ANALYSIS OF PLATELET INDICES AND ITS PREDICTIVE VALUE IN DIAGNOSIS OF THROMBOCYTOPENIA

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ABSTRACT

BACKGROUND
Thrombocytopenia can be due to decreased production of platelets in bone marrow or due to increased destruction. Aetiology of thrombocytopenia can be evaluated by determination of platelet indices, whether it is hypoproductive or hyperdestructive type. Platelet indices (MPV, PDW, P-LCR, Platelet-Crit) are simple, cost-effective, non-invasive and reliable tool to differentiate types of thrombocytopenias.

The main objective of the study was to evaluate the variation and relationship of platelet indices in hypoproductive and hyperdestructive thrombocytopenia cases.

MATERIALS AND METHODS
Automated Haematology Analyzer Sysmex XN-1000i is used to assess platelet indices. 70 cases of thrombocytopenia were included in our study. Inclusion and exclusion criteria applied.

RESULTS
This study includes 70 patients of thrombocytopenia over a period of 2 months, who were classified into hypoproductive (32 cases) and hyperdestructive (38 cases). The mean platelet count in hypoproduction group was (56.5 ± 18.5) x 103/mm and mean platelet indices were MPV= (10.6 ± 0.12) fL, PDW= (10.6 ± 0.13) fL, P-LCR= (28.93 ± 1.3) % and Platelet-Crit (0.05 ± 0.02). The mean platelet count of hyperdestructive group (41.3 ± 15.3) x 103/mm, mean platelet indices MPV= (11.4 ± 0.89) fL, PDW= (12.2 ± 2.16) fL, P-LCR= (36.1 ± 5.3) % and Platelet-Crit (0.05 ± 0.01).

CONCLUSION
The combined interpretation of MPV, PDW and P-LCR by automated cell counters can be very useful parameters in differentiating thrombocytopenias due to various aetiologies. Platelet indices showed inverse relationship with platelet count as they are increased in hyperdestructive type and shows linear relationship in hypoproliferative type. Platelet-Crit is a less sensitive parameter to differentiate these thrombocytopenias.

KEY WORDS
Hyperdestructive Thrombocytopenia, Hypoproductive Thrombocytopenia, Platelet Large Cell Ratio, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet-Crit (PCT).

BACKGROUND
Thrombocytopenia is an important clinical finding in hospitalised patients. During evaluation it is essential to identify the aetiology of thrombocytopenia, whether it is due to hypoproduction or hyperdestruction, as this will have effect over the proper management of the patients.[1]

Bone marrow aspiration is the gold standard method for assessing the cause of thrombocytopenia, but this procedure is invasive, time consuming and carries an overt risk of bleeding diathesis in critical cases.[2] Of late with the use of automated cell counters various platelet indices such as mean platelet volume, platelet distribution width and platelet-crit and P-LCR can be measured with a simple complete blood count and may provide some valuable data[3] in estimating aetiology of thrombocytopenia.

Aim
To evaluate variation in platelet indices to differentiate hypoproductive type from hyperdestructive thrombocytopenias.

MATERIALS AND METHODS
This is a cross-sectional study done for a period of 2 months in a tertiary care centre, comprises of total 70 cases. Diagnosis was established based on clinician diagnosis. Some are already diagnosed relapsed cases and for some cases bone marrow aspiration was done to establish the diagnosis.

Platelet count and various platelet indices like Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Platelet-Crit (PCT) and platelet large cell ratio of the thrombocytopenic patients were done in Automated

Haematology Analyzer Sysmex XN-1000i. Correlation with routine peripheral smear findings of particular cases was done. Significant clinical details and available investigations including serological results of the patients were included. The gender was not taken into interpretation, as the range of platelet indices are almost same for both gender.

Inclusion Criteria
All patients with a platelet count of less than 1,50,000/mm3, confirmed by peripheral blood smear and bone marrow aspiration.

Exclusion Criteria
Patients on antiplatelet drugs and other medications causing thrombocytopenia were excluded.

Automated Haematology Analyser XN 1000i Works on Three Basic Principles[4] as Follows:
- Fluorescence Flow Cytometry using a semiconductor laser (639 nm): evaluates physiological and chemical characteristics of cells as the blood sample is aspirated and measured, diluted to the specified ratio and stained and a semiconductor laser beam is emitted. The forward scattered light is received by the photodiode and the lateral scattered light and lateral fluorescent light are received by the photomultiplier tube. This light is converted into electrical pulses.

    Forward scattered light provides information on blood cell size and lateral scattered light provides information on the cell interior (nucleus) and by measuring the intensity of the fluorescence emitted you can obtain information of RNA/ DNA content of the cell.
- Impedance-based sensor for cell detection and analysis using Hydrodynamic Focussing for RBC and PLT analysis: diluted sample is injected into RBC PLT detector which passes through the middle of aperture via hydrodynamic focussing and causes an electrical resistance recorded as impedance pulse. Cell size is proportional to pulse height. With the help of this detection, principle RBC and platelet histograms are generated.
- Sodium lauryl sulfate Haemoglobin Method- cyanide-free Haemoglobin analysis.

Platelets are differentiated from non-platelet fragments and red blood cells with the help of (log-normal) distribution curve generated from the initial platelet histogram.

Optical or impedance method generates platelet distribution curve from which platelet indices like Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and the platelet large cell ratio can be derived (Table 1).[5-7]

Mean platelet volume is the measurement of average size of the platelet reported in femtolitre (fL) and is available on most haematology analysers.[5,6,8]

Platelet distribution width is the measurement of variation in size of platelets. Either calculated from platelet distribution width at 20% frequency level or as the standard deviation of platelet volume divided by MPV × 100.[6] Platelet indices which are derived from platelet histogram are highly specific with different analysers having different reference ranges.[6]

Platelet large cell ratio (P-LCR) measures the percentage of platelets larger than 12 fL.[5,6]

Platelet indices that are calculated from platelet distribution curve are influenced by the factors that affect the platelet count like interference from cells or cell fragments, insufficiency in detecting platelet clumps and giant platelets.

Erroneous result of platelet indices like overestimation of MPV, increased value of PDW and increased platelet large cell ratio (P-LCR) occur when red blood cells are misclassified as platelets.

It is difficult to have platelet distribution curve in patients with severe thrombocytopenia and hence calculation of platelet indices is not possible in such cases.[6]

Blood Sample Collection
Blood samples obtained from vein puncture are collected in EDTA anti-coagulant vacutainer in 1.5 mg/ 1 mL ratio of whole blood. Thoroughly mix the sample immediately after collecting into the vacutainer to prevent clot formation, then the sample is registered into Sysmex XN 1000i automated haematology analyser.

A total of 70 samples were selected for the study, gender was not taken into account as the range of platelet indices are almost the same for both gender.

The specific parameters included in the study were PLT count, MPV, PDW, P-LCR and PLT-crit.

Anticoagulant used for platelet count can affect the calculation of platelet indices. For example K2 or K3 Ethylenediaminetetra Acetic Acid (EDTA) leads to an increase in MPV from 7.9% within 30 mins to 13.4% over 24 h when it is measured by impedance and decreases MPV by 10% when calculated by an optical method.[6,8] Other variables for example PDW and the fraction of large cells[8] apart from MPV are also affected by this time delay. Processing the sample as soon as possible, preferably within 120 mins is recommended to avoid erroneous results.[9] EDTA can also lead to agglutination of platelets, which results in a falsely low platelet count (pseudo-thrombocytopenia). Therefore, each laboratory should possess methods to detect this.[7]

Samples Quality Control Checking System
In this system, platelet histogram errors flag was carefully inspected to eliminate unreliable erroneous flagged results. The various defining criteria are as follows:

MPV is average size of the platelets in Blood. MPV= (mean ± SD) (fL), MPV= (9.7 ± 1.48) fL.

Variation in platelet size comprising of both small platelets and giant/ mega platelets is an indication of hyperproduction of platelets in bone marrow is calculated as PDW (Mean ± SD) %, PDW= (14.46 ± 1.68) %.

Platelet-Crit is a measure of total platelet mass. Normal range of PCT is 0.1 - 0.31%.

P-LCR increases in conditions with lead to increase peripheral destruction of platelets than those with decrease production. Hence, P-LCR has inverse relation to platelet count and it is directly related to PDW and MPV. P-LCR= (mean ± SD) (%); P-LCR= (33.40 ± 2.96) %.

Data Collection and Analysis
Details of the patients like clinical history, presenting complaints, treatment details and previous haemogram reports if any- above data are collected from medical records.

Quantitative data analysis of the platelet indices was done. Mean and standard deviations were calculated. Measured

platelet parameters between the two groups of thrombocytopenic patients were statistically tested by unpaired 't' test to obtain the P-value. Statistically significant result is considered, when P-value obtained is less than 0.05.

RESULTS

Our study was done on 70 of thrombocytopenic patients who were classified into hypoproductive and hyperdestructive group. In the hypoproductive we had 32 cases and in the hyperdestruction there were 38 cases. Measured platelet parameters were statistically tested using unpaired test. The distribution of cases in each subgroup and comparison of the distribution with similar studies were shown in Table 2.

The mean platelet count in the hypoproduction group is $(56.5 \pm 18.5) \times 10^3$/mm and in the hyperdestruction group is $(41.3 \pm 15.3) \times 10^3$/mm, having a P-value of 0.0004. Mean of platelet indices like mean MPV in the hypoproduction group is 10.6 ± 0.12 and in the hyperdestruction group is 11.4 ± 0.89 resulting in a significant P-value of < 0.0001. Mean PDW in the hypoproduction group is 10.6 ± 0.13 and in the hyperdestruction group is 12.2 ± 2.16 having a P-value of <0.0001. Mean P-LCR in the hypoproduction group is 28.93 ± 1.3 and in the hyperdestruction group is 36.1 ± 5.3 with a P-value of < 0.0001. The mean PCT in the hypoproduction group is 0.05 ± 0.02 and in the hyperdestruction group is 0.05 ± 0.01 having a P-value of 1. Mean values of different platelet indices and P-value in hyperdestruction thrombocytopenia and hypoproduction thrombocytopenia were shown in Table 3.

Parameter	Coulter Principle	Benefits	Drawbacks	Applications
Platelet count	Calculated usually by impedance method in automated flow cytometric analysis	Quick, simple and often requires less amount of blood	Less accurate and has high % CV in severe thrombocytopenia. This test is highly reliable on procedure used for calculation and often fails to detect platelet clumps and giant platelets	Indicative of thrombocytopenia, but in general has no relationship with risk of bleeding in thrombocytopenic patients[10-12]
MPV- Mean platelet volume	Derived value obtained from the platelet distribution curve	Quick, simple and often requires less amount of blood, commonly used	Value varies with platelet distribution curve, as it is derived from it	Indicative of bone marrow failure as a cause of low platelet count.[13-18] Decrease MPV value along with platelet count of $< 20 \times 10^9$/L[19] result in bleeding manifestation
PDW- Platelet distribution width	Derived value obtained from the platelet distribution curve	Quick, simple and often requires less amount of blood	Value varies with platelet distribution curve, as it is derived from it	No data available to correlate bleeding risk in thrombocytopenic patients
P-LCR- Platelet large cells ratio	Derived value obtained from the platelet distribution curve	Quick, simple and often requires less amount of blood	Value varies with platelet distribution curve, as it is derived from it	No data available to correlate bleeding risk in thrombocytopenic patients

Table 1. Clinical Efficacy and Features of Platelet and Platelet Indices

Aetiologies	Total Number of Cases in Present Study (%)	Katti et al[20] Total Cases (%)	Shaheena et al and Vimal et al[1] Total Cases (%)
Hypoproduction			
Severe anaemia	05 (7.1%)		
Megaloblastic anaemia	15 (21.4%)	08 (8%)	11 (9.2%)
Leukaemia	06 (8.5%)	06 (6%)	2 (1.7%)
CKD	06 (8.5%)		
Others			13 (10.8%)
Hyperdestructive			
ITP	11 (15.7%)	4 (4%)	3 (2.5%)
Dengue	08 (11.4%)	29 (29%)	26 (21.7%)
Malaria	05 (7.1%)	24 (24%)	8 (6.7%)
Chronic liver disease	05 (7.1%)	4 (4%)	20 (16.7%)
Sepsis	01 (1.4%)	4 (4%)	6 (5%)
Others	08 (11.4%)	21 (21%)	31 (25.8%)
Total	**70**	**100**	**120**

Table 2. The distribution of Thrombocytopenia cases in each subgroup and comparison of the distribution with Similar Studies

Platelet Indices	Hypoproduction	Hyperdestruction	P Value
Platelet count (mean ± SD) (×10³/mm3)	(56.5 ± 18.5)	(41.3 ± 15.3)	0.0004
MPV (mean ± SD) (fL)	10.6 ± 0.12	11.4 ± 0.89	<0.0001.
PDW (mean ± SD) (fL)	10.6 ± 0.13	12.2 ± 2.16	<0.0001
P-LCR (mean ± SD) %	28.93 ± 1.3	36.1 ± 5.3	<0.0001
PCT (mean ± SD) %	0.05 ± 0.02	0.05 ± 0.01	1

Table 3. Mean Values of different Platelet Indices and P-value in Hypoproduction Thrombocytopenia and Hyperdestruction Thrombocytopenia

DISCUSSION

Thrombocytopenia is defined as platelet count below 150,000/mm³. It is a frequent complication in patients admitted in an emergency care,[21] hence its early detection is important for proper management of the patients. Hence, the methods to distinguish between the types of thrombocytopenia like hypoproductive due to reduced production and hyperdestructive thrombocytopenia is necessary. Automated Haematology Analyzer used for the measurement of platelet indices has many advantages over manual estimation as it is very simple, quick and inexpensive test which also eliminates the observer bias.[22,23] Further in manual method, a delay between collection of blood and smear preparation may change the platelet morphology and also artifactual increase in platelet diameter can occur because of increased adhesiveness with flattening of the platelets on the smears.[24]

Though these parameters have been available in past from the routinely used blood cell counters in the laboratory, their exact role in application to clinical diagnosis has still not been fully established.[25] In our study thrombocytopenia is divided into hypoproductive and hyperdestructive type based on the aetiology.

In our study, PLT indices (MPV, PDW, P-LCR, PCT) measured by Sysmex XN 1000i very well correlated with other published studies.

Shaheena Parveen et al and Mourouguessine Vimal et al found a significant low (10.17 ± 1.3) mean MPV in the hypoproduction group than in the hyperdestruction group (12.3 ± 0.9), which is comparable to our study.

Study done by Numbenjapon et al conclude that MPV was significantly high in hyperdestruction thrombocytopenia group than hypoproductive thrombocytopenia. In hyperdestructive thrombocytopenia, bone marrow compensates actively for the platelet loss and start releasing young larger platelets ("left shift"), which tend to decrease in size during its 7 - 10 days lifespan.[26]

Ntaios et al found an increased MPV and PDW in Immune Thrombocytopenic Purpura (ITP).

Kaito et al similarly found a substantial increase in MPV and PDW in ITP than in aplastic anaemia.

Few studies found a high PDW can also result in hyperdestructive thrombocytopenia, because of the release of heterogeneous population of platelets which vary in their size (Anisocytosis).[27,28]

PCT is a representation of volume percent of platelets and its value is not altered by severity of thrombocytopenia of either hypoproductive or hyperdestructive aetiology. In this study, we did not find any major difference in PCT among the two subgroups.

CONCLUSION

The mean platelet volume is useful in discriminating the hypoproductive and hyperdestructive thrombocytopenia. Thus, interpretation of platelet indices can help the patients to avoid unnecessary invasive investigations like bone marrow aspiration and unnecessary platelet transfusion. Platelet indices showed inverse relationship with platelet count as they are increased in hyperdestructive type and show linear relationship in hypoproliferative type. Platelet-Crit is less sensitive parameter to differentiate these thrombocytopenias.

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