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Utility of automated cell counter histogram in diagnosis of thrombocytopenia and pseudothrombocytopenia

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ABSTRACT

Background: Automated hematology analyzers have become mainstream of complete blood count (CBC) during the past two decades; they are potential, more accurate to produce results of CBCs. However, often automated analyzers can give false results too, specially, falsely low platelet count due to platelets aggregates, which have to be confirmed on peripheral smears. **Aims and Objectives:** The aim and objective of the study was to diagnose thrombocytopenia (TCP) cases and differentiate them from pseudothrombocytopenia (PTCP) cases by analyzing platelet histogram and to determine the sensitivity and specificity of platelet histograms for diagnosing TCP and PTCP. **Materials and Methods**: The present study was conducted in the Department of Pathology at Shyam Shah Medical College, Rewa, M.P., after obtaining ethical clearance from the Institutional Ethics Committee. It was prospective study conducted for a period of 15 months from January 2021 to May 2022 on 1000 samples. **Results:** The sensitivity and specificity of platelet histogram flags and presence of multiple peaks to diagnose TCP and PTCP cases were calculated as 73.60% and 93.60%, respectively. The present study also interpret that mean platelet volume and platelet distribution width cannot use as indicator to differentiate between TCP and PTCP. **Conclusion:** The present study concludes that analysis and interpretation of histogram more specifically platelet histogram flagging provide clue for early detection of PTCP cases prior peripheral smear examination and also helpful in differentiating them from true TCP cases. These platelet histogram flagging can be used as a screening parameter for the detection of PTCP and these are helpful in preventing unnecessary stress for clinician, patients, and their relatives. However, the peripheral smear examination will remain the gold standard to differentiate TCP from, PTCP.

Key words: Ethylenediaminetetraacetic acid; Thrombocytopenia; Pseudothrombocytopenia

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INTRODUCTION

Histogram is graphical representation of data analyzed by hematology analyzer, in which size of particle or cells plotted on X-axis and number of cells plotted on y-axis. In three- part differential hematology analyzer, platelet histogram, RBC histogram, and WBC histogram generated. Normally platelet histogram lies between 2 and 25 fl, starts from baseline and should end on baseline. The peak of curve provides a parameter called mean platelet volume (MPV), indicates average size of platelet in given sample. The deviation of platelet histogram results in platelet upper (PU) discriminator and platelet lower discriminator flags at upper discriminator and lower discriminator, respectively. Analysis of theses flags can help in the early detection of pseudothrombocytopenia (PTCP).

PTCP was 1st time reported in 1969,[1] it is relatively common findings in clinical laboratories. It is well-known *in vitro* phenomenon. It is defined as falsely low platelet

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count given by automated hematology analyzers, while the actual platelet count is within normal range for the same patient. The reason behind falls low platelet count is that the hematology analyzers count the platelet clumps (CLP) as a single giant platelet or as small lymphocytes. Its prevalence is reported to vary between 0.1% and 2% among hospitalized patients[1-3] and 15–17% among out patients evaluated for isolated thrombocytopenia (TCP).[4,5] It can lead to diagnostic error, overtreatment, and further unnecessary testing. Clinical consequences with potential life-threatening events (Unnecessary platelet transfusion, inappropriate treatment including corticosteroids) are still observed when PTCP is not readily detected.[6-9]

Ethylenediaminetetraacetic acid (EDTA) is introduced in 1950s in laboratories. Since then, it is widely used as anticoagulant in laboratories. One of the most important disadvantages of EDTA is that it causes platelet aggregation. Most often the clumping is caused by the presence of agglutinating anti-platelet antibodies that react with platelets in blood. Platelet clumping leads to PTCP, which has to be examined and confirmed by microscopic examination of peripheral smear of that sample.

The present study is conducted to find out cases with falsely low platelet. It can be only revealed by conventional blood smear inspection and consequent correlation with different values produced by automated hematology analyzers. Visual evaluation of blood smears is regarded as gold standard for the detection of EDTA-induced PTCP.

Aims and objectives

1. To diagnose TCP and pseudo-TCP using automated cell counter histogram
2. To determine the sensitivity and specificity of platelet histograms for diagnosing TCP and PTCP.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology at Shyam Shah Medical College, Rewa, M.P., after obtaining ethical clearance from the Institutional Ethics Committee vide no IEC/MC/2020/463. It was prospective study conducted for a period of 15 months from January 2021 to May 2022 on 1000 samples. All samples were collected from different wards of Sanjay Gandhi Memorial and Gandhi Hospital Rewa (M.P.). Samples were collected in EDTA vials and were processed in automatic hematology analyzer (SYSMEX KX-21). Platelets parameters, changes in platelet histogram such as PU flag, and multiple peaks CLP flag were studied in the samples having platelets count less than 1 lakh were selected and smears prepared for same.

After drying, smears examined in microscope at low power and oil immersion. If platelet count on peripheral smears is found to be <1 lakh, then it will be counted as true TCP and if the platelet count on peripheral smears is found to be more than 1 lakh, then it will be counted as PTCP. After counting platelets on peripheral smears, consequently, we have correlated various changes in platelet histogram such as PU flags and multiple peaks (CLP flag), with peripheral smears findings. Sensitivity, specificity, positive predictive value, and negative predictive value of platelet histogram were calculated using SPSS software.

Inclusion criteria

1. Platelet count <1 lakh
2. Known cases of immune thrombocytopenic purpura
3. Patients with prolonged bleeding time.

Exclusion criteria

1. Platelets count more than 1 lakh
2. Known cases of leukemia
3. Clotted sample
4. Dilute sample
5. Patients with deranged coagulation profile.

RESULTS

In our study, it seems that, out of 1000 cases, only 53% (530/1000) of cases showed true TCP, whereas 47% (470/1000) cases are belong to PTCP category (falsely low platelet count given by cell counter) and it is merely a laboratoy artifact, which is primarily due to EDTA-induced platelet clumping. Most of the PTCP cases showed abnormal platelet distribution curve such as the presence of multiple peaks (CLP flag) or presence of PU flag (curve not end at base line). These changes are shown below in the cases which are observed during study.

Cases of PTCP observed during study

Case 1

Figure 1.

Case 2

In the present study, it is observed that the cases in which platelet count is falsely low, i.e., PTCP cases most of them shows multiple peaks/Pu flag in platelet distribution curve which comprises 44% (440/1000) of all studied samples and 93% (440/470) of all PTCP cases (Table 1). The present study also interpret that most of the true TCP cases, i.e., 73% (390/530), did not showed any of above given abnormality in platelet distribution curve; however, it is also observed that 26% (140/530) of true TCP cases showed multiple peaks/PU flag in platelet distribution curve of complete histogram [Figure 2].

In the present study, it is observed that only 19% (100/530) of total cases of TCP shows increase in MPV, while remaining 81% (430/530) cases of TCP have MPV of normal range. Similarly, 19% (90/470) cases of PTCP showed increase in MPV, while remaining 81% (380/470) of cases did not show change in MPV (Table 2). Thus, according to the present study, MPV cannot be used as an indicator to differentiate TCP from PTCP.

In the present study, it is observed that 64% (340/530) cases of TCP showed increase in platelet distribution width (PDW), whereas in 36% (190/530) of cases, it remains in normal range. Similarly, 72% (340/470) cases of PTCP showed increase in PDW, whereas in remaining 28% (130/470) cases, PDW remains in normal range (Table 3). Thus, according to the current study, it seems that PDW alone cannot be used as a marker to differentiate TCP from PTCP.

After analyzing various changes in platelet distribution curve such as PU flag and the presence of multiple peaks and their correlation with platelet count given by automated hematology analyzer and platelet count on peripheral smears, the sensitivity, specificity, positive predictive value, and negative predictive value were found to be 73.60%, 93.60%, 92.85, and 75.90, respectively.

DISCUSSION

Among various parameters of platelet histogram (MPV, PDW, PU flags, and multiple peaks), we assessed that the presence of multiple peaks (CLP flag) and PU flag have potential ability to detect the PTCP cases. The sensitivity of platelet histogram is found to be 73.60% and specificity is 93.60%, positive predictive value is 92.85, and negative predictive value is 75.90. The sensitivity and specificity may vary on different automated hematology analyzers, to our knowledge, there is no study done on SYSMEX KX 21 analyzers; however, few studies done on different analyzers are summarized in Table 4 with their sensitivity and specificity.

Almost similar study conducted by Hawkins et al.,[10] using Sysmex XE-5000 analyzer on 600 EDTA-anticoagulated blood specimens, The sensitivity of platelet abnormal distribution flag and platelet CLP flag was found to be 42% and 57%, respectively. The specificity of platelet abnormal distribution flag and platelet CLP flag was 57% and 99%, respectively.

A study Conducted by Schuff-Werner et al.[11] considered platelet distribution curve and changes observed in it, like curve is flattened and serrated like a saw blade. Sensitivity was calculated at 33.2%, specificity at 97.3%, and efficiency at 96.7%. The sensitivity of platelet histogram in this study is far low (33.2%) as compare to our study (73.60%), but specificity is nearly similar to the present study, which is 93.60% in the present study.

In a study by Xu et al.,[12] on the samples with low platelet counts showed that the total sensitivities of the platelet, CLP

Table 1: Correlation of thrombocytopenia and pseudothrombocytopenia with presence of multiple peaks/PU flag

TCP				PTCP			
PU flag/multiple peaks present (CLP)		PU flag/multiple peaks absent (CLP)		PU flag/multiple peaks present (CLP)		PU flag/multiple peaks absent (CLP)	
Number	Percent	Number	Percent	Number	Percent	Number	Percent
140	14	390	39	440	44	30	3

TCP: Thrombocytopenia, PTCP: Pseudothrombocytopenia, CLP: Clump flag

Table 2: Correlation of thrombocytopenia and Pseudothrombocytopenia with MPV

TCP				PTCP			
Normal MPV		Increased MPV		Normal MPV		Increased MPV	
Number	Percent	Number	Percent	Number	Percent	Number	Percent
430	81	100	19	380	81	90	19

MPT: Mean platelet volume

Table 3: Correlation of thrombocytopenia and pseudothrombocytopenia with PDW

TCP				PTCP			
Normal PDW		Increased PDW		Normal PDW		Increased PDW	
Number	Percent	Number	Percent	Number	Percent	Number	Percent
190	36	340	64	130	28	340	72

PDW: Platelet distribution width, TCP: Thrombocytopenia, PTCP: Pseudothrombocytopenia

Table 4: Comparison of sensitivity and specificity of histogram in different type of studies

Study conducted by	Year	Parameters included	Sensitivity (%)	Specificity (%)
Hawkins et al.	2016	PAD	For PAD- 42%	83
		CLP	For CLP- 57%	99
			For PAD and Or CLP=73	82
Schuff-Werner et al.	2020	Platelet distribution curve and IMI	83	63.3
Xu et al.	2022	Platelet clumps flag and platelet abnormal flag	80 and 100	94 and 91
Present study	2020-22	Platelet distribution curve and its PU flag	73.60	93.60

PAD: Platelet abnormal distribution, CLP: Platelet clump flag, IMI: Immature cell informationm, PU flag: Platelet upper discriminator flag

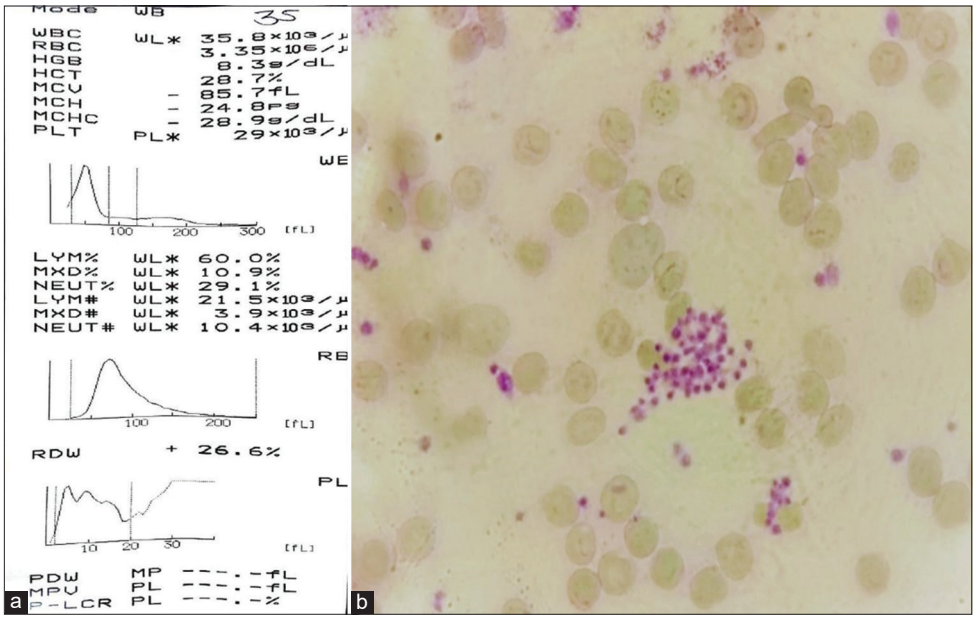

Figure 1: (a) Platelet count in this histogram is 29×10³/μL and platelet distribution curve shows multiple peaks and not ending at base line (PU flag). (b) Peripheral smear (Leishman stain, ×100): - Peripheral smear of corresponding case shows multiple CLP of platelets, platelet count found to be 190×10³/μL, and total leukocyte count found to be 18×10³/μL. Thus, from above given histogram and peripheral smear findings, we can say that it is a case of PTCP

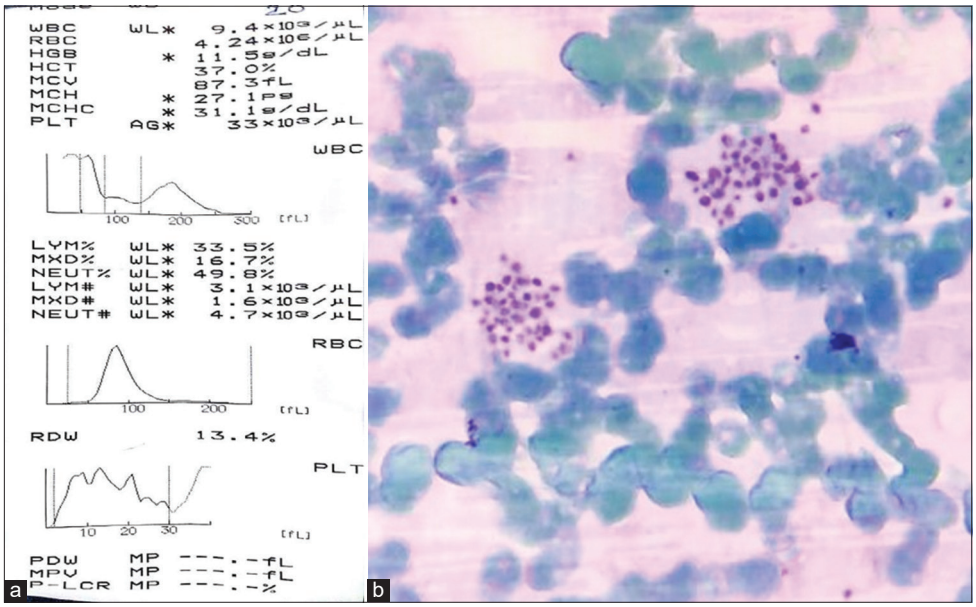

Figure 2: (a) In above Histogram, the platelet distribution curve shows multiple peaks (saw tooth appearance of graph) and platelet distribution curve does not ending at base line (PU flag). (b) Peripheral smear (Leishman stain, ×100): - The corresponding peripheral smear shows large CLP of platelets. Platelet count of this case on peripheral smear is found to be 180000 per cubic mm. Thus, from above given histogram and peripheral smear findings, we interpret that it is a case of PTCP

and platelet abnormal flags on platelet histogram/platelet distribution curve were 80.01% and 100%, respectively.

Limitations of the study

This study is done on Sysmex KX-21 automated analyser, the results may vary on different model of automated hematology analysers. So multicentric studies, with large sample size are needed to establish the sensitivity and specificity of platelet histogram flagging for detection of thrombocytopenia and pseudothrombocytopenia.

CONCLUSION

The present study concludes that analysis and interpretation of histogram, more specifically platelet histogram flagging, provide clue for early detection of PTCP cases prior peripheral smear examination and also helpful in differentiating them from true TCP cases. These platelet histogram flagging can be used as a screening parameter for the detection of PTCP and these are helpful in preventing unnecessary stress for clinician, patients, and their relatives. However, the peripheral smear examination will remain the gold standard to differentiate TCP from PTCP.

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