Ethylenediaminetetraacetic Acid (EDTA) – Induced Pseudothrombocytopenia – A Routine Challenge

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ABSTRACT

Introduction:
Pseudothrombocytopenia is a condition in which false platelet count occur. EDTA – dependant – pseudothrombocytopenia is because of agglutination of anti-platelet antibodies due to its reaction with platelet in EDTA – anticoagulant blood.

Objective:
To see the effect of ethylenediaminetetraacetic acid (EDTA) on platelet clumping in patients with pseudothrombocytopenia.

Materials and Methods:
This was a prospective study conducted at Department of Pathology, Indus Medical College, Tando Muhamamd Khan over a period of 6 months. Patients with pseudothrombocytopenia on Hematology Analyzer were cross-checked with standard microscopy for confirmation of pseudothrombocytopenia. The samples were also collected in citrate and heparin tubes for evaluation. The data was analyzed using SPSS 24.0.

Results:

Conclusion: Patients showed significant effect of EDTA anticoagulant on platelet clumping. Patients with thromcytopenia that do not correlate clinically, must be cross-checked by standard microscopy for confirmation of pseudothrombocytopenia.

Keywords: Pseudothrombocytopenia, thrombocytopenia, EDTA, anticoagulant, platelets.

Introduction

Pseudothrombocytopenia is defined as falsely reduced count of platelets with absence of clinical findings such as ecchymoses or petechiae which is caused by inadequate measurements. Platelet clumping and presence of giant platelets are the main causes of falsely decrease in platelet counts. [(1)]

Platelet clumping is most commonly seen when blood is collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant. This is caused by anticoagulant dependant agglutinins, which include immunoglobulins (IgM, IgA and IgG). [(1-2)] Clumping of platelets depends on time and also relies upon use of instrumentation for automated counting of platelets. [(3-5)] Among all cases of isolated thrombocytopenia in hospitalized patients, pseudothrombocytopenia accounts between 0.01 - 2% cases. [(1)] In absence of clinical

features, evaluation of decreased platelets number is necessary. Visual examination by peripheral blood smear will be accurate to rule out the presence of pseudothrombocytopenia so that unnecessary treatment can be avoided. [2] In such circumstances, use of alterative anticoagulation may help in more accurate estimation of platelet estimation. [2, 6] Therefore, this study is designed to evaluate the effect of EDTA on platelet clumping, resulting in pseudothrombocytopenia.

Materials and Methods

This was a prospective study conducted at Department of Pathology, Indus Medical College Hospital Tando Muhammad Khan. The study was conducted for period of 6 months (February 2020 to July 2020). Blood samples were collected in EDTA tubes and evaluated by automated haematology analyzer. Patients with low platelet counts without any clinical features of thrombocytopenia were included. Peripheral blood smears were performed for all cases of thrombocytopenia. Wright's stain was used for preparation of slides and examined by 2 pathologists.

Patients with false low count of platelets due to clumping were filtered. Additional blood samples were collected in heparin tubes and sodium citrate tubes. Correction for sodium citrate dilution was performed by multiplication of obtained number with multiplication factor 1.1 (n x 1.1). These samples were also automated haematology analyzer. After 4 hours of collection, the samples were again analyzed for the evaluation of time dependant influence. The data was analyzed using SPSS 24.0.

Results

A total of 90 patients were selected for the study. Patients were aged between 16 to 60 years. Females were more prevalent as compared to males with male to female ratio of 1:1.8 (Figure 1). Mean age of patients was 41.49 ± 5.3 years. Platelet counts were evaluated from samples collected immediately in all anticoagulants (Table 1) and after 4 hours (Table 2).

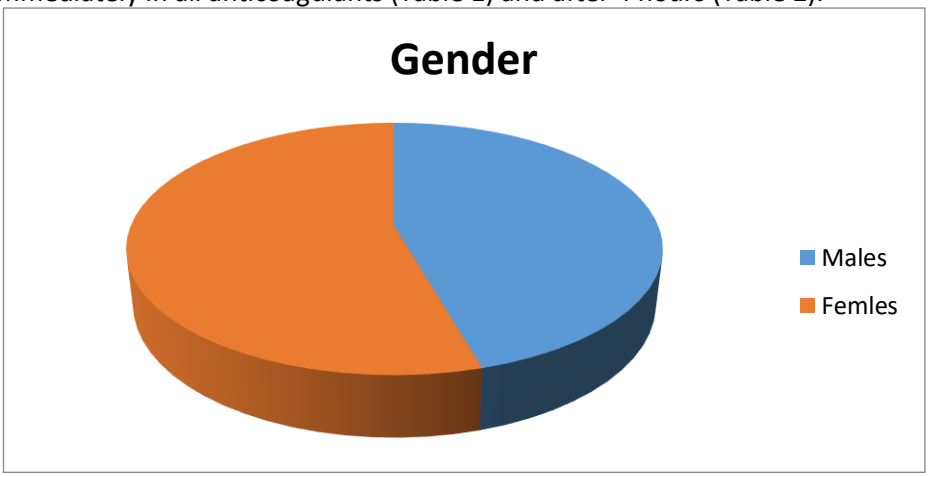

Figure 1: Gender Distribution (n=90)

Table 1: Values of Platelets in Various Anticoagulants Immediately After Collection (n=90)

Anticoagulants	Minimum	Maximum	Mean
EDTA	25,000	1,38,000	1,01,000
Citrate	47,000	3,51,000	1,57,000
Heparin	33,000	2,27,000	1,23,000

Table 2: Values of Platelets in Various Anticoagulants after 4 Hours of Collection (n=90)

Anticoagulant	Minimum	Maximum	Mean
EDTA	49,00	1,18,000	58,000
Citrate	44,000	2,43,000	1,38,000
Heparin	31,000	2,03,000	1,08,000

Discussion:

Pseudothrombocytopenia can be caused by various reasons such as inadequate sampling of blood, clumping or satelllitism of platelets or due to giant platelets. Clumping of platelets may also be due to EDTA – anticoagulant or cold agglutinin. Anti-platelet antibodies are the main cause of pseudothrombocytopenia due to EDTA. [1, 3-4] The activator for production of antibodies is not known and can rise in response to different antigen, followed by cross-reactivity with platelets causing agglutination. [2] The complete and exact mechanism of antibody reaction with antigens on membranes of platelets is not understood. It is postulated that when EDTA is mixed with platelets, persuade a conformational variation in membrane that results in ''neoantigens'' exposure to which antibody binds. [2, 5] In patients with Glanzmann thrombasthenia, there is no reaction of platelets with these antibodies which suggests that glycoproteins on platelet membrane, GpIIb or GpIIIa, which are not present in these patients, may be ''neoantigens'' in pseudothrombocytopenia. [6] It is postulated that the binding site for antibody to GpIIb is usually not visible in GpIIa-GpIIIa complex and so the complex must separate before binding of antibody occur. [7] The EDTA concentration, drugs, temperature and pH may fluctuate the dissociation of complex. [8] it is also been alleged that clumping of platelets induced by EDTA can be separated by mixing calcium chloride in order to re-associate the complex of GpIIb-GpIIIa and sodium heparin to maintain the anticoagulant effect for exact platelet count estimation. [9, 14] Likewise, the count of platelets in pseudothrombocytopenia has been performed by adding aminoglycosides. [1, 9-10]

In current study, the pseudothrombocytopenia caused by EDTA was more prevalent in females than in males. Similarly, Berkman et al showed more female predominance in his study. [2] In our study, the samples anticoagulated with EDTA revealed decreased count of platelets and mean count of platelets as compared to heparin and citrate anticoagulated samples. Citrate is considered to have better anticoagulant activity for reduction of pseudothrombocytopenia in various studies. [1, 9, 11] In few studies, the count of platelets was increased in magnesium sulphate anticoagulated samples as compared to EDTA. [9] The routine estimation of mean platelet volume (MPV) is performed in by majority of automated haematology analyzers and is very useful to differentiate idiopathic thrombocytopenic purpura (ITP) or other etiologies of thrombocyropenia from pseudothrombocytopenia. [2, 12] Few studies also support the time dependant effect of EDTA anticoagulation which also leads to change in mean platelet volume (MPV). [1, 9]

In our study, decrease in platelet counts was seen in EDTA samples as compared to samples anticoagulated with heparin and citrate after 4 hours of collection. These findings are also supported by other studies. [1, 2, 9, 13] Clumping of platelets seen on peripheral film is helpful in diagnosis of pseudothrombocytopenia caused by EDTA. [9-10, 14-15] The term ''EDTA – dependant

thrombocytopenia'' was used by Berkman for the condition of in vitro aggregation of platelets with the use of blood anticoagulated with EDTA. [2] Various other terms were also used by other researchers, including ''anticoagulant – induced pseudothrombocytopenia'' by Schrezenmeir and ''laboratory diseases'' by Gschwandtner et al. [16-17]

Conclusion

EDTA-anticoagulation showed significant platelet clumping which cause pseudothrombocytopenia. Peripheral blood smears are usually not evaluated by standard microscopy and they remain ignored if histograms or warning flags of automated haematology analyzers are not evaluated adequately. Due to this ignorance, pseudothrombocytopenia caused by EDTA is undiagnosed which ultimately leads to unwanted investigations, unnecessary blood transfusions and hold back of emergency surgical procedures. In these circumstances, patients may suffer discomfort and extra cost. Peripheral blood smears should be evaluated properly for the evaluation of clumping and/or aggregation of platelets in patients with no clinical picture of thrombocytopenia.

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