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EVALUATION OF EDTA DEPENDENT PLATELET CLUMPING RESULTING IN PSEUDO THROMBOCYTOPENIA

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Abstract

Background: *Pseudo thrombocytopenia is defined as a low platelet count, caused by EDTA dependent platelet aggregation. Pseudo thrombocytopenia has no clinical significance. It misdiagnosis may lead to unnecessary diagnostic tests and treatment.*

Objective: *To evaluate the frequency of EDTA dependent pseudo thrombocytopenia in a laboratory setting.*

Methodology: *It is a cross sectional study and enrolled 500 participant's samples. The both male and female came for complete blood count (CBC) was initially enrolled. Later on only participants had low platelet count in EDTA vaccutainers were enrolled for further analysis. After taking verbal informed consent, about three to five milliliter blood was taken in EDTA and sodium citrate vaccutainers separately. The platelet count was estimated through automated hematology analyzer. The data was analyzed through (SPSS v.25.0).*

Results: *From total of 240 participants, more male participants (60.41%) were observed as females (39.58%). The mean (± standard deviation) age was 26.66±5.031. It has been observed that the 31.25% participants had low platelet count when measuring in EDTA vaccutainer. While in sodium citrate vaccutainer only 10.0% had low platelet count. It has been found that sodium citrate vaccutainer is more reliable for platelet count estimation (p=0.001).*

Conclusion: *EDTA dependent PTCP is a laboratory artifact that has serious effects for patients. In the present study, sodium citrate anticoagulant effectively corrected EDTA dependent PTCP in 21.25% of the patients.*

INTRODUCTION

Thrombocytopenia is usually termed as low platelet amount concentration than the normal range (1). It is also known as true thrombocytopenia (2). On the other hand, pseudo thrombocytopenia (PTCP) is not a disease rather it is a sampling or laboratory problem or errors that falsely causes low platelets to count during laboratory examination (3). There could be several reasons for PTCP. One of the most common reasons that lead to PTCP is the formation of platelet clumps in the patient's blood sample (4). Clumping in the sample may be due to; various sampling errors, the late addition of anticoagulant after drawing blood sample, improper mixing of anticoagulant, vigorous mixing of sample in anticoagulant, presence of anti-platelet autoantibodies in anticoagulant, late examination of the sample, collection and examination of the sample at a temperature lower than 37'C and the most important is Ethylenediaminetetraacetic acid (EDTA) mediated clumping (5, 6)Different vials are used for platelets count containing different anticoagulants. These include EDTA Vial, sodium citrate vial, and heparin vial. EDTA is the most commonly used vial having a purple top. It is universally used for complete blood cells analysis (7). Although these are not ideal for complete blood profiles these can give better results. Sodium citrate can reduce platelet clumping up to two times while heparin can reduce platelet clumping rate more efficiently. But in the case of PTCP, these two also cannot give 100% results (8).

However, in rare cases, platelets agglutination occurs due to EDTA (9). This phenomenon occurs in vial because of some anti-platelet auto-antibodies. Immunoglobulin G or immunoglobulin M is the auto-antibodies that can act on the surface of glycoprotein (gp) IIB or IIIA present on the surface of platelets. These auto-antibodies present in EDTA anticoagulants can expose epitope surfaces of gp IIB/IIIA of platelets that can then react to form clumps (10). If any patient present with thrombocytopenia, but have no clinical symptoms like bleeding, PTCP should be diagnosed before any other diseases (3). Otherwise, additional costs and risks associated with further diagnostic testing may occur (4). This study mainly focuses on the incidences of EDTA-dependent platelet clumping leading PTCP in a laboratory setting.

Material and Methodology

It is a cross sectional study and enrolled 500 participant's samples. The non-probability convenient sampling technique was followed. The both male and female came for complete blood count (CBC) was initially enrolled. Later on only participants had low platelet count in EDTA vaccutainers were enrolled for further analysis. The patient had any other chronic disease, clotted samples, and dengue patient samples were excluded. After taking verbal informed consent, about three to five milliliter blood was taken in EDTA and sodium citrate vaccutainers separately. The platelet count was estimated through automated hematology analyzer (MINDRAY BC 5000). The data was recorded on a excel sheet and statistical analysis was performed through Statistical Package for the Social Sciences (SPSS v.25.0). The data was analyzed descriptively and analytically.

Results

In this study two hundred and forty participants were finally enrolled and their platelet count was analyzed to identify PTCP. From total of 240 participants, more male participants (60.41%) were observed as females (39.58%). The ages of participants were between 10 to 40 years. The mean (± standard bdeviation) age was 26.66±5.031. The age groups were made and observed (Table 1).

Table 1: Characteristics of study variables

Variables	Frequency (%)
Gender	
Male	145 (60.41%)
Female	95 (39.58%).
Age (Years)	
10-20	22 (9.16%)

21-30	160 (66.66%)
31-40	58 (24.16%)

In this study, platelets count was observed in EDTA and sodium citrate vaccutainer to estimate the frequency of PTCP in participants. According to the laboratory guidelines, the normal ranges of platelets count is 150-450 x10^9/L. On estimation it has been observed that the 31.25% participants had low platelet count when measuring in EDTA vaccutainer. While in sodium citrate vaccutainer only 10.0% had low platelet count. The ANOVA test was applied to estimate the values of platelet count in EDTA and sodium citrate vaccutainers (Figure 1). It has been found that sodium citrate vaccutainer is more reliable for platelet count estimation (p=0.001). The p-value of ≤0.005 was considered statistically significant.

Table 2: Frequency and percentage distribution of platelets count in different vaccutainers

Platelet count (x10^9/L)	EDTA vaccuatiner	Sodium citrate vaccutainer
Less than the normal range	75 (31.25%)	24 (10.0%)
Normal	165 (68.75%)	216 (90.0%)

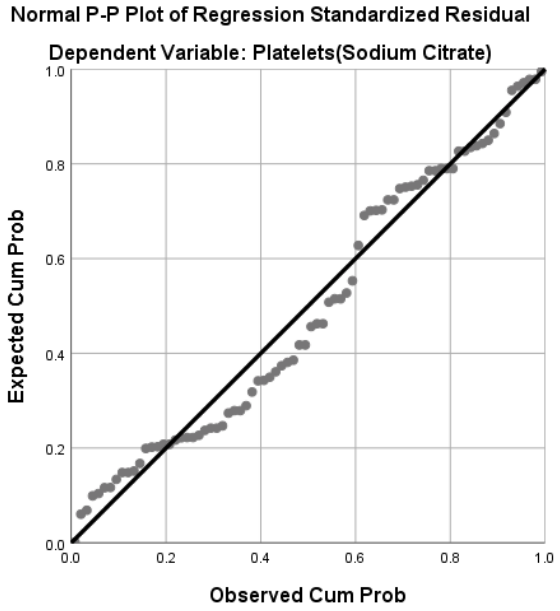

Figure 1: Analysis of variance for EDTA and sodium citrate vaccutainer for platelet count estimation

Discussion

EDTA dependent PTCP is an artifact that was produced in vitro and has major consequences for clinical practice (11). In order to rectify this laboratory error, a number of different approaches are now being investigated. In present study platelet counts were found to be significantly greater in samples processed with EDTA anticoagulant than in samples treated with sodium citrate. In the present study this was the 21.25% of the total patients.

A study by Weber et al. in 2021 enrolled seventeen paired sets of blood samples in EDTA and sodium citrate vaccutainers. The platelet count was assessed at baseline and at multiple intervals, extending up to a maximum of four hours. The baseline citrate platelet counts were predominantly lower than the EDTA counts, consistent with the results of present study. They also discovered that citrate sample-based platelet counts exhibited less consistency than EDTA sample-based platelet counts over time, with optimal stability lasting up to one hour post-sample collection

(12). A recent report from Pakistan evaluated the effectiveness of three distinct anticoagulants for the rectification of chronic PTCP in patients. They could not resolve platelet clumping despite utilizing EDTA, sodium citrate, and lithium heparin anticoagulants. Moreover, they advised that in instances of multicoagulant-resistant PTCP, fresh blood samples should be obtained in a syringe for manual platelet counting through a Neubauer chamber (13).

A study by Sharif F et al., enrolled 151 patients with EDTA dependent PTCP, and samples were taken from both EDTA and sodium citrate tubes. The median platelet count in blood samples treated with EDTA anticoagulant was 85,000/ul (interquartile range, 65,000-114,000). The median platelet count in sodium citrate blood samples was 78,100/ul (interquartile range: 55,000–100,100). Overall, blood samples processed in sodium citrate tubes had lower platelet counts than EDTA samples. Sodium citrate anticoagulant was effective in resolving EDTA-PTCP in 47 (31.1%) of instances. However, in the vast majority of cases (104 out of 151, 68.9%), the platelet count obtained from the EDTA blood sample was greater. The median platelet count difference measured by both tubes was 19500/ul (range: 1000–130800/ul) (14). The present study findings have practical application, which is the study's strength. In several centers, sodium citrate is used instead of EDTA, with varying results, as described above. The present study findings provide compelling evidence against the use of sodium citrate for this purpose. However, the data was collected from a single center and is constrained due to the retrospective study methodology. We urge that further prospective studies be done to identify alternate approaches for EDTA dependent PTCP correction in order to avoid the associated clinical concerns.

Conclusion

EDTA dependent PTCP is a laboratory artifact that has serious effects for patients. In the present study, sodium citrate anticoagulant effectively corrected EDTA dependent PTCP in 21.25% of the patients. The present study findings do not support the use of sodium citrate instead of EDTA for the treatment of PTCP.

Conflicts of interest: None.

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REFERENCES

1.Reusser P, Osterwalder B, Speck B. [EDTA-dependent pseudo-thrombopenia]. Schweiz Med Wochenschr. 1986;116(45):1566-9.
2.Yoneyama A, Nakahara K. [EDTA-dependent pseudothrombocytopenia--differentiation from true thrombocytopenia]. Nihon Rinsho. 2003;61(4):569-74.
3.Lardinois B, Favresse J, Chatelain B, Lippi G, Mullier F. Pseudothrombocytopenia—a review on causes, occurrence and clinical implications. Journal of clinical medicine. 2021;10(4):594.
4.Lin J, Luo Y, Yao S, Yan M, Li J, Ouyang W, et al. Discovery and Correction of Spurious Low Platelet Counts due to EDTA-Dependent Pseudothrombocytopenia. Journal of Clinical Laboratory Analysis. 2015;29(5):419-26.
5.Xu P, Fang K, Chen X, Liu Y, Dong Z, Zhu J, et al. The flagging features of the Sysmex XN-10 analyser for detecting platelet clumps and the impacts of platelet clumps on complete blood count parameters. Clinical Chemistry and Laboratory Medicine (CCLM). 2022;60(5):748-55.
6.Waxman C, Lind T. Blood sample collection and handling. Advanced monitoring and procedures for small animal emergency and critical care. 2023:699-715.
7.Saigo K, Sakota Y, Masuda Y. [EDTA-dependent pseudothrombocytopenia: clinical aspects and laboratory tests]. Rinsho Byori. 2005;53(7):646-53.
8.Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. Clinical Chemistry and Laboratory Medicine. 2012;50(8):1281-5.

9.Zhang Y, Chen B, Feng J, Yu W, Liang W, Li M, et al. Effects of time, anticoagulant and detection channel on platelet count in ethylenediaminetetraacetic acid (EDTA)-dependent pseudothrombocytopenia. PeerJ. 2025;13:e19103.
10.Feys H. Insights into thrombotic thrombocytopenic purpura by monoclonal antibody-based analysis of the Von Willebrand factor cleaving protease, ADAMTS-13. 2006.
11.Amir E, Latif AQ, Anwar M, Rahat N, Khalid G, Anwar R. EDTA related Pseudothrombocytopenia (PTCP) Incidence and Resolution of Clinically Threatened Artifact. Pakistan Journal of Medical & Health Sciences. 2023;17(04):166-.
12.Weber D, Nakashima MO. Platelet count in sodium citrate-anticoagulated whole blood: Comparison to EDTA-anticoagulated results and stability over time. International Journal of Laboratory Hematology. 2021;43(1).
13.Waseem N, Hanif B, Jabbar J. Multicoagulant resistant pseudothrombocytopenia. JPMA. 2021;71(9):2247-9.
14.Sharif F, Junaid A, Ashraf K, Ijaz M, Saeed M, Farhat T, et al. Evaluation of sodium citrate anticoagulant for the resolution of EDTA-dependent pseudo thrombocytopenia. Journal of Ayub Medical College Abbottabad. 2023;35(4):544-8.