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Original Research Article

The Estimation of Platelet Count from a Peripheral Smear on the Basis of the Platelet: Red blood cell Ratio: Prospective Study

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Abstract

Introduction: Accurate platelet counts is of utmost importance in clinical practice and for patient management. There are several method for counting platelet used in hematology laboratory. These methods are manual method using counting chamber, Examination of a peripheral blood smear (PBS) and automated hematology analyzers**.** Present Study was conducted with the objectives of estimation of platelet count indirectly from peripheral blood smear (PBS) on the basis of platelet: red blood cell (RBC) ratio and using automated RBC count; and to verify the reliability of this method in comparison to automated platelet counts.

Method: A prospective study was conducted in a tertiary care hospital on 250 Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples. Each blood sample was processed for platelet count estimation with automated hematology analyzer and Leishman's stained PBS examination. The number of platelets per 1000 erythrocytes was multiplied by the automated RBC ($x10^6$ cells/microlitre) to give an approximate manual count ($x10^3$ cells/ microlitre). Two paired t-test was used for comparison of the two methods.

Result: The Pearson's correlation test shows significant positive correlation for platelet count estimation by two methods. (r =0.9975).

Conclusion: Platelet count estimation based on platelet/RBC ratio is a simple and reliable method to estimate platelet counts from peripheral smears and it should be proposed as a method of reference.

Keywords: Platelet Count, Thrombocytopenia, platelet: RBC ratio.

Introduction

Automated hematology analyzers is routinely used for performing complete blood counts. But the accuracy of this method in predicting the platelet count in patients with thrombocytopenia is questionable [1,2]. Since Platelets play a key role in hemostasis and thrombosis. Platelet count is one of the critical parameters in patient care. Thus Accurate and reproducible platelet counts is essential for patient management.

The International Council for Standardization in Hematology (ICSH) and the International Society of Laboratory Hematology (ISLH) have recommended a method based on the measurement of platelet/RBC ratio with fluorescent labeled platelets in fluorescent flow cytometer as the reference method for platelet counting in peripheral blood [3]. But this method is expensive and cannot be performed routinely in developing countries.

A routine method for counting platelets in a peripheral smear is by taking the average of platelets in ten oil immersion fields and multiplying it by 15000 or 20000 [4,5]. But this method has its own drawbacks.

Objectives

1. Estimation of platelet count from PBS by counting the number of platelets per 1000 RBC in PBS and then calculating the platelet count on the basis of platelet: RBC ratio using the automated RBC count.
2. To verify the reliability of technique by comparing the platelet count obtained by this method with the automated platelet count.

Materials and Methods

A prospective study conducted in a tertiary care hospital on blood samples received in Central Pathology laboratory(CPL) during February 2020 to march 2020. Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples sent to CPL for complete blood count(CBC) from subjects of any age and gender, and with any diagnosis during the study period were included in the study.

Hemolysed and clotted samples were excluded.

Sample size: 230.

The samples were analyzed by two methods:

1. Automated hematology analyzer Mindray BC 2800 using impedance method to get complete blood count (CBC).
2. Air dried thin smears were made from all samples and stained with Leishman stain.

These PBS were examined under light microscope using x100 oil immersion lens. In a monolayer zone of the smear, platelets were counted simultaneously with RBC till 1000 RBC were counted. Number of RBCs counted by counting the RBCs in one quarter of oil immersion fields and multiplying it by four.

The number of platelets per 1000 RBC thus obtained was multiplied by automated RBC count in 10^6/uL to get an estimation of platelet count in 10^3/uL.

Data was processed using SPSS Program v17.

Results

Two sample T test was done. Automated platelet count ranged from 11 - 830 x 10^3/microlitre and had a mean value of 208 x 10^3 /microlitre. Platelet count estimated by the method used in this study had a range of 20- 800 x 10^3/microliter and the mean was 220 x 10^3 /microlitre.

P value <0.05 was considered as statistically significant. R value was 0.9975 and T value was 14.52. Hence, two sample T test showed no significant difference between the two methods.

Pearson correlation of two methods was done and gave a value of R = 0.9975 and P value of < 0.00001. Thus the two methods are highly positively correlated.

Discussion

Accurate and reproducible platelet counts are essential for the management of thrombocytopenic patients at risk of bleeding 1. Platelet count is important to evaluate the risk of occurrence of spontaneous bleeding in a patient. If there is confidence in the platelet count values at low levels, it is possible to reduce platelet transfusions to those that are clinically necessary [6].

Many methods for counting platelets have been published and the number of alternative methods is due to difficulties in counting small cells which are activated easily, aggregate and the difficulty in differentiating platelets from extraneous matter [6].

With the development of sophisticated automated blood cell analyzers, the

proportion of blood count samples which require a blood smear has steadily diminished. Nevertheless, examination of blood smear is a crucial diagnostic tool. Analysers using the standard impedance measurement are able to provide an accurate platelet count upto $20x10^3$/uL. False increases in platelet count will occur when red cell or white cell fragments, microcytic red cells, immune complexes, bacteria or cell debris are included in the count [7]. False decrease in platelet count will occur in the presence of large platelets and if there is platelet clumping [6].

An estimation of whether the platelet count is normal, low or high and any abnormalities of platelet morphology can be made out by examining a PBS.

It is a standard procedure that all abnormal platelet values generated by cell counters should be confirmed by manual examination of Leishman stained PBS [8,9].

Each time the automated count is erroneous, the platelet count must be systematically estimated from blood smears because even the most expensive and effective machine is not able to replace human judgement [10].

Some authors recommend calculating the average number of platelets counted in 10 oil immersion fields; the adequate values are included between 8 to 20 platelets per field [11-13]. The average number of platelets is then multiplied by a factor of 20,000 for wedge preparations or 15,000 for monolayer preparations in order to obtain and estimate the platelet count per micro liter, but this method is approximative and does not give the real number of platelets.

In our laboratory, we estimate the platelet count indirectly by using the automated RBC and calculating the platelet count on the basis of the red cell: platelet ratio in a stained blood film. Platelet counts by this method were not significantly different from automated platelet counts.

Using same method, Theml H and also Brahimi et al have estimated the number of platelets relative to 1000 RBC and found it to be reliable [14,15].

The traditional 'gold standard' method for platelet count was manual phase contrast microscopy [15-17]. But, this method is time consuming and imprecise at low counts. It still offers a relatively inexpensive, simple and viable means to enumerate platelets in nonspecialized laboratories [6].

Now, the International Reference Method (IRM) for platelet count is flow cytometry [3,18,19]. The proposed International Society of Laboratory Haematology (ISLH) reference method uses specific monoclonal antibodies to platelet cell surface antigens (e.g., anti CD41 and anti CD61) which are conjugated to a fluorescent substance. Flow cytometric analysis of the ratio of fluorescent platelets to non-fluorescent red cells gives a highly accurate and precise platelet counts [18,19]. This offers a suitable comparison for platelet count methods.

Conclusion

Platelet count estimated from PBS based on platelet: RBC ratio were not significantly different from platelet count estimated by automated hematology analyser. This is a reliable technique and can be used for microscopic validation of automated platelet count.

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