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ORIGINAL ARTICLE

EVALUATION OF SODIUM CITRATE ANTICOAGULANT FOR THE RESOLUTION OF EDTA-DEPENDENT PSEUDO THROMBOCYTOPENIA

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Background: EDTA-dependent pseudo thrombocytopenia (EDTA-PTCP) refers to a falsely low platelet count occurring in the presence of ethylene diamine tetra-acetic acid (EDTA) anticoagulant during blood sample collection, which results in the formation of platelet clumps in vitro. This phenomenon has significant clinical implications, including unnecessary administration of platelets. Our study aims to evaluate the efficacy of sodium citrate anticoagulant for the resolution of EDTA-PTCP. **Methods:** This retrospective study was conducted in the haematology laboratory of Shifa International Hospital (SIH), Pakistan. Patients with pseudo thrombocytopenia (i.e. platelet count less than 150,000/ul with platelet clumps seen on peripheral smear) were included in this study if they had blood samples drawn in both EDTA and sodium citrate tubes less than 48 hours apart. Data was analyzed using IBM® SPSS Software Version 22. **Results:** A total of 151 study participants were included in this study. The mean age was 48.95±20.69 years and the majority were female (52.3%). Wilcoxon signed-rank test showed that there was a statistically significant difference in platelet count measured in both tubes (Z = -3.223, *p*=0.001). Overall, blood samples processed in sodium citrate tubes showed lower platelet count than EDTA samples. Sodium citrate anticoagulant was able to correct EDTA-PTCP in 47 (31.1%) of the cases. **Conclusion:** Sodium citrate anticoagulant was only able to resolve one-third of our EDTA-PTCP cases. Our findings do not support the use of sodium citrate as a suitable alternative for correction of EDTA-PTCP.

Keywords: Platelets; Pseudo thrombocytopenia; EDTA; Sodium citrate; Diagnosis

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INTRODUCTION

Pseudo-thrombocytopenia (PTCP) refers to a falsely low platelet count which can occur due to various reasons such as the presence of clots in blood samples, platelet satellitism and platelet size variation.[1] A major cause of PTCP is the use of ethylene diamine tetra acetic acid (EDTA) anticoagulant used during blood sample collection. EDTA is the most commonly used anticoagulant in routine haematology testing. However, it can induce the formation of platelet clumps in vitro, a phenomenon known as EDTA-dependent pseudo-thrombocytopenia (EDTA-PTCP).[2]

Previous studies have shown that spuriously low platelet count due to EDTA-induced platelet aggregation can be seen in 0.1% of the general population as well as in up to 15% of patients being evaluated for thrombocytopenia.[3,4] EDTA-PTCP is also seen more frequently in patients with a history of hospital admission, autoimmune diseases, viral infections and recent immunization.[5] The proposed mechanism of EDTA-PTCP is that EDTA mediates a conformational change in platelet structure which exposes glycoprotein IIb/IIIa (Gp IIb/IIIa) on the surface membrane. Pre-existing antibodies present in blood bind to this receptor, inducing platelet activation and aggregation.[6]

EDTA-PTCP is an in-vitro artefact with significant clinical implications. Hence it is imperative to take preventive and corrective action in such cases. Falsely low platelet count has prompted clinicians to transfer their patients to intensive care units, posing an unnecessary burden on hospital resources and patient's finances.[7] Ceran *et al*. highlighted how EDTA-PTCP may lead to unnecessary platelet transfusion. This poses a risk to patient safety by exposing them to the adverse events associated with transfusion, as well as putting constraints on the availability of platelet units.[8] Zhong *et al*. reported a case in which EDTA-PTCP led to the misdiagnosis of Immune Thrombocytopenia (ITP) and non-indicated administration of dexamethasone.[9] Furthermore, Sudha *et al*. reported a case in which EDTA-PTCP led to the performance of bone marrow biopsy, an invasive procedure which could have been avoided.[10]

Various methods have been recommended for the resolution of pseudo-thrombocytopenia. One such technique is the use of an alternative

anticoagulant other than EDTA during blood sample collection.[11] A study from Pakistan published in 2021 reported that trisodium citrate anticoagulant led to a statistically significant improvement of platelet count in samples with EDTA-PTCP.[12] The rationale for using another anticoagulant is that anti-platelet antibodies which result in platelet clumping would not form in the absence of EDTA. However, the alternative anticoagulant must be suitable for measuring all parameters of the complete blood count. Therefore, our study aims to evaluate the efficacy of sodium citrate anticoagulant for the correction of EDTA-PTCP.

MATERIAL AND METHODS

This is a retrospective study conducted in the haematology laboratory of Shifa International Hospital (SIH), Pakistan. SIH is a 550-bed quaternary care hospital, accredited by the Joint Commission International (JCI) and regularly participates in external quality assurance (EQA) activities conducted by the College of American Pathologists (CAP). On average, the department of haematology receives approximately 800 blood samples for complete blood counts (CBC) per day. The frequency of EDTA-PTCP reported in our laboratory is less than 1% of all blood samples received for CBC testing.

Patients were recruited for this study based on the inclusion and exclusion criteria given below.

Inclusion criteria:

All of the following criteria were required for inclusion in this study:

- Patients of all age groups who underwent CBC testing at SIH between March and November 2022.
- CBC performed on a sample in an EDTA tube shows pseudo thrombocytopenia (i.e., platelet count less than 150,000/ul with platelet clumps seen on peripheral smear).
- Blood samples were drawn in both K3-EDTA and 3.2% sodium citrate tubes (Greiner Bio-one, Austria) less than 48 hours apart.

Exclusion criteria:

- True thrombocytopenia in the absence of platelet clumping (occurring due to various medical and haematological disorders)
- Any history of platelet transfusion
- CBC only available in one vial (either EDTA or sodium citrate)
- In the pre-analytical phase, samples with insufficient blood volume were excluded. This was done to control the confounding effect of an inappropriate ratio of blood to anticoagulant volume.

Blood samples were processed on Sysmex automated analyzer XN 9000, with all CBC indices including platelet count measured through an impedance channel. Impedance is the most common modality available in haematology analyzers. Peripheral smears were examined on the blood samples in both EDTA and sodium citrate tubes.

There is a dilutional impact on certain CBC parameters when blood is collected in a sodium citrate tube. Therefore, a multiplication factor of 1.1 was applied to the values of haemoglobin, haematocrit, total leucocyte count and platelet count determined from the samples processed in a sodium citrate tube. This correction factor was not applicable for mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), which are derived parameters.[13]

Data has been analyzed using IBM® SPSS Software Version 22. Mean and standard deviation are calculated for quantitative variables which include age and values of CBC parameters. Frequencies and percentages are reported for qualitative variables, namely gender. Shapiro-Wilk test is being applied to determine whether the CBC parameters follow a normal distribution. Independent samples T-test is used to compare CBC parameters of EDTA and sodium citrate tubes for normally distributed variables whereas non-parametric tests namely the Wilcoxon signed-rank test and Mann-Whitney U test have been used in case of skewed data. A p-value of <0.05 is considered statistically significant for all data analysis.

This study was commenced after receiving ethical approval from the Institutional Review Board of SIH. Due to the retrospective nature of this study, funding was not required.

RESULTS

A total of 151 study participants with EDTA-PTCP were included during the study period who had samples drawn in both EDTA and sodium citrate tubes. The majority of the study participants were female (79 participants, 52.3%). The mean age was 48.95±20.69 years. Figure 1 shows the frequency of EDTA-PTCP according to age distribution.

Figure 2 shows the typical peripheral smears of our patients with EDTA-PTCP. These smears were prepared from the blood samples drawn in EDTA tube.

Figure 3 shows a smear prepared from a sample processed in a sodium citrate tube, still exhibiting small platelet clumps.

Paired samples t-test showed a statistically significant difference in haematocrit and MCV measured in blood samples from both tubes ($p<0.05$). Whereas haemoglobin, MCH and total leucocyte count were similar in both tubes. Table 1 shows the mean and standard deviation of the aforementioned CBC parameters.

Shapiro-Wilk test was applied which showed that platelet count measured in both tubes did not follow a normal distribution. The median platelet count within blood samples with EDTA anticoagulant was 85,000/ul (interquartile range: 65,000–114,000). Whereas the median platelet count in sodium citrate blood samples was 78,100/ul (interquartile range: 55,000–100,100). Hence, non-parametric analysis was performed. Wilcoxon signed-rank test showed that there was a statistically significant difference in platelet count measured in both tubes (Z = -3.223, *p*=0.001). Overall, blood samples processed in sodium citrate tubes showed lower platelet counts than EDTA samples. Sodium citrate anticoagulant was able to resolve EDTA-PTCP in 47 (31.1%) of the cases. However, in the majority of the cases (104 out of 151, 68.9%), the platelet count measured from the EDTA blood sample was higher. The median difference in platelet count measured by both tubes was 19500/ul (range: 1000 to 130800/ul). Table-2 summarizes the characteristics of patients who showed improvement in platelet count with sodium citrate anticoagulant as compared to those in whom EDTA-PTCP was not resolved with sodium citrate.

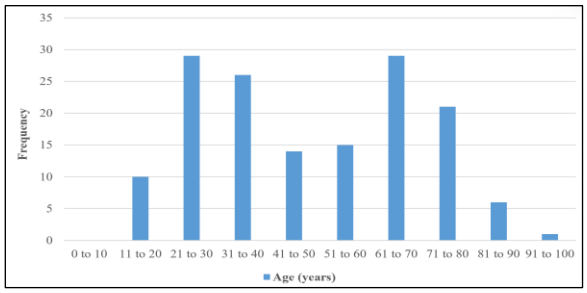

Figure-1: Frequency of EDTA-PTCP according to age

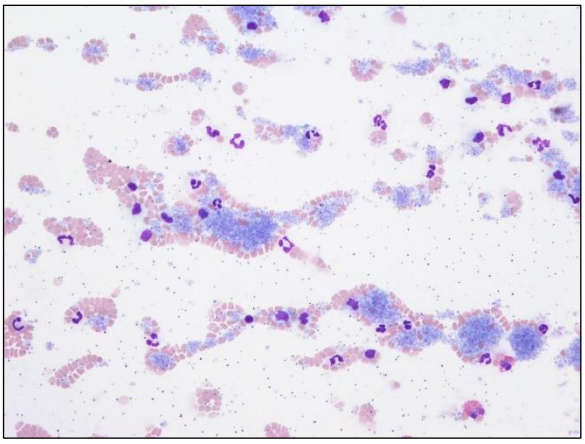

Figure-2: EDTA-PTCP (light microscope image taken at 20x)

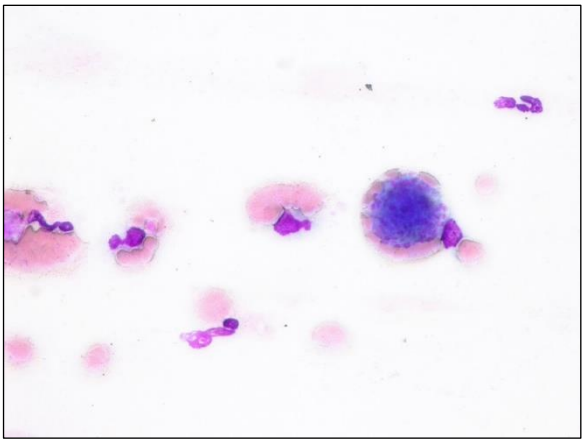

Figure-3: Peripheral smear of blood sample collected in sodium citrate tube, showing persistence of small platelet clumps. (light microscope image taken at 40x)

Table-1: Comparison of red cell indices and total leucocyte count in EDTA and sodium citrate tubes

Cbc parameter	Edta tube (Mean±SD)	Sodium citrate tube (Mean±SD)	*p*-value
Haemoglobin (g/dl)	11.79±2.17	11.84±2.50	0.673
Hematocrit (%)	36.02±6.44	36.73±7.45	0.04
MCV (fl)	84.05±7.45	85.67±7.89	<0.001
MCH (pg)	27.62±3.05	27.64±2.92	0.786
Total leucocyte count (/ul)	8658.21±5517.43	8615.77±5433.75	0.872

Table 2: Comparison of samples which showed improvement in platelet count versus no improvement with sodium citrate. **Mann-Whitney U test

	Platelet improvement with sodium citrate (n=47)	No improvement with sodium citrate (n=104)	*p*-value
Age (MEAN ± SD, Years)	45.30±20.20	50.61±20.80	0.142
Gender Males n (%) Females n (%)	 22 (30.6) 25 (31.6)	 50 (69.4) 54 (68.4)	 0.885
EDTA platelet count (Median & IQR,/uL)	71000 (42000–96000)	90500 (69000–117750)	<0.001**

Mann- Whitney U test showed that in samples which showed improvement in platelet count with sodium citrate anticoagulant, the baseline EDTA platelet count was significantly lower than in those samples which did not show resolution of EDTA-PTCP (U = 1567, *p*<0.001).

DISCUSSION

EDTA-PTCP is an in-vitro artefact with significant clinical implications. Several interventions are being evaluated to resolve this laboratory error. Our study investigates the use of sodium citrate anticoagulant for the resolution of EDTA-PTCP. We found this technique to be effective in only one-third of our patients. The majority of our patients (68.9%) showed higher platelet counts in samples processed with EDTA anticoagulant than in samples processed with sodium citrate.

In a study published by Weber *et al* in 2018, seventeen paired sets of blood samples were simultaneously collected in EDTA and sodium citrate tubes. Platelet count was analyzed at baseline as well as at several intervals reaching up to a maximum of four hours. The baseline citrate platelet counts were lower than EDTA counts in the majority of the cases, similar to the findings of our study. They also reported that citrate sample-based platelet counts were less consistent than EDTA sample-based platelet counts over time, with the ideal stability lasting up to one hour of drawing the sample.[14]

In another study conducted in Korea, ten patients with pseudo-thrombocytopenia were assessed. After evaluating different methods to resolve EDTA-PTCP in these patients, the authors concluded that the addition of amikacin to EDTA anticoagulant was the most effective technique.[15] Use of sodium citrate anticoagulant resulted in platelet count correction in 50% of the cases, as compared to 31.1% seen in our study.

A recent report from Pakistan assessed the efficacy of three different anticoagulants for the correction of persistent pseudo-thrombocytopenia in a single patient. They were unable to resolve platelet clumping despite the use of EDTA, sodium citrate and lithium heparin anticoagulants. Furthermore, they recommended that in case of multicoagulant-resistant pseudo thrombocytopenia, fresh blood samples may be collected in a syringe for manual platelet count using a Newbauer chamber.[16]

Our results confirm the findings of these three aforementioned studies,[14–16] with a much larger sample size as compared to the previous studies. This makes our results highly representative of the true scenario.

Starting from the time of blood sample collection, overfilling of sample tubes and strenuous venipuncture can induce platelet activation and aggregation in vitro.[1] Thus EDTA-PTCP may be prevented through good phlebotomy practices during the pre-analytical stage.

A simple technique that is widely used to disaggregate platelet clumps is to incubate the sample at 37 °C before testing on an automated haematology analyzer.[17] This method is particularly useful in resource-limited settings where alternative anticoagulants may not be available. Another first-line method is the use of a vortex mixer. Cho *et al* reported that vortex mixing before CBC analysis was able to resolve EDTA-PTCP in 27 out of their 28 patients (96%).[18] The major advantage of these two techniques is that blood re-sampling is not required.

Alternative anticoagulants such as magnesium sulphate and lithium heparin are also being investigated for the resolution of EDTA-PTCP. It is hypothesized that excess magnesium prevents platelet clumping by counteracting the effect of calcium and maintaining the stability of the Gp IIb/IIIa complex.[19] However, the problem with using alternative anticoagulants is that they may not reliably measure other parameters of the CBC. Our results showed that despite applying a correction factor to account for the dilutional effect of sodium citrate on routine CBC indices, haematocrit and its derived parameter MCV were significantly different from the values obtained from EDTA blood samples. A recent study also showed that the quality of Leishman-stained slides from blood samples collected in magnesium sulphate tubes was not satisfactory.[20]

In laboratories where a fluorescence channel is available for analysis of haematological analytes, fluorescence-based platelet counts in samples with EDTA-PTCP are significantly higher than the platelet count measured by the impedance channel.[21] However this technique is expensive and not available in resource-limited settings. Lastly, one more method that has been explored more recently involves the addition of amikacin to blood samples before testing. Amikacin and kanamycin supplementation has proven to be highly effective in dissociating platelet clumps.[22,23] The Utility of this technique is again limited due to lack of availability.

The strength of this study lies in the practical value of our findings. Sodium citrate is being used as an alternative to EDTA in many centres, with variable results as described above. Our results provide clear evidence to recommend against the use of sodium citrate for this purpose. However, data has been collected from a single center and is limited by the retrospective study design. We recommend that further, prospective studies should be conducted to investigate alternative methods for the correction of EDTA-PTCP, to prevent the associated clinical implications.

CONCLUSION

EDTA-PTCP is a laboratory artefact with significant consequences for patients. In our study, sodium citrate anticoagulant was able to correct EDTA-PTCP in one-

third of the cases. Our findings do not support the use of sodium citrate in place of EDTA for the resolution of pseudo-thrombocytopenia.

Conflict of interest: All authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

FS: Study design, data analysis, write-up. AJ, KA: Study design, write-up. MI, MS: Data collection, literature review, manuscript editing. TF, NR, MUR: Data collection, manuscript editing.

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