Original Article

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Pathology Section

Evaluation of Thrombocytopenia in Megaloblastic Anemia by Platelet Indices and Megakaryocytes- Comparison with Hypoproduction and Hyperdestruction

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ABSTRACT

Introduction: Thrombocytopenia may result from many mechanisms such as: marrow hypoplasia (decreased megakaryocytes), ineffective thrombopoiesis (normal to increased megakaryocytes) and increased destruction of platelets (increased megakaryocytes). The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in some studies, whereas ineffective thrombopoiesis has been proposed as the mechanism in others.

Aim: This study was taken up to study the platelet indices in thrombocytopenia secondary to megaloblastic anemia, hypoproduction and hyperdestruction. And also aimed to evaluate the discriminative function of platelet indices in megaloblastic anemia in comparison with hypoproductive and hyperdestructive causes of thrombocytopenia and to correlate platelet indices with bone marrow megakaryocyte cellularity.

Materials and Methods: Platelet indices in 32 cases of thrombocytopenias of megaloblastic etiology were compared with platelet indices of 31 cases of marrow proven hypoproductive thrombocytopenias (aplastic anemia, hypoplastic anemia, acute leukemia) and 32 cases of hyperdestructive thrombocytopenias (Immune thrombocytopenia). Descriptive analysis was used and comparison among means of platelet indices in all the groups was done with one way ANOVA using Scheffe's test. Categorical data was analyzed using Chi-square test. Platelet indices and bone marrow megakaryocytes were analyzed and correlated in each group. A p-value of less than 0.05 was considered statistically significant.

Results: The mean values platelet indices were significantly higher ($p<0.05$) in the hyperdestructive group [PDW(16.6fL), MPV(12.1fL), P-LCR(42.3%)] compared to the hypoproductive group [PDW(11.8fL), MPV(10.9fL), P-LCR(31.5%)]. Whereas, mean values of PDW (14.7fL) and MPV (11.6fL) in the megaloblastic group showed a significantly higher value (p-value<0.05) than hypoproductive group but no statistical significant difference was seen compared to hyperdestructive group (p-value>0.05). The mean P-LCR (37.4%) in megaloblastic group was intermediate between both the other groups with a significant statistical difference (p-value<0.05).

Conclusion: Both hypoproduction and ineffective thrombopoiesis are the underlying pathomechanisms in megaloblastic thrombocytopenia as evidenced by the marrow findings and platelet indices. Platelet indices are of significant discriminative value in differentiating the various causes of thrombocytopenias. We hereby infer that megaloblastic thrombocytopenia is to be included as a separate category apart from hypoproliferative and hyperdestructive groups.

Keywords: Ineffective thrombopoiesis, Mean platelet volume, Platelet distribution width, Platelet large cell ratio

INTRODUCTION

Platelet count of less than 1.5 lakh/mm^3 defines thrombocytopenia. It may result from many mechanisms such as: marrow hypoplasia (decreased megakaryocytes), ineffective thrombopoiesis (normal to increased megakaryocytes) and increased destruction of platelets (increased megakaryocytes) [1-3].

Distinction between these categories is made by bone marrow examination. Hyperdestructive thrombocytopenia is a result of extramedullary platelet destruction with normal or increased bone marrow production, e.g., immune thrombocytopenic purpura (ITP), secondary ITP and disseminated intravascular coagulation. Hypoproductive thrombocytopenias are caused by decreased bone marrow production because of primary or secondary bone marrow diseases such as aplastic anemia, acute leukemia, myelodysplastic syndrome and post chemotherapy [3].

Studies in literature have shown the utility of platelet indices

in identifying the pathomechanism in various causes of thrombocytopenia. There is increasing evidence that platelet indices, such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Platelet Large Cell Ratio (P-LCR), have a significant role in the discrimination between hypoproductive and hyperdestructive thrombocytopenia [3-5]. MPV is postulated as surrogate marker of bone marrow activity. High MPV suggests increased megakaryocytic activity and low MPV indicates marrow suppression [6]. PDW measures the variability in the size of platelets. P-LCR is a ratio of large platelets to total platelet count. P-LCR is directly related to PDW and MPV whereas it is inversely related to platelet count [6].

The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in some studies, whereas ineffective thrombopoiesis has been proposed as the mechanism in others [1,3,5-8].

MATERIALS AND METHODS

This is a retrospective analytical study undertaken in the Department of Pathology, JSS Medical College and Hospital, Mysuru, India, over a period of two years, starting from May 2014 till April 2016. The study involved 95 cases of thrombocytopenia satisfying the inclusion criteria, based on the etiology and divided them into three categories: thrombocytopenia secondary to megaloblastic anemia, hypoproduction and hyperdestructive causes.

Inclusion Criteria: Total 32 cases of marrow proven thrombocytopenias of megaloblastic etiology with low serum vitamin B12/folate levels (<200pg/ml) and platelet count of <1.5 lakh/cumm with available platelet indices were selected and 31 cases of marrow proven aplastic anemia and acute leukemia with platelet count of <1.5 lakh/cumm with available platelet indices constituted hypoproductive thrombocytopenia. All the remaining 32 cases of immune thrombocytopenia with platelet count of <1.5 lakh/cumm with available platelet indices were included in the category of hyperdestructive thrombocytopenia.

Exclusion Criteria: Cases with unavailable platelet indices were excluded from the study.

Subjects: A total of 136 cases who underwent bone marrow biopsy over a period of two years from May 2014 to April 2016 were studied. Out of these, 95 cases satisfying the inclusion criteria were selected and grouped into above mentioned three categories.

Sample Processing: The bone marrow aspiration sample and blood sample were collected in EDTA vacutainer whereas, the bone marrow biopsy was collected in formalin and decalcified. The marrow aspiration smears were stained with Leishman's stain and the trephine biopsy sections were stained with Haematoxylin and Eosin. The hematological parameters such as platelet count, MPV, PDW, P-LCR were estimated by automated analyzer Sysmex XN-1000 (Sysmex America, Inc. in Lincolnshire, Illinois) and the results were computed. The average time taken from collection of sample to start processing in the analyser is 40 minutes in our hospital. Adequacy of megakaryocytes in bone marrow aspiration was assessed as follows: normal (one megakaryocyte per one to three low-power fields), decreased (one megakaryocyte per five to ten low-power fields) or increased (more than two megakaryocytes per low-power field) [9,10]. The bone marrow aspiration findings were confirmed after assessment of megakaryocytes in trephine biopsy sections. Platelet indices and bone marrow megakaryocytes were analysed and correlated in each group.

STATISTICAL ANALYSIS

All the data was entered in Microsoft excel sheet for analysis. Analysis was done using Microsoft Excel 2013 and SPSS 20. Pearson correlation was applied for correlation of parameters. Platelet indices and bone marrow megakaryocytes were analyzed and correlated in each group. A p-value of less than 0.05 was considered statistically significant. Descriptive analysis was used and comparison among means of platelet indices in all the groups was done with one-way ANOVA using Scheffe's test. Categorical data was analyzed using Chi-square test.

Ethics: This study was approved by institutional ethical committee.

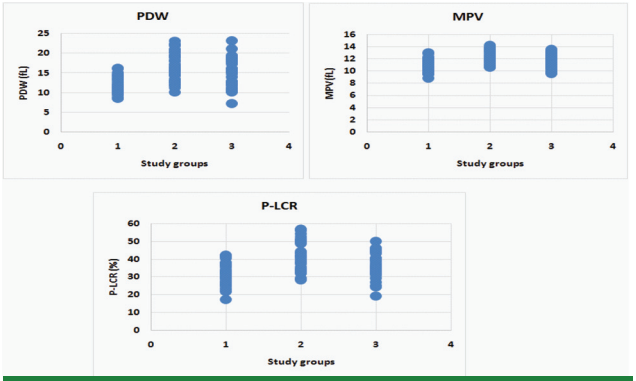
RESULTS

The mean age of patients in hypoproductive, hyperdestructive and megaloblastic groups was 42.8, 49.1 and 47.3 years. Male to female ratio in our study in hypoproductive, hyperdestructive and megaloblastic groups was 1:1.2, 1.13:1 and 1:1.46 respectively. The mean values of platelet count in hypoproductive, hyperdestructive and megaloblastic groups were 48,290/cumm, 61,470/cumm and 76,910/cumm respectively [Table/Fig-1].

Parameter	Study Groups	Number of cases	Mean	Std. Deviation
Platelet count (Lakh/mm^3)	Hypoproduction	31	0.482	0.34
	Hyperdestruction	32	0.614	0.31
	Megaloblastic	32	0.769	0.40
	Total	95	0.623	0.37
PDW(fl)	Hypoproduction	31	11.852	1.98
	Hyperdestruction	32	16.691	3.55
	Megaloblastic	32	14.741	3.87
	Total	95	14.455	3.78
MPV (fl)	Hypoproduction	31	10.926	1.02
	Hyperdestruction	32	12.159	1.15
	Megaloblastic	32	11.631	1.06
	Total	95	11.579	1.18
P-LCR(%)	Hypoproduction	31	31.519	6.49
	Hyperdestruction	32	42.359	8.77
	Megaloblastic	32	37.428	7.25
	Total	95	37.161	8.71

[Table/Fig-1]: Mean values of platelet count and platelet indices.

Distribution of PDW, MPV and P-LCR among cases in the three study groups is shown in [Table/Fig-2].The mean values of platelet indices were significantly higher (p<0.05) in the hyperdestructive group [PDW(16.6fL), MPV(12.1fL), P-LCR(42.3%)] compared to the hypoproductive group [PDW(11.8fL), MPV(10.9fL), P-LCR(31.5%)] [Table/Fig-1&3]. Whereas, mean values of PDW (14.7fL) and MPV (11.6fL) in the megaloblastic group showed a significantly higher value (p-value<0.05) than hypoproductive group but

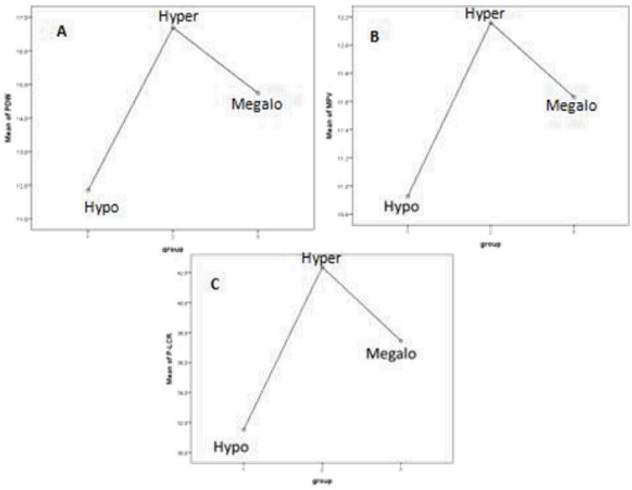


[Table/Fig-2]: Distribution of PDW, MPV and P-LCR among cases in the three study groups-1-hypoproduction, 2-hyperdestruction and 3-megaloblastic thrombocytopenia.
1-hypoproduction, 2-hyperdestruction and 3-megaloblastic thrombocytopenia

Dependent variable	Study groups (mean value)	Study groups	Mean Value	p-value
PDW(fl)	Hypoproduction (11.85)	Hyperdestruction	16.69	<0.001
		Megaloblastic	14.74	0.003
	Megaloblastic (14.74)	Hypoproduction	11.85	0.003
		Hyperdestruction	16.69	0.062
MPV(fl)	Hypoproduction (10.93)	Hyperdestruction	12.16	<0.001
		Megaloblastic	11.63	0.040
	Megaloblastic (11.63)	Hypoproduction	10.93	0.040
		Hyperdestruction	12.16	0.156
P-LCR(%)	Hypoproduction (31.52)	Hyperdestruction	42.36	<0.001
		Megaloblastic	37.42	0.011
	Megaloblastic (37.42)	Hypoproduction	31.52	0.011
		Hyperdestruction	42.36	0.038

[Table/Fig-3]: One-way ANOVA by Scheffe's test to compare the means of platelet indices between the groups.
One-way ANOVA by Scheffe's test: p value< 0.05-statistically significant

no statistical significant difference was seen compared to hyperdestructive group (p- value>0.05). The mean P-LCR (37.4%) in megaloblastic group was intermediate between both the other groups with a significant statistical difference (p-value<0.05) [Table/Fig-3&4].



[Table/Fig-4]: Dot-plot of means of platelet indices in each group.
A-means of PDW, B-means of MPV, C-means of P-LCR . Hypo- thrombocytopenia secondary to hypoproduction.Hyper- thrombocytopenia secondary to hyperproduction. Megalo-thrombocytopenia secondary to megaloblastic anemia

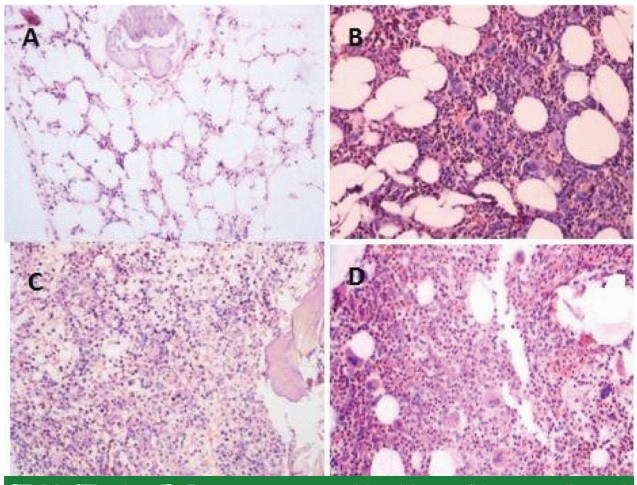
Bone marrow trephine biopsy in all the acute leukemia and aplastic anemia cases of hypoproductive group showed decreased megakaryocytes [Table/Fig-5&6a]. Increased megakaryocytes were a common finding in all the cases of immune thrombocytopenia in hyperdestructive group [Table/Fig-5& 6b]. While, the megaloblastic group had mixture of cases with normal, increased and decreased megakaryocytes [Table/Fig-5&6c,6d].

DISCUSSION

Platelet count below 1.5lakh/mm^3 defines thrombocytopenia but does not alone explain the underlying pathomechanism unless a bone marrow examination is done which shows decreased production of megakaryocytes, ineffective thrombopoiesis or increased peripheral destruction. Since bone marrow examination is invasive and expensive, the usefulness of other parameters estimated by the automated analysers are being investigated which include platelet indices such as PDW, MPV and P-LCR. The combined interpretation of MPV, PDW & P-LCR can prove to be very

Categories	No. of cases	Megakaryocytes			X^2 value DF=4	p-value
		Decreased	Normal	Increased		
Hypoproduction	31	31(100%)	0	0	86.55	0.0001
Hyperdestruction	32	0	0	32(100%)		
Megaloblastic	32	10(31.2%)	8(25%)	14(43.7%)		
Total	95	41(43.1%)	8(8.4%)	46(48.4%)		

[Table/Fig-5]: Bone marrow megakaryocyte cellularity in the three study groups.



[Table/Fig-6a-d]: Bone marrow trephine biopsy (Hematoxylin and Eosin x100). (a) Decreased megakaryocytes in aplastic anemia; (b) Inreased megakaryocytes in immune thrombocytopenia; (c) Decreased megakaryocytes in megaloblastic anemia; (d) Inreased megakaryocytes in megaloblastic anemia.

useful parameters to differentiate thrombocytopenias of various etiologies.

Hyperdestructive thrombocytopenia (ITP) can be precisely differentiated from hypoproliferative type (acute leukemias, aplastic anemias) based on platelet indices such as MPV,PDW & P-LCR as proved in many studies [3,4,8]. All the three platelet indices were significantly higher in the hyperdestructive group compared with the hypoproductive group which is consistent with other studies by Katti et al., Elsewefy et al., Baig et al., and Barrios et al., [3-5,11].

In a study by XU et al., though significant differences were observed in platelet count, MPV and PDW between patients with ITP and patients with bone marrow failure, they concluded that MPV and PDW do not have enough predictive efficiency for the diagnosis of bone marrow failure in thrombocytopenic patients [12].

Since, bone marrow findings in cases of thrombocytopenia give a definite diagnosis of the underlying pathomechanism, bone marrow study is frequently asked in cases of thrombocytopenia. The findings of decrease in mega-karyocytes in aplastic anemia and leukemia, and increase in the number of megakaryocytes in immune thrombocytopenia were consistent with other studies [9,13,14].

The number of megakaryocytes in megaloblastic anemia was normal, increased and decreased in 25%, 43.7% and 31.2% of cases. Similar results were seen in a study by Gupta et al., with normal, increased and decreased megakaryocytes in 8.3%, 58.3%, 33.3 % of cases respectively [10]. Choudhary et al., found absent, decreased normal and increased megakaryocytes in 2.4% 16.0%, 47.4% and 34.2% of cases respectively [9].

The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in many studies where bone marrow shows decreased megakaryocytes and the platelet indices are studied including cases of megaloblastic anemia under the category of hypoproduction [3,5-8]. Whereas, ineffective thrombopoiesis has also been proposed as a mechanism, where marrow shows normal to increased megakaryocytes [1]. The findings of normal, increased and decreased megakaryocytes in our study support the hypothesis of both hypoproduction and ineffective thrombopoiesis in the causation of thrombocytopenia in megaloblastic anemia.

This is further supported by the fact that mean values of MPV, PDW and P-LCR in megaloblastic group were significantly different ($p<0.05$) from the hypoproductive group. This difference can be explained by hypothesising the underlying pathomechanism as ineffective thrombopoiesis in addition to hypoproduction by also taking the bone marrow findings into consideration. If the mechanism was only hypoproduction, all cases in megaloblastic group should have shown decreased megakaryocytes. Hence, we conclude that platelet indices can be considered as the mirror image of mechanism of thrombocytopenia.

LIMITATIONS

Cases where platelet indices were unavailable were not included in the study.

CONCLUSION

Based on our results of platelet indices and bone marrow findings, mixed mechanisms are operative in the causation of thrombocytopenia in megaloblastic anemia. We hereby infer that megaloblastic thrombocytopenia is to be included as a separate category apart from hypoproliferative and hyperdestructive groups. Bone marrow examination still remains the gold standard for discriminating hypoproductive thrombocytopenias from the hyperdestructive causes and platelet indices are of significant discriminative value in differentiating the various causes of thrombocytopenias. Standardized measurements for platelet indices might significantly increase the utility of platelet indices in the diagnosis and understanding of pathomechanism of various clinical conditions.

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