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ORIGINAL RESEARCH

COMPARISON BETWEEN MANUAL PLATELET COUNT AND AUTOMATED PLATELET COUNT IN THROMBOCYTOPENIA PATIENTS

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ABSTRACT

Introduction: Nowadays, platelet counting is employed as a routine method owing to the emergence of dengue fever from the past few decades. Manual methods yield varying outcomes while sometimes automated methods produce inappropriate outcomes. Hence, the goal of this study is to evaluate the diagnostic accuracy of manual modality of platelet count with respect to automated analyzers. Additionally, this study was also analyzed the correlation between manual and automated analyzer.

Materials and Methods: The current study was carried out in the Department of Pathology, Employee's State Insurance corporation (ESIC) Hospital, Sarojini Nagar, Lucknow (U.P.) on a group of 600 patients (375 female and 225 male) including both indoor and outdoor settings from 1st November to 30th November, 2021 in a one month duration. The blood samples were collected in tubes containing K3-EDTA (tri-potassium ethylenediamine-tetra-acetic acid). Manual method of platelet counting was performed by slide method with Leishman stain while automated counting was carried out by using Mindray BC-6200, 5 part auto-hematology analyzer.

Results: A total of 600 patients have studied with the mean platelet count in automated analyzer was 1.16±0.99 $\times 10^3$ /µL and by manual method with Leishman stain was 1.23±1.03 lacs /mm^3. A positive correlation was observed between automated analyzer and manual platelet count (r=0.837, p=0.00).

Conclusion: When the platelet counts are very low, manual method of platelet counting should be done carefully to exclude clumping or irregular distribution of platelets.

Keywords: Automated cell counter, Dengue, Platelets, EDTA.

INTRODUCTION

Platelets are small cytoplasmic protrusion (2-4µm in diameter), anucleate with discoid morphology, short-lifespan (7-10 days) circulating blood cells, originated from megakaryocytes especially in the bone marrow.[1] Routinely, around 10^{11} platelets are produced daily to maintain the normal platelet count in the bloodstream of healthy individuals which is $150\text{-}450\times10^3$ platelets/µl.[2,3] Platelets play an essential role in homeostasis and arterial thrombosis.[4] Platelets are also implicated in various physiological and patho-physiological processes.[5] Platelet counts are a crucial examination to diagnose the hemorrhagic disease and in the management of patients.[6] Recently, estimation of platelet count is frequently recommended especially during dengue fever season. Despite this, regular platelet count is required in pregnancy triggered bacterial sepsis, hypertension, leukemia and malaria and in patients with chemotherapy.[7] Platelet count is a very important pathological analysis of blood, but it requires accurate and economical modality. Platelets are counted by two approaches i.e. manual method and automated method. Manual method by using diluting fluid like 1% ammonium oxalate in neubaur chamber and also slide method with Leishman stain, these method are simple, economical, and suitable if done in proper manner.[8] The outcomes of platelet count are equivalent to automated modality except in case of very low platelet counts. Momodu et al., found that platelet count by automated modality produces better outcomes in contrast to manual modality.[9] ISLH (International society for laboratory in hematology) and ICSH (International council for standardization in hematology) recommend the estimation of platelet count as a reference modality for calibration of automated analyzer, but it requires an experienced individuals and flow cytometer.[6,10] Sometimes, automated approaches may produce inappropriate outcomes especially in EDTA (ethylenediamine-tetra-acetic acid) samples.[11,12] One of the studies demonstrated that the automated modality overestimates the platelet counts in comparison to manual platelet analyzer.[13] Hence, the goal of this study is to evaluate the diagnostic accuracy of manual modality of platelet count with respect to automated analyzers. Additionally, this study was also analyzed the correlation between manual and automated analyzer.

MATERIALS AND METHODS

The current study was carried out in the Department of Pathology, Employee's State Insurance corporation (ESIC) Hospital, Sarojini Nagar, Lucknow (U.P.) on a group of 600 patients (375 female and 225 male) including both indoor and outdoor patients from 1st November to 30th November, 2021 in one month duration. The blood samples were obtained from all age group patients, handled confidentially and labeled with name, age, sex and serial number of that patient. Blood samples were collected from the venous blood after applying tourniquet and transferred blood into the tubes containing EDTA (ethylenediamine-tetra-acetic acid) and immediately mixed that blood sample with anticoagulant in the EDTA tube. Blood samples were randomly categorized into three groups: Group A- Thrombocytopenia having low platelet count (<1.5 lacs/mm^3) patients, Group B- Normal platelet count (1.5-4.5 lacs/mm^3) and Group C- Thrombocytosis having high platelet count (>4.5 lacs/mm^3). The counting of platelets were performed within 4 hours of collection of blood samples. Blood samples without clotting inside the EDTA tubes were included for this study while EDTA tubes with blood clotting were excluded. Manual method of platelet counting was performed

by slide method in which smears were prepared from EDTA blood, air dried and stained with Leishman stain. After staining where RBCs were just touching to each other, at that place platelets were counted under oil immersion lens (100x) from Olympus cx21i microscope in 10 fields and multiplied that platelets count to 20,000. Automated counting of platelets was carried out by using Mindray BC-6200 auto-hematology analyzer 5 part (also count immature cells and nucleated RBCs) counter by following instruction provided by the manufacturer.

STATISTICAL ANALYSIS

The data was analyzed by SPSS 21.0 version after entering into Microsoft excel sheet. Mean value of quantitative variables were calculated and compared two independent sample "t" tests were employed for comparison of quantitative data. Correlation analysis was done by Pearson correlation method to see the association between two variables. A p-value of <0.05 was considered to be statistically significant.

RESULTS

A total of 600 patients (375 male and 225 females) have studied with the mean platelet count in automated was $1.16\pm0.99\times10^3$ /µL and by manual method was 1.23±1.03 lacs /mm3 (**Table-1**). 600 patients were randomly categorized into three groups to compare the platelet counts by automated and manual platelet analyzer. Group "A" comprised of 101 patients with platelet counting ranging from <1.5 lacs/mm3, Group "B" comprised of 493 patients with platelet counting ranging from 1.5-4.5 lacs/mm3 and Group "C" comprised of 06 patients with platelet counting ranging from >4.5 lacs /mm3 (**Table-1, figure 1a and 1b**). **Distribution of CV (coefficients variation) among the groups is assessed.** In the current study, samples from all three groups were analyzed and CV (coefficient of variation) was calculated (table 2). Automated and manual method was compared for all 3 groups. A significant result was observed for automated vs manual method for normal group (p=0.02) and thrombocytosis group (p=0.04).

Comparison of platelet count among the different groups: Platelet counts were compared between normal to thrombocytopenia and normal vs thrombocytosis group (figure 6). It was observed that the mean platelet counts among the normal group was 1.54 lacs/mm3 while thrombocytopenia group had 0.431.54 lacs/mm3 (p<0.0001) and thrombocytosis group has 4.61 lacs/mm3 (p<0.0001).

Table 1: Number of cases with different condition such as Normal platelet count, Thrombocytopenia (low platelet count) and Thrombocytosis (high platelet count).

Groups		Number of Patients (600)	Platelet Count
Group A	Thrombocytopenia	101	<1.5 lacs/mm^3
Group B	Normal platelet count	493	1.5-4.5 lacs/mm^3
Group C	Thrombocytosis	06	>4.5 lacs/mm^3

Figure 1: Platelets (a) Platelet clumps under oil immersion (b) Large platelets under oil immersion (Leishman Stain, 100x).

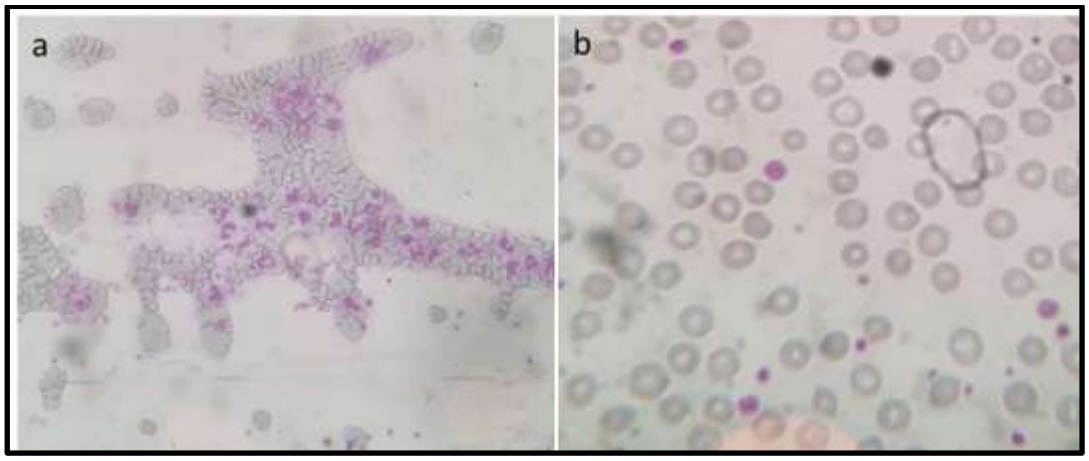


Table 2: Distribution of CV (coefficients variation) in all three groups

Groups		Automated	Manual	Statistical analysis	
		CV	CV	t-test	p-value
Group A	Thrombocytopenia	34.77	27.05	1.54	0.063
Group B	Normal	35.59	30.46	2.2	0.025
Group C	Thrombocytosis	17.08	9.9	1.96	0.04

Figure 2: Group-A scatter Plot of Platelet count -automated and manual method (r=0.0631, p=0.530). Pearson correlation was done.

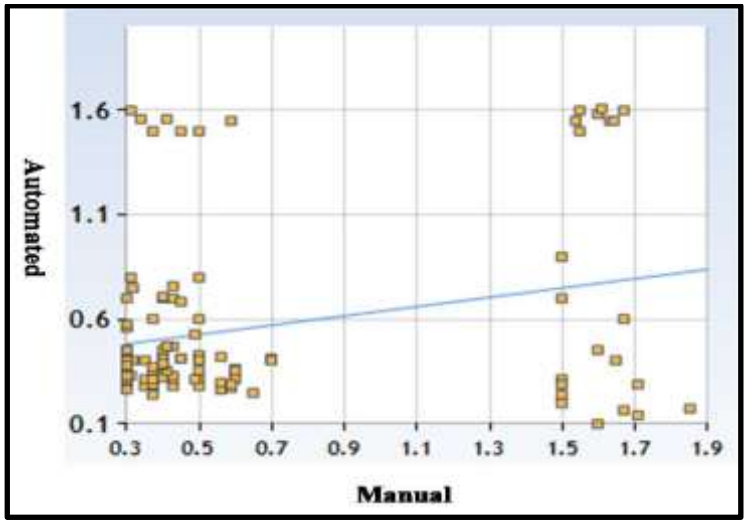


Figure 3: Group-B scatter Plot of Platelet count -automated and manual method (r=0.135, p=0.002). Pearson correlation was done.

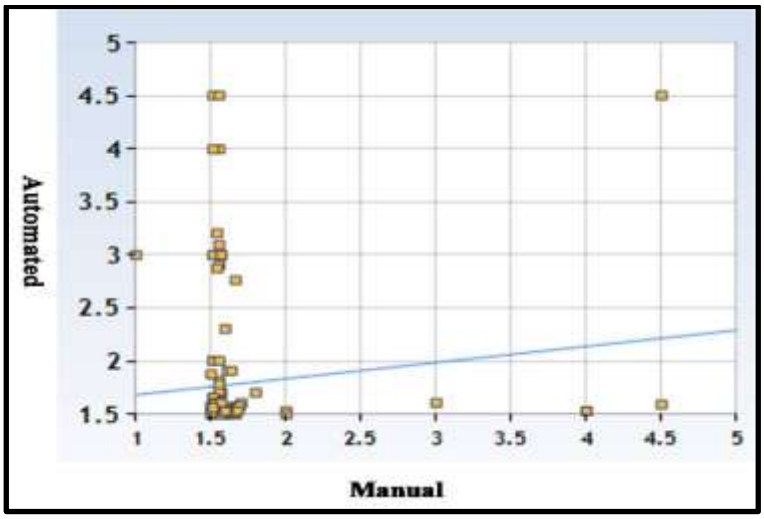


Figure 4: Group-C scatter Plot of Platelet count -automated and manual method (r=0.082, p=0.04). Pearson correlation was done.

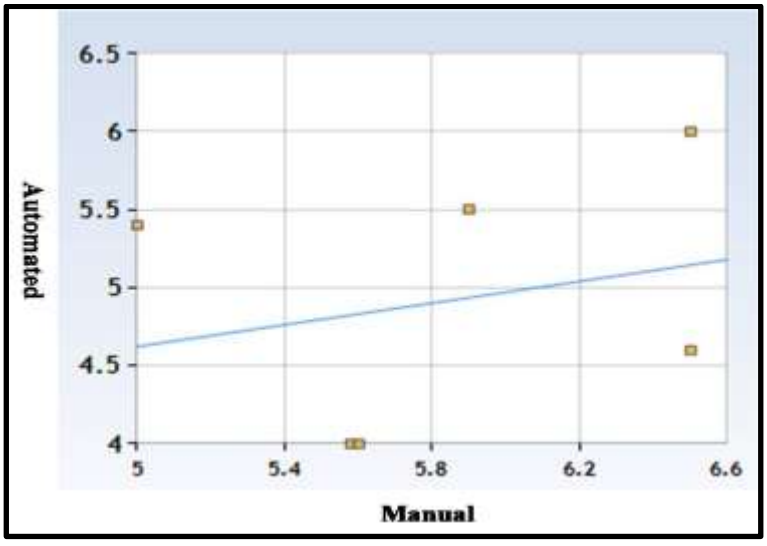


Figure 5: Scatter plot of few automated platelet count of patients (14.85%) of Group-A and platelet count under microscope (r=0.084, p=0.049). Pearson correlation was done.

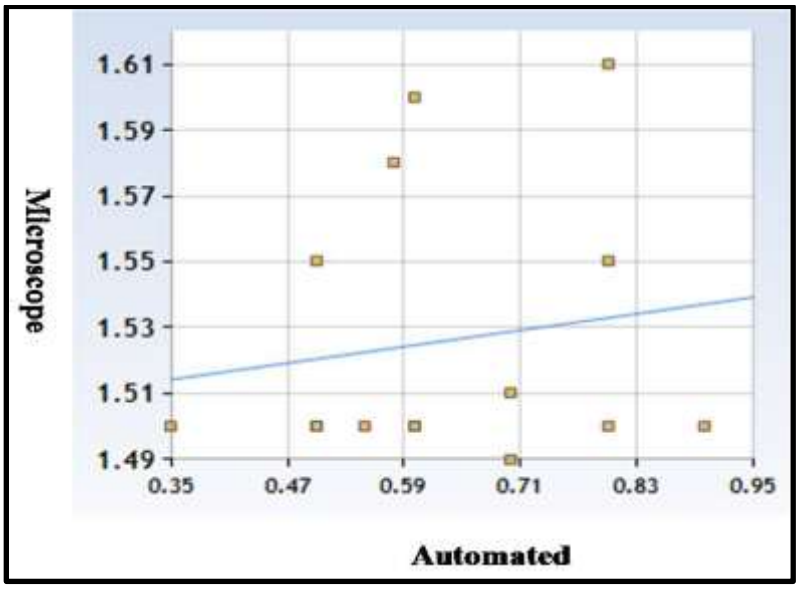
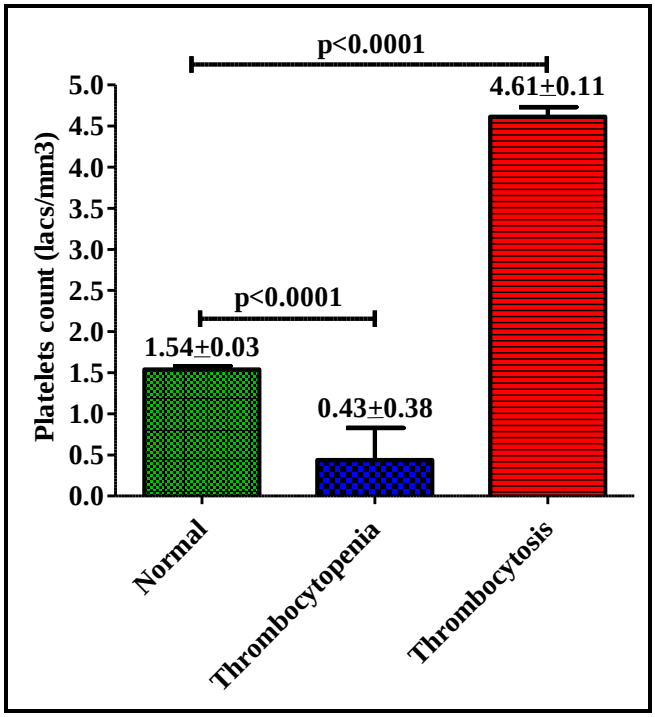


Figure 6: Comparison of platelet count among the different groups (Normal vs Thrombocytopenia and Normal vs Thrombocytosis). Student t test was used to compare the groups



DISCUSSION

The platelets circulate in the blood as small disc and are derived from megakaryocytes in the bone marrow. Megakaryocyte constitutes <1% of myeloid cells in the bone marrow. One megakaryocyte can give rise to one thousand to three thousand platelets. The platelets are

about 3µm in diameter and are nonnucleated. The life span of normal platelet is about seven to twelve days and is destroyed by splenicmacrophages. The normal range of platelets count in healthy human being is 1.50 to 4.50 lacs platelets per µl. The thrombocytopenia is one of the critical conditions where patients platelet count decreases below the normal range.Platelet counting is regularly performed in the hematology laboratory through various methods like manual platelet counting by hemocytometer, automated platelet counting, immuno-platelet counting, peripheral blood smear mediated platelet counting and radioisotope technique for platelet counting. Counting through manual have a high accuracy but the platelet numbers are variable and platelet counting through automated analyzer must be cross checked because particle with similar size (platelet clumps and aggregates, fragments of WBC, giant platelets and microcytes) also scatter the light resulting in false positive outcomes[14] and false positive outcomes can also obtained even through accurate and expensive hematology analyzers. Several researchers analyzed the outcomes associated with both manual and automated platelet counting methods.[15-16] Anitha et al., reported the non-significant differences between automated and manual platelet analyzer.[17] Another similar study also found a non-significant relationship between automated and manual platelet analyzer.[18] A study conducted by Momani et al., reported the non-significant relationship was also observed between automated and manual platelet analyzers.[19] Several lines of evidence also reported the significant relationship between manual and automated platelet counting.[20,21,22] One of the studies reported only marginal differences between these methods of platelet counting.[23] Anchinmane et al., demonstrated the strong relationship between automated and manual platelet analyzer.[24] In our study, the mean platelet count in automated was $1.16\pm0.99\times10^3$ /µL and by manual method was 1.23±1.03 lacs /mm3. We compared automated and manual method for all 3 groups. A significant result was observed for automated vs manual method for normal group (p=0.02) and thrombocytosis group (p=0.04). In this study a positive correlation was observed between automated analyzer and manual platelet count (r=0.837, p=0.00). Bakhubaira S in 2013 also concluded that significant positive correlation is present between the manual and the automated counting methods of platelets and recommended that platelet count is not varied when done by manual or automated methods, but in every method, it should be accompanied by platelet estimate by manual method, especially with abnormal counts.[25] In our study, platelet counts were compared between normal to thrombocytopenia and normal vs thrombocytosis group. It was observed that the mean platelet counts among the normal group was 1.54 lacs/mm3 while thrombocytopenia group had 0.431.54 lacs/mm3 (p<0.0001) and thrombocytosis group has 4.61 lacs/mm3 (p<0.0001). Aashna et al. in 2009 conducted a study in thrombocytopenic patients, they assessed platelet count by automated analyzer, showed an inverse relation with Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW).[26] They concluded that automated hematology analyzer is crucial for quick and accurate complete blood count evaluation but all blood samples that show abnormal results or low platelet counts on analyzers should be confirmed by manual count on peripheral smear. The platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) can point to the underlying pathology especially in cases of thrombocytopenia. Jangbhadur Singh et al. in 2020 concluded that platelet count by manual method using chamber for counting as well as by traditional method using peripheral blood

smears for platelet counting are validated as alternative and reliable methods of platelet counting and we haveresults more or less similar findings.[27]

CONCLUSION

A positive correlation was observed between manual method and automated analyzer in platelet count but in few of the thrombocytopenic patients there was significant difference in platelet count because of platelet clump or irregular distribution. Automated analyzers produce immediate results but if there are very low platelet counts or abnormal platelet histogram, we should ensure platelet count with a slide method under a microscope. In conclusion, manual platelet counting should be employed in thrombocytopenic patients before giving the final report.

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