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Research Article

Utility of Peripheral Blood Smear in Thrombocytopenic Patients for Platelet Count Estimation

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ABSTRACT

Background: In most of the laboratories, the Hematology analyzer is utilized to count platelets in patient blood samples. Although Hematology analyzers normally produce an accurate platelet count, their accuracy has been brought into question while enumerating low platelet counts, platelet abnormalities, or platelet-like fragment interference [6]. Peripheral blood film is highly useful in the diagnosis of unexplained thrombocytopenia i.e pseudo-thrombocytopenia and also in monitoring the therapeutic response.

Materials & Methods: Platelet count in analyser and peripheral smear examined for the patients with low platelet count in analyser. The statistical analysis was done by using pearson correlation coefficient.

Results: We studied 15 patients with thrombocytopenia i.e. < 150 x 103/uL platelet count on automated cell counter. We observed. The mean platelet count on automated analyzers was 0.67± 0.24 lakhs/cu.mm whereas the mean platelet count verified on peripheral smear was 1.09± 0.45lakhs/cu.mm with a significant difference between the two groups.

Conclusion: platelet count estimation by PBS method is reliable and statistically significant when compared to haematology analyser especially in pseudo thrombocytopenia cases.

Keywords: Platelet Count, Automated Hematology Analyser, Peripheral Blood Smear Thrombocytopenia, Pseudo-Thrombocytopenia, Platelet Clumps.

INTRODUCTION

Platelets are non-nucleated discoid 1-3µ cells, produced in bone marrow megakaryocytes by fragmentation of cytoplasm[1]. Platelets serve both structural and molecular functions in blood clotting[2].Normal platelet count in healthyperson is 1.5-4.0 lakh/mm3 of blood[3].The accurate plate count estimation has an important role in diagnosis and treatment of thrombocytopenia cases. The reliability of platelet count is highly desired where the platelet transfusion is necessary. Thrombocytopenia is commonly associated with various conditions like bacterial sepsis, terminal liver diseases, renal failure, leukemia, malignancy, after chemotherapy etc. [4, 5]

In most of the laboratories, the hematology analyzer is utilized to count platelets in patient blood samples.Although hematology analyzers normally produce an accurate platelet count, their accuracy has been brought into question while enumerating low platelet counts, platelet abnormalities, or platelet-like fragment interference[6].

Currently, the majority of automated hematology analyzers count platelets using optical density and electronic impedance concepts [7].

The Analyser gives flags for platelet clumps, fragmented RBCs, giant platelets, and small RBCs which can interfere with platelet count, hence accurate count cannot be generated. In such cases, manual verification of platelet count is of utmost importance for critical care and evaluation of thrombocytopenic patients which can lead to life-threatening bleeds [8].

Peripheral blood film is highly useful in the diagnosis of unexplained thrombocytopenia i.e pseudo-thrombocytopenia and also in monitoring the therapeutic response [9]. It can be used as the quality control for the verification of results generated by automated instruments [10].

METHOD AND MATERIALS

The present study was carried out in the Department of Laboratory, BIRRD Hospital Tirupati, over the period of 18 months (March 2024 – august 2025). Inclusion criteria were all the samples of the patients having thrombocytopenia with platelet count less than 150,000/uL on automated blood cell counter. The inadequate samples, haemolysed samples and clotted samples were excluded from the study. Venous blood samples were collected for all the patients in ethylenediaminetetraacetic acid (EDTA) vacutainers tube and were stored at room temperature until analyzed within two hours. Each blood sample was mixed properly for 10 minutes with automated mixer. The platelet count estimation done by processing blood samples in an automated hematology analyzer Mindray BC-5000 automated cell counter. The hematology analyzer calibration, quality control as well as the maintenance were done as recommended by the manufacturer. The blood samples with low platelet count on hemato-analyzer were used to prepare air dried blood smear and was stained manually with Leishman's stain as per standard procedure. The PBS was then examined under light microscopy with x100 oil immersion lens. In a Leishman's stained peripheral blood preparation, platelet can be identified as small purple coloured bodies with irregular borders. The average number of platelets was calculated and was multiplied by fifteen thousand. In an ideal zone of blood peripheral film, each platelet on an average 100x oil immersion field represents 15,000 platelets / µl, estimating final platelet count. [11] Qualitative variables were described as frequency, and quantitative variables were measured as mean and standard deviation and keeping the 95% confidence interval and p-value of <0.05.

RESULTS

We studied 15 patients with thrombocytopenia i.e. < 150 x 103/uL platelet count on automated cell counter. We observed 47% of the patients were male and rest 53% were female with male: female of 0.8:1 (Figure 1). Most of the patients belonged to the age group of <35 years & 56-65 years i.e. 34% followed by the age group of 35-45 years i.e. 20% (Figure 3).The mean age was found to be 46 years. The mean platelet count on automated analyzers was 0.67± 0.24 lakhs/cu.mm whereas the mean platelet count verified on peripheral smear was 1.09± 0.45lakhs/cu.mm with a significant difference between the two groups (p-value <0.0001) (Table 1).

Table 1. Mean and Standard Deviation Values of Platelet Estimation by Manual Peripheral Blood Smear Examination and Automated Cell Counter

Method	N	Range	Mean	SD	Median	IQR
Analyzer	15	0.12-0.94	0.67	0.24	0.72	0.54-0.87
Peripheral smear	15	0.35-2.00	1.09	0.45	1.00	0.78-1.5

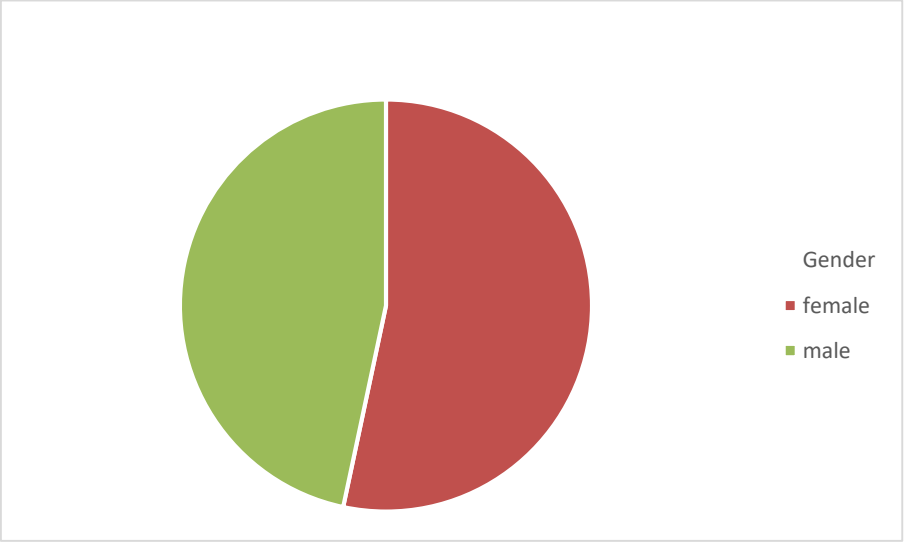

Figure 1. Gender Wise Distribution of the Patients.

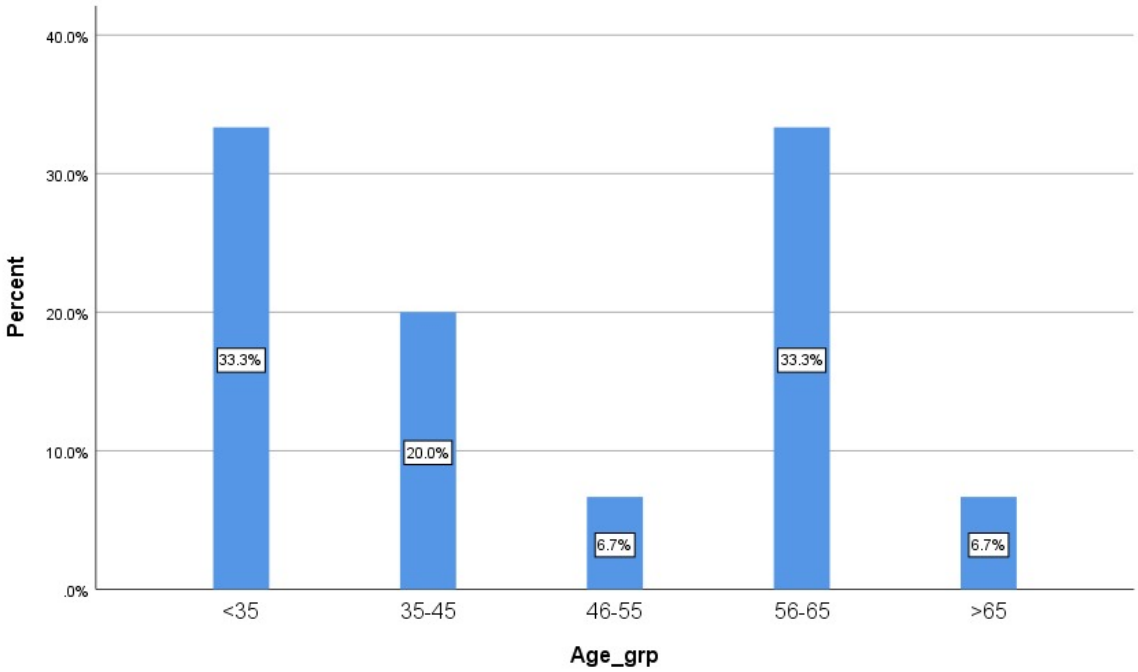

Figure 2. Age Wise Distribution of the Patients.

On manual examination, we observed that 33% of the patients which were previously diagnosed as thrombocytopenic on automation were found to be adequate in manual method i.e. pseudo-thrombocytopenia cases (Figure 3).

We also evidenced that when examined on peripheral smear the actual platelet count was actually higher than automated count for most of the thrombocytopenic cases (Figure 4)

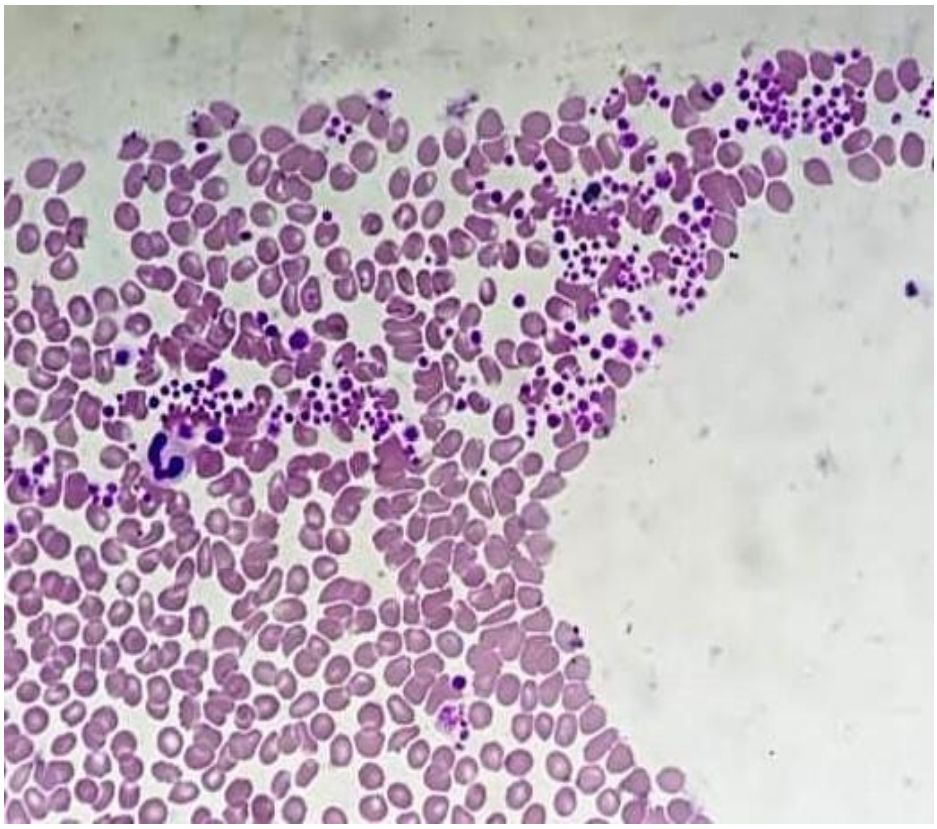

Figure 3. PBS Showing Platelet Clumps.

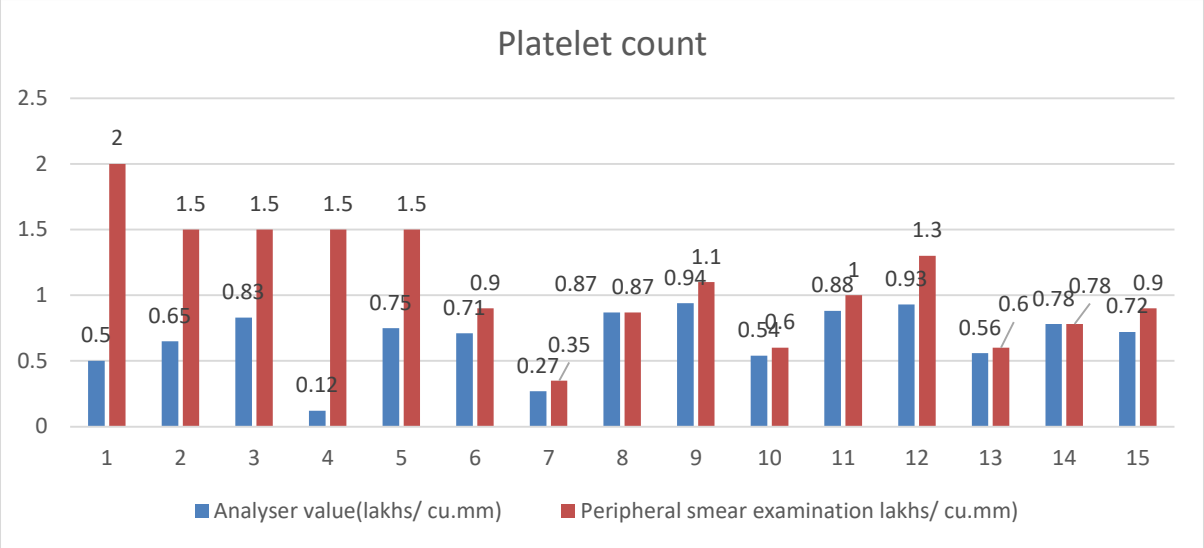

Figure 4. Comparison of Platelet Counts on Automated Hematology Analyzer and Manual Method

DISCUSSION

Over the last many years, significant improvements have been made in automated haematology analyzers used for both analytical purposes as well as the whole blood cells description. Because of it, manual procedures have been gradually losing their importance in haematology[12].Although hematology analyzers often produce precise platelet counts especially in healthy individuals, their precision is doubtful while estimating low platelet counts, in the context of giant platelet, platelet clumps, fragmented RBCs, small RBCs and platelet satellitism. [1] When an automated platelet count is low or flagged, platelet count estimation from the manual method by examining blood smears should be the gold standard, since no machine, no matter how costly or effective, can completely replace human judgment. [13]

We observed that out of of 15 patients, 47% were male and rest 53% were female with male: female of 0.8:1. Prajapati AK [7] studied out of 100 patients, 63% were male and rest 37% were female with male: female of 1.7:1. Tariq et al., (2023) [14] studied 60 adult patients including 31 females and 29 males with an approximate male-female ratio of 1:1. Castromayor et al., (2019) [13] also observed the 384 adult patients with thrombocytopenia, with an approximately 1:1 ratio based on sex. [13] Another Indian study from Assam, Gogoi et al., (2018) [15], observed 797 thrombocytopenic patients with 71% male and 29% female (male: female = 2.44:1).

Most of the patients in the present study belonged to the age group of <35 & 56-65 years (34% each group) followed by the age group of 35-45 years (20%) with mean age of 46 years. Prajapati AK [7] study showed most of the patients belonged to the age group of 30-40 years (34%) followed by the age group of 20-30 years (23%) with mean age of 38.8 years. Gogoi et al., (2018) [15] showed similar observation with mostly (22.5%) belonged to the age group of 30–40 years followed by 20–30 years age group i.e. 19%. Tariq et al., (2023) [14] evidenced the mean age of 43.7 years. However, Castromayor et al., (2019) [13] stated that thrombocytopenia was most common between 6th to 8th decades of life affecting 36% of patients.

The present study showed the mean platelet count on automated analyzers was 0.67± 0.24 lakhs/cu.mm whereas the mean platelet count verified on peripheral smear was 1.09± 0.45lakhs/cu.mm. Prajapati AK [7] study showed the mean platelet count on automated analyzers was 85.46 ± 38.81 x 103/uL whereas on peripheral smear was 92.13 ± 38.30×103/uL with a significant difference between the two groups (p-value <0.0001). It's mostly because of presence of giants platelets in the samples. Tariq et al., (2023) [14] observed the mean platelet count on automated analyzers was 58 ± 28×109 / L whereas the platelet count verified on peripheral smear was 117±13×109 /L with a significant difference (p-value of <0.001).However, they also included the samples in their study showing platelet clumps apart from giant platelets. Castromayor et al., (2019) [13] evidenced that the mean of the automated platelet levels was approximately 76 ± 45 x 109 /L while the manual platelet count

results was 170 ± 99 x10 9 /L with a significant difference with p value < 0.05.

In our present study, we noted 33% Pseudo thrombocytopenia cases, whereas prajapati AK[7] noted 10% pseudo-thrombocytopenia cases. Gogoi et al., (2018) [15] also documented similar 9.8% pseudo-thrombocytopenic cases in their study. However, Tariq et al., (2023) [14] showed 42% pseudo-thrombocytopenic patients owning to the inclusion of the cases with platelet clumps apart from giant platelets.

CONCLUSION

Thrombocytopenia affects both male and female sex almost equally mostly middle aged people. In thrombocytopenia, it is crucial to confirm automated analyzer platelet count by examining peripheral smear, especially in samples with low platelet count before treatment. It may prevent patients from unnecessary further investigations and treatment. Thus,the peripheral smear examination remains the gold standard method for accurate platelet count estimation.

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