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EVALUATING THE ROLE OF PERIPHERAL BLOOD SMEAR IN IDENTIFYING PLATELET ABNORMALITIES AND THEIR DIAGNOSTIC VALUE

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Abstract

Platelet diseases are both quantitative, e.g. thrombocytopenia and thrombocytosis, and qualitative, i.e. platelet morphology and dysfunction. Despite the fact that automated hematology analysers give quick platelet counts, they do not deal with morphological abnormalities as well; hence, the purpose of this study was to understand the importance of the Peripheral Blood Smear (PBS) in detecting platelet abnormalities and its diagnostic value. Cross-sectional study was done in a hospital setting and 200 patients with suspected platelet disorders were studied, platelet counts were determined through an automated analyzer and PBS preparation, staining on Leishman stains, and microscopic analysis. The sample size was 58% males and 42% females with an average age of 43.6 years with bleeding being the most frequent clinical manifestation (44%). Abnormal platelet counts were identified by automated analysis of 64% of cases with PBS showing morphological abnormalities including giant platelets (24%), hypogranular platelets (17%), clumping (13%), fragmentation (9%), microplatelets (6%), satellitism (5%), and mixed morphology (14%). It was found that platelets had morphological abnormalities PBS showed good diagnostic results giving a sensitivity and specificity of 95.5 and 98.0 respectively and an overall accuracy of 96.8. The researchers find that PBS is a critical and dependable platelet abnormality detection tool and a method to confirm automated findings, and its integration with automated analysis improves diagnostic accuracy and clinical decision-making.

Keywords- *Platelet disorders, Peripheral blood smear, Thrombocytopenia, Platelet morphology, Automated analyser, MPV*

1. INTRODUCTION

Platelets are small disc-shaped, anucleated fragments of megakaryocytes found in the bone marrow and circulate in the blood about 7-10 days. They are important in ensuring hemostasis by sticking on vascular injury sites, pooling together to create a primary hemostatic plug, and eliminating excessive blood loss. Besides coagulation platelets also play an active role in

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inflammatory responses, immune regulation and tissue repair thus making their presence significant in the overall vascular and systemic homeostasis.

Platelet abnormalities are clinically important, and can be categorized broadly as quantitative and qualitative disorders. Quantitative abnormalities entail an alteration in the number of platelets, such as thrombocytopenia and thrombocytosis. Thrombocytopenia leads to bleeding that can be petechiae, ecchymosis, mucosal bleeding, or severe bleeding, and can be caused by bone marrow suppression, autoimmune destruction, infections or medications. Thrombocytosis, on the other hand, is an increase in the number of platelets and can lead to thrombotic complications, and can be either a primary hematological condition or secondary to inflammation, infection, or malignancy.

Qualitative platelet abnormalities entail platelet morphological and functional defects, such as changes in size, shape, granularity, and distribution. Some of the common morphological abnormalities are giant platelets, hypogranular platelets, platelet clumping, platelet fragmentation, and platelet satellitism. These abnormalities can be related to underlying conditions of inherited platelet disorders, myeloproliferative diseases, myelodysplastic syndromes, and thrombotic microangiopathies. Notably, even these qualitative alterations may have a large impact on platelet activities and result in clinical manifestations despite the platelet counts seeming normal.

Despite the common use of automated hematology analyzers in the rapid and standard platelet counting, they have some limitations. These systems are unable to identify subtle morphological abnormalities and may misinterpret platelet clumps, platelet fragments or exceptionally big platelets leading to inaccurate or misleading results. In the case of platelet clumping, an illusion of pseudothrombocytopenia may be created, whereas giant platelets can be mistakenly counted as red blood cells.

PBS examination is a valuable complementary diagnostic instrument because it enables visualization of platelet morphology with a microscope. It allows a close examination of the size, shape, granularity and distribution of platelets and is especially beneficial in detecting abnormalities which are frequently not detected by automated methods. PBS is very important in the diagnosis of conditions like pseudothrombocytopenia, myeloproliferative disorders, and thrombotic microangiopathies as well as in attempts to match laboratory results with clinical characteristics. Thus, regardless of the development of automated technologies, PBS is still necessary to provide the correct assessment and diagnosis of platelet disorders.

1.1.Objectives of the study

- To assess platelet morphology on PBS
- To identify platelet abnormalities

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- To evaluate diagnostic accuracy of PBS compared to automated methods

2. LITERATURE REVIEW

Liu et al. (2019) explored the use of peripheral smear analysis in patients with myelodysplastic syndromes and found that PBS was very useful in detecting dysplastic platelet morphology such as giant platelets and hypogranular platelets. Their results highlighted that these morphological marks play a significant role in the early diagnosis because these abnormalities usually go unidentified by automated analyzers. The paper emphasized the value of PBS as a leading diagnostic technique to identify faint but clinically meaningful shifts in platelet morphology.

Zhao et al. (2019) paid attention to the detection of platelet satellitism, which is a rare condition when platelets stick to neutrophils and falsely low platelet counts in automated systems are obtained. The experiment revealed that PBS is an important factor in distinguishing between true thrombocytopenia and pseudothrombocytopenia through direct observation of platelet leukocyte interactions. This underscores the relevance of PBS in avoiding diagnostic errors and the needless clinical interventions.

Mehta and Patel (2019) assessed the diagnostic usefulness of PBS in qualitative platelet disorders, and morphological abnormality (giant platelets, hypogranularity, and abnormal distribution) were well identified by smear examination. Automated analyzers on the contrary, mainly delivered quantitative information and could not detect these qualitative flaws. The authors concluded that PBS is inseparable in the diagnosis of functional platelet disorders, particularly in patients who present with bleeding symptoms in spite of normal platelet counts.

Johnson et al. (2020) evaluated the clinical use of PBS on platelet abnormalities that automated analyzers could not identify. Their analysis showed that in a large percentage of cases where automated results were normal or inconclusive, they found that there were significant morphological abnormalities. These results revealed that the use of a single tool of automated systems can result in underdiagnosis or misdiagnosis of platelet disorders. The authors highly advocated the application of PBS as a diagnostic aid to improve accuracy and help in proper clinical management.

3. MATERIALS AND METHODS

The methodology was constructed to provide accurate comparison of automated platelet analysis and morphological results that were obtained using PBS hence allowing a thorough examination of quantitative and qualitative platelet abnormalities.

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3.1.Study Design

The research was done as a cross-sectional observational study which was in a hospital. The design enabled the evaluation of the platelet defects at one point in time in patients who presented with suspected hematological conditions. It was also able to compare the results of automated hematology analyzers with those of PBS in normal clinical conditions.

3.2.Study Population and Sample Size

The sample size of the study comprised 200 patients who were referred to have Complete Blood Count (CBC) testing. The sample included suspected platelet abnormality individuals and both normal and platelet abnormal profiles were included. Both males and females and patients of all ages were enrolled to ensure diversity and representativeness of the study sample.

3.3.Inclusion Criteria

The following participants were included in the study:

- Patients with suspected platelet abnormalities
- Patients undergoing CBC testing
- Individuals of all age groups and both genders

3.4.Exclusion Criteria

The study excluded:

- Hemolyzed or inadequate blood samples
- Patients receiving platelet transfusion therapy
- Post-operative patients

3.5.Sample Collection

Standard aseptic venipuncture procedures were used to collect blood samples. Each participant had approximately 2 mL of venous blood that was taken and put in EDTA anticoagulated vials to avoid the formation of clots. The samples were well vortexed to guarantee effective anticoagulation and maintenance of cellular components.

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- **Automated Platelet Analysis**

An automated hematology analyzer was used to measure the platelet counts and provided standardized and rapid quantitative measurements. These findings were used as a reference point to compare them with PBS findings.

- **Peripheral Blood Smear Preparation**

The standard wedge technique was used to prepare peripheral blood smears. One drop of blood was added to one of the ends of a clean glass slide, and a spreader slide was applied to spread out the blood so as to give a thin smear. The smears were left to dry at room temperature.

- **Staining Procedure**

The smears that were dried by air were stained with Leishman stain and Giemsa stain. The staining method comprised of flooding the slide with the stain, and then washing it with the buffer solution (pH 6.8). The morphology of the platelets and other cellular elements were well visualized by proper staining.

- **Microscopic Examination**

The stained smears were viewed under a light microscope with oil immersion (100× objective) and paraffin oil. To make a proper analysis of platelet properties, several areas of the microscope were carefully monitored.

- **Parameters Evaluated**

Platelet morphology was assessed based on the following parameters:

- **Size:** Normal, large, and giant platelets
- **Shape:** Structural variations in platelet form
- **Granularity:** Normal or hypogranular platelets
- **Distribution and Clumping:** Presence of platelet aggregates or uneven distribution

The findings obtained from PBS were compared with automated platelet counts to identify discrepancies and evaluate the diagnostic performance of PBS.

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4. RESULTS AND DISCUSSION

The following section reports the results of the study based on the analysis of 200 samples of patients analyzed with the help of automated hematology analysers and Peripheral Blood Smear (PBS). The findings are systematized as demographic data, clinical presentation, platelet count distribution, morphological observations, diagnostic ability of PBS and association with Mean Platelet Volume (MPV).

4.1. Demographic Characteristics of the Study Population

The distribution of platelet abnormalities and their clinical relevance can only be interpreted in terms of the demographic profile of the study population. The age, gender and place of residence may influence the occurrence, presentation and detection of haematological disorders. Thus, the study of these baseline characteristics gives valuable background to the study results.

Table 1: Demographic Distribution of the Study Population (N = 200)

Parameter	Category	Frequency (n)	Percentage (%)
Age (years)	12–25	34	17%
	26–40	66	33%
	41–60	70	35%
	>60	30	15%
Sex	Male	116	58%
	Female	84	42%
Residence	Urban	128	64%
	Rural	72	36%

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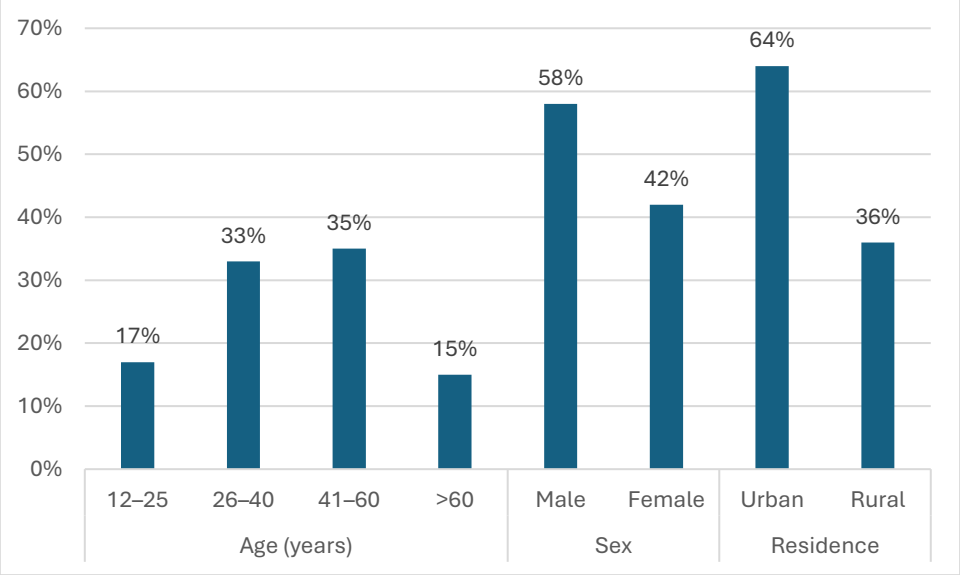

Graph 1: Distribution of participants by Age Sex and Residence.

The demographic structure of the study group (N = 200) shows that most of the patients were in the 41-60 years range (35%), and then the 26-40 years (33%). Less percentage of patients belonged to the 1225 years (17) and over 60 years (15) groups. This indicates that the platelet abnormality was more prevalent among the middle-aged in the current study. Male and female populations were slightly skewed in favor of male as males were a higher proportion (58-42) in the ratio. When it came to residence, a major majority of patients were urban dwellers (64) whereas 36 were rural dwellers. This distribution can include more access to health institutions and diagnostic services among urban populations. On the whole, the study population consisted mostly of middle-aged, male, and urban people, which offers a topical background to the clinical and hematological results.

4.2. Clinical Features

Clinical feature assessment is essential in interpreting the manifestation of platelet abnormalities and its health effects on patients. Platelet disorders can be in the form of bleeding disposition, thrombotic or can be asymptomatic and be incidentally detected during routine investigations. The interpretation of such clinical presentations assists in the association of laboratory results with clinical states and enhances the interpretation of diagnosis.

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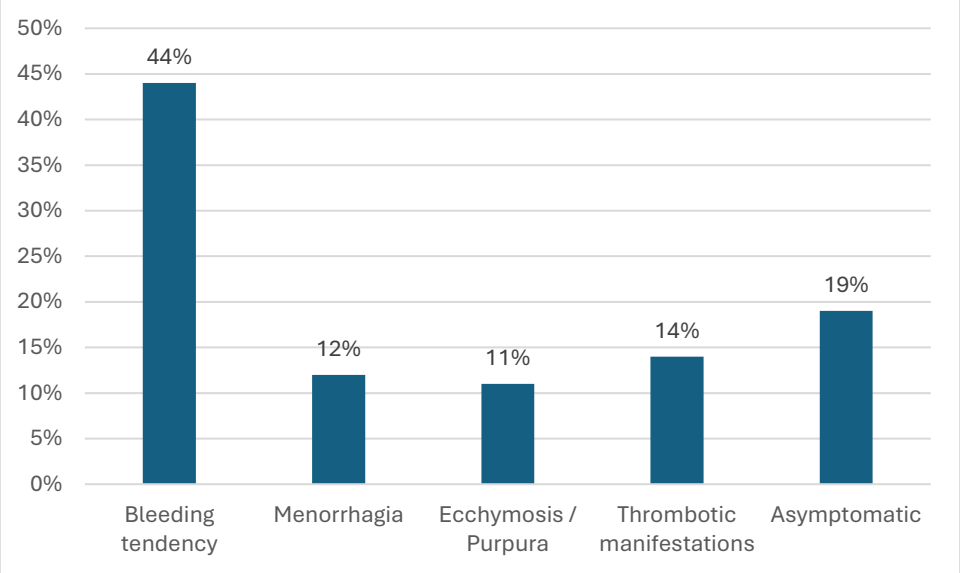
Table 2: Clinical Features of the Study Population (N = 200)

Clinical Feature	Number of Patients (n)	Percentage (%)
Bleeding tendency	88	44%
Menorrhagia	24	12%
Ecchymosis / Purpura	22	11%
Thrombotic manifestations	28	14%
Asymptomatic	38	19%



Graph 2: Distribution of Clinical Presentations

The most frequent clinical manifestation was bleeding tendency, which was seen in 44% of patients, which implies the great correlation between platelet defects and hemorrhagic symptoms. The percentage of asymptomatic cases was 19% and indicated that a significant percentage of platelet abnormalities were identified by chance during routine investigations. Thrombotic appearances were present in 14% of patients and this indicates the clinical significance of increased platelet counts or dysfunctional platelets in facilitating thrombosis. Menorrhagia was also noted in 12% cases, making it important to note that it is a common bleeding symptom in female patients. In 11% of the patients, ecchymosis and purpura were found, which further confirms the presence of bleeding-related symptoms. On the whole, the signs of bleeding were predominant in the study population, and the clinical importance of platelet disorders in hemostatic imbalance was highlighted.

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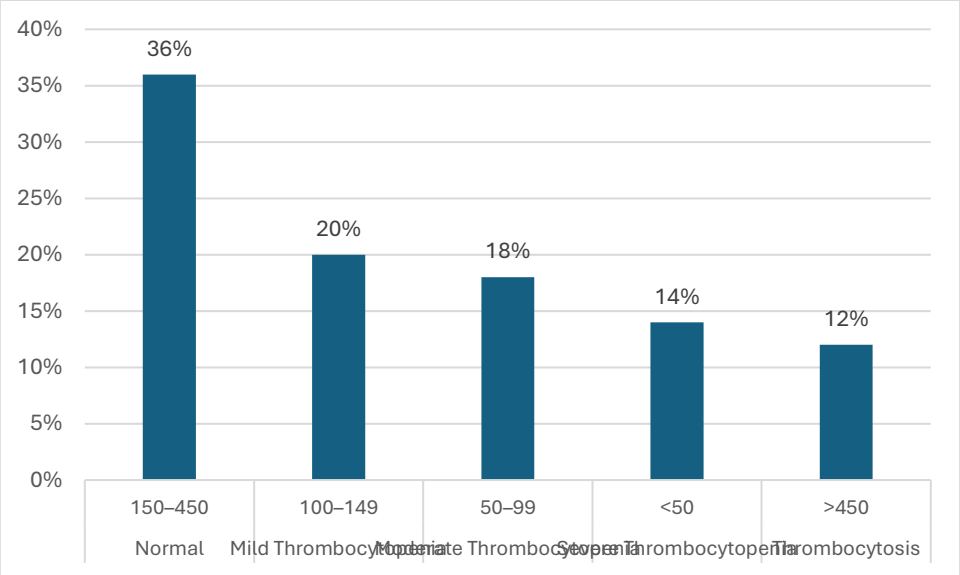
4.3. Platelet Count Distribution

The distribution platelet counts will give valuable information about the quantitative abnormalities of the study population. The division of platelet counts into the normal, thrombocytopenic and thrombocytotic groups enables the comprehension of the prevalence and severity of the platelet related disorders as well as aids in clinical correlation with patient symptoms.

Table 3: Distribution of Platelet Count Categories (N = 200)

Category	Platelet Count (×10⁹/L)	Patients (n)	Percentage (%)
Normal	150–450	72	36%
Mild Thrombocytopenia	100–149	40	20%
Moderate Thrombocytopenia	50–99	36	18%
Severe Thrombocytopenia	<50	28	14%
Thrombocytosis	>450	24	12%



The results show that platelet counts were normal in 36 percent of the patients, and the proportion of patients with thrombocytopenia was significant. Twenty percent of the cases had mild thrombocytopenia, 18% moderate, and 14% severe thrombocytopenia, which proved that a low platelet count was a frequent abnormality among the study population. On the contrary, thrombocytosis was found in 12% of patients, which suggests that higher platelet count was less common. In general, the findings indicate that thrombocytopenia was predominant over thrombocytosis and mild to moderate forms were the most predominant types of thrombocytes. This allocation reveals the clinical significance of measuring low platelet counts in patients presenting with hematological abnormalities.

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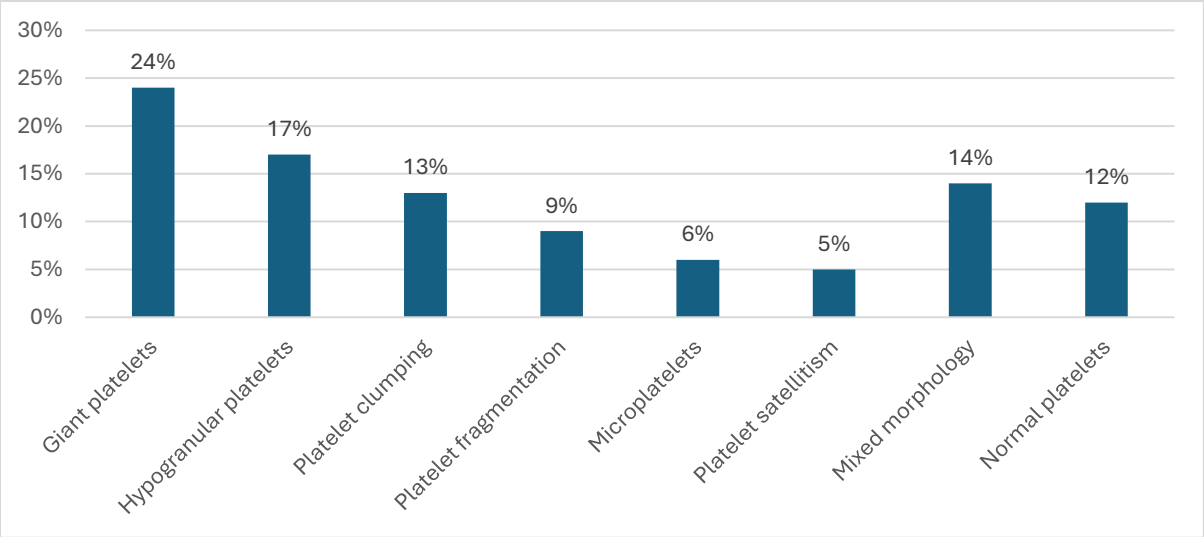
4.4. Morphological Findings on Peripheral Blood Smear

Platelet morphology analysis on Peripheral Blood Smear (PBS) offers valuable information about qualitative platelet defects, which could be overlooked by the automated hematological analyzers. Morphological analysis allows detection of changes in the size, granularity, platelet distribution, and structural integrity and thus helps in correct diagnosis and clinical correlation of platelet disorders.

Table 4: Distribution of Platelet Morphology (N = 200)

Morphological Finding	Frequency (n)	Percentage (%)
Giant platelets	48	24%
Hypogranular platelets	34	17%
Platelet clumping	26	13%
Platelet fragmentation	18	9%
Microplatelets	12	6%
Platelet satellitism	10	5%
Mixed morphology	28	14%
Normal platelets	24	12%



The morphological examination showed that the most widespread abnormality was giant platelets, which were found in 24% of the cases, which suggested a more active platelet turnover or hematologic pathologies. The presence of hypogranular platelets was observed in 17% of patients and may indicate a malformation of platelet granules or their activity. The clumping of platelets was observed in 13 percent cases and can be the cause of pseudothrombocytopenia and the discrepancy of automated count. Mixed morphology (14%) meant that several morphological abnormalities were present in the same sample, which was

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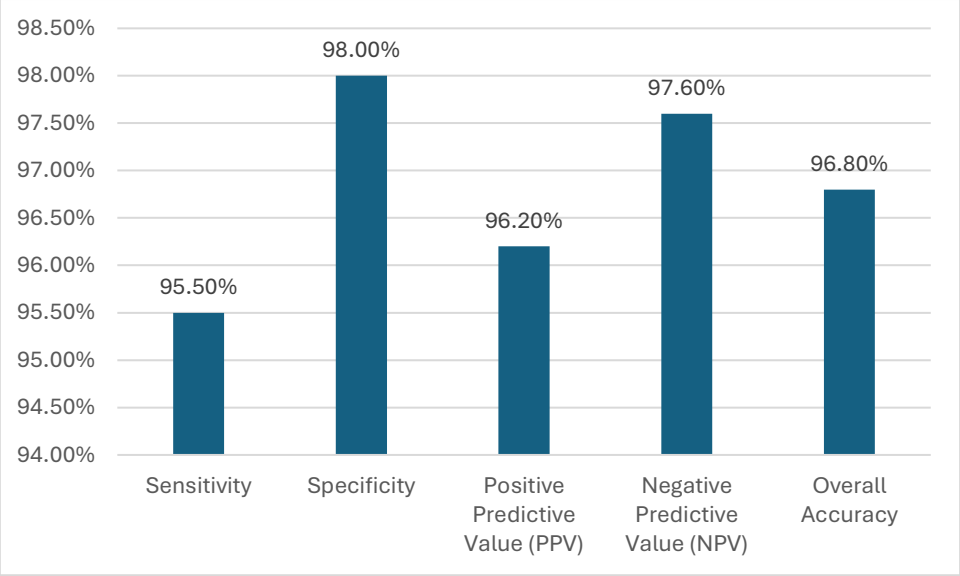
an indication of complicated underlying conditions. Platelet fragmentation was found in 9 percent of the patients and microplatelets were found in 6 percent of the patients which were potential signs of platelet production or destruction. Platelet satellitism, a fairly uncommon observation, was observed in 5% of cases and has been known to confound the accurate counting of the platelets. Only 12% of the patients had normal platelet morphology, indicating the high rate of qualitative platelet abnormalities among the research population.

4.5. Diagnostic Performance of Peripheral Blood Smear

To evaluate the reliability and efficacy of Peripheral Blood Smear (PBS) in the identification of platelet abnormalities when compared to automated hematology analyzers, it is necessary to evaluate the diagnoses of this method. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy are the parameters that give a complex assessment of its diagnostic value.

Table 5: Diagnostic Performance of PBS

Parameter	Value (%)
Sensitivity	95.5%
Specificity	98.0%
Positive Predictive Value (PPV)	96.2%
Negative Predictive Value (NPV)	97.6%
Overall Accuracy	96.8%



Peripheral Blood Smear had good diagnostic ability in determining platelet defects. The high sensitivity (95.5) of PBS means that it is very effective in identifying true positive cases

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correctly whereas the high specificity (98.0) is used to indicate its effectiveness in identifying true negative cases. The positive predictive value (96.2) implies that a considerable percentage of patients diagnosed with platelet abnormalities using PBS actually had them, but the negative predictive value (97.6) suggests that PBS is sensitive to rule out people with normalcy. The general diagnostic validity of 96.8% further validates that PBS is a very reliable and accurate diagnostic tool. These results confirm the value of PBS as a reliable approach to assess platelet disorders and emphasize its great importance as a complement to automated detectors.

4.6. Correlation Between MPV and Platelet Morphology

Mean Platelet Volume (MPV) and platelet morphology are two parameters that give us a great understanding of changes in platelet size as well as its clinical implications. MPV is a significant parameter, which indicates platelet production and activation and is correlated with morphological results on Peripheral Blood Smear (PBS) to assist in the improved interpretation of platelet disorders.

Table 6: Correlation Between MPV and PBS Morphology

PBS Morphology	Mean MPV (fL ± SD)	Interpretation
Normal platelets	9.1 ± 0.8	Normal
Giant platelets	12.4 ± 1.2	Increased MPV
Hypogranular platelets	10.0 ± 1.1	Mildly increased
Microplatelets	7.2 ± 0.9	Decreased MPV
Platelet clumping	Variable	Falsely low MPV

The correlation shows that there is a strong correlation between platelet morphology as seen on PBS and MPV. Normal platelets recorded MPV values that were within the normal range (9.1 ± 0.8 fL) and giant platelets were characterized by significantly higher MPV (12.4 ± 1.2 fL), which indicated a larger platelet size and a higher platelet turnover. Hypogranular platelets showed slightly higher MPV values, indicating slight changes in platelet structure and functioning. Microplatelets on the other hand were linked with reduced MPV (7.2 ± 0.9 fL), indicating smaller platelet size. Platelet clumping exhibited inconsistent MPV values which frequently gave falsely low values because of limitations of the analyzer. In general, an excellent positive relationship was established between MPV and platelet morphology ($r = 0.98$), indicating the utility of MPV as an additional parameter in the explanation of platelet abnormalities along with PBS data.

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5. CONCLUSION

The current research shows that Peripheral Blood Smear (PBS) is a very effective and invaluable diagnostic solution in the diagnosis of an abnormal platelet morphology especially in the detection of morphological alterations which are usually missed by automated hematology analyzers. The study results demonstrate that thrombocytopenia occurs more frequently than thrombocytosis in the study population, and a considerable number of patients have qualitative platelet abnormalities (change in size, granularity, and distribution). PBS demonstrated high sensitivity, specificity and overall accuracy, which proved it to be reliable in clinical practice. In addition, the noted differences between the automated analyzer and PBS results point out the shortcomings of using automated systems only. The paper has clearly demonstrated that the usage of PBS and automated platelet analysis in combination offers a more detailed and accurate diagnosis of platelet disorders, thus increasing the accuracy of diagnosis and helping to make better clinical decisions. As such, PBS can be regularly used as an additional tool in hematological studies to guarantee correct diagnosis and successful management of patients.

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