Bavani S et al /J. Pharm. Sci. & Res. Vol. 11(4), 2019, 1506-1509

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Influence of Red Cell Microcytosis in the Accuracy of Platelet Counting by Automated Methods

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Abstract

Background: Accurate assessment of platelet count is an important element in the diagnosis and treatment process for hemostasis disorder.

Aims: The aim of this study is to determine the influence of red cell microcytosis in the accuracy of platelet count and to evaluate the accuracy of platelet count by automated impedance and optical methods in red cell microcytosis.

Materials and Methods: A total of 103 samples with mean corpuscular less than 80 fl were examined in this study. The samples were analyzed using Sysmex XN -1000 analyzer, and manual count using hemocytometer was performed for each sample.

Results: There is a strong significant correlation between PLT-I and PLT-M (N=103, r=0.86); PLT-O and PLT-M (N=103, r=0.92); and PLT-I and PLT-O (N=103, r=0.95). Analysis from the data suggested that spurious platelet count may be contributed by PLT-I methods in microcytosis samples. There is a significant influence in the accuracy of platelet count in microcytosis samples.

Conclusions: This finding indicates that the platelet count in impedance method is overestimated as compared to the platelet optical method in microcytosis samples.

Keywords: Platelet, microcytosis, impedance, optical, hemocytometer

INTRODUCTION

The three main components of blood in a human body are red blood cells, white blood cells, and platelet. Platelet plays a fundamental role in homeostasis and works together with coagulation factors to stop bleeding by clumping and clotting at the damaged blood vessels site. Platelet is the smallest blood corpuscles measuring between 1-3 µm in diameter or with a cell volume of 2-20 fl. and appears as dark purple spots on a stained blood smear. Platelet counts indicate the number of platelet in a person with normal range of about 150,000-450,000 platelets in each microliter of blood. Average platelet volume from blood samples will be elevated over time after blood collection and cause changes in the shape of the platelet [1]. Relatively the change in volume is stable for one to three hours after the collection of blood samples. This is the most suitable time to perform platelet count using electronic instruments because the platelet will be uniformly in size with minimal morphological changes.

Accurate assessment of platelet counts can be done using three commonly used methods in the laboratory. These methods consist of hemocytometer counting, impedance counting, and optical counting. Hemocytometer count is a manual counting method where the diluted blood sample is evenly distributed on the hemocytometer slide and observes under the microscope. Currently most laboratories use automated counters to count the number of platelets. Two customary methods of automated counting are the impedance and optical counting. In both methods, collected blood is diluted and counted by passing the samples through an electronic counter [2]. Usually the instrument is programmed to count only particles within the proper range of size for platelets that passes through the electronic counter.

However, microcytosis in blood samples may cause overestimation of platelet counts when using automated counters. Microcytosis define as the present of numerous microcytic red blood cells with its mean corpuscular volume (MCV) less than 80 fl. Blood samples which usually drawn from patients with thalassemia, iron deficiency anemia, hemolytic autoimmune anemia, and malignant neoplasm contains lot of fragmented and microcytic red blood cells which interferes with the platelet counts. The impedance method widely overestimated platelet counts in microcytosis samples as compared to the result by optical method [3]. In severe microcytosis cases, impedance platelet count is not always reliable [4]. The results from platelet counts suggested that optical method is the preferred method in microcytic anemia when using an automated analyzer [5]. The optical method is superior to the impedance method in calculating platelet count in samples with low MCV values [6]. Wrong fitted curves may be generated when there is a presence of extremely low volume of red blood cells. Red blood cells fragments which often observed in patients with malignancies as part of microangiopathic process are also recognized as a cause of erroneous platelet counts [7].

In a condition such as patient with high leucocyte count, automated counting may yield an unusually low platelet counts because some platelet may be filtered out by white blood cells before the samples are counted. In contrast, if the red blood cells in the sample have burst or hemolysed, its fragments will be falsely counted as platelets. This will consequently increases the platelet counts and produces anomalous result. It is important to overcome this problem to avoid unnecessary treatment and platelet transfusion. The present study was therefore planned to determine the influence of red blood cells microcytosis in the accuracy of platelet counting by automated methods and to evaluate the accuracy of platelet counts by the automated methods.

MATERIALS AND METHODS

This cross-sectional study was conducted on randomly selected blood samples sent to the Hematology Laboratory, Hospital Putrajaya, Malaysia for a period of 30 days (one month) after due approval from the institute Ethics committee.

Blood sampling and automated counting

103 whole blood samples collected in EDTA (Ethylenediaminetetraacetic acid) vials were obtained from the hematology laboratory. A total of 46 male and 57 female blood samples from different age group were randomly selected for the study. A complete blood count was measured within 4-6 hours after collection using Sysmex XN-1000 analyzer. The samples were then subjected to counting with the PLT-I (Platelet count impedance) and PLT-O (Platelet count impedance optical) methods using the same analyzer.

Manual counting using hemocytometer

The manual counting is the "gold standard" for platelet count. PLT-M (Platelet count manual) was done using hemocytometer with 1% ammonium oxalate in order to verify the results by the automated analyzer. The blood sample was diluted in 1:200 of 1% ammonium oxalate and incubated for 5 minutes. Then, the blood sample was distributed evenly into the counting chamber. The chamber was left undisturbed for 10-15 minutes in a petri dish with moist gauze to inhibit evaporation. Platelets in the entire central squares of the counting chamber (within the 25 medium squares) were counted under the microscope at 40x objective.

The total number of platelet was determined using this formula;

$$\text{Total Platelet Count} = \frac{\text{No. of cells counted x Dilution Factor x Depth Factor}}{\text{Area counted}} = \frac{n \times 10 \times 200}{1}$$

Platelet concentration

The platelet concentrations from the three counting methods of PLT-M, PLT-I, and PLT-O were compared for significant differences. This measurement was examined with respect to the changes in MCV (50-59 fl, 60-69 fl, and 70-79 fl) to determine the influences of red cells microcytosis in platelet counting, and which automated platelet counting method produce more accurate result.

Statistical analysis

The collected data was analyzed using SPSS v. 23. The association between the three counting methods was tested using Pearson correlation test and ANOVA for comparison within each group. A $P < 0.05$ was considered as statistically significant.

RESULTS

A total number of 103 blood samples included in this study have the MCV values of less than 80 fl. There are 12 blood samples (11.7%) with MCV of 50-59 fl; 31 blood samples (30.1%) with MCV of 60-69 fl; and 60 blood samples (58.3%) with MCV of 70-79 fl respectively.

Results from the Pearson correlation test indicated significant correlation between the platelets counting methods. The correlation between PLT-M and PLT-I methods was calculated at $r = 0.86$ ($P < 0.05$) (Figure 1). PLT-M and PLT-O methods recorded a significant correlation of $r = 0.92$ ($P < 0.05$) (Figure 2), whereas the PLT-I and PLT-O also shows another significant correlation of $r = 0.95$ ($P < 0.05$) (Figure 3).

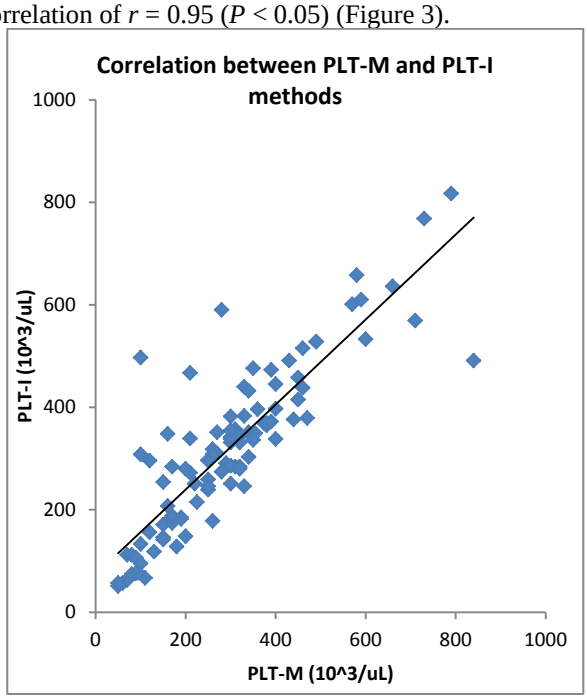

Figure 1: Correlation between platelet counts using PLT-M and PLT-I methods.

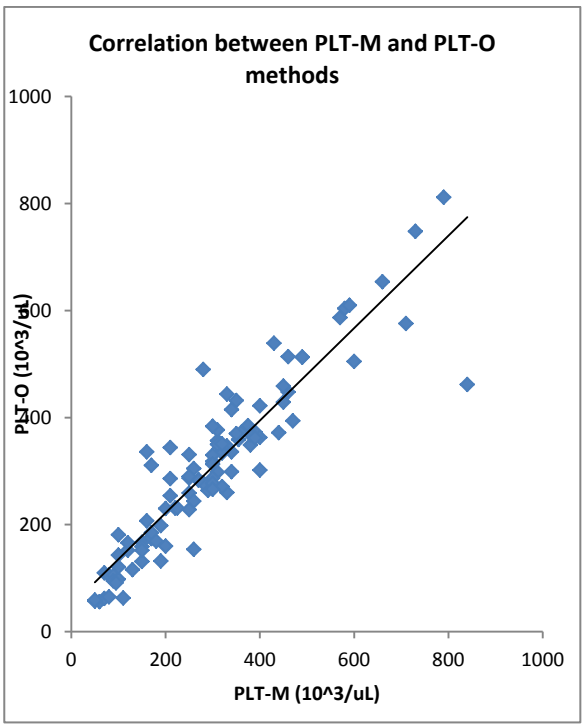

Figure 2: Correlation between platelet counts using PLT-M and PLT-O methods.

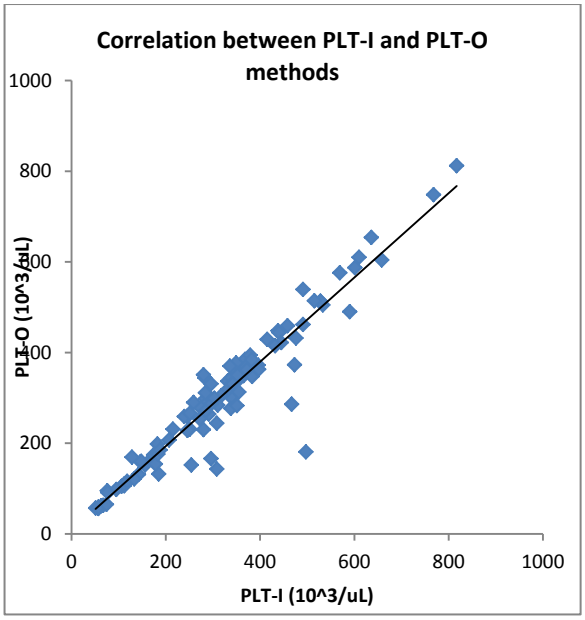

Figure 3: Correlation between platelet counts using PLT-I and PLT-O methods.

However, the PLT-I counting methods demonstrated an overestimated platelet counts in microcytosis samples with MCV <80 fl as compared to PLT-M and PLT-O methods. This was signified by mean (± standard deviation, [SD]) values of the platelet counts in Table 1.

In contrast, the platelet counts recorded using the PLT-O method was closer to those obtained with PLT-M method as the "gold standard" in the microcytosis samples (Figure 4).

Table 1: Mean values of platelet counts using different methods in microcytosis samples with different MCV.

MCV (fl)	*n*	x 10^3/uL; mean ± SD		
		PLT-M	PLT-I	PLT-O
50-59	10	379.0 ± 209.89	441.5 ± 143.71	377.5 ± 179.38
60-69	29	307.1 ± 129.35	324.0 ± 121.16	316.8 ± 122.8
70-79	54	275.2 ± 157.46	298.7 ± 154.37	287.7 ± 148.62

The differences in mean platelet counts between PLT-I and the other two counting methods implicate that spurious platelet count may have been released by the PLT-I methods in microcytosis samples. This implies a significant influence in the accuracy of platelet counts in microcytosis blood sample.

DISCUSSION

Counting platelet using hemocytometer chamber is the "gold standard" to verify the degree of accuracy of the automated platelet count. However, this method has some limitation in terms of its use particularly with the issues of operator competency, time consuming, and complication in preparing the procedure. It is also not practical for laboratories with high workload. Thus far, no consensus exists regarding which automated method gives more reliable and accurate platelet counts. Several studies suggested that the impedance method produced the best platelet count in samples from patient undergoing chemotherapy [8,9].

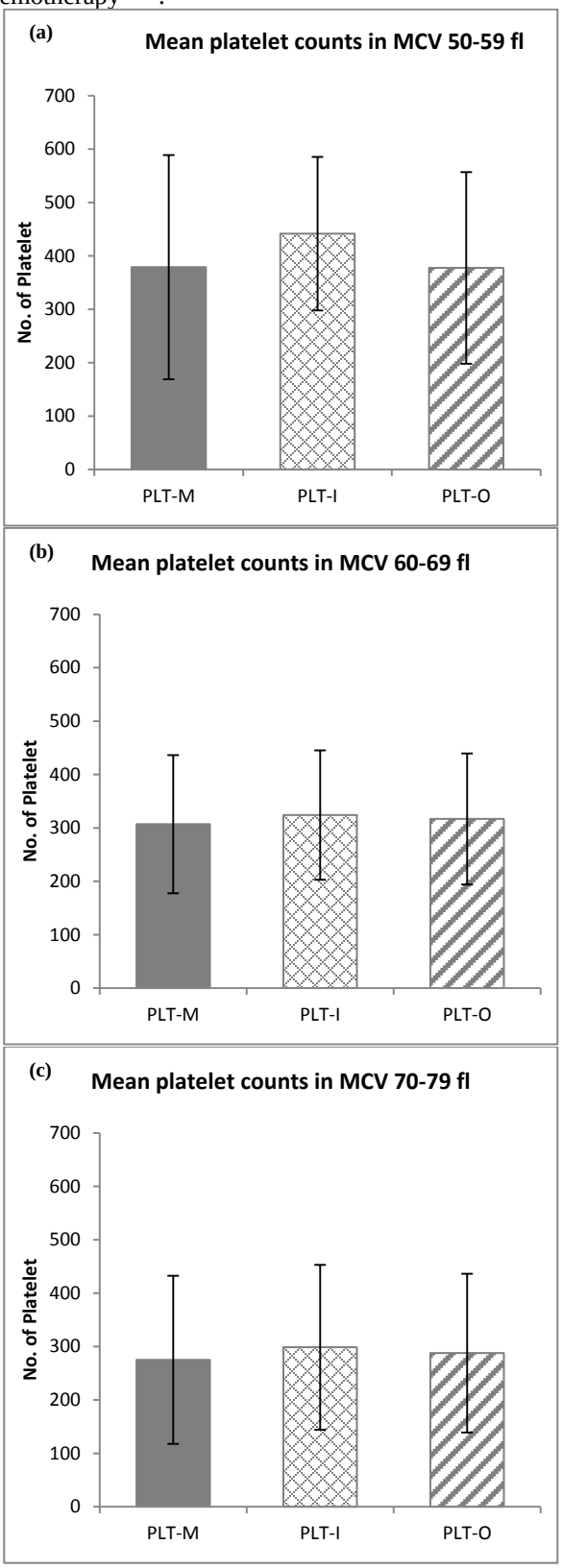

Figure 4(a-c): Comparison between mean platelet counts of different MCV groups using PLT-M, PLT-I and PLT-O counting methods. The data is expressed as mean ± SD.

In this study, the impedance method showed increased platelet counts as compared to the optical and manual counts in microcytosis samples. But the most important finding is the identification of MCV 50-59 fl as the cut-off threshold which may affect the accuracy of the platelet counts. The results suggested that platelet counts within the MCV 50-59 fl threshold may have been overestimated by the PLT-I counting method. It was reported that the MCV threshold that affects the accuracy of platelet counts is <70 fl [10]. Microcytes that appears in the blood of patients with anemia due to iron deficiency interferes with the PLT-I counting method to a various degree [11]. Optical methods are found to be less susceptible to interference of non-platelet elements such as microcytes. The impedance method may provide biased results when red blood cells fragments or macrothrombocytes are present. This present study had a limitation particularly on the quantity of samples according to the MCV groups. Nevertheless, the outcome points toward the importance of having an accurate platelet count in microcytosis blood samples.

CONCLUSION

This current study showed that the PLT-I method is affected by the present of microcytes in severe microcytosis samples and makes the accuracy of the results doubtful. On the other hand, the PLT-O method gives more reliable results which is closer to the "gold standard" method and produced higher consistency in counting platelet accurately in microcytosis samples. The PLT-O method is simple, rapid and does not require specialized and expensive equipment. In conclusion, it is proposed that the PLT-O method to be used as an alternative solution to the conventional method of using hemocytometer for platelet counting.

Financial support and sponsorship

The author gratefully acknowledges Universiti Kuala Lumpur and the Final Year Project course for providing financial support.

Conflicts of interest

There are no conflicts of interest.

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