibodies that react with platelets in blood anticoagulated with EDTA.
Materials & Methods: - This is a prospective study over a period of 3 years in VIMSAR Burla. The inclusion criteria for selecting pseudothrombocytopenia patients was unexpectedly low platelet count with clumping of platelets in peripheral blood smear. The samples were collected in EDTA, also in sodium citrate & heparin for evaluation and then compared.
Results: - A total of 51 patients aged between 16 to 80 years were found to have pseudothrombocytopenia. The mean platelet count in EDTA anticoagulated blood of patients with pseudothrombocytopenia was lower than that in citrate and heparin. Four hours of collection showed further decrease in platelet count in EDTA samples in contrast to the sample anticoagulated with citrate or heparin.
Conclusion: - Patients with low platelet count not correlating with clinical presentation must undergo peripheral blood smear examination to prevent spurious thrombocytopenia.

KEYWORDS

EDTA- dependent Pseudothrombocytopenia, Peripheral blood smear,Sodium citrate, Heparin.

INTRODUCTION: -

Pseudothrombocytopenia is the term used when there is falsely low platelet count but absence of clinical features like petechiae or ecchymoses, result from incorrect measurements. Most common cause of incorrect platelet counting is due to presence of giant platelet or clumping.**[1]**

Most commonly clumping of platelets seen when blood is collected into ethylene diamine tetracetic acid (EDTA) anticoagulant. It is caused by anticoagulant dependent agglutinins.These are immunoglobulins of IgG, IgA& IgM subtypes.**[1,2]**

Platelet clumping is dependent on time& also varies with the type of instrumentation used for automatic counting.**[3,4,5]**

On evaluation for isolated thrombocytopenia, EDTA-dependent Pseudothrombocytopenia prevalence is between 0.01-2% among hospitalized patients.**[1]** The identification of Pseudothrombocytopenia is possible when there is low platelet count in absence of significant clinical features. Peripheral blood smear examination should be done in case of necessity. **[2]** Alternative anticoagulants can be used for more accurate platelet counts.**[1]** It is very important to identify Pseudothrombocytopenia for accuracy of clinical assessment and avoiding unnecessary treatment.**[2,6]**

MATERIALS & METHODS

The study was conducted in Department of Pathology VIMSAR,Burla from April 2017 to March 2020. All the hospitalized patients who gave their sample for CBC or TPC in EDTA anticoagulated vial were evaluated. The inclusion criteria for our study was unexpected low platelet count of less than $140x10^9$/L not correlating with clinical feature or positive flagging for platelet aggregate in automated routine hematology analyser. For such cases blood smears were prepared, Leishman stained and examined for platelet clumping or aggregates.

Then, after confirmation of Pseudothrombocytopenia, the patients were asked for additional blood samples that were collected in sodium citrate and heparin anticoagulated collection tubes. Mathematical correction for dilution by sodium citrate was done by multiplying the obtained value by multiplication factor 1.1 (n x1.1). These samples were measured in parallel by automated hematology analyser. For evaluation of time dependent influence, the analysis of platelet count was done after four hours of collection in all anticoagulants tubes. SPSS version 20 and word excel were used for statistical analysis.

RESULTS

A total of 51 patients aged between 16 to 80 years were found to have Pseudothrombocytopenia during the study period. Average age for female patients was 60 years and male patients was 62 years. Male: Female ratio in our study was1:1.4.EDTA anticoagulated blood sample had platelet counts from $21x10^9$/l to $139x10^9$/l. Peripheral blood smear prepared from EDTA anticoagulated sample showed clumping of platelets.

Citrated blood sample from the same patients had platelet count from $42x10^9$/l to $330x10^9$/l and heparinized blood sample had platelet count $30x10^9$/l to $220x10^9$/l.Mean platelet count in EDTA anticoagulated blood sample was 102 $x10^9$/l,in citrated blood it was150 x 10^9/l & in heparinized sample 120 x 10^9/l**.[Table-1]**

EDTA anticoagulated blood sample had lower platelet count & mean platelet count than citrated and heparinized blood sample.In post four hours of collection, platelet count decrease further in EDTA sample in contrast to citrated& heparinized sample**.[Table-2]**

DISCUSSION:-

Spuriously low platelet counts may be caused by multiple reasons like improper blood sampling technique, platelet clumping or satellitism or giant platelets. Platelet clumping may be due to cold agglutinin or EDTA associated. EDTA dependent Pseudothrombocytopenia is due to antiplatelet antibodies.**[1,3,4]**

The trigger for antibody production is unknown and may arise in response to an unrelated antigen, and the antibody may then cross-react with platelets resulting in agglutination.**[2]** The exact mechanism of the reaction of antibodies with platelet membrane anti- gens is not known. It appears that EDTA, when added to platelets in vitro, induces a conformational change in the membrane resulting in the exposure of

"neoantigens" to which the antibodies bind**. [2,5]**In Glanzmann thrombasthenia patients, platelets do not react with these anti- bodies suggesting that the platelet membrane glycoproteins (GP) IIb or IIIa, which are missing in these platelets, may be the "neoantigen" in PTCP**. [6]** It has been postulated that the antibody binding site of the GP IIb is normally hidden in the GP IIb- IIIa complex and that the complex must dissociate before antibody binding may occur**.[7].** Many drugs, concentration of EDTA, pH, and temperature may affect complex dissociation**. [8]** Recently it has been postulated 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 for correct estimation of platelet counts**.[9,14]**Similarly,the platelet count in cases of PTCP has been done by the addition of an aminoglycoside antibiotic (e.g kanamycin) **.[1,9,10]**

Incidence of EDTA dependent Pseudothrombocytopenia was more in female as compared to males in our study. Berkman et al also found that female more commonly affected**.[2]**

In present study, EDTA anticoagulated samples showed lower platelet count & mean platelet count than sample anticoagulated with heparin and citrate.Citrated samples show higher platelet value than heparin. Citrate is found to be better anticoagulant to reduce Pseudothrombocytopenia also in other studies**. [1,9,11]** Some studies show that mean platelet count was increased in sample anticoagulated with magnesium sulphate than in EDTA sample**.[9]**Mean platelet volume is recorded routinely by most automated blood cell counters and is an extremely useful means of differentiating ITP or other types of thrombocytopenia from EDTA-PTCP in the apparently thrombocytopenic patient**. [2,12]**Some other studies say EDTA anticoagulation also leads to time dependent changes of mean platelet volume**.[1,9]**

In this study, platelet counts decreased dramatically in EDTA sample, in contrast to the sample anticoagulated with citrate and heparin after four hours of collection. Similar results also observed in other studies. **[1,2,9,13]**

In our study, clumping of platelets was seen in peripheral blood smear examination in patients with low platelet counts on automated cell counter.Platelet aggregates were more in EDTA sample than in citrated and heparinized sample**.[9]** Platelet clumping in peripheral blood smear help to diagnose EDTA dependent pseudothrombocytopenia in other studies. **[9, 10,14,15]** Berkman et al use the term EDTA dependent thrombocytopenia for the phenomenon of in vitro-platelet aggregation using EDTA anticoagulated blood sample**.[2]** The term anticoagulant induced PTCP is proposed bySchrezenmeir et al and Gschwandtner et al use term a 'laboratory disease' for it**.[16,17]**

CONCLUSION-

Blood smears are not routinely evaluated by visual inspection and remains unnoticed if warning flags and histograms of hematology analyzers are not interpreted correctly. Because of that, EDTA-PTCP remain undiagnosed which 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 So,. 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.

Table-1-Platelet values in different anticoagulants immediately after collection

Anticoagulants	Minimum value	Maximum value	Mean
EDTA	21,000	1,39,000	1,02,000
Citrate	42,000	3,30,000	1,50,000
Heparin	30,000	2,20,000	1,20,000

Table-2-Platelet values in different anticoagulant after 4 hours after collection

Anticoagulants	Minimum value	Maximum value	Mean
EDTA	4500	1,22,000	54,868
Citrate	41,000	2,35,000	1,34,180
Heparin	29,000	2,00,000	1,08,090

REFERENCES-

1. Shrestha A, Karki S. Evaluation of EDTA induced Pseudothrombocytopenia and effect of alternative anticoagulants. Journal of pathology Nepal 2014; vol-v: 626-629.
2. Neille Berkman, YossefMichaeli, Reuven or and AmiranEldor. EDTA dependent Pseudothrombocytopenia; A clinical study of 18 patients and a Review of literature. American J of Hematology 36:1991:195-200.
3. John P.Greer, John Foerster, George M. Rodgers, FrixosParaskevas, BertilGlader, Daniel A. Arber, Robert T. Means, Jr., editors. Wintrobe's Clinical Hematology. Vol 2 12th edition. Lippincott Williams and Wilkins: 2012.2606pp.
4. Veenhoven WA, Van Der Schans GS, Huiges W, Metting-Scherphuis HE, Halie MR, Nieweg HO: Pseudothrombocytopenia due to agglu tinins. Am J Clin Pathol 72:1005-1008, 1979.
5. Onder 0, Weinstein A, Hoyer LW: Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. Blood 56:177-182, 1980.
6. Pegels JG, Bruynes ECE, Engelfriet CP, Kr von dem Borne AEG: Pseudothrombocy topenia: An immunological study on platelet anti- bodies dependent on ethylene diamine tetra-acetate. Blood 59: 157- 161, 1982.
7. Van Vliet HHDM, Kappers-Klunne MC, Abets J: Pseudothrombocy- topenia: a cold autoantibody against platelet glycoprotein GPIIb. Br J Haematol 62501-51 1, 1986.
8. Girmann G, Pees H, Scheurlen PG: Pseudothrombocytopenia and mexiletine. Ann Intern Med 100:767, 1984.
9. Werner PS, Steiner M, Fenger S, Gross HJ, Bierlich A, Dreissiger K. Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. Brithish Journal of Haematology2013;16:684-92.
10. CHae, H Kim, Lim JM, Oh EJ, KimY, Ham K. Novel method to dissociate platelet clumps in EDTA-dependent pseudothrombocytopenia based on the pathophysiological mechanism. Clinical Chemistry and laboratory Medicine 2012;50:1387-91.
11. Wu Wei, Guo Ye, Zhang Lin, Cui Wei, Li Wei, Zhang Shuo. Clinical utility of automated platelet clump count in the screening for ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia. Chinese Medical Journal 2011;124:3353-7.
12. Sassier P: The relation of platelet size and count: Its importance in diagnosing platelet disorders. Am J Clin Path 83:275-280, 1985.
13. Fitzgerald LA,PhilipsDR. Calcium regulation of the platelet membrane glycoprotein IIb-IIIacomplex. The Journal of Biological Chemistry 1985;260:11366-74. PMID 3161896.
14. Momani A, Khasawnch R. Anticoagulant-Induced PseudoThrombocytopenia: A case report. JRMS 2012;19:73-5.
15. Yoneyama A, Nakahara K. EDTA-dependent pseudothrombocytopenia--differentiation from true thrombocytopenia. Nihon Rinsho2003;61:569-74. PMID:12718077
16. Sakurai S, Shiojima I, Tanigawa T, Nakahara K. Aminoglycosides prevent and dissociate the aggregation of platelets in patients with EDTA-dependent pseudothrombocytopenia. British journal of Haematology1997;99:817-23.
17. SchrezenmeirH, Muller H, Gunsilius E, Heimpel H, SeifriedE. Anticoagulant induced pseudothrombocytopenia and pseudoleucocytosis. Thrombohaemostasis1995;73:506-13.
18. Gschwandtner M.E, Siotrzonek P, Boddinger C et al . Documented sudden onset of pseudothrombocytopenia. Annals of Hematology 1997;74:283-5.