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Original Research Article

The Study of Megakaryocytes in Bone Marrow Aspiration Smears in Patients with Thrombocytopenia

A Choudhury[1], G Mishra[1], JK Behera[1], H Soren[1], P Mishra[1], J Nayak[2]

[1]Assistant Professor, Department of Pathology, MKCG Medical College & Hospital, Brahmapur.

[2] Associate Professor, Department of Pathology, MKCG Medical College & Hospital, Brahmapur.

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Corresponding author: Dr Pratibha Misra

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Abstract

Background: Thrombocytopenia is a common hematology condition for which bone marrow aspiration is indicated. Thrombocytopenia is encountered in various hematological disorders including MDS as well as non-MDS. Dysmegakaryopoiesis is characterized by various megakaryocytic alterations in bone marrow aspirates and include both dysplastic and non-dysplastic features.

Methods: A prospective study was conducted in cases of non-MDS related thrombocytopenia and the bone marrow aspirates were studied morphologically. The cases of thrombocytopenia (platelet count <150,000 per microliter or <150 x 10^9/L or <1.5 K/cumm) was done by automated platelet count and were further confirmed manually in peripheral smear. Bone marrow aspiration was done in confirmed thrombocytopenia cares and the morphology of megakaryocytes was studied.

Results: A total of 98 cases of thrombocytopenia were included in the present study predominated by megaloblastic anemia. 43 cases, followed by acute leukemia, 30 cases (AML-16, ALC-14), Aplastic anemia (12 cases), ITP (11 cases) and multiple myeloma (2 cases). Dysmegakaryopoesis in the form of nuclear separation, bare nuclei, micromegakaryocytes, emperipolesis, and vacuolization were observed in the present study. The evaluation of megakaryocyte alternation in morphology in cases of thrombocytopenia can provide more knowledge to the pathogenesis of various hematopoietic disorders.

Keywords: Dysmegakaryopoiesis, emperipolesis, bone marrow aspiration

Background

Thrombocytopenia is a common haematological condition for which bone marrow aspiration is indicated [1]. Thrombocytopenia is encountered in various haematological disorders including MDS as well as NON-MDS [2]. A diversity of factors can contribute to anomalous platelet counts; one of these is inappropriate platelet production. Thrombocytopenia (platelet counts less than 150,000/μl) can lead to inadequate clot formation and increased risk of bleeding [2]. Immune thrombocytopenic purpura (ITP) is a very common cause of thrombocytopenia. Various other haematological conditions such its megaloblastic anemia, aplastic anemia and leukemias can also present with thrombocytopenia [2]. Dysmegakaryopoie-

sis is characterized by various megakaryocytic alterations in bone marrow aspirates and include both dysplastic and non-dysplastic features.

Dysplastic features of megakaryocyte morphology include multiple separated nuclei, micro megakaryocytes (3-6 times larger than RBC with 1-2 lobes, mature nucleus and lower nuclear to cytoplasmic ratio) and hypo granular forms (with little or no granules). Non dysplastic features of megakaryocytes include immature forms (with basophilic cytoplasm, high nuclear to cytoplasmic ratio and no nuclear lobation), emperipolesis (intact hematopoietic cells within cytoplasm) cytoplasmic budding, vacuolization and bare nuclei without cytoplasm [1].

A defect in any stage of megakaryocytopoiesis can lead to dysmegakaryocytopoiesis and thrombocytopenia. Wright demonstrated in normal and abnormal human physiology that changes in platelet number were associated only with changes in the megakaryocytes. Their number increases as the demand for platelets rises.

It is necessary to assess megakaryocyte numbers (Quantity) as well as its morphology (Quality). In films of an aspirate quantity can only be a subjective assessment that megakaryocytes are decreased, normal or increased.5 Normal (1 megakaryocyte / 1-3 low-power fields), increased (>2 megakaryocytes / >3 low power field), or decreased (1 megakaryocyte / 5 -10 low-power fields).

When haemopoiesis is normal, megakaryocytes do not form clusters of more than two or three cells. Larger clusters of megakaryocytes are seen in regenerating marrow, following chemotherapy and bone marrow transplantation, and also in various pathological states and this feature is diagnostically useful.

Methods

A prospective study was conducted IN Pathology Department of M.K.C.G. medical College, Berhampur in cases of NON-MDS related thrombocytopenia & the bone marrow aspirates was studied morphologically.

Study period: 2 years (2018-2020)

Inclusion Criteria:

All patients with platelet counts <1,00,000/μL with/without bleeding manifestations.

Exclusion Criteria

1. Inadequate material
2. Patient with thrombocytopenia where bone marrow aspiration is not indicated.
3. Non-cooperative patients.
4. Bleeding manifestations other than thrombocytopenia.
5. Pseudothrombocytopenia cases
    - Platelet agglutination
    - Satellitosis
    - Phagocytosis by other cell

Results

The present study was carried out in the haematology section of department of pathology, M.K.C.G. Medical College and Hospital. (X Total of 98 cases of thrombocytopenia were included in the present study predominated by megaloblastic anemia 43 cases followed by Acute leukemia 30 cases (AML 16 cases, ALL 14 cases), Aplastic anemia 12 cases, ITP 11 cases and Multiple myeloma 2 cases).

Out of 98 cases of thrombocytopenias those were studied during period of two years, 43 (43.8% were from the megaloblastic anemia, 30 (30.6%) from acute leukemia, 11 (11.2%) from ITP, 12 (12.2%) from aplastic anemia, 2 (2.04%) from multiple myeloma.

Table 1: Incidence of diseases in the present study

Name of the Disease	No. of Patient	Percentage	Special investigations for confirmation of BMA diagnosis
Megalobastic anaemia	43	43.8%	Serum B12 and Folic acid assays
Acute Leukemia	30	30.6%	Cytochemistry
AML			
ALL			
ITP	11	11.2%	By exclusion of secondary thrombocytopenia
AA	12	12.2%	Bone marrow biopsy
MM	2	2.04	Bone marrow biopsy
Total	98	100	

Table 2: Age incidence of present study

Age in years	**No. of Cases**	**Percentage**
1 - 10 Yrs.	11	11.22
11 - 20 Yrs.	29	29.59
21 - 30 Yrs.	10	10.20
31 - 40 Yrs.	12	12.20
41 - 50 Yrs.	23	23.46
51 - 60 Yrs.	7	7.14
61 - 70 Yrs.	5	5.10
71 - 80 Yrs.	1	1.02
Total	98	100

In this present study, there were two peaks between 11-20 years and 41-50 years of age.

Table 3: Sex incidence in present study

Gender	No. of Cases	Percentage
Male	52	53.06%
Female	46	46.94
Total	98	100

Thrombocytopenia was commonly seen in males as compared to females with wide range of age distribution.

Table 4: Quantitative megakaryocyte changes observed in this study

Cases	**Normal**	**Increased**	**Decreased**	**Absent**	**Number of cases**
MGA	37	2	4	-	43
AA	-	-	12	-	12
ITP	1	10	-	-	11
AML	2	-	14	-	16
ALL	-	-	14	-	14
MM	1	-	1	-	2
Total	41	12	45	-	98

Statistical analysis by χ^2 test of the quantitative results revealed χ^2=121.54, P < 0.05 - Significant.

Tables 5: Morphological alterations of megakaryocytes in various conditions

Case	Nuclear Separation / Hyper Segmented	Bare nuclei	Emperipolesis	Vacoulization	Hypolobated form	Immature form	Micromegakaryocytes	Normal
Megaloblastic anemia	35	4	1	1			1	43
Acute myeloid leukemia	3	--	--	--	--	--	--	16
Acute lymphoblastic anemia	--	--	--	--	--	--		14
Aplastic anemia	1	--	--	--	--	--	--	12
Multiple myeloma	--	--	--	--	--	--	--	2
ITP	4	--	--	--	8	11	4	11
TOTAL								98

Table 6: Comparison of dysplastic feature of megakaryocyte

Study	**Dysplastic**
Parul *et al* [4]	Hypolobation ,hypogranular
Choudhary *et al* [1]	Mostly separated nucleoli
Neelima *et al*	Micromegakaryocyte (57.2%)
Present study	Nuclear separation, Hypolobated form micromegakaryocytes

Table 7: Comparison of non-dysplastic feature

Study	**Non dysplastic features**
Muhury *et al*	84.2% bare nuclei , 94% young(immature)
Choudhary *et al*	30.3% bare nuclei , 94% young(immature)
Present study	36.36% bare nuclei, 100% young(immature)

In the present study of the total 98 cases, 30 were acute leukemia presenting with thrombocytopenia, showing preponderance of AML. This finding correlates well with the studies conducted by Kulshrestha et al, Muhury et al, Kibria et al except Rahim et al in which ALL was predominant.

Conclusion

The most common cause of thrombocytopenia in the present study was Megaloblastic anemia followed by acute leukemia, aplastic anemia and Immune thrombocytopenic purpura (ITP). Dysplastic megakaryocytes were commonly seen in ITP and megaloblastic anemia and should be looked for in a suspected case of MDS. Dysplastic features in megakaryocytes were observed in various etiologies of non MDS related thrombocytopenia. Further comparative study with increased sample size including cases of MDS should be done to understand the occurrence of dysplastic megakaryocytes in various non-MDS related thrombocytopenias.

Megakaryocyte changes studied in various haematological conditions in bone marrow aspirate associated with thrombocytopenia were statistically significant suggesting that megakaryocytes though forming a small percentage of cells in Bone marrow need to

be given equal importance as that given to erythroid and myeloid cells in BMA evaluation.

An attempt was made in this present study to elucidate the changes in the megakaryocyte in numerical and morphological as well.

Morphologic changes in megakaryocyte seen in MDS were also seen in various non MDS condition which should also be considered during diagnostic evaluation.

Further studies on the evaluation of megakaryocytic alteration and their contribution to thrombocytopenia can provide knowledge to the pathogenesis of numerous hematopoietic disorders that may identify clinical applications of the newer strategies to regulate platelet count and functioning of platelets.

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