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ORIGINAL ARTICLE

EDTA related Pseudothrombocytopenia (PTCP) Incidence and Resolution of Clinically Threatened Artifact

ERUM AMIR[1], AMTUL QUDDOS LATIF[2], MOHAMMAD ANWAR[3], NOSHABA RAHAT[4], GULNAZ KHALID[5], RAFIA ANWAR[6]
[1]*Assistant Professor of Pathology, Karachi Medical & Dental College, Karachi*
[2]*Assistant Professor of Clinical Pathology, Jinnah Postgraduate Medical Centre*
[3]*Associate Professor & Head of the Department, Basic Medical Sciences & Blood Bank, JPMC, Karachi*
[4]*Associate Professor & Head of the Department, Pathology, Basic Medical Sciences Institute, JPMC.*
[5]*Professor & Head of the Department Pathology , KM&DC.*
[6]*Associate Professor of Pathology, KM&DC*
Correspondence to Dr. Erum Amir Email: erumamir09@gmail.com

ABSTRACT

Background: EDTA related pseudothrombocytopenia (PTCP) is immune mediated, time and temperature dependent spurious low platelet counts commonly observed in laboratory practices, as an in vitro phenomenon. Microscopic evaluation of every sample is not possible due to immense workload hence PTCP is overlooked mostly due to immense work load .Vortex mixer is practically feasible tool often used in laboratories to obtain the accurate platelet counts of the suspected samples by using same EDTA anticoagulated samples.
Aim: To evaluate the frequency of EDTA related pseudothrombocytopenia in OPD of tertiary care centre and analyze the vortexing for resolution of PTCP.
Methodology: This was case control study conducted in clinical pathology department of JPMC in collaboration with ICU from November 2022 to January 2023. Total 3000 samples of CBC were analyzed. 150 patients with platelet count less than 150μl , with abnormal histograms and platelet clumps on smear were enrolled as cases of PTCP. 150 healthy individuals with match age and gender recruited as controls. Sample was subjected to vortex for 2 min at 3000rpm Samples were reanalyze ,clumps dissolution recheck by smear.
Results : Prevalence of isolated thrombocytopenia was 33%, with 15% cases of EDTA related PTCP, platelet clumps seen in all PTCP cases on smear, 87% clumps were resolved completely with significantly increased platelet counts post vortexing with p value <0.05.
Conclusion: Vortexing has proved as a promising, rapid and a cost effective method to deal with PTCP, save patient from needless investigations and invasive procedures.
Keywords: Thrombocytopenia, Pseudothrombocytopenia, Ethylene diamine tetra acetic acid, Vortex, Platelet histograms,

INTRODUCTION

Thrombocytopenia is platelet counts below the normal reference range of 150,000/µl to 400,000 µl.It could be an incidental finding observed during complete blood count (CBC) evaluation. Platelet counts below 100,000 /µl are related with high risk of spontaneous bleeding. Pseudothrombocytopenia is suspected if clinical manifestations do not correlate with low platelet counts[12]. Pseudo thrombocytopenia (PTCP) is immune mediated, time and temperature dependentspurious low platelet counts, commonly observed in laboratory practices, as an in vitro phenomenon[7]. The potential etiological factors could bepre-analytical errors such as poor mixing of sample, time lapse betweensample collection and processing, use of different anticoagulants such as Ethylinediamine tetra acetic acid (EDTA), sodium citrate, heparin, large size platelets and platelet satellitism[17]. EDTA is so far the commonest cause of PTCP in the samples run on auto analyzers, initially detected by Gowland in 1969. EDTA dependent PTCP is an analytical error revealed after the introduction of automated hematological analysis in clinical pathology, defined and described specifically soon afterward[11,3].

EDTA is the preferably used anticoagulant, suitable for cell counting and microscopic evaluation of cell morphology on peripheral smear due to its ability of preservation of cellular morphology[2].It maintains the fluidity of whole blood that makes electronic cell counting much easier. EDTA is basically a Ca^{++} chelator that initiates conformational changes in platelets and exposes the cyto-adhesive glycoprotein IIb/IIIa receptors on the platelet's plasma membrane and thus antiplatelet IgM and IgG antibodies recognize these epitopes and platelet clumps are formed[1,3].

The prevalence of PTCP has been estimated at around 0.03–0.27% in admitted patients whereas reportedly found to be on the higher side as 17% in out-patients[15]. In addition to healthy individuals PTCP is also associated with certain pathological conditions like autoimmune disorders, cardiovascular and liver diseases, malignancies, sepsis, viral infections and also due to certain medications[12]. Peripheral smear is regarded as gold standard technology for recognition of platelet aggregates. PTCP remains overlooked during routine sample processing as microscopic evaluation of peripheral smears is skipped due to immense workload, whereas warning flags and platelet histograms shown by hematology analyzers are also misinterpreted[11].

Till date there is no consolidated guideline to identify and investigate this underdiagnosed and misleading laboratory artifact[4,5]. Nevertheless few studies recommend simple and cost effective measures, focused primarily to avoid the falsely low platelet counts, save time, resources and delayed reporting of the results[14]. The commonest strategy followed is to redraw the sample in alternative anticoagulants such as citrate and sodium heparin.However this is not only inconvenient for the patient but also increases the reporting time[16]. Vortexing is a manual method used in few laboratories for resolution of the PTCP. Sparse literature review is available about the standardized utilization of vortex regarding time and speed that otherwise might result in red blood cell fragmentation and false rise in platelet. Only a handful of studies have mentioned the significance of this simple technique for duration of 2 minutes at speed of 3000 rounds per minute (rpm) for the resolution of PTCP.

METHOD

Sample size: Using an online calculator on the Epiwebsite , the sample size was determined with a confidence interval of 95%(standard value of 1.96), the prevalence of the in vitro analytical artifact from a prior study (0.99%) and an error rare of 5% (standard value of 0.05).
Place of study: The present study was conducted in clinical pathology department of Jinnah Postgraduate Medical Center

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(JPMC). All ethical perspectives of the research were fulfilled in this study.

Duration of study: This was a case control study performed within a time period of 3 months from 1st November 2022 to 31stJanuary 2023.

Study population: The mixed patient's population of different specialties presented to OPD of Jinnah Postgraduate Medical center (JPMC).

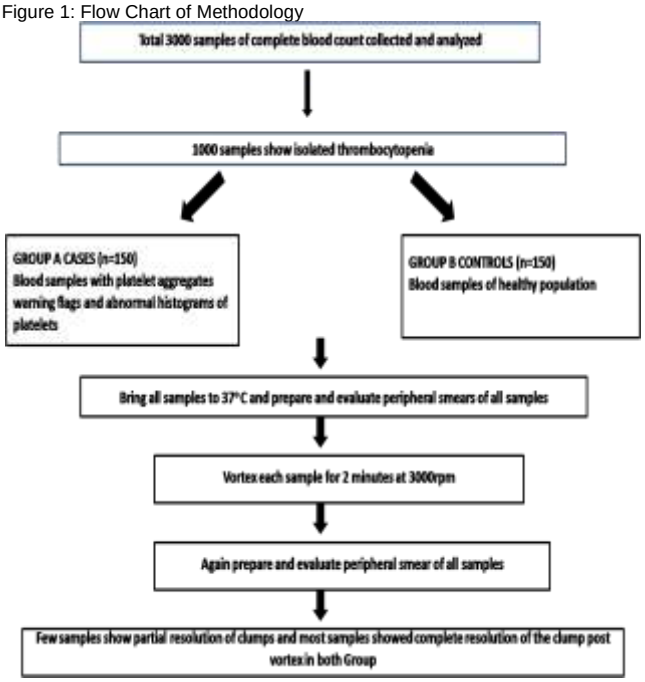
Ethical approval: This study was performed after approval was taken from institutional review board(IRB) of JPMC Karachi via a letter NO-F-2-82/2022-GEN/JPMC, dated 8-10-2022.

Inclusion criteria: Target population that was recruited comprised of two groups, Group A included samples of patients from OPD while Group B included control samples from the healthy population of employees of JPMC lab. Age and gender of controls were matched with cases. Patients of both genders between the age of 17 to 80 years were selected.

Exclusion criteria: Patients with any hemostatic disorder, leukemia, malignancies and chronic disorders were excluded. Patients admitted in wards of JPMC were not included in the study.

Data collection procedure: Whole blood samples with requisition of CBC were collected in EDTA containing purple top vacutainer and verified for pre analytical sample acceptance and rejection protocols of processing, accordingly analyzed in SYSMEX XN 1000 analyzer(Sysmex Corporation, Kobe, Japan) The laboratory is accredited by the ISO15189 standard for analysis of cell counts and perform regular internal and external quality control. Total 3000 samples of CBC were collected out of which 1000 samples with isolated thrombocytopenia were segregated. Among these samples with isolated thrombocytopenia, the analyzer exhibited warning flags and abnormalplatelet histograms in 150 samples. All of these samples were labeled as cases with suspected pseudo thrombocytopenia and selected as group A While 150 healthy controls were selected randomly from employees of clinical lab JPMC as group B.

Figure 1: Flow Chart of Methodology



Validity of instrument: Vortex device was calibrated using a tachometer, having the speed established and set at 3000 rpm.Samples from both groups were spinned for 2 minutes by vortex mixer at 3000 RPM.

Sample analysis: Peripheral smear of each sample was prepared and evaluated microscopically to confirm the presence of platelets clump of both groups. All 150 samples of Group A showed clumps on microscopy hence confirming pseudo thrombocytopenia. Whereas only few controls of Group B showed platelet clumps. Samples from both groups were spinned for 2 minutes by vortex mixer at 3000 RPM. Samples were analyzed by Sysmex XN 1000, peripheral smear prepared and evaluated. After vortexing, these samples were reanalyzed on Sysmex XN-1000 for checking platelet count and then were re-confirmed by microscopic analysis of the blood films to assess if the platelet clumps were resolved or not. Microscopy was performed by one person using Nikon B-50 40x objective. 30 fields were investigated. The number of clustered platelets in each aggregate in the 30 fields was registered and PTCP reported.

Statistical analysis: The statistical analysis was performed by using the statistical package in social sciences (SPSS) version 23. P value of <0.05 was considered as significant with 95% Confidence Interval (CI).

RESULTS

A total of 3000 blood samples through OPD were analyzed. 1000(33.3%) cases had isolated thrombocytopenia. All samples that exhibited warning flags and abnormal platelet histograms were considered as suspected cases of pseudothrombocytopenia. Peripheral blood smear review was done, all samples which showed platelet aggregates were considered as cases of pseudothrombocytopenia (n=150) that is 15% of total , 850(85%) samples didn't show any platelet aggregates on smear examination, were labeled as true thrombocytopenia (Table 2). Counts with percentages reported for baseline qualitative data set by applying Pearson Chi Square test, means with standard deviation reported the quantitative data set. P value of < 0.05 was considered statistically significant. Independent sample t test and paired sample t test were applied to compare the pre and post vortexed samples between cases and control groups and compare within cases and within controls groups respectively.

Insignificant difference was found in age and gender between cases and control group. Schistiocytes found in only 6% and 4% samples of cases and controls which was not statistically significant. Significant platelet clumps were found in samples of control group. Significant percentage of platelet clumps were seen on peripheral smear evaluation in cases as compared to controls, 130 (87%) out of 150 showed complete resolution post vortexed whereas 20(13.3%) showed partial resolution. Platelet clumps were resolved in 9(81.8%) samples after vortexing in control group (Table 1).

Table 1: Baseline characteristics of the cases and controls

Characteristics	Cases	Control	P value
Gender			
Male	100(66.7%)	100(66.7%)	1.000
Female	50(33.3%)	50(33.3%)	
Schistiocytes			
Yes	9(6%)	6(4%)	0.435
No	141(94%)	143(96%)	
Clumps before vortex seen	150(100%)	6(4%)	
Clumps before vortex not seen	0	143(96%)	0.001
Clumps totally resolved after vortex	130(86.7%)	9(81.8%)	0.651
Clumps partially resolved after vortex	20(13.3%)	2(18.2%)	

P value < 0.05 was considered significant Using Pearson Chi Square test

Table 2: Frequency of pseudothrombocytopenia in the patients of isolated thrombocytopenia

Total cases of isolated thrombocytopenia	1000(100%)
Cases with true thrombocytopenia	850 (85%)
Cases of pseudothrombocytopenia	150 (15%)

The mean platelet count of patients with PTCP was 63.52±31.79 before subjecting to vortexing was significantly lower than platelet count of healthy control group which was 286.73±79.09. Mean platelet count of samples of the PTCP cases obtained after rotation with vortex for 2 min at the speed of 3000rpm was 156.72±66.67 significantly less than mean platelet value of control group 287.32±79.09 (Table 3). The platelet counts were raised significantly post-vortexing both in cases and controls (Table 4).

Table 3: Comparative analysis between cases and controls before and after vortexing

	cases	controls	P value
Platelet count pre vortexed samples	63.52+/-31.79	286.73+/-79.09	.001
Platelet count post vortexed	156.72+/-66.67	287.32+/-79.09	.003

P value < 0.05 was considered as statistically significant by using independent sample t test

Table 4: Comparative analysis of the pre and post vortexed samples within cases and controls

	Cases	**Controls**
Number of samples	150	150
Platelet count before vortexing	63.52+/-31.79	286.73+/-79.09
Platelet count after vortexing	156.72+/-66.67	287.32+/-79.09
P value	.005	.141

P value of <0.05 considered as statistically significant by using paired sample t test

DISCUSSION

The automated hematology analyzers are integrated for the upgradation of current laboratory setup and enhancement of diagnostic practices, widely utilized for clinical as well as researchs' perspective. Complete blood count is the most frequently requested investigation by clinicians in all health care systems[1]. Even though auto-analyzers are calibrated and quality assurance performed according to manufacturer's instructions, almost every laboratory experiences few specimens with inaccurate results for one or more CBC parameters. These unreliable reports draw detrimental effects on patientcare and must be addressed timely and professionally[20]. EDTA dependent pseudothrombocytopenia is a relatively common laboratory artifact caused by in vitro platelet aggregation secondary to immune mediated exposure and cohesion of cryptic antigen on the surface of platelet with non-specific anti-platelet antibodies[9,10].

In this study pseudothrombocytopenia was identified in patients of isolated thrombocytopenia by microscopic evaluation of peripheral smear for the presence of platelet aggregates. Peripheral smear examination is the gold standard for the confirmation of allegedly low platelet count reported by analyzers in samples anti-coagulated with EDTA. The prevalence of EDTA associated PTCP in general outpatient population is 5% in our study which increased upto 15% within cases of isolated thrombocytopenia, in accordance with the cited literature[15,17]. In contrast Zhang et al reported prevalence of 49% PTCP whereas a local study has documented much higher prevalence of PTCP that is 75%[10,9] . Gender wise, there is no difference found in frequency of PTCP in the present study which contradicts results from a similar study by Pullen et al and Shresta et al who found PTCP more common in females as compared to males .As this is a case-control study, so we recruited equal number of patients from both males and females from diseased and healthy population and that could be the logical explanation of no influence of gender on prevalence of PTCP in our study. Lippi et al depicted comparable findings[17].

In this study we followed the protocol and segregated the samples reported as low platelet counts with alarm flags and abnormally crenated platelet histograms. To the best of our knowledge, so far there is no recommended guideline to obtain the reliable results on these specimens. Practically applicable approaches to achieve reliable results are heating the samples upto 37^{0}C, alternate anticoagulant, and use of vortex. Vortex mixer is a practically feasible tool often used in laboratories to obtain the accurate platelet counts of the suspected samples by using same EDTA anti coagulated sample. We subjected the samples with suspected thrombocytopenia to vortexing and rerun the samples on analyzers. Platelet counts were substantially increased in the PTCP cases comparable to controls after vortexing through reduction in the platelet aggregates. Undercontrolled time and speed of this experimental study, our findings demonstrated that vortexing is an effective strategy applied to optimize platelet count in routine lab practices. There are insufficient publications on vortex as reliable manoeuvre for dissipation of platelet clumps. Similarly Romario et al and Tantanate et al reported vortex as compelling technique to deal with PTCP[7,16].

More importantly our study highlighted that by setting standard speed and time, vortexing imparted the detrimental impact on erythrocytes as minimal. Schistiocytes reported after vortexing in samples of diseased as well as healthy group, strongly aligned with the observations of Romario et al[7.] Controlled time and speed in this experimental study played vital role in preserving the structure of red blood cells. Control group showed insignificant rise in platelet counts in contrast to the cases due to the less number of platelet aggregates needed to be resolved in controls. 86.7% of platelet aggregates were resolved by vortexing in this experimental study whilst partial dissociation occurred in 13.3% cases of PTCP. Complete disaggregation of 85% of the platelet clumps was stated by Mourad et al which also corresponds to our data findings[21].

CONCLUSION

PTCP is a complex phenomenon frequently observed in laboratories leading to inaccurate generation of reports . It can be resolved with minimum resources and efforts after timely identification of this in vitro mechanism. Present study successfully demonstrated the standardized application of vortex in resolution of platelet clumps by setting speed at 3000 rpm for 2 minutes. Additionally this study determined Vortexing produces almost no detrimental effect on erythrocytes cell membrane in majority of PTCP cases. Larger fraction of clumps were resolved byvortexing at controlled time and speed without red blood cell fragmentation .Vortexing has proved as a promising, rapid and a cost effective method to deal with PTCP and facilitates the lab personnel in timely dispatch of results with corrected cell counts , avoid sample recollection and save patient from needless investigations, invasive procedures and wastage of money. We suggested that by integrating vortex in algorithms designed to figure out the platelet aggregates detected in routine samples , it would no longer be a challenge for the laboratories to mete out accurate reports.

Clinical Significance of pseudothrombocytopenia: It is a laboratory finding benign in nature. The patient with PTCP are not at risk of bleeding nor do they require any medical intervention like transfusions. It can lead to erroneous diagnosis, misinterpretation of a clinical condition and unnecessary lab investigations. Pseudo thrombocytopenia may also induce psychological stress on the patients.

Contribution of authors: EA: Presented the main idea, did the main write-up, and approved the final version to be published, **AQL:** Designed the study and revised it critically for the important intellectual content and supervised the study, **MA:** Provide concept, drafted the article and study design and main methodology, analyzed and interpret the data, **NR & GK:** Sampling, acquisition, and analysis of data, **RA:** Sampling, acquisition of data

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Conflict of interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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Declaration of Patient Consent: The authors certify that they have obtained all appropriate patient consent forms describing the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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