ORIGINAL RESEARCH PAPER

Volume-8 | Issue-5 | May-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE ANALYSIS OF TWO METHOD OF ENUMERATION OF PLATELET COUNT IN THROMBOCYTOPENIC PATIENT: MANUAL AND ELECTRICAL IMPEDANCE.

Pathology

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ABSTRACT

Thrombocytopenia is one of the critical parameters in patient management. Therefore, it is very important that laboratories assess platelet counts with utmost accuracy. Traditional microscopic methods are very intense with variable results and are highly dependent on the individual training. Recent developments in automated peripheral blood differentials using a computerized system have shown many advantages as a viable alternative. In reference to above context, this study was conducted with following objectives: To compare the estimation of platelet count done by manual method and by electrical impedance method in thrombocytopenic patient and asses the merits in clinical practice. This was a crossectional study carried out on 100 Patients with platelet count <1,50,000/cu mm over a span of 1 year. The platelet counts were done by automated analyser, using microscopy and on haemocytometer. Statistical software SPSS 2 and independent T tests were used to compare the variables between the groups. P value of <0.05 was considered significant. The overall mean platelet count by the electrical impedance method and manual method was 76.6 ± 34.7 x 109/L and 70.3 ± 36.93 x 109/L respectively. The P value for automated method versus manual method was 0.44,suggesting that there was no significant variation in the platelet counts between the manual methods when compared with the electrical impedance method. The study also inferred the method of manual counting using haemocytometer as preferable method of choice in case samples with very low platelet count on automated analyser using electrical impedance.

KEYWORDS

Thrombocytopenia, Electrical impedance, Haemocytometer, Platelet count

INTRODUCTION:

Thrombocytopenia is associated with many diseases such as malaria, dengue, pregnancy-induced hypertension, cytotoxic therapy in haemopoietic malignancy etc., and is one of the critical parameters in patient management at risk of bleeding. (1,2,3) It is well-known that thrombocytopenia is one of the critical parameters in patient management. Therefore, it is very important that laboratories assess platelet counts with utmost accuracy. The normal range of platelet count in a healthy individual is 150000 - 400000/µL. (1,2,3)In patients with severe thrombocytopenia obtaining accurate and precise platelet counts by automated analysers is still challenging especially when a clinical decision has to be made for platelet transfusion.(4) Therefore, finding the reliable method to assess low platelet counts in thrombocytopenic patients becomes of paramount importance for clinical decisions. The platelet in peripheral blood is heterogeneous with respect to size, density, and staining characteristics. Their morphology also varies greatly depending on the methods by which they are examined, and the anticoagulant used. (1)

The common methods of platelet estimation are:

1. Manual counting using counting chamber
2. Evaluation on the peripheral smear
3. Assessment using the automated cell counters.

Peripheral smear examination have been commonly used for platelet estimation in settings of low platelet count. It also provides additional assessment of platelet size, shape, granulation, and analysis of phenomena such as aggregation or platelet satellitism.(5)The methods used for automated platelet counting are impedance, optical scatter, optical fluorescence, and immunologic flow cytometry. Up to date, the only "Gold Standard" in platelet counting available to assess any degree of accuracy of the automated count has been the manual phase-contrast microscopic method. (6)The manual method itself has significant limitations in terms of performance, particularly in the area of imprecision. In 2001, a joint Task Force of the International Society of Laboratory Hematology and the International Council for Standardization in Haematology recommended a new immunologically based reference method but it is not available in all laboratories. (3,7)Although hematology analyzers usually provide reliable platelet counts with good precision, however in some clinical situations interference with the automated count can occur, requiring manual method of platelet estimation. Situation requiring manual count include the presence of red cell fragments, microcytic red cells, apoptotic white blood cell fragments, giant platelets and platelet clumps.(8,9,10)Recent studies, mainly focusing on the counts of low levels of platelets, demonstrated that automated counts were not as accurate in severely thrombocytopenic samples. Traditional microscopic methods are very intense with variable results and are highly dependent on the individual training. Recent developments in automated peripheral blood differentials using a computerized system have shown many advantages as a viable alternative. But some cases like morphology abnormality and very low platelet count in not give accurate results.(11,12,13) The Consensus Conference on Platelet Transfusion Therapy of the National Institute of Health, reported that there was a lack of reproducibility and a variability in platelet counts at low levels. This fact is a great problem in recommending a standard threshold for platelet transfusion in thrombocytopenic patients. Thus, higher precision and accuracy in platelet counting is required in thrombocytopenic patient. (14)

AIM OF THE STUDY:

To compare the estimation of platelet count done by manual method and by electrical impedance method in thrombocytopenic patient.

OBJECTIVES OF THE STUDY:

1. To compare the manual and electrical impedance method for the counts of thrombocytes.
2. To know the merits of manual method and electrical impedance method in estimation of thrombocytes.
3. To compare the merits of application in clinical practice.

MATERIALS AND METHODS:

Study design: Crossectional study
Study site: Central laboratory, Department of Pathology, AVBRH, Sawangi, Wardha ,MH
Sample size: 100

Study duration: 1years

Recruitment characteristics: The following criteria were used for inclusion or exclusion for patients in present study.

- **Inclusion criteria:**

All ages
Platelet count <1.50,000/cu

- **Exclusion criteria:**

Hemolyzed samples, clotted samples
Data of age and sex of patients were taken from the laboratory forms and whenever possible by interviewing the patient directly along with clinical evaluation.All Ethylenediaminetetraacetic acid (EDTA) anticoagulated samples of patients (OPD/IPD), received in the central laboratory were processed for complete blood count. Samples was examined by the trained hematology technicians , processed by the Automated counter using the Sysmex® and manually by the hemocytometer at the same time, in addition to platelet estimate by the thin blood film, stained with Giemsa's Stain.

Technical issues:

- **Automated Platelet Count**

Platelets were analyzed in automated counters by electrical impedance methods. The principle is that the blood sample is aspirated and measured to predetermined volume, diluted at a specific ratio, and fed into each transducer. The transducer chamber has 2 min holes called aperture. Blood cells suspended in the diluted sample are passed through an aperture causing a change in the direct current resist between electrodes. These analyzers using electrical impedance determines numbers of various cellular elements. Each cellular component (WBC, RBC or Platelet) generates a channelized pulse that is proportional to its size and volume, which are sorted based on the size to determine final counts for each cellular component. The pulse with a volume between 2-20 fL is considered and counted as platelets. Coulter analyzers will provide "flagging" if abnormal platelet size (i.e., giant platelets or platelet clumping) is encountered, which will then prompt to review a slide using standard microscopy.(8)

- **Assessment of Platelet Count on Giemsa 's Stained Smear (15)**

Platelets were counted on peripheral smear stained with Giemsa stain using standard methodology. The platelets were counted under oil immersion objectives in an area were the red blood count (RBC) morphology is well made out (RBC's are separated without overlapping).The calculation is done by; the average number of platelet in an oil immersion field multiplied by 20 thousand.

- **Assessment of Platelet Count on haemocytometer**
- **Procedure-**

1. Well-mixed blood was diluted 1 : 100 in diluting fluid(ammonium oxalate), and the vial containing the suspension is rotated on a mechanical mixer for 10–15 minutes.
2. The hemocytometer was filled in the usual fashion, using a separate capillary tube for each side.
3. The chamber was covered with a Petri dish for 15 minutes to allow settling of the platelets in one optical plane. A piece of wet cotton or filter paper was left beneath the dish to prevent evaporation.
4. The platelets appeared round or oval and frequently have one or more dendritic processes. Their internal granular structure and a purple sheen allow the platelets to be distinguished from debris, which is often retractile. Ghosts of red cells that have been lysed by the ammonium oxalate are seen in the background.
5. Platelets were counted in 10 small squares five on each side of the chamber. If the total number of platelets counted was less than 100, more small squares were counted until at least 100 platelets had been recorded.

Platelet count (per μL) = (Number of cells counted/Number of squares counted) × Dilution x 250.

Statistical analysis: Data was recorded in recording format. The collected data was put in master chart group and tabulated according to age sex and various other criteria using excel sheet. Statistical software SPSS 2 and independent T tests were used to compare the variables between the groups. P value of <0.05 was considered significant

RESULT:

The present study titled" comparative analysis of two method of enumeration of platelet count in thrombocytopenic patient: manual and electrical impedance" was carried out in the Department of Pathology, has been described for its results as below-

A total of 100 samples in EDTA tubes, received in central laboratory during the study span who met the inclusion criteria were recruited. 49 were females and 51 were males. Mean age of the patients was 30. 2years.The samples of 100 patients were divided in to three groups on basis of degree of thrombocytopaenia i.e. mild (101-150 x 10^9 / L), moderate (51-100 x 10^9 /L) and severe (<50 x 10^9 /L) by automated analyser. Among total samples, 60 samples were grouped as mild thrombocytopenia (101-150 x 10^9 / L), 31 samples were grouped as moderate (51-100 x 10^9/L) and 9 samples were grouped as severe (<50 x 10^9/L). Table 1

TABLE 1:DISTRIBUTION OF SAMPLES ON BASIS OF DEGREE OF THROMBOCYTOPENIA

No. of samples (n=100)	Degree of thrombocytopaenia (10^9/L)
60	101-150 (mild)
31	51-100 (moderate)
9	<50 (severe)

The platelet count by automated counter showing falsely low platelet counts (n=10) were found to have scattered platelet clumps or giant platelets on peripheral smears.

The mean platelet count by automated analyser using electrical impedance and manual method using haemocytometer for mild, moderate and severe thrombocytopenia is depicted in Table 2.

TABLE 2:MEAN PLATLET COUNT BY METHOD OF ELECTRICAL IMPEDENCE AND HAEMOCYTOMETER

No. of samples (n=90)	Degree of thrombocytopenia (109/L)	Mean platelet count by electrical impedance (109/L)	Mean platelet count by manual method (109/L)
50	101-150 (mild)	120± 20	122± 21
31	51-100 (moderate)	75± 19	69± 10
9	<50 (severe)	35± 16	20± 12

The overall mean platelet count by the electrical impedance method and manual method was 76.6 ± 34.7 x 10^9/L and 70.3 ± 36.93 x 10^9/L respectively. The P value for automated method versus manual method was 0.44,suggesting that there was no significant variation in the platelet counts between the manual methods when compared with the electrical impedance method.

DISCUSSION:

In the present study, when comparing the mean platelets count by both methods to the age and gender of the studied patients, it was found that the estimated mean platelets count did not show significant difference between the two methods in males as well as in females. (7)

The overall mean platelet count by automated analyser using electrical impedance and manual counting did not show any significant difference statistically. The similar observations were also inferred by Sinha et al, Bakhubaira S, Al Hosni et al and Gao Y et al.(5,7,9,15) However, the present study observed that automated analyser using electrical impedance overestimated the platelet count in severe thrombocytopenic patient which may possibly affect the clinical decisions of platelet transfusions when they are indicated. Mohamed Rachid et al conducted a study which compared the mean platelet count by the optical (23 ± 13 × 10^9/l), impedance (37 ± 17 × 10^9/l) , immunological (21 ± 12 × 10^9/l) and manual method (22 ± 13 × 10^9/l) in blood samples with platelet count <50 x 10^9/l.(4) The study inferred that impedance method showed significantly elevated numbers of platelet count as compared to optical, immunological and manual methods. These findings were also similar to the studies by Sinha et al, Bakhubaira S, Anitha k et al and Olivera et al.(7,8,14) The automated analysers shown the rapid and reliable full blood counts based on their linearity limits but showed inaccurate and poorly reproducible at enumerating platelets in severe thrombocytopenia which may be attributed to secondary interference from cells or materials of a similar size to platelets or due to light scatter like in the case of fragmented RBCs, microcytic RBCs, lipemic samples, debris in patients taking cytotoxic drugs or in the presence of giant platelets or platelet clumps.The present study has shown that manual methods are reliable to validate the counts using automated analyser specially in blood samples of severe thrombocytopenia (<50,000 x10^9 / l). The

literature in the past has recommended manual platelet counting using haemocytometer as a reference method for estimating platelet count. Hanseler et al using the H1 counter, claimed that for counts of less than 30,000 platelets per μL, the automated counting should be replaced by the manual chamber procedure. (13)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. 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. But It is also essential that pathology laboratory personals and clinicians who rely on platelet counts for various scenarios in day to day practice understand the limitations of the instrumentation in use and the measurement uncertainty of automated platelet counters. (12)The accurate platelet counting still remains a challenge which subjects the interest in developing an improved reference procedure to enable optimization of automated platelet counting. Our study further emphasizes that there is a clear requirement to develop suitable external quality control material to improve the calibration of analysers for counting severely thrombocytopenic samples. In the absence of external calibration, the results of this study indicate that automated analyser, by tending to overestimate the platelet count, potentially result in the under-transfusion of platelets at the decision thresholds for prophylactic platelet transfusions

Limitation:

The present study as such did not come across significant limitation except for small sample size. Other aspects that could affect these methods like platelet volume, underlying disease, method used to prepare the blood film have also not been taken into consideration in our study. Larger sample studies with more stringent criteria are recommended to throw light on the same.

CONCLUSION :

The present study concluded estimation of platelet by automated analyser using electrical impedance and manual counting using haemocytometer as equivalent statically. The study infers the method of manual counting using haemocytometer as preferable method of choice in case samples with very low platelet count on automated analyser using electrical impedance.

Recommendation:

This study recommends that platelet count is not varied when done by manual method or automated analyser using electrical impedance method, but in cases of samples with severe thrombocytopenia on automated analyser, it should be accompanied by manual counting by haemocytometer.

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