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Comparative Analysis of Impedance and Optical Platelet Counting Techniques in Correlation with Peripheral Smear Examination in Thrombocytopenic Pregnant Women

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Abstract

Background: Thrombocytopenia affects 7–12% of pregnant women, which may increase the risk of maternal bleeding. Accurate platelet counting is crucial in obstetrics regarding drugs and transfusion decisions. Impedance and optical-based counting methods are used in automated haematology analyzers. However, their reliability can be affected by large and giant-sized platelets common in pregnancy. Platelet counting on Peripheral smear under light microscope is time-consuming, but it has remained the reference standard for confirming low counts.

Objectives: To assess the correlation between platelet counting by peripheral smear and both the impedance and optical-based methods in thrombocytopenic pregnant women.

Methodology: A prospective cross-sectional study was conducted at Haematology Lab, PIMS Hospital Islamabad on 176 thrombocytopenic pregnant women. Platelet count was measured by peripheral smear examination, impedance and optical methods on the same EDTA sample. Data was analyzed using SPSS v20 with Spearman correlation assessment between the peripheral smear method with both the automated methods. Correlation was also done based on Mean Platelet Volume (MPV) and the degree of thrombocytopenia.

Results: Platelet counting by Peripheral smear examination showed a very strong correlation with optical counts (r=0.96) as compared to impedance (r=0.72, p<0.001). In the cases with higher MPV>12fL, optical correlation was greater than impedance method (r=0.95 vs 0.59, p=0.017). Optical method had a much better correlation as compared to impedance in mild (r=0.91 vs 0.28) and moderate (r=0.75 vs 0.26) thrombocytopenia.

Conclusion: Impedance method is unreliable in thrombocytopenia cases with higher MPV. Optical method is more reliable in mild to moderate thrombocytopenia. In very low platelet count, both methods showed similar results.

Keywords: Platelet count, peripheral smear, impedance, optical, thrombocytopenia, Pregnancy.

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Introduction

Several physiological changesare induced by pregnancy, comprising of variations in hematological parameters. Remarkably, there are changes in platelet counts, often leading to decrease in count (platelet counts <150,000/μL) in some pregnant women. The occurrence is 7-12%. This condition can rise due to greater plasma volume than before, hemodilution, and changes in platelet production and consumption[1,2] Precise assessment of platelet levels is fundamental, as thrombocytopenia in pregnancy can result in noteworthy complications in both mother and fetus, including hemorrhagic happenings.

Platelet counting can be done using different methods: counting on peripheral smear, impedance-based methods, and optical techniques.[3,4] Peripheral blood smears are examined microscopically to detect and calculation of platelets directly. While this technique is laborious, it offers several benefits, for example the capacity to directly evaluate platelet count, their morphology as well as identify defects, which provides a more broad view of platelet strength.[3]

Impedance-based platelet counting operates using electrical opposition to check the size and number of platelets as they pass through a small hole. Although this method is generally fast, it has noteworthy limitations, mostly in pregnancy. Big or huge platelets, which are commonly observed during this period, may be misinterpreted as red blood cells (RBCs) by the machine because of their size, leading to falsely low platelet

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counts. Similarly, if red cells are fragmented, microcytic or lipid particles are present in the sample they may be calculated as platelets, thus giving ahigher platelet count. Optical methods involve considering light scattering to count and sizing platelets. This technique offers the benefit of providing comprehensive information about platelet properties, including size and presence of granules, which can help in the identification of various platelet problems. Optical methods are typically quicker than manual counting by microscopy, can handle greater sample volumes and remain unchanged by larger platelets, microcytes or fragmented red cells. [5,6]

Given these factors, comparing the efficiency of these methods versus platelet counting by peripheral smear in thrombocytopenic women who are pregnant is vital. Identifying which automatic technique associates best with peripheral smear examination will augment diagnostic accuracy and patient care.

This article aimed to explore these comparisons in-depth, shedding light on the most effective platelet counting methods for managing thrombocytopenia during pregnancy. The rationale of my study was to evaluate the accuracy of impedance and optical methods in order to determine which automated method (impedance or optical) provides best results that are more closely aligned with peripheral smear counts, particularly in the context of platelet size variations observed in pregnancy; to analyze how the presence of larger or giant platelets, commonly observed during pregnancy, affects the accuracy of impedance-based and optical platelet counting methods. Furthermore, it may help in providing evidence-based recommendations for healthcare providers regarding the most reliable platelet counting method for use in pregnant women with thrombocytopenia. This may allow them to plan the delivery, reserve or transfuse platelet concentrates as needed, and follow appropriate anesthesia protocols. The objective of our study was to compare and correlate automated Platelet Counting Methods (Impedance and optical) with the Peripheral smear method in thrombocytopenic pregnant women.

Methodology

It was a Prospective Cross sectional study conducted at the Pathology Department, Haematology section, Pakistan Institute of Medical Sciences (PIMS), Islamabad for a duration of three months from November 2024 to January 2025.

Sample size was calculated by WHO Calculator, as follows: Level of significance = 5%, Power of test = 80%, Population standard deviation =0.5; Test value of population: mean value of platelet count by automated method = 1.89, Test value of population: mean value of platelet count by manual method = 2.04 [8] Sample size came out to be 175. The sampling technique used was Consecutive Non-probability Sampling. Pregnant women in their third trimester of pregnancy, those having a platelet count of less than 150,000/µL as determined by preliminary testing, were included in the study. Patients having a history of hematological Disorders or chronic Medical illnesses (e.g., liver disease, chronic renal failure) were excluded from the study.

After approval from the Hospital Ethics Committee, the study was conducted on pregnant females fulfilling the exclusion and inclusion criteria. Freshly collected venous blood sample (approximately 2-3 ml) using standard venipuncture techniques in EDTA anticoagulant tubes, along with patient's demographic details was sent by the gynaecologists from obstetric clinics to the Pathology Department/hematology section for platelet count assessment. Informed consent was obtained from each participant before data collection begins. Initial assessments involved collecting demographic data, such as age, medical history and any previous platelet counts. For the microscopic platelet count, a peripheral blood smear was prepared from the collected blood sample within 3 hours of collection, stained with Giemsa stain, and examined under a light microscope. Platelets were counted in a defined number of fields (i.e. 10 fields on 100X objective) to calculate the platelet count per microliter of blood[3]. The same blood sample was processed using a calibrated hematology analyzer Mindray BC-6200 for impedance and optical based platelet counting, following the manufacturer's instructions for sample preparation and analysis. The impedance method detects changes in electrical resistance as platelets pass through an aperture, while the optical method measures light scattering as platelets pass through a laser beam. Both platelet count output from this analyzer were recorded. All results from the three methods (peripheral smear, impedance-based, and optical) were documented in a standardized data

collection form, ensuring that data collection is conducted in a blinded manner. MPV, PDW were also recorded. Pathologists performing the counts on Peripheral smear were unaware of the automated results to minimize bias. Quality control measures were implemented to ensure accuracy, including running controls with known platelet counts alongside study samples and regularly calibrating the automated analyzers according to manufacturer guidelines.

The data was entered and analyzed using SPSS version 20. Quantitative variables such as age of patient, Platelet count values obtained from each method were measured as Mean ± Standard Deviation. Both impedance based and optical methods were compared with the platelet count obtained on peripheral smear. Data was analyzed with Spearman correlation assessment between the peripheral smear method with both the automated methods. Correlation was also done based on Mean Platelet Volume (MPV) and the degree of thrombocytopenia. A p value less than 0.05 was considered significant.

Results

Out of 176 subjects, the mean age of patients was 28.6 ± 5.67 years (age range=15-45 years). The data was non-parametric, so spearman correlation was used to find out correlation between the peripheral smear method vs impedance method and peripheral smear method vs the optical method of platelet counting. The correlation coefficient (r) was calculated and evaluated as follows: r>9 very strong correlation, r=0.7-0.9: strong correlation, r=0.5-0.7: moderate correlation, r=0.3-0.5: weak correlation and r<0.3: very weak correlation. The correlation between the two methods with manual method is shown in table I.

Correlation was then calculated in the similar manner based on the degree of thrombocytopenia. Thrombocytopenia was classified on the basis of platelet count as mild (100-149 x 10^3/ul), moderate (50-99 x 10^3/ul), Severe (21-49 x 10^3/ul) and very severe (≤20). Number of cases in these classes was 80,65,22 and 9 respectively. Based on the degree of thrombocytopenia, the correlation was calculated as depicted Table II.

Correlation was also done based on the Mean platelet volume (MPV). On the basis of MPV, two groups were made group 1 MPV<12 fl, and group 2 MPV>12 fl. Table III, figure 1 represent this correlation, as follows:

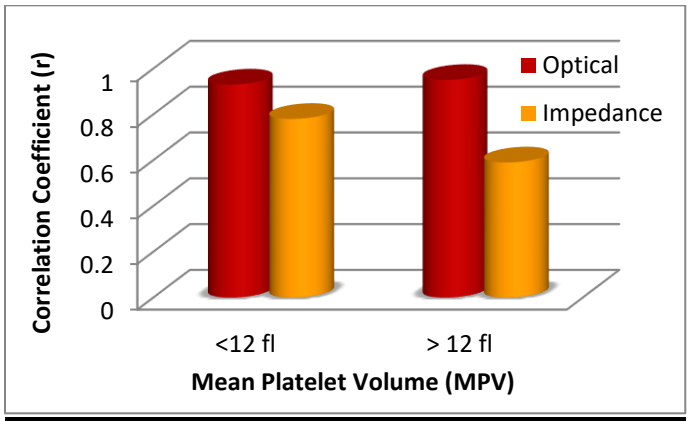

Figure 1. Effect of MPV on method correlation.

Table I: Correlation of Peripheral Smear Method with both Impedance and Optical Methods.

Correlation parameters	Correlation coefficient (r)	Strength of correlation	p-value	Fisher's r-to-z transformation
Peripheral Smear & Optical	0.962	Very strong	<0.001	9.90
Peripheral Smear & Impedance	0.72	Strong		

Table II: Correlation of Peripheral Smear Method with both Impedance and Optical Methods based on degree of thrombocytopenia.

Degree of thrombocytopenia	Correlation coefficient (r) between Peripheral Smear method and optical based method	Correlation coefficient (r) between Peripheral Smear method and impedance based method	Fisher's r-to-z transformation
Mild	0.91	0.28	7.69
Moderate	0.75	0.26	3.93
Severe	0.81	0.52	1.69
Very severe	0.84	0.79	0.25

Table III: Correlation of Peripheral Smear Method with both Impedance and Optical Methods based on MPV Groups

MPV Groups	Correlation coefficient (r) between Peripheral Smear method and optical based method	Correlation coefficient (r) between Peripheral Smear method and impedance based method	P value
MPV <12 fl	0.94	0.78	0.017
MPV >12 fl	0.95	0.59	

Discussion

Platelet count assessment is important clinically especially in pregnant females as it will determine treatment strategies in them. Peripheral Smear platelet count remains gold standard for accurately diagnosing thrombocytopenia. Many recent studies have compared Peripheral Smear platelet count with automated platelet count in thrombocytopenic pregnant females. Study done by Zakar et al compared Peripheral Smear platelet count with automated platelet count in thrombocytopenic pregnant females to assess eligibility for neuraxial anaesthesia. They observed that Peripheral Smear platelet count should be a preferred test as it helped more pregnant females to use the neuraxial block during labour.[7] Chaturvedi V et al also observed that Peripheral Smear platelet count is simple, reliable and cost-effective method especially in pregnant females.[8]

Study done by Balakrishnan A et al also concluded that Peripheral Smear platelet count should be the gold standard for platelet count estimation.[9] Pathak et al compared platelet estimation by neubauer, peripheral smear and automated method. They observed no statistically significant difference among these methods for platelet estimation. However, they observed that in thrombocytopenic patients automated method may underreport the platelet count and neubauer and Peripheral Smear platelet count should be used for confirmation of actual platelet count in thrombocytopenic patients.[10] However few studies have shown that both automated and manual method for platelet estimation are equivalent.[11,12]

The current study compared Peripheral Smear platelet counts with automated impedance and optical methods in pregnant women with low platelet counts. It also observed that how large platelets in pregnancy affect the accuracy of automated machines. The aim was to help doctors choose the most reliable method for safe delivery, transfusion planning, and anaesthesia care. In current study Peripheral Smear method showed a very strong correlation with optical counts ($r=0.96$) as compared to impedance ($r=0.72$, $p<0.001$). Optical method had a much better correlation as compared to impedance in mild ($r=0.91$ vs 0.28) and moderate ($r=0.75$ vs 0.26) thrombocytopenia. In the cases with higher MPV>12fL, optical correlation was greater than impedance method ($r=0.95$ vs 0.59, $p=0.017$). Study done by Pedra et al observed that optical method showed higher precision than impedance method in thrombocytopenic cases.[13] Another study compared optical, impedance and fluorescence methods for platelet estimation. It was observed that optical method was more reliable and this method showed minimal bias as compared to other two methods in that study.[14]

Study done by Ayed B et al estimated platelet count in microcytic anemia comparing both impedance and optical method. It was observed that impedance method overestimated the platelet counts at low mean corpuscular volume. They suggested that in situations of microcytosis optical method should be used so that severe thrombocytopenia is not missed.[15] As impedance method estimates the platelet count by the size as the cells pass through the aperture of instrument, and it may miscalculate small red cells or platelet clumps. Thus, thrombocytopenia may be missed by this method.[16]

The study done by Bhagwat et al tested whether optical platelet counting and digital slide review give correct platelet numbers in difficult cases where routine machines may be confused by other cells and the analyzer shows red flag for platelet analysis. The study showed that optical platelet counting was more reliable and accurate than impedance counting in thrombocytopenia with platelet interference. As MPV increases, the difference between impedance and optical counts becomes more significant.[17]

Similarly, other studies also showed that impedance method gives falsely high platelet count due to interference with red cell fragments or lipid particles.[18,19] Study done by X Hu et al also compared accuracy of both methods in platelet estimation in cases of interference of small red blood cells or platelet clumps. Their study showed that optical method of platelet counting gives better correlation to microscopy than impedance method in such situations. It was also observed that optical method has good repeatability and has better anti-interference ability.[20]

Study done by MK So observed that optical method of platelet counting gave results closer to the true platelet count in samples with platelet clumping due to EDTA.[21] Similar results were obtained by Y Bao in EDTA induced pseudothrombocytoenia.[6] A study by Wang Z et al compared optical and impedance platelet counting methods in immune-related thrombocytopenia. They

found that optical platelet counts were more accurate than impedance counts. When mean platelet volume (MPV) was below 13 fL, both methods gave similar results, however, when MPV was 13 fL or higher, platelet counts measured by the impedance method were lower. These findings are in line with the present study, which also showed better correlation with optical platelet counts at higher MPV values.[22]

Conclusion

The Peripheral Smear method closely matched the optical platelet counts, suggesting that optical counting gives results more reliable and consistent with Peripheral Smear estimation than the impedance method. Impedance method is unreliable in thrombocytopenia cases with higher MPV. Optical method is more reliable in mild to moderate thrombocytopenia. In very low platelet count, both methods showed similar results.

Therefore, it must be concluded from our study that in thrombocytopenic pregnant women, optical based platelet counting is more reliable overall as compared to impedance method, and must be specially requested so that results can be provided timely and reliably so that appropriate interventions can be undertaken if required.

LIMITATIONS:

- The study was conducted on a limited sample size, which may affect the generalizability of the findings.
- Peripheral Smear platelet counting is subjective and operator-dependent, leading to potential inter-observer variability.

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