ORIGINAL ARTICLE

FREQUENCY OF PSEUDOTHROMBOCYTOPENIA IN OUTDOOR PATIENTS: ROLE OF TRISODIUM CITRATE IN CORRECTING IT

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Background: Pseudothrobocytopenia (PTCP) is the name given to spuriously low platelets count given by automated haematology analyser in an otherwise normal and asymptomatic patient having normal platelets count. This may lead to unnecessary investigations and anxiety. The present study was planned to know the frequency of this condition. **Methods:** This cross-sectional, quantitative analytical study was conducted at a tertiary health care facility (Department of Pathology Ayub Medical College Abbottabad) in collaboration with a private sector reference laboratory and research centre for blood diseases (Abbott Clinical Laboratory) over a period of three years. Citrated blood samples of all the participants with low platelet count on CBC (EDTA blood), examination of their Giemsa stained blood film along with a detailed history and complete physical examination. **Results:** As much as 30/15,000 participants had PTCP on initial testing, with a frequency of 0.2%, comprising 14 males and 16 females with male to female ratio 0.875:1. Ten (10) patients had mild, 14 had moderate, and 6 had severe PTCP which was corrected with citrate in 96.66% patients initially. **Conclusion:** Frequency of PTCP was 0.2%, affecting all the age groups with female predominance and the differences between testing on EDTA and citrate was statistically significant ($p<0.05$), signifying the role of citrate in correcting it.

Keywords: Pseudothrombocytopenia, thrombocytopenia, complete blood counts, platelets, EDTA, Citrate, Platelet aggregation

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INTRODUCTION

Complete blood count (CBC) is one of the common tests performed routinely in patients seeking medical advice and increasing use of haematology analysers have made it much easier than before. It has however given rise to spuriously low platelet count in some patients. This is a benign condition given the name 'pseudothrombocytopenia' (PTCP) sometimes leading to anxiety. Pseudothrombo-cytopenia (PTCP) is spuriously low platelet count when an Ethylenediaminetetraacetic acid (EDTA) blood sample is tested for complete blood count on an automated haematology analyser. It is defined as an inaccurately low estimate of the number of platelets in a sample of blood that is caused by clumping of the platelets in the laboratory sample rather than by a disease that affects platelet production or destruction.[1] It is not something new as it was described for the first time in 1969 by Gowland and co-workers as agglutination of platelets by a serum factor in the presence of Ethylenediaminetetraacetic acid (EDTA), used as an anticoagulant.[2] Later on it was seen with other anticoagulants including sodium citrate, lithium heparin, disodium oxalate and hirudin.[3] Patients with pseudothrombocytopenia are usually asymptomatic. Blood report showing thrombocytopenia is worrisome for the patient and the doctor. It may cause unnecessary treatment delay, unnecessary or overtreatment, unwanted platelet transfusion, or delay in essential procedures.[4] Some other aspects of pseudothrombocytopenia have also been reported in the literature. It has been reported in patients with systemic lupus erythematosus and lupus nephritis.[5,6]

Recently, pseudothrombocytopenia has been reported in COVID-19 patients.[7] Pseudothrombocyto-penia has been reported useful in the diagnosis of scrub typhus.[8] Platelet aggregates is the hallmark of pseudothrombocytopenia which are not counted by the analyser as platelets but probably as leukocytes. Platelet histogram can provide some clue to this and a well-stained blood film is confirmatory.[9]

Considering the paucity of research on this important clinical entity, the present study was planned to see the frequency of this condition to increase its awareness in health care workers.

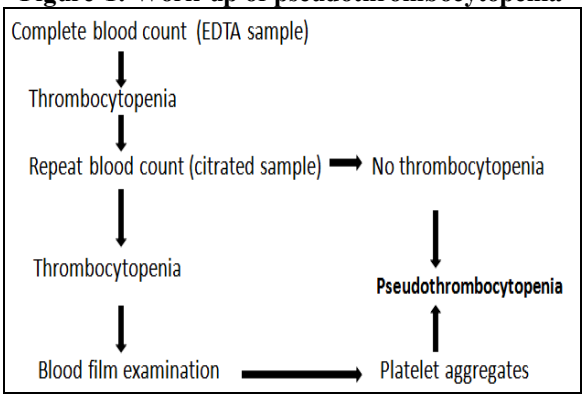
MATERIAL AND METHODS

This was a cross-sectional, quantitative analytical study conducted at a tertiary care health facility (Department of Pathology Ayub Medical College Abbottabad) in collaboration with a private laboratory and research centre for blood diseases (Abbott Clinical Laboratory). The objective of the study was to see the frequency of PTCP in EDTA blood and role of trisodium citrate in correcting it. The study was conducted after taking an informed written consent from each participant. As much as 15,000 patients

were initially recruited in the study by non-random convenience sampling technique over a period of three years (June 2018 to June 2021), referred to laboratory for the work-up of thrombocytopenia noted on complete blood count report (EDTA blood). After history and physical examination, a fresh citrated venous blood sample was taken in the laboratory for repeat blood counts from those patients whose blood counts revealed thrombocytopenia on the initial testing in some other laboratory. Repeat blood count was performed on Mindray BC 500 as the first step. Those with normalization of platelet counts on citrated blood samples were labelled as pseudothrombocytopenia. A well-stained blood film (May Grunewald Giemsa) examination was performed next on samples with low platelets for platelet aggregates by an experienced haematologist as the second step. Blood samples with normalization of platelet count on citrated blood and/or platelet aggregates on blood film, were included in the study. Those without platelet aggregates on blood film, already diagnosed with thrombocytopenia or diseases known to cause thrombocytopenia were excluded from the study. Patients on treatment for thrombocytopenia were also excluded from the study. Work-up of thrombocytopenia is depicted in Figure-1. Classification of pseudo-thrombocytopenia as mild, moderate and severe was done on the analogy of classification of true thrombocytopenia.[10]

The patients were divided into three groups depending upon the platelet count by EDTA method. Descriptive statistic was used for independent *t*-test, and $p \leq 0.5$ was considered significant.

Figure-1: Work-up of pseudothrombocytopenia



RESULTS

As much as 30/15,000 (0.2%) patients showed PTCP (Table-1). Three (10%) were <20 years old, 5 (16.7%) >60 years, and the remaining 22 (73.3%) aged 20–60 years. Fourteen participants were male and 16 were female with male to female ratio 0.875:1 (Table-2).

Ten (10) patients had mild, 14 moderate, and 6 patients were with severe PTCP on initial testing (Table-3). Descriptive statistics for platelet count on EDTA and citrate sample revealed *t*-value as -13.19, and p=0.00001 (Table-4). One blood sample (citrate) revealed thrombocytopenia. Platelets aggregates were confirmed on examination of Giemsa stained blood film. The sample was repeated with citrate in a pre-warmed tube kept at 37 °C throughout the testing period and PTCP disappeared (Table-5).

Table-1: Frequency of pseudothrombocytopenia

Total number of participants	15,000
Participants with PTCP	30
% participants with PTCP	0.2%

Table-2: Age and gender-wise distribution of cases (n=30)

			Male		Female	
Age (Years)	**No.**	**Percent**	**No.**	**Percent**	**No.**	**Percent**
<20	3	10.0	2	66.7	1	33.3
20–39	10	33.3	4	40.0	6	60.0
40–60	12	40.0	5	58.3	7	41.7
>60	5	16.7	3	60.0	2	40.0

Male:Female= 0.875:1

Table-3: Severity of pseudothrombocytopenia

Severity	**Platelets $\times 10^3/\mu L$**	**No.**	**Percent**
Mild	100–149	10	33.3
Moderate	50–99	14	46.7
Severe	<49	6	20.0

Table-4: Descriptive statistics

Platelet count $\times 10^3/\mu l$	**EDTA sample**	**Citrate sample**
Max	144	155
Min	15	290
R	129	135
Mean±SD	82.9±38.0	206.5±34.37

t-value= -13.19, p=0.00001

Table-5: PTCP with EDTA versus Citrate

PTCP (EDTA sample, room temperature)	30
PTCP (Citrate sample, room temperature)	1
Correction of PTCP by citrate	29 (96.66%)

DISCUSSION

The frequency of PTCP was 0.2% in the present study. This is in accordance with the findings of earlier research.[10] A similar study revealed higher incidence of pseudothrombocytopenia (17%) due to delay in the examination of blood and lack of blood film examination facility.[11] This is contrary to our findings, as these two factors were excluded in the present study. A study revealed pseudothrombocytopenia in as much as 15.3% patients.[12] Our findings are different from the results of this study. The findings of another study conducted on pseudothrombocytopenia are comparable to our findings with an incidence of 0.27%.[13] In another study the incidence of pseudothrombocytopenia was 0.15%. Our results are comparable to this study too.[14]

Some risk factors have been identified showing higher association with PTCP.[15] In the present study higher association was seen with the increasing age. This is in accordance with the findings

of study under reference. Our findings of higher incidence in male are also different from the results of this study. All the participants of present study were asymptomatic and did not have any recent or previous history of bleeding. This is in accordance with the findings of earlier studies.[16]

None of the participants of present study received platelet transfusion on the basis of EDTA based blood report. This is contrary to what has been reported previously.[17] In the present study pseudothrombocytopenia was resolved by trisodium citrate except one patient in which blood sample was kept at 37 °C throughout the testing period. A similar strategy was adopted in a previous study.[18]

Prevalence of PTCP depends upon study population. In general population it is up to 0.1%. Higher frequency has been observed in hospitalized and patients already having true thrombocytopenia.[19–21] Pseudothrombocytopenia has been reported mostly in the form of scattered case reports.[22–26] A few organized studies or review articles have also highlighted this important clinical condition.[12] Viral infections have been described as a possible cause of pseudothrombocytopenia in a case series.[27] The PTCP has been reported in blood donors.[28] We did not come across any such case. The present study is one of the pioneer research work in our country on pseudothrombocytopenia.

CONCLUSION

Frequency of PTCP was 0.2%, affecting all the age groups with female predominance. The differences between testing on EDTA and citrate were statistically significant, signifying the role of citrate in correcting it.

LIMITATIONS OF STUDY

Sample size was small compared to the research conducted previously by the others.

RECOMMENDATIONS

Patients presenting with thrombocytopenia should always be advised blood film examination to exclude possibility of pseudothrombocytopenia.

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MAK: Literature review, data analysis, critical revision
NG: Literature review, proof reading, drafting
SM: Literature review, proof reading, drafting
SB: Data analysis, Drafting, Revision

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