



COMPARISON OF PLATELET COUNTS SHOWN BY AUTOMATIC HAEMATOLOGY ANALYSER AND COUNTS MEASURED MANUALLY IN POPULATION OF MOUNTAINOUS HILLY REGION OF CHENAB VALLEY ATTENDING OPD ON ROUTINE BASIS IN GMC DODA ASSOCIATED HOSPITAL

Sumat-ul –Khurshid¹, Nazia Tabassum^{2*}

¹Assistant Professor Department of Pathology GMC Doda J&K

²Registrar Department of Pathology GMC Doda J&K

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Corresponding author: Dr. Nazia Tabassum Sheikh

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Abstract

In clinical practice it is very important to perform the platelet counts. The estimation of platelet counts from peripheral blood smears is an accurate method and provides adequate quality assurance in traditional methods as the automated cell counters are not available at all hospital setups especially in rural areas. In modern era use of automated analyzers based on impedance technology has resulted in improvement of accuracy and helps in measurement of platelet indices such as Mean Platelet Volume (MPV), Plateletcrit (PCT) and Platelet Distribution Width (PDW). However, both the methods have certain limitations.

Platelet counts were estimated on a 5-part Differential Automated Hematology Analyzer Mindray Model (BC-5130) and manually on Leishman stained peripheral blood Smear.

Aims & Objectives: 1. This study was conducted to compare platelet counts by peripheral blood smear method and automated method in patients attending OPD on routine basis in a mountainous hilly region of J&k .

2. It also aims to study the relation, if any, between the platelet counts (automated) and platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) and to assess the possible role of these parameters in certain defined situations.

Materials and methods: A prospective study was conducted in a tertiary care hospital on 300 Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples. Samples were evaluated by 5-Part differential automated hematology analyser using impedance counting method and by examination of peripheral blood smear method (PBF).

Results: In thrombocytopenic patients, the platelet counts assessed by automated analyzer revealed an inverse relation with Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) and showed large granular and giant platelets on PBS.

Conclusion: Automated hematology analyzer is essential for immediate and accurate complete blood count evaluation but blood samples showing erroneous results or low platelet counts on analyzer should be confirmed on peripheral blood smear. The platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) can point to the underlying pathology especially in cases of thrombocytopenia.

Keywords: Automated analyser, Peripheral blood smear, MPV, PDW, Large granular platelets.

Introduction

It has been proved that Platelets are more difficult to count than red blood cells or white cells.¹ The normal range of platelet count in a healthy individual ranges from 1.5 to 4.5 lakhs/mm³.² When the platelet counts are more than 20,000/mm³, clinical symptoms are mild, e.g. easy bruising. With platelet count less than 10,000/mm³, the risk of life-threatening hemorrhage like intracranial or GIT bleeding increases significantly.³ The Clinical bleed may result from either numerical deficiency due defect at production level, or increased degradation at peripheral level or platelet function defect.

The common methods of platelet estimation are:

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

Manual platelet counting in the Neubauer chamber, by means of a phase-contrast microscope⁴ has been recommended as the reference method for assessing the platelet number by the International Committee for Standardization in Hematology (ICSH 1984)⁵ However, it is a time consuming method which usually results in high levels of variability.

The traditional method of estimating platelet counts from peripheral blood smears is a fairly accurate method and provides adequate quality assurance. The average numbers of platelets counted per oil immersion field are

multiplied by 15,000 to yield a platelet count estimate per μl .⁶

As Automation has afforded high precision and accuracy for platelet counting in normal individuals.⁷ But automated counting is still controversial in thrombocytopenic or other patients in which other small particles could generate electrical or optical signals that resemble platelets, such as debris and red cell fragments.⁸ Most counters use the principle of electrical impedance or optical signals for counting the platelets using the particle size for counting them.⁹ On the other hand, the presence of large platelets beyond the normal size range can lead to underestimation of the platelet counts.¹⁰ The use of multiple light scatter parameters rather than impedance alone has improved the ability to discriminate platelets.¹¹ Platelet count related indices are being estimated in addition to routine parameters.

The most significant among these are Plateletcrit (PCT), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) as together they measure the functional integrity of platelets. Platelet activation, which is seen as platelet swelling and shape change, is ultimately reflected as an increase in MPV and PDW.¹² Mean platelet volume (MPV) has been reported to be useful in differentiating thrombocytopenia due to peripheral platelet destruction from that resulting from reduced platelet synthesis. This measurement may also be used to evaluate bone marrow suppression and recovery in patients on chemotherapeutic regimens.¹³ In thrombocytopenic patients, especially those with hematological neoplasms, ITP, febrile neutropenia and acute febrile illnesses (dengue, malaria, etc.) increasing the accuracy of reporting platelet counts would definitely augment clinical decision making.

The Present study was conducted to compare platelet counts by peripheral blood smear method and automated method and to find relation between the platelet count (automated) and platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) and to assess the possible role of these parameters in certain defined situations in patients attending OPD on routine basis in a mountainous hilly region of J&K India in GMC DODA.

MATERIALS AND METHODS:

This is an observational prospective study conducted in the department of Pathology in hilly mountainous region of J &K India in GMC Doda over a period of 2 months.

Inclusion criteria:

1. Only those patients were included in whom both CBC and PBF were done simultaneously.
2. Those patients where platelet counts were done as a part of PBF where CLINICIANS were in doubt of accuracy of platelet counts by hematology analyser.

3. Patients with platelet counts less than $1\text{lakh}/\text{mm}^3$ on CBC

Exclusion criteria:

1. All those patients were excluded from the study where CBC and PBF were not done simultaneously.
2. All those patients where on CBC platelet counts were more than $1\text{lakh}/\text{mm}^3$ but we included those cases where clinicians ordered a manual platelet counts without assessing CBC FIRST.
3. Hemolysed blood samples, and clotted samples in case when patient was not willing to give repeat sample.

Study Period:

It was a prospective study over a period 2 months from 12th October 2019 to 12th December 2019.

Study population:

This study included 300 cases of CBC and PBF samples.

All the OPD samples coming from various clinical departments to the Pathology Department for CBC were followed. The EDTA anticoagulated venous blood samples (1.5mg of the anhydrous salt per ml of blood) of patients received in the laboratory were evaluated by 2 techniques for platelet estimation:-

1. Automated method by Trained Senior hematology Technician on 5-Part differential analyser (Mindray Model BC-5130)
2. Manually by making thin Peripheral blood smear, stained with Leishman stain and analysed by Pathologists.

Automated Method for the Assessment of Platelet Counts :

Each sample of blood was thoroughly mixed on an automated mixer and a complete blood counts including the platelet count was obtained using 5-part Differential Auto Hematology Analyzer Mindray (BC-5130). Platelets were counted & sized in automated counter by electrical impedance method (also known as Coulter Method).¹⁴

Assessment of Platelet Count on Leishman Stained Peripheral Blood Smear:

Blood smears were made from the venous blood samples collected in EDTA containing purple topped vacutainer tubes and stained with Leishman's stain following standard protocol.¹⁵

The number of platelets, on an average, per oil immersion field in a count of successive ten oil immersion fields was calculated and multiplied by 15,000 for rough calculation of platelet counts. It has been proved to be a reliable method for the estimation of platelets and was used to yield a platelet count estimate in lakhs/ mm^3 .¹⁶

Normal Platelet count ranges from $150 \times 10^9/\text{L}$ to $450 \times 10^9/\text{L}$.¹⁷ A platelet count less than $150 \times 10^9/\text{L}$ was defined as thrombocytopenia while a count more than $450 \times 10^9/\text{L}$ was defined as thrombocytosis. Thrombocytopenia was further subdivided into mild (platelet count $100 \times 10^9/\text{L}$ to $<150 \times 10^9/\text{L}$), moderate (platelet count $50 \times 10^9/\text{L}$ to $<100 \times 10^9/\text{L}$) and severe (platelet count $<50 \times 10^9/\text{L}$).¹⁸ The Mean Platelet Volume

(MPV) and the Platelet Distribution Width (PDW) were also measured and the set ranges of these parameters were given by the auto analyzer Mindray (BC-5130). MPV was defined as the measurement of the average size of platelets in blood as calculated by the machine while the PDW was used as a measure of platelet anisocytosis.¹⁹

RESULTS

This prospective study of 2 months duration included total 300 cases; Out of which majority of patients were in the age group of 21-30 years i.e 116 cases(38.66%) followed by 31-40 years i.e 41cases(13.66%).(Table-1).Males constituted 151 cases and females constituted 149 cases. A nearly equal distribution of male and female patients was observed in this prospective observational study with male to female ratio of 1.01:1 (Table -2)

On Automated CBC Analyser 5-part differential Mindray (BC-5130) platelet counts of 300 patients were done and it was found that:

1. <50000 platelets/mm³ were found in 61(20.33%) cases (severe Thrombocytopenia)
2. 50000-1lakhs/mm³ were found in 176(58.66%) cases(Moderate Thrombocytopenia)
3. 1Lakh-1.5lakhs /mm³ were found in 39(13%) cases(Mild Thrombocytopenia)
4. 1.5-4.5 lakhs/mm³ were found in 22(7.33%) cases(Normal Platelet counts)
5. >4.5 lakhs/mm³ were found in 2 (0.66%) cases (Thrombocytosis) (Table-3)

On Peripheral Blood Film (PBF) method it was found that:

1. <50000 platelets/mm³ were found in 7(2.335%) cases (severe Thrombocytopenia)
2. 50000-1lakhs/mm³ were found in 18(6%) cases(Moderate Thrombocytopenia)
3. 1Lakh-1.5lakhs /mm³ were found in 20(6.66%) cases(Mild Thrombocytopenia)
4. 1.5-4.5 lakhs/mm³ were found in 253(84.33%) cases(Normal Platelet counts)
5. >4.5 lakhs/mm³ were found in 29(0.66%) cases (Thrombocytosis) (Table 4)

Table 1: AGE WISE DISTRIBUTION OF PATIENTS IN YEARS(n=300)

AGE IN YEARS	NUMBER OF CASES	Percentage
0-10	20	6.66%
11-20	40	13.33%
21-30	116	38.66%
31-40	41	13.66%
41-50	30	10%
51-60	15	5%
61-70	20	6.66%
71-80	13	4.33%
81-90	5	1.66%
TOTAL	300	100%

Table 2: SEX WISE DISTRIBUTION OF CASES(n=300)

Males	Percentage	Females	Percentage	Total	Percentage
151	50.33%	149	49.66%	300	100%

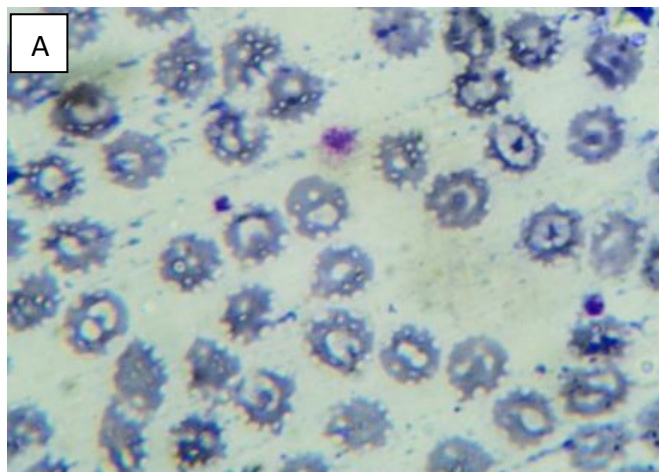
Table 3: PLATELET COUNTS ASSESSED BY 5-PART DIFFERENTIAL MINDRAY ANALYZER (BC-5130)(n= 300)

Platelet counts	Number of cases	Percentage
Severe Thrombocytopenia (<50000)	61	20.33
Moderate Thrombocytopenia 50000-1lakh	176	58.66%
Mild Thrombocytopenia 1lakh-1.5 lakhs	39	13%
Normal Platelet counts 1.5 lakh-4.5lakhs	22	7.33%
Thrombocytosis >4.5 lakhs	2	0.66%
Total	300	100%

Table 4: PLATELET COUNTS ASSESSED BY PBF IN PATIENTS(n=300)

Platelet counts	Number of cases	Percentage
Severe Thrombocytopenia (<50000)	7	2.335%
Moderate Thrombocytopenia 50000-1lakh	18	6%
Mild Thrombocytopenia 1lakh-1.5 lakhs	20	6.66%
Normal Platelet counts 1.5 lakh-4.5lakhs	253	84.33%
Thrombocytosis >4.5 lakhs	2	0.66%
Total	300	100%

IMAGES:



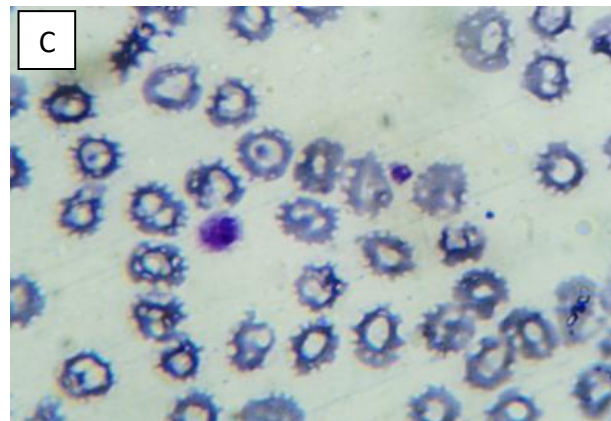
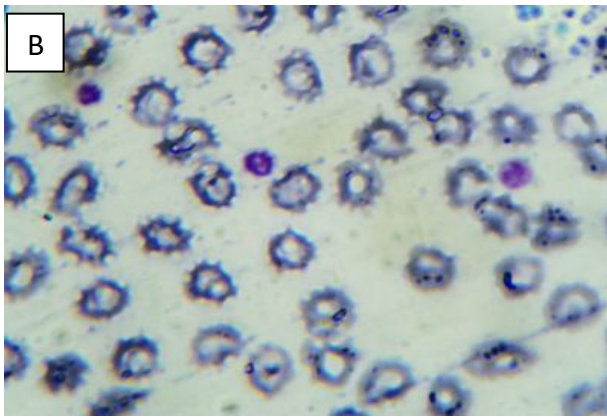


Figure 1(A,B,C): Photomicrograph showing large granular swollen platelets against RBC background.

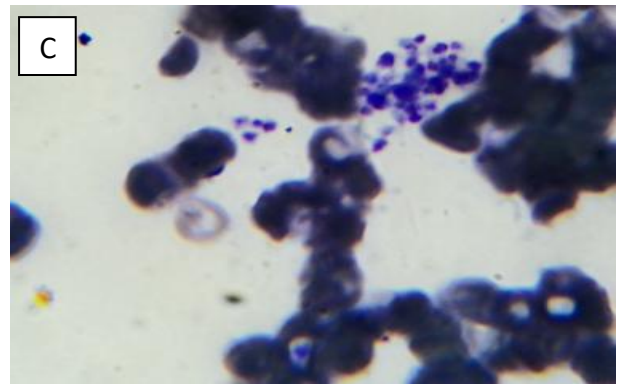
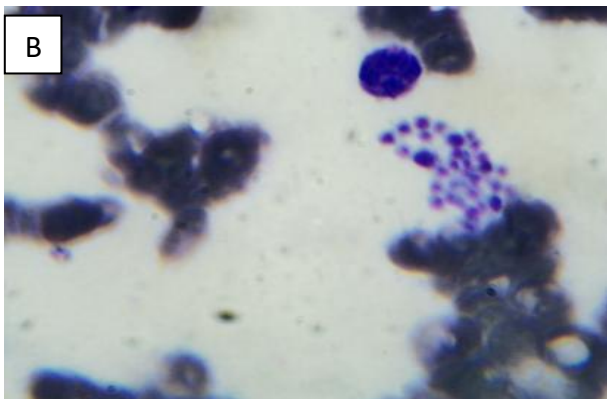
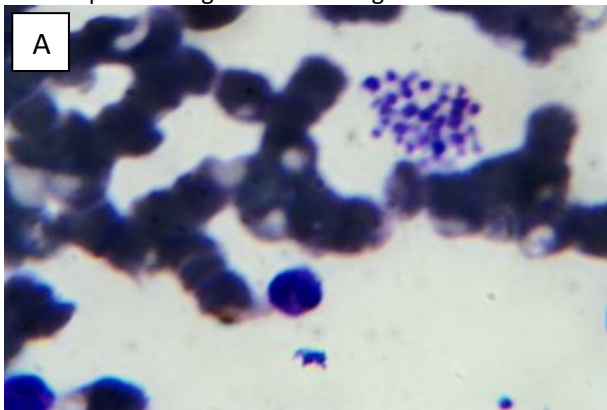


Figure 2 (A,B,C): Photomicrograph showing large clumps of platelets on PBF(100x/oil immersion)

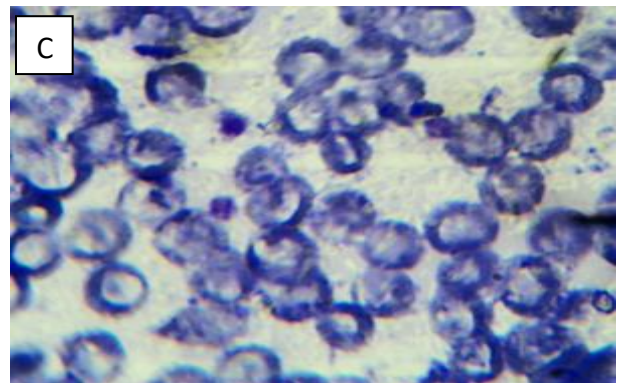
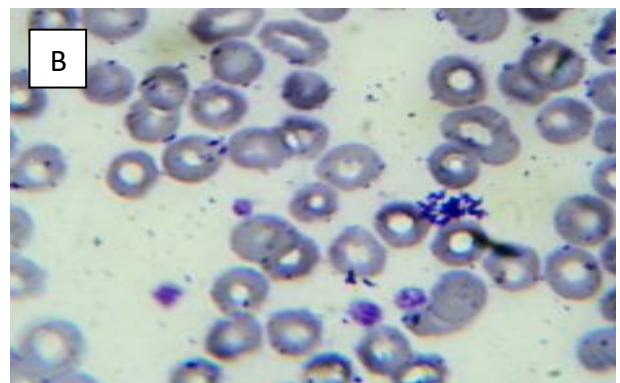
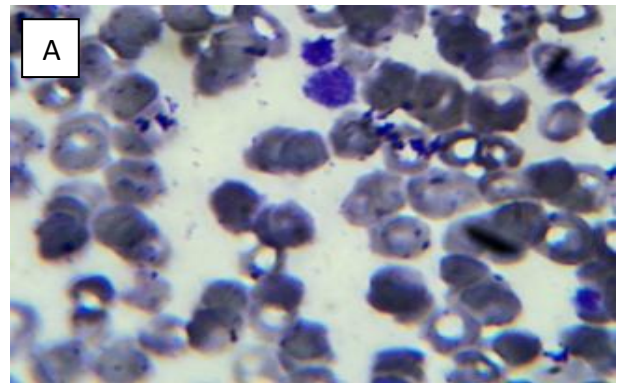


Figure 3(A,B,C): Photomicrograph showing giant platelets and macro-ovalocytic blood picture on PBF(100X/Oil immersion)

DISCUSSION:

This prospective study done over a period of 2 months duration from 12th October to 12th December 2019 included 300 cases of CBC and PBF.

On Automated CBC Analyser 5-part differential Mindray (BC-5130) platelet counts of 300 patients were done and it was found that:

1. <50000 platelets/mm³ were found in 61(20.33%) cases (severe Thrombocytopenia)

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3. 1Lakh-1.5lakhs /mm³ were found in 39(13%) cases (Mild Thrombocytopenia)

4. 1.5-4.5 lakhs/mm³ were found in 22(7.33%) cases (Normal Platelet counts)

5. >4.5 lakhs/mm³ were found in 2(0.66%) cases (Thrombocytosis) (Table-3)

On Peripheral Blood Film (PBF) method and we found that:

1. <50000 platelets/mm³ were found in 7(2.335%) cases (severe Thrombocytopenia)

2. 50000-1lakhs/mm³ were found in 18(6%) cases (Moderate Thrombocytopenia)

3. 1Lakh-1.5lakhs /mm³ were found in 20(6.66%) cases (Mild Thrombocytopenia)

4. 1.5-4.5 lakhs/mm³ were found in 253(84.335%) cases (Normal Platelet counts)

5. >4.5lakhs/mm³ were found in 2(0.66%) cases (Thrombocytosis) (Table 4)

On CBC analyser majority of the patients showed platelet counts in the range of 50000-1lakhs ie 176(58.66%) cases and on PBF majority of the patients showed platelet counts in the range of 1.5-4.5lakhs i.e. 253(84.33%) cases that too on lower side i.e platelet counts of 1.1lakhs to 2lakhs were found in 212(70.66%) cases. This showed that in this mountainous hilly region of J&k majority of the population has platelet counts in low range. This could be due to:

1. Genetic reasons
2. Megaloblastic and Dual Deficiency Anemia as a part of Pancytopenia in Vitamin B-12 deficiency patients in major population of Chenab valley. This could be due to differences in the dietary habits of the people of this belt because :

- Majority of the people of this belt are below poverty line
- Majority of the people of this belt are pure vegetarians (Vegans)

3. All most all of our blood samples were collected in EDTA tubes which is known to consume platelets if the blood sample is not processed within 2 hours and hence platelet

count on CBC are less. In addition EDTA inhibits platelet staining Of PBF smears leading to further decrease in counts.

On CBC Analyser Platelet counts of 50000-1 lakhs were found in 176(58.66%) cases and on PBF platelet count of 50000-1lakhs were found in 18(6%) cases. This DIFFERENCE could be due to:

1. Many large granular swollen (activated platelets) (Fig -1) found in this belt of Mountainous hilly region of J&K. which could not be interpreted by CBC Analyser as platelets,
2. Large clumps of platelets as seen on Peripheral blood film. (Fig- 2) and platelet Sattelitism
3. Few giant platelets of the size of RBC in population of Chenab valley which could not be interpreted by CBC Analyser as platelets. (Fig -3)

On CBC analyser <50000 platelets were found in 61 patients and on PBF <50000 platelets were found in 7 patients. Platelet count of less than <50000 in 7 patients on both CBC Analyser and PBF was because of medical condition in these patients; two of them were suffering from dengue fever, two from malaria and rest of the 3 patients were referred to a tertiary care centre in GMC Jammu and were lost to our follow up so the cause of thrombocytopenia in these patients could not be made out

Conclusion:

Thus, it can be concluded that a better quality hematology analyzer is crucial for quick and accurate complete blood counts. On the other hand all those blood samples which show abnormal results or low platelet counts on analyzers should be confirmed by manual count on peripheral blood smear. The platelet indices such as Mean Platelet Volume (MPV), Plateletcrit and Platelet Distribution Width (PDW) are the additional features provided by the analyzers that can indicate the pathogenesis of altered platelet count especially in cases of thrombocytopenia. Thus, the present study is an attempt, on how to correct ourselves and the machine so that it is beneficial for patient care. It also teaches us the unnecessary panic which is created by the clinicians on reading just platelet count on a CBC report is not justified. Clinicians should examine all the parameters including Platelet indices (MPV, PDW and Plateletcrit) to arrive at Conclusion.

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