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Thrombocytopenia Vs Pseudo thrombocytopenia Will indices help us?

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ABSTRACT

Thrombocytopenia is defined as a platelet count of less than 1.5 lakhs/μL of anticoagulated blood. Platelet clumping is a common laboratory phenomenon resulting in pseudo thrombocytopenia that complicates platelet counts reporting. Some of the important causes are EDTA dependent agglutination and aggregation secondary to platelet activation, improper collection techniques, or delayed mixing with anticoagulants. Unfortunately, this pseudo thrombocytopenia is not well detected by the auto analyzers. In tropical countries like India, where dengue and malaria are endemic and important causes of thrombocytopenia, recognizing pseudo thrombocytopenia and minimizing the errors in platelet enumeration becomes vital. In this study, we compared various platelet indices between true thrombocytopenia and pseudo thrombocytopenia cases and found that only MPV is statistically significant. Hence, low platelet counts given by the automated hematology analyzer should be verified by a well stained peripheral smear and management should not go only by platelet parameters from the automated analyzer. This will prevent unnecessary referrals and platelet transfusions for the patient and thereby improve patient care.

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INTRODUCTION

Platelet clumping is a common laboratory phenomenon resulting in pseudo thrombocytopenia (Tan *et al.*, 2016). Pseudo thrombocytopenia is defined as falsely low platelet counts on automated analyzers. The important causes described in the literature are improper techniques/delayed mixing with anticoagulants, EDTA dependent agglutination, aggregation secondary to platelet activation, etc. (Bizzaro, 2009). Pseudo thrombocytopenia may lead to significant costs and discomfort to the patient due to needless diagnostic testing and unnecessary transfusions (Lau *et al.*, 2004; chia Hsia, 2012; Chacko *et al.*, 2013). Hematology analyzers count the platelet clumps as a single giant platelet or as small lymphocytes in the white blood cell gate and flag as thrombocytopenia. Often, pseudo thrombocytopenia remains unnoticed because blood smears are not routinely evaluated by visual inspection and warning flags as well as histograms of hematology analyzers are not interpreted accurately. The aim of this study is to compare and correlate various platelet indices in thrombocytopenia and pseudo thrombocytopenia in EDTA anticoagulated blood samples.

MATERIALS AND METHODS

This was a prospective study done over a period of 5 months in the Department of Hematology and Clinical Pathology, Saveetha Medical College and Hospi-

Table 1: Comparison of Mean age and platelet values between case groups

Parameters	True Thrombocytopenia	Pseudo thrombocytopenia	p-value
Age	46.56 (± 33.16)	44.51 (± 35.78)	>0.05
Platelet count	81230(±34352)	81237(±34586)	>0.05

Table 2: Mean platelet value and platelet indices of different groups

Parameters	Control	True thrombocytopenia	Pseudo thrombocytopenia
Platelet count (cells/μl)	290200±65493	81230(±34352)	81237(±34586)
PDW (fl)	11.16(±3.73)	14.09(±6.37)	14.46(±7.30)
MPV (fl)	9.82(±1.72)	11.18(±2.44)	11.51(±2.61)
PLCR (%)	23.51(±13.78)	34.56(±18.26)	36.54(±19.66)
PCT (%)	0.28(±0.12)	0.09(±0.079)	0.09(±0.08)

Table 3: Comparison of mean values of different parameters in-between control group and case groups

Parameters	Group	Mean value (±2SD)	p-value
MPV	Control	9.82(±1.72)	
	True thrombocytopenia	11.18(±2.44)	<0.0001
	Pseudo thrombocytopenia	11.51(±2.61)	<0.0001
PDW	Control	11.16(±3.73)	
	True thrombocytopenia	14.09(±6.37)	<0.0001
	Pseudo thrombocytopenia	14.46(±7.30)	<0.0001
PLCR	Control	23.51(±13.78)	
	True thrombocytopenia	34.56(±18.26)	<0.0001
	Pseudo thrombocytopenia	36.54(±19.66)	<0.0001
PCT	Control	0.28(±0.12)	
	True thrombocytopenia	0.09(±0.079)	<0.0001
	Pseudo thrombocytopenia	0.09(±0.08)	<0.0001

MPV-Mean Platelet Volume, PDW – Platelet Distribution Width,
P-LCR – Platelet large Cell ratio, PCT – Plateletcrit

Table 4: Comparison of mean values of different parameters in case groups

Parameters	True Thrombocytopenia	Pseudo Thrombocytopenia	p-value
MPV	11.18 (± 2.44)	11.51 (±2.61)	<0.05
PDW	14.09(± 6.37)	14.46(±7.30)	>0.05
P-LCR	34.56(±18.26)	36.5364 (±19.66)	>0.05
PCT	0.0897 (± 0.079)	0.093(±0.082)	>0.05
MPV/Platelet	0.0002029((± 0.00039)	0.0002028(±0.000356)	>0.05
MPV/PCT	190.96(± 411.92)	193.21(±431.93)	>0.05
PDW/Platelet	0.000257(±0.0005383)	0.0002449(±0.00039118)	>0.05
PDW/PCT	237.2059(±503.272)	230.54(± 499.62694)	>0.05

MPV-Mean Platelet Volume, PDW – Platelet Distribution Width,
P-LCR – Platelet large Cell ratio, PCT – Plateletcrit

tal. The study was done on all blood samples submitted for complete blood counts.

Inclusion criteria

1. K_2 , EDTA anticoagulated venous samples

2. Platelet count less than 1.5 lakhs /μL

Exclusion criteria

1. Platelet count more than 1.5 lakhs/μL

2. Samples were taken from veins that had an intravenous line (as it may be diluted by IV fluid)

3. Lysed samples were identified during processing.

4. Samples with visible clots

Complete blood count with platelet indices estimation was done in well-mixed anticoagulated blood samples using Sysmex XN 1000 Hematology 6 part analyzer. Peripheral smear was done for all the cases flagged by the analyzer as having low platelet counts. Staining was done using Leishman stain and manual estimation of platelet count was done by a pathologist who was blinded to the analyzer values.

Samples that showed low platelet count both in the automated hematology analyzer and peripheral smears were classified as true thrombocytopenia. Samples that showed low platelet count in the automated hematology analyzer and normal platelet count in the peripheral smears were classified as pseudo thrombocytopenia.

Platelet indices of an equal number of cases, whose platelet counts were matched at a regular class intervals of 10,000 cells/ μL from 0 to 1.49 lakhs cells/μL, were compared from two study groups. The gender and the age in both groups were matched.

About 113 cases were selected in each group and PDW, MPV, P-LCR and PCT were compared in both the groups. 100 control cases, whose platelet count was normal during the study period, were taken and their mean platelet indices were taken as laboratory reference. Statistical analysis was done using IBM SPSS v23.0.

RESULTS AND DISCUSSION

Mean age of true thrombocytopenia and pseudo thrombocytopenia groups were 46.5 years (range 18-84 years) and 44.5 years (range 5-90 years), respectively. M: F ratio in both the groups was 1.8:1. Gender distribution and age distribution of true thrombocytopenia and pseudo thrombocytopenia are presented in Figures 1 and 2.

Both age and platelet count were well matched among both the case groups ($p>0.05$). Comparison of mean age and platelet count between both study groups are presented in Table 1.

During the study period, about 3756 complete blood counts and smear studies were done and the prevalence of pseudo thrombocytopenia was estimated to be 3%. The mean platelet count and the mean platelet indices of all the groups were given in Table 2.

In our study, when compared to control cases, we found that MPV, PDW, P-LCR, is significantly higher in both the case groups ($p<0.0001$). PCT was significantly lower in both the case groups ($p<0.0001$). Comparison of mean values of different parameters in-between control group and case groups is presented in Table 3.

In our study, we found that PDW, P-LCR, MPV/Platelet, MPV/PCT, PDW/ Platelet, PDW/PCT, were not significantly correlated between true thrombocytopenia and pseudo thrombocytopenia, whereas MPV is significantly lower in true thrombocytopenia group ($p <0.05$). A comparison of the mean values of different parameters in case groups is presented in Table 4.

Pseudo thrombocytopenia is caused by platelet clumping, which is an uncommon and often unrecognized phenomenon that results in errors in the interpretation of platelet counts (Bizzaro, 2009). In this study, the incidence of smear confirmed pseudo thrombocytopenia was 3%, which is higher when compared to other studies in the literature, which had 0.1-2% (Cohen *et al.*, 2000; Bartels *et al.*, 1997). We compared various platelet indices between smear confirmed true thrombocytopenia and smear confirmed pseudo thrombocytopenia cases and found that only MPV is statically significant ($p<0.05$).

Investigating the cause of platelet clumping includes

1. Verification of the method by which the blood was drawn,
2. The proper technique has been followed during collection
3. Has the sample mixed properly immediately after collection
4. Has the sample promptly stored and transported to the laboratory
5. If suspected EDTA induced clumping, repeat the test in a blood sample in sodium citrate
6. If clumping persists, test the blood sample collected in heparin

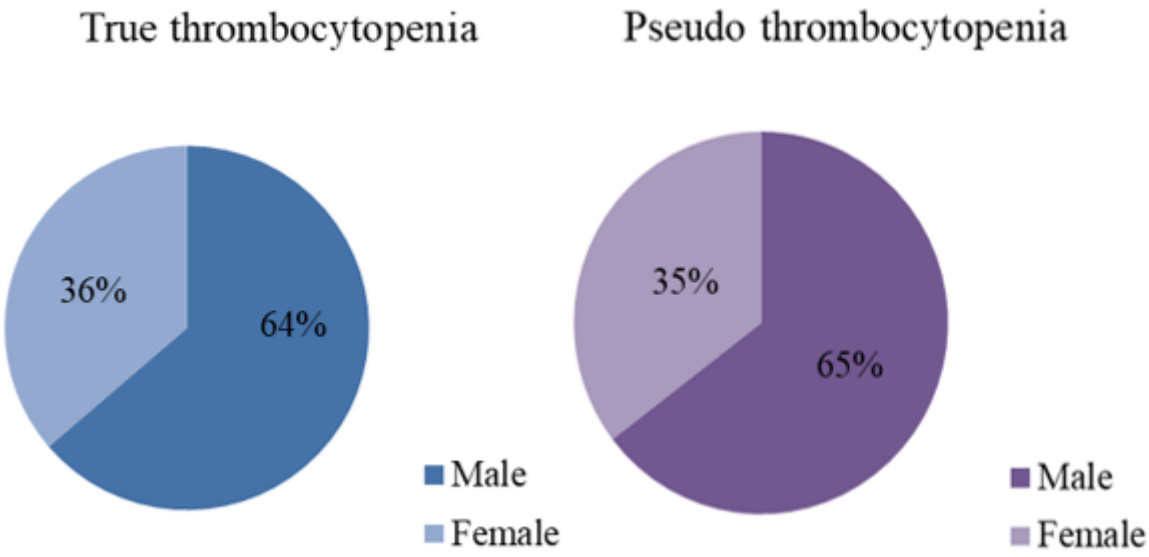

Figure 1: Gender-based case distribution in both the groups

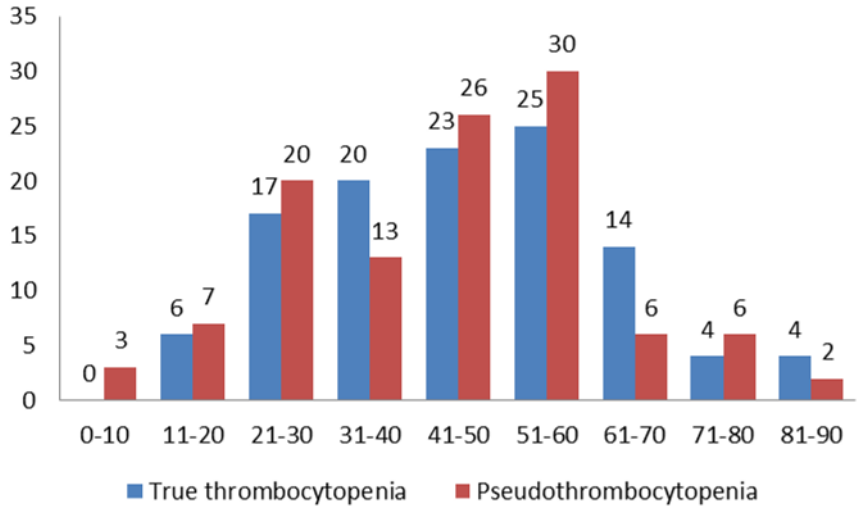

Figure 2: Age-based case distribution in both the groups

7. If not possible, obtain a sample in ammonium oxalate and count platelets utilizing a hemocytometer grid, as per described methods (Bizzaro, 1995).

A recommended approach for pseudo thrombocytopenia is, if the instrument estimated low platelet count, it is advisable not to give the platelet result with clumps and recommend the above-mentioned steps. If the instrument estimates within or above normal platelet count, a count may be given with an added comment noting the presence of platelet clumps as it suggests that the true count likely to be higher (Tan *et al.*, 2016).

CONCLUSIONS

Pseudo thrombocytopenia is an uncommon condition, but it can occur due to improper sample collection and EDTA induced clumping. Though modern hematological analyzers "flag" platelet clumps, it is important to do manual verification of the counts using peripheral smear. This can prevent unnecessary investigations/ platelet transfusions for the patient and thereby improving patient care.

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