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Original Research Article

Utility of peripheral blood smear in platelet count estimation

Vyankatesh T. Anchinmane, Shilpa V. Sankhe*

Department of Pathology, Tomo Riba Institute of Health and Medical Sciences, Naharlagun, Arunachal Pradesh, India

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***Correspondence:**
Dr. Shilpa V. Sankhe,
E-mail: vyankatesha78@gmail.com

ABSTRACT

Background: There are several methods of platelet count used in hematology laboratory. These methods are manual counting, automated hematology analyzer counting, platelet count estimation by peripheral blood smear (PBS) method etc. Many diseases such as dengue, malaria, pregnancy induced hypertension etc. may leads to severe thrombocytopenia. Timely and precise diagnosis of platelet count plays very crucial role in critical care management of thrombocytopenia cases. The present study was undertaken to estimate platelet counts by PBS method and correlate them with results from automated hematology analyzer method.
Methods: Study included one hundred randomly collected blood samples in EDTA anticoagulant vacutainer tubes. Each blood sample was processed for platelet count estimation with automated hematology analyzer and Leishman's stained PBS examination. The statistical analysis was done by using Pearson's correlation test to access the agreement between both the methods.
Results: The Pearson's correlation test showed significant positive correlation for platelet count estimation between both the methods. (r =0.9789).
Conclusions: Platelet count estimation by PBS method is reliable and statistically significant when compared to hematology analyzer method. PBS platelet estimation method can be taken as early and rapid procedure for platelet assessment in critical severe thrombocytopenia cases. This method is simple, cheaper and can be done in rural hospital setup where automation is not available.

Keywords: Hematology analyzer, Platelet count, PBS

INTRODUCTION

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.[1,2]

In most of the laboratories, the hematology analyzer is utilized to count platelets in patient blood samples. However, varying platelet count results may be observed with different hematology analyzer for the same blood sample, which makes the comparison very difficult. The other methods for estimation of platelet count are manual counting by counting chamber (e.g. Neubauer's counting chamber), PBS examination, immunoplatelet counting and radioisotope labelling technique.[3,4]

PBS is highly informative hematological tool for RBC's, WBC's and platelets morphology and counting. PBS screening is commonly used for disease diagnosis and its progress and therapeutic response. It has been reported that platelet count estimation can be obtained by multiplying the average number of platelets in body part

of PBS (where RBC's do not overlap each other) with $20x10^9$/L. The platelet count results obtained by this method of PBS examination had no statistically significant difference when compared with hematology analyzer platelet count results for same blood samples.[5,6]

In the present study, we analyzed the platelet count results obtained by hematology cell analyzer and PBS examination in randomly selected blood samples.

METHODS

A tertiary care hospital-based study was conducted. The blood samples were collected from 100 patients who were admitted in hospital and receiving medical treatment. The patient's selection was done by simple random sampling with any medical diagnosis.

All the venous blood samples were collected in EDTA vacutainer tube. The samples were stored at room temperature until they were analyzed within four hours. The hemolytic samples, clotted samples and inadequate blood samples were excluded from the study.

Each blood sample was properly mixed with automated mixer for ten minutes. The platelet count was obtained by processing blood samples in fully automatic hematology analyzer ERMA AGD 210. The analyzer was maintained and calibrated as recommended by manufacturer.

After processing the blood samples with hematology analyzer, the same blood samples were used to prepare air dried PBS. Then the PBS was stained manually with Leishman's stain. The PBS was examined under light microscopy with x100 oil immersion lens.

The blood samples were excluded from the study if PBS of respective blood sample showed platelet aggregates or giant platelets. The platelets were counted in ideal zone of PBS where RBC's did not overlap each other and there was fairly even distribution of WBC's and platelets. The average number of platelets in an ideal zone of Leishman's stained PBS was multiplied by twenty thousand. The each platelet in an ideal zone, in an average oil immersion field represents 20,000 platelets per µl. Thus, the platelet count estimation was done from PBS.[5,7]

The statistical analysis was done for test performances and their comparisons by using coefficient of variation (CV), linear regression and mean differences with SPSS software.

RESULTS

The platelet count results of one hundred randomly selected hospital patients blood samples obtained from hematology analyzer and PBS examination were compared with each other. The comparison was done irrespective of age, sex and specific disease of patients.

The assessment for the measurements of central tendency (mean and median) and variation (range and standard deviations) for all one hundred blood samples, platelet count results by both the methods, are as shown in Table No.1.There was no any significant statistical difference for these parameters. The platelet count estimated by PBS method used in this study had a range of 6 to $58x10^4$ platelets per µl. The hematology analyzer platelet count ranged from 5 to $55x10^4$ platelets per µl. The PBS platelet count estimation method showed mean ($20.25x10^4$ platelets per µl) and median ($18x10^4$ platelets per µl) when compared to hematology analyzer platelet count method (mean $19.13x10^4$ platelets per µl and median $16.5x10^4$ platelets per µl respectively). The standard deviations of platelet count in the whole blood by hematology analyzer method and PBS method were $9.4062x10^4$ and $9.2084x10^4$ platelets per µl respectively. (Table 1).

Table 1: Measurement of central tendency and variation for methods of platelet count estimation.

Method	N	Range ($X10^4$/µL)	Mean ($X10^4$/µL)	Median ($X10^4$/µL)	SD ($X10^4$/µL)
Hematology analyzer	100	5- 55	19.13	16.5	9.4062
PBS	100	6- 58	20.25	18	9.2084

Table 2: Linear regression analysis for methods of platelet count estimation.

Reference method	Testing method	Correlation	Slope	Y-intercept
Hematology analyzer	PBS	0.9789	1.000	-1.120

The Pearson's correlation test was applied with platelet count by hematology analyzer as 'reference method' and platelet count by PBS method as 'testing method'. The Pearson's correlation test result showed slope of 1.000 and y-intercept of -1.120. The coefficient of correlation of the linear regression for analysis of platelet count estimation for PBS method and hematology analyzer method, was r = 0.9789 which indicates that these

methods have excellent positive correlation with each other for platelet count results (Table 2).

DISCUSSION

The precise, accurate and reliable assessment of platelet count is required to avoid unnecessary platelet transfusion in the treatment of severe thrombocytopenia patients. The accuracy is also needed after platelet transfusion treatment to check the therapeutic response.

The platelets circulate in the blood as small disc and are derived from megakaryocytes in the bone marrow. Megakaryocytes constitutes <1% of myeloid cells in the bone marrow. One megakaryocyte can give rise to one thousand to three thousands platelets. The platelets are about 3µm in diameter and are non-nucleated. The life span of normal platelet is about seven to twelve days and is destroyed by spleen macrophages. In wet preparations, platelets appear as colourless, discoid or elliptical refractile bodies. In Leishman's stained PBS, platelets appears as light blue coloured, round, oval or rod shaped structures. Platelets are multifunctional and plays key role in hemostasis, thrombosis and wound repair. The normal range of platelets count in healthy human being is $15x10^4$ to $40x10^4$ platelets per µl. The thrombocytopenia is one of the critical conditions where patients platelet count decreases below the normal range.[8,9]

The platelet count is commonly done in laboratories by evaluation of PBS, Neubauer's chamber counting or by automated hematology analyzer. The platelet counting is more difficult as compared to RBC's count and WBC's count. Until recently, the Brecher and Cronkite platelet counting method described in 1950, was considered as reference method while comparing with platelet counting by semi-automated and automated methods. These manual phase contrast microscopic method was considered as gold standard which had significant limitations in view of imprecision. This method results are highly variable and depends upon individual training. Few researchers mentioned that the risk of error estimation is upto 10% to 20% for this manual platelet counting method.[9]

In the year 2001, a joint task force of ISLH and ICSH recommended a new immunological based reference method for platelet counting. This method utilizes monoclonal antibodies to platelet surface antigens conjugated to suitable flurophore. This method permits the possible implementation of new reference method for calibration of hematology analyzers.[3]

The platelet count with hematology analyzer is usually precise. In hematology analyzers, the principle of particle impedance is utilized which was first described by Wallace H. Coulter in 1954. The hematology analyzer platelet count accuracy is compromised while processing blood samples with low platelet counts or with blood samples with abnormal platelets morphology like giant platelets or blood samples having presence of non platelet particles like RBC, WBC fragments. The accuracy of platelet count is also compromised in hematology analyzer due to inadequate calibrations and lack of adequate quality control material.[10,11] Despite of advances in hematology automation, the PBS has its own importance in hematology laboratories for validating results of other methods for platelet counting. Till date, even the accurate and best quality hematology analyzer also cannot replace the PBS evaluation.[12]

In the present study, it was found that the estimated mean platelet count by PBS method and hematology analyzer method didn't show significant difference. There was significant positive correlation was found in both these methods when Pearson's correlation test was applied. ($r = 0.9789$) The platelet multiplication factor utilized in this study was $20x10^4$ platelets per µl. The similar results were obtained by Malok M et al and Gao Y et al.[7,5]

Webb et al and Bajpai et al had reported slightly better results with $15.0x10^4$ platelets per µl as platelet multiplication factor instead of $20x10^4$ platelets per µl.[13,14] The different platelet counting sensitivity mechanisms of hematology analyzers, quality control issues of hematology analyzers and issues related to trained qualified PBS examining personal might be the reasons of different views of researchers for different platelet multiplication factor.[10,15] The PBS platelet count estimation method gives approximate platelet count and not the exact one and is the drawback of this method. However, PBS method precisely comments on adequacy of platelet count in patient blood samples and hence, this method has upper hand over other methods in diagnosing thrombocytopenia cases and avoiding unnecessary platelet transfusions.

CONCLUSION

PBS is reliable, cheap and cost effective method that can be used for estimation of platelet count in hematology laboratory. It has advantage over other platelet counting methods in ease of performance and no need of laboratory automation facilities. The PBS platelet count method is very useful in remote area laboratories. This method also important for verification of platelet count obtained from automated hematology analyzer. The platelet multiplication factor of $20x10^4$ platelets per µl with 100x oil immersion objective lens can be used for interpretation of adequacy of platelets in PBS. More comparison studies are necessary to determine the accuracy of platelet multiplication factor in view of different sensitivities of automated hematology analyzers for platelets counting.

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