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EDTA DEPENDENT ARTIFACTUAL THROMBOCYTOPENIA AND THE EFFECT OF ALTERNATE ANTICOAGULANT.

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ABSTRACT

BACKGROUND- Artifactual thrombocytopenia is a condition in which there is falsely lowered platelet count in the patients who have thrombocytopenia but the absence of any sign of bleeding like petechaie or echymoses . This false low platelet count due to Use of EDTA is called as pseudothrombocytopenia, which is a type of artifactual thrombocytopenia. The aim of this study was to compare the platelet count in EDTA blood samples showing psuedothrombocytopenia and by using other alternative anticoagulants.

MATERIAL AND METHOD- This study was conducted in the haematology department of the central laboratory of the NIMS medical college and hospital, Jaipur. OPD and IPD patient were evaluated for thrombocytopenia caused by use of EDTA anticoagulant in whole blood sample. Patients having low platelet count and with clumping or aggregates on peripheral blood film were selected. Samples of same patient were taken for further evaluation using alternative anticoagulant like sodium citrate and heparin.

RESULTS— A total of 50 patients within the age group of 20 to70 years were selected who has pseudothrombocytopenia. EDTA anticoagulated blood sample has platelet count in the range of $20x10^9$\l to $149x10^9$\l and samples from same patient anticoagulated with citrate has platelet count in the range of $41x10^9$\l to$312x10^9$\l and heparin anticoagulated sample has platelet count in the range of $29x10^9$\l to $210x10^9$\l. platelet counts decreased dramatically in EDTA anticoagulated samples as compare to citrate and heparin anticoagulated samples post four hour of collection.

Conclusion:- Manual microscopic examination of the peripheral blood films should be done in every case with low platelet count for platelet clumping or aggregation, or in isolated thrombocytopenia flaaged in hematology analyser. Alternate anticoagulant must be used for accurate platelet count and precise diagnosis.

KEYWORDS : Artifactual thrombocytopenia, thrombocytopenia, EDTA, anticoagulant

INTRODUCTION-

Thrombocytopenia is the most common cause of bleeding. Thrombocytopenia occurs due to many reasons main are- artifactual thrombocytopenia, deficient platelet production, increased platelet destruction and abnormal distribution or pooling within the body.

Pseudothrombocytopenia is a condition in which there is falsely lowered platelet count in patients who have no signs of bleeding like petechiae or echymoses. Artifactual thrombocytopenia is falsely low platelets, platelets are not counted properly and accurately and the most common cause being platelet satellitism and giant platelets[1].

It is caused by anticoagulant dependent agglutinins. These agglutinins are IgG, IgA and IgM immunoglobulins subtype which should be considered in patients who have thrombocytopenia but the absence of petechiae or any other bleeding signs or symptoms. This commonly occurs when EDTA is used as anticoagulant. Platelet clumping is time dependent and also depends on the type of automated counter used.[2] EDTA dependent pseudothrom bocytopenia (EDTA-PTCP) is a common problem amongnst laboratories.

MATERIAL AND METHOD:-

This study was performed in haematology department of central laboratory of NIMS medical college and hospital, Jaipur . All OPD and IPD patients whose CBC sample Came in as whole blood in EDTA vial during study period were evaluated and the criteria for selecting pseudothrombocytopenia (PTCP) was low platelet counts of less than $150x10^9$/l or positive flagging for platelet aggregates in system horiba pentrax 60 hematology analyser.

Blood smears were prepared of such cases using leishman stain (Romanowsky stain) and after manual microscopic blood film examination PTCP was confirmed by the presence of platelet clumps or aggregation. After confirmation of PTCP the patient was asked for additional blood samples using sodium citrate and heparin as anticoagulant and measured in parallel by automated routine haematological analyser (Horiba pentra 60) (5 part 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.

RESULT:- A total of 50 patients aged between 20 to 70 years were found to have pseudothrombocytopenia during the study period. Males accounted for 30% and females 70% with a M:F of 1:2.3.Platelet counts with EDTA anticoagulated samples ranged from $20x10^9$/l to $149x10^9$/l and samples from same patients anticoagulated using sodium citrate ranged from $41x10^9$ /l to $312x10^9$ /l and heparin anticoagulated showed platelet count ranging from $29x10^9$ /l to $210x10^9$ /l (fig.1).

The mean platelet count in EDTA- anticoagulated blood of individuals with PTCP was $104x10^9$/l whereas the mean platelet count in citrate-anticoagulated samples was $151x10^9$/l and 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 anticoagulated samples show higher value as compared to heparin. 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)

Table1- platelet value in different anticoagulants instant and after 4 hours

ANTICOAGULANT	MINIMUM VALUE	MAXIMUM VALUE	MEAN
EDTA	20200.00	149000.00	104296.00
CITRATE	41000.00	31200.00	151320.00
HEPARIN	29000.00	210000.00	123560.00

EDTA after 4 hours	4000.00	141000.00	53647.00
Citrate after 4 hours	40000.00	232000.00	131060.00
Heparin after 4 hours	29000.00	198000.00	106940.00

Discussion:-

Ethylenediaminetetraacetic acid (EDTA) is commonly used anticoagulant for blood cell counts estimation.

EDTA-PTCP is an in-vitro phenomenon due to formation of antiplatelet antibodies that cause platelet clumping in blood[1] .In this study EDTA-PTCP was diagnosed by examination of peripheral blood film for microscopic platelet aggregates or clumping in patients with low platelet count on Coulter cell counter.

EDTA-PTCP was diagnosed and confirmed by seeing platelet aggregates in smears in different literatures as well[3-6]. In this study, PTCP diagnosed from EDTA anticoagulated samples showed lower platelet count than samples anticoagulated with citrate and heparin. Samples anticoagulated with Citrate show higher platelet count than heparin anticoagulated samples. Werner et al found that citrate is superior to EDTA anticoagulant to reduce PTCP[4]. 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[7] . A higher incidence of PTCP was seen in females in this study as well.

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[4].

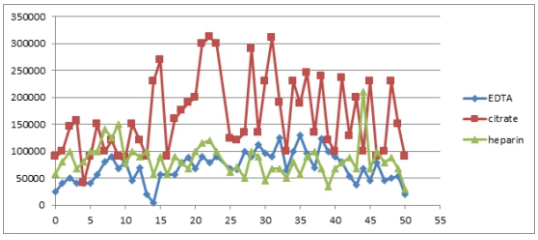

figure 1: Platelets count in different anticoagulants measured immediately after sample collection

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.[4] 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[8].

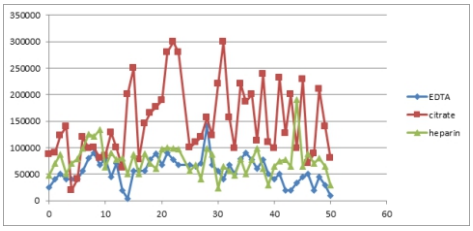

Figure 2: Platelets count in different anticoagulants measured after 4 hrs of sample collection.

Blood films are also not routinely evaluated by visual inspection and remains unnoticed because warning flags and histograms of hematology analyzers are not interpreted correctly. So, undiagnosed EDTA-PTCP 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. The reliable and timely identification of this artifact is essential, since there are high chances that it may be confused with other life threatening platelet disorders, or otherwise leads to inappropriate clinical and therapeutic decision making.[9]

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 changesof mean platelet volume (MPV)[4,10,11].

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.[4,13] The addition of an aminoglycoside antibiotic (e.g kanamycin) has similarly been used to count platelets in cases of PTCP.5,14 Gschwandtner et al referred PTCP as a 'laboratory disease[15] and Schrezenmeir et al proposed that the phenomenon of in vitro-platelet aggregation should be collectively called as anticoagulant induced PTCP[16].

CONCLUSION:-Peripheral blood films should be examined for platelet clumping/aggregation in cases which shows no clinical signs of thrombocytopenia 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 testing and expenditure of the patient.

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