COMPARISON PLATELETS COUNT BY AUTOMATED AND MANUAL HAEMOCYTOMRTER METHODS

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ABSTRACT

Background: Manual platelet counting in the Neubauer chamber, by means of a phase-contrast microscope, has been recommended as the reference method for assessing the platelet number by the International Committee for Standardization in Hematology (ICSH -1984). platelet counts as a reference for the calibration of hematology analyzers has been a continuing problem due to varying results. **Methods:** Fifty-Five blood samples in EDTA-anticoagulant vacutainer tubes were obtained and randomly divided into 3 group; Group A-normal control (n =34), Group b thrombocytopenia (n = 14) and Group C high platelets count thrombocytosis (n = 6). Each blood sample were analyzed for platelet count by manual microscopy, and automated hematology analyzer. The agreement between the two methodologies were assessed using the paired t-test and correlation coefficient analyses. **Results:** The coefficient of variation values significantly differed in Group A and were high for Automated cell counter. High positive correlation was also observed between Automated cell counter values and manual method (r=0.935, p=0.001) in group b was not significantly correlated. **Conclusion:** Based on the results observed in thrombocytopenia, manual platelet count using by haemocytometry may be promising if platelets are very low and giant platelets.

INTRODUCTION

Platelet count estimation is an important element of the diagnostic and treatment process. Platelets are small a nucleate cell fragments adapted to adhere to damaged blood vessels, aggregate on one another and facilitate the generation of thrombin.[1] In patients with abnormal thrombocytes where platelet transfusion is required, the reliability of the platelet count is highly desired and necessary to provide appropriate treatment, Hematology analyzers are intended to count (patient) blood samples.[2] Many blood centers use these analyzers to perform quality control on the blood components that they produce.The platelets are counted with hematology analyzers, but varying results among different hematology analyzers are observed, making comparisons very difficult. Measurement of platelet counts using automated hematology analyzers is usually quite precise and accurate. However, the accuracy of automated platelet counts can be compromised when measuring very low platelet counts or in the presence of interference from non-platelets particle or platelet abnormality.[2] 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 but this method is expensive and cannot be performed routinely in developing countries. A traditional method for counting platelets in a peripheral smear which has been in use for a long time is by taking the average of platelets in ten oil immersion fields and multiplying it by 15000 or 20000. But this method has its own drawbacks.[3]

Manual platelet counting in the Neubauer chamber, by means of a phase-contrast microscope,[4,5] has been recommended as the reference method for assessing the platelet number by the International Committee for Standardization in Hematology (ICSH -1984).[6]

Aim of the study The aim of this study was to determine the reliability and accuracy of the Beckman Counter 3000 plus automated system with manual microscopic count (hemocytometer) method. this study was performed to compare the platelets count by different method which can be used manually and automated.

METHODS AND MATERIALS

Patients studied The study was conducted on a group of Fifty five patients (20 males and 35 females) randomly

selected from hashim salama clinic, Age and gender of the patients were taken from the lab requests without meeting the patients.

The study was dealing with blood samples from the lab. All the obtained data were handled confidentially, instead of reporting the name of patients, each sample was given a unique numeric identifier. laboratory assays Blood Samples

Blood samples were obtained from 55 patients who were randomly divided into three groups. Group A(n = 35) had the patients with normal count, Group B(n = 14) had the patients with thrombocytopenia and group C(n= 6) had the patients with thrombocytosis. Venous blood specimens were collected and within 20 seconds from blood sampling, blood was transferred to a tube containing ethylene diamin tetraacetic acid (EDTA), and within 4 hours a whole-blood count was performed using an automated cell counter and manual method. Notation was made, if clots were seen in the blood sample or if the amount of blood in the tube was grossly inadequate such that a disproportionately high concentration of EDTA would be present; these samples were excluded from the study.

Automated Analyzer Platelet Counts After thorough mixing of each blood sample on an automated mixer for 10 min, a complete automated blood count was performed using BC- 3000 plus auto hematology analyzer which was maintained and calibrated as recommended by the manufacturer.

Manual Platelet Estimation Platelet count done by using hemocytometer (improved Neubaure chamber), Whole blood is diluted with a 1% ammonium oxalate solution. The isotonic balance of the diluents is such that all erythrocytes are lysed while the leukocytes, platelets, and reticulocytes remain intact. The standard dilution for platelet counts is 1:100. This dilution is prepared using the sahli pipette.

The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemacytometer. The cells are allowed to settle and then are counted in a specific area of the hemacytometer chamber under the microscope. The number of platelets is calculated per μL (10^9 /L) of blood.

Statistical analysis The data was entered into Excel spreadsheet (Microsoft, Redmond, Washington) but was exported to SPSS 20.0 for data analysis. Averages were reported for quantitative variables. Two-independent sample t-test was applied to compare quantitative values. Pearson-correlation was also applied to observe correlations. A p-value of <0.05 was considered to be statistically significant.

RESULTS

We have studied fifty five patients (20 male and 35 females) mean age (45±12 years). Platelet count by manual method was 222.73± 138.1 x 10^9 /l and by automated method was 249.11± 154.3 with p value of 0.001 (Table 1). There was statistically significant difference between two methods. Fifty -five patients randomly divided into three groups were compared for platelet count by automated method and manual cell count method by using hemacytometer. Group-A: Consisted of 35 patients with normal platelet count values ranging from 150-450 x 10^9/l

Group-B: - consisted of fourteen patients ranging from 15,000 to 140x 10^9/l and Group-C: consisted of six patients with high platelet count ranging from 500 -990 x 10^9/l.
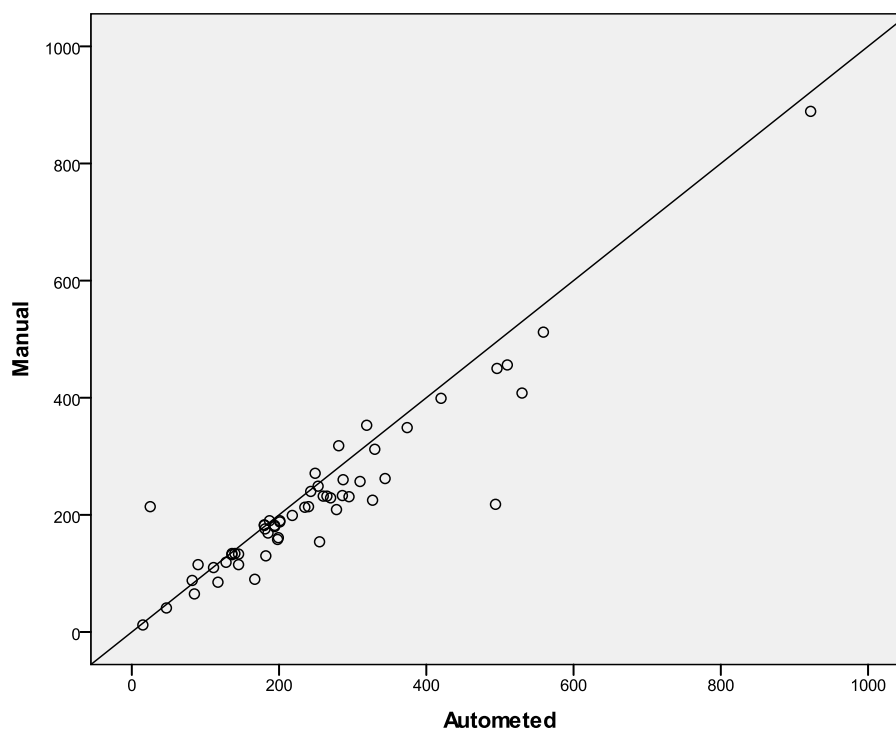
In the present study, all group samples were analyzed and coefficient of variation was calculated by statistical method.

Group A samples were estimated by Automated cell counter and manual method by number chamber, significant difference was observed in the coefficient of variation between automated cell counter and manual method (24.9 vs. 30.4 , p=0.001).

In group B, no significant difference was observed in the coefficient of variation between cell counter result and manual method (44.5 vs. 36.9 p=0.014). However, in group C, coefficient of variation values were significantly high for automated cell counter as compared to manual method values (28.5 vs 37.9, p=0.019). When correlations were applied, positive correlation was observed between Automated cell counter values and manual method (r=0.935,p=0.001).

Table 1: Platelets estimation by manual and automated methods.

Parameters	Manual methods	Automated methods	t-test	p-value
Platelets count	222.73±138.1	249.11±154.3	3.542	0.001

Correlation 0.9355 p 0.001

Table 2: Distribution of Coefficient of Variation between Different Groups.

Groups	Platelets count x 10⁹/l	Automated method (CV)	Manual Method (CV)	Stat	
				t-test	p-value
Group A	normal count (150-450)	24.9	30.4	4.43	0.001
Group B	Thrombocytopenia ≤ 140	44.5	36.9	1.75	0.104
Group C	Thrombocytosis ≥ 450	28.5	37.9	7.23	0.019

Fig. 1: Scatter plot of platelet count manual and automated method (R = 0.935, p = 0.001).

In our study there was no significant difference between manual and automated count only in case of thrombocytopenia. Discussion, conclusion

There were different methods of platelet count in hematology laboratory and these methods were manual counting, automated cell counting, platelet count by peripheral blood smear, immunoplatelet counting and radioisotope labeling technique for platelet counting. Many authors had given their thoughts on the methods of platelet counting.

Reliability of platelet count by comparison with manual method was studied by Lawrence J.B.[7] Kun also concluded the discrepancy of platelet numbers between automated blood cell analysis and manual counting in patients with thrombocytopenia.[8]

This study was conducted to compare the platelet estimation by manual count (improved neubar champers counting) and automated method.

Platelet count by manual method was 222.73± 138.1 x 10⁹ /l and by automated method was 249.11± 154.3 with p value of 0.001. There was statistically significant difference between two methods.

Earlier methods to enumerate platelets were inaccurate and irreproducible. The manual count is still recognized as the gold standard or reference method, and until very recently the calibration of platelet counts by the manufacturers of automated cell counters and quality control material was performed by this method.[9] However, it is time-consuming and results in high levels of imprecision. The introduction of automated full blood counters using impedance technology resulted in a dramatic improvement in precision. However, impedance counts still have limitations as cell size analysis cannot discriminate platelets from other similar-sized particles. More recently, light scatter or fluorescence methods have been introduced for automated platelet counting, but there are still occasional cases where an accurate platelet count accepted by laboratories and method was considered to give better reproducibility than the conventional microscopic count.[10]

Thrombocytopenia which is mean low platelets count and patient may need urgent platelets transfusion we note there was no statistical difference between manual and automated method since to get an accurate platelet count by the use of an automated hematology analyzer may be complicated by the presence of particles of similar size and/or light scatter properties (microcytic red cells,

white blood cell fragments) and by giant platelets and platelet clumps or aggregates.[11]

Even the most expensive and accurate hematology analyzers are not designed to eliminate peripheral blood film evaluation, and microscopic validation of platelet counts.

Although platelet count is a daily routine laboratory test, the estimation techniques seem to have not been validated. This is due to the fact that the methods of validation of the diagnostic tests were finalized during the second half of the 20th century and researchers are tempted to validate the new methods first, especially the less widespread. Even if the manual platelet numeration, using a counting chamber, remains the technique of reference, it consumes more time and requires a phase-contrast microscope, which is not always available in routine laboratories.[10]

In our result we note there was no significant difference between platelets count by manual method and automated method in all case of thrombocytopenia only.

In this study, on the basis of results, it is recommend that a manual platelet count using haemocytometer should be performed if platelets is very low and giant platelets.

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