Original Article

A Study to See Alterations in Morphology of Megakaryocytes in Bone Marrow Aspiration in Cases of Thrombocytopenia

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ABSTRACT

The term thrombocytopenia is used when the platelet count is less than 150,000/microliter. It can be encountered in various hematological disorders i.e non-myelodysplastic hematological conditions to myelodysplastic syndrome.

Introduction: The present study was undertaken to calculate the prevalence of various conditions that are associated with thrombocytopenia and to see the alterations in megakaryocytes morphology in bone marrow in various cases of thrombocytopenia.

Methods: This study was a prospective series of 63 bone marrow aspiration conducted in Bir Hospital, NAMS in patients who presented with thrombocytopenia.

Statistical Analysis: The distribution of various morphological changes in cases on non-myelodysplastic conditions and myelodysplastic syndrome were compared using Chi-square test. A p-value less than 0.05 was considered significant.

Results: In the present study, the commonest cause for thrombocytopenia for which bone marrow examination was sought was megaloblastic anemia, followed by acute leukemia and mixed maturation.

In non-myelodysplastic condition such as immune thrombocytopenia showed immature megakaryocytes, bare megakaryocytes, emperipolesis and cytoplasmic vacuolations in the megakaryocytes. Similar morphological features was observed in myelodysplastic syndrome as well.

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

Keywords: Megakaryocytes, Thrombocytopenia, Bone marrow

Introduction

Platelets are formed and released into the bloodstream by precursors cells called megakaryocytes that are derived from the haemotopoietic stem cells, which evolve from the multipotential haemagioblast. Mature megakaryocytes give rise to circulating platelets by the acquisition of the cytoplasmic structural and functional characteristic necessary for platelet action[1], reaching cell sizes <50-100 microns in diameter and ploidy ranging up to 128 N.[2] The hallmarks of megakaryocytes maturation are endoreduplication and expansion of cytoplasmic mass.[3] For the release of thousands of platelets from single megakaryocytes, it requires an intricate series of remodeling events. So, any abnormalities in this process can give rise to clinically significant disorders. Various factors can contribute to abnormal platelet counts, the most common one being inappropriate platelet production. The term thrombocytopenia means platelet count less than 150,000/ microliter can give rise to

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inadequate clot formation and increased risk of bleeding.[4] The thrombocytopenia can be encountered in various non-myelodysplastic conditions and myelodysplastic syndromes.[5]

Various studies have highlighted the dysplastic morphology of megakaryocytes in thrombocytopenia associated with myelodysplastic syndrome. Myelodysplastic syndrome are a heterogenous group of clonal hematopoietic stem cell disorders characterized by cytopenia(s), dysplasia in one or more cell lineage, bone marrow failure and increased risk of transformation to acute myeloid leukemia.[6] Megakaryocytes alteration have also been documented in some bone marrow aspiration in non-myelodysplastic syndrome.

The present study was undertaken to calculate the prevalence of various conditions associated with thrombocytopenia and to assess the megakaryocytic alterations in various cases of thrombocytopenia. The alteration can be dysplastic and non-dysplastic. Statistical analysis was applied to see if there was a significant difference in megakaryocytic alteration existed in non-myelodysplastic and myelodysplastic conditions.

Methods

This was a prospective study of 63 cases of bone marrow aspiration of patient who presented with thrombocytopenia i.e platelet count less than 150,000/mm3 from November 2021 to October 2022 at Department of Pathology, National Academy of Medical Sciences (NAMS), Bir hospital, Kathmandu, Nepal. Written consent were obtained from all patients and confidentiality maintained throughout the study. Ethical approval was taken from IRB, NAMS.

The clinical details, including history, physical findings, complete blood counts, peripheral blood examination and other relevant laboratory investigations required were noted. The automated platelet count was further confirmed manually on peripheral blood smear which was stained in Leishmans' stain according to the guidelines in practical hematology. In subject with thrombocytopenia, the bone marrow aspirate from the posterior superior iliac spine were stained with Giemsa stain and examined under light microscope. (Olympus Pentahead BX53 model). Cases fulfilling the definition of thrombocytopenia but lacking marrow particle were excluded from the study.

The number of megakaryocytes was rated as following. Normal, if 1 megakaryocyte per 1 to 3 low power fields, increased, if more than 2 megakaryocytes per low power fields and decreased, if 1 megakaryocytes per 5-10 low power fields.[6]

Megakaryocytes morphology were studied with a 100X objective. Normal megakaryocytes have 4-16 nuclear lobes. Dysplastic features of megakaryocytes included multiple separated nuclei, micromegakaryocytes and hypogranular forms whereas non-dysplastic features included platelet budding, cytoplasmic vacuolation, immature forms, bare megakaryocytic nuclei, hypolobated forms, and emperipolesis. Micromegakaryocytes are defined as megakaryocytes whose size is similar to a large lymphocytes or monocytes and having single or bilobed nucleus whereas immature megakaryocytes are young megakaryocytes with scant bluish cytoplasm and devoid of nuclear lobe separation, occupying the most of the cell.[7] Hypogranular forms are megakaryocytes which have pale grey or water clear cytoplasm with sparse or no granules. The emperipolesis was considered depending on the engulfment of any one type of hematopoietic cells. The number and morphology of the megakaryocytes were assessed and documented. The data was analysed with SPSS version 16.

Results

The total number of 63 bone marrow aspirates cases that had thrombocytopenia were enrolled in this study. Male were 41 and female were 22. The ratio of male to female ratio 1.8:1.The peak age of presentation of thrombocytopenia was in 4th to 5th decade of life followed by 2nd to 3rd, 5th to 6th and 6th to 7th decade. The most common clinical features seen in case of thrombocytopenia was pancytopenia 40 cases, followed by bicytopenia 18 cases and thrombocytopenia 5 cases.

Table 1: Distribution of age in cases of thrombocytopenia

Age group in years	No. of cases	Cases in %
>15-24	05	07.93
25-34	09	14.28
35-44	07	11.11
45-54	17	27.0
55-64	09	14.28
65-74	10	15.87
75-84	06	09.52
>85	------	--------
Total	63	100

Table 2: Common causes for thrombocytopenia

BM/Clinical diagnosis	No. of cases	Percentage
Megaloblastic anemia	17	27.0
Acute Leukemia	09	14.2
Mixed maturation	08	12.69
Plasma cell myeloma	07	11.11
Hypocellular marrow	07	11.11
Myelodysplastic syndrome	03	04.76
Immune thrombocytopenia	03	04.76
Chronic lymphocytic leukemia	03	04.76
Chronic Myeloid Leukemia	03	04.76
Hairy Cell Leukemia	01	01.58
Hypersplenism	02	03.17

Megakaryocytes were normal in 4 cases of megaloblastic anemia and two cases each of multiple myeloma and hypocellular marrow. Decreased megakaryocytes were seen 13 cases of megaloblastic anemia followed by 8 cases of acute leukemia and 5 cases each of multiple myeloma and hypocellular marrow. Megakaryocytes were increased in 7 cases with mixed maturation and cases of immune thrombocytopenia. In 2 cases of hypersplenism observed, there were normal megakaryocytes.

Table 3: Number of Megakaryocytes in different causes of thrombocytopenia

Causes of thrombocytopenia	No. of Megakaryocytes/LPF					
	Normal (1MK/1-3LPF)		Increased >2MK/LPF		Decreased 1MK/5-10LPF	
	No.	%	No.	%	No.	%
Megaloblastic anemia	4	24	0	0	13	76
Acute leukemia	1	12	0	0	8	88
Mixed maturation	1	12	7	88	0	0
Plasma cell myeloma	2	28	0	0	5	72
Hypocellular marrow	2	28	0	0	5	72
Myelodysplastic syndrome	1	34	0	0	2	66
Immune thrombocytopenia	1	34	2	66	0	0
Chronic lymphocytic leukemia	1	34	0	0	2	66
Chronic Myeloid Leukemia	1	34	0	0	2	66
Hairy cell Leukemia	0	0	0	0	1	2.6
Hypersplenism	2	100	0	0	0	0
TOTAL	16		9		38	

In the present study, it was found that megakaryocytes were decreased in 76% and normal in 24% cases of megaloblastic anemia. Similarly, in acute leukemia, megakaryocytes were decreased in 88% and normal in 12 % cases. In cases of immune thrombocytopenia, increased megakaryocytes were seen in 66% and normal in 34%.

Cases with mixed maturation showed increased megakaryocytes in 88% and normal in 12% of cases.

Among the different morphological alterations of megakaryocytes in bone marrow, hypolobated forms were seen mainly in megaloblastic anemia i.e 14 cases, followed by acute leukemia, mixed maturation, multiple myeloma, chronic lymphocytic leukemia and chronic myeloid leukemia 3 cases in each. Nuclear lobe separation were seen in 8 cases of megaloblastic anemia followed by 3 cases each of mixed maturation and multiple myeloma and chronic lymphocytic leukemia.

Bare megakaryocytes were seen in 4 cases of megaloblastic anemia followed by 3 cases each of acute leukemia, immune thrombocytopenia and chronic myeloid leukemia. Immature forms were seen mainly in acute leukemia i.e 4 cases followed by Myelodysplastic syndrome and immune thrombocytopenia 3 cases each. Emperipolesis was seen in 2 cases of immune thrombocytopenia. Cytoplasmic vacuolization were seen in myelodysplastic syndrome and immune thrombocytopenia 2 cases each.

Table 4: Altered morphology of megakaryocytes for different causes of thrombocytopenia

	MA	AL	MM	MY	MDS	ITP	CLL	CML	HCL	HS	TOTAL
Hypolobated	14	3	3	3	1	1	3	3	2	2	35
Immature		4	2	1	3	3					13
Micromegakaryocyte						1		3			4
Hypogranular			2			1					3
Emperipolesis					1	2					3
Nuclear lobe separation	8	2	3	3		2	3		2	2	25
Platelet budding						1					1
Bare megakaryocyte nuclei	4	3	2	2	1	3		3			18
Cytoplasmic vacuolisation					2	2					4

(MA: Megaloblastic anemia, AL: Acute leukemia, MM: Mixed maturation, MDS: Mylodysplastic syndrome, ITP: Immune thrombocytopenia, CLL: Chronic lymphocytic leukemia, CML: Chronic myeloid leukemia, HCL: Hairy cell leukemia, HS: Hypersplenism)

Discussion

Thrombocytopenia is frequently encountered in complete blood count examination for which bone marrow examination is done to look for any alteration in megakaryocytes number and morphology. The bone marrow aspirate is stained using Giemsa stain to see any alteration in megakaryocytes number and morphology. This help to diagnose underlying hematological disorder.

In the present study, thrombocytopenia was seen in all age groups with a minimum age of 15 years to maximum age of 80 years. Male to female ratio is 1.8:1. This similar to study done by Vinayakamurthy et al.[7]Out of 63 cases of thrombocytopenia, maximum number of cases i.e 17 cases i.e 27% were of 4th to 5th decade of life. This was followed by 9 cases of 2nd to 3rd , 5th to 6th and 6th to 7th decade of life.

Thrombocytopenia was more common in male 43 cases and female 20 cases. The commonest cause of thrombocytopenia for which bone marrow examination was sought was megaloblastic anemia 17 cases (26.56%) followed by acute leukemia 9 cases [7] (14.06%). Our findings are similar to study done by Muhury et al.[5] who found acute leukemia the most common cause for thrombocytopenia which may be due to infiltration of the marrow by the leukemic cells.

Other causes were mixed maturation 8 cases (12.50%), plasma cell myeloma 7 cases (11.0%) and hypocellular marrow 7 cases (11%). Least cases were seen of hypersplenism 2 cases(3.1%) and Hairy cell leukemia 1 case (1.5%).

Among the varied morphological features observed, hypolobated megakaryocytes were in 40%, nuclear lobe separation in 32%, bare megakaryocyte nuclei in 22% cases of the megaloblastic anemia. Similarity exist in study observed by Wickramasinghe et al.[8] Similarly, acute leukemia cases showed immature megakaryocytes in 30.4%, followed by bare megakaryocytes 16.6% and nuclear lobe separation in 8% of cases.

Multiple myeloma showed hypogranular form in 66.66%, followed by immature form 15.3%, nuclear lobe separation in 12%, bare megakaryocyte nuclei 11.11% and hypolobated forms in 8.5%. Hypolobated form was observed in almost all cases like megaloblastic anemia, acute leukemia, multiple myeloma, mixed maturation, immune thrombocytopenia, leukemia and hypersplenism. This attributed to diminished DNA synthesis and increased ploidy leading to nuclear maturation defect.

Myelodysplastic syndrome also showed various alterations like cytoplasmic vacuolation in 50%, emperipolesis in 33%, hypolobated forms in 8.5%, immature form in 7.69% and bare megakaryocyte nuclei in 5.5%.

Cases of chronic myeloid leukemia also showed alterations in the form of micromegakaryocytes, hypolobated forms and bare megakaryocytic nuclei.

Megakaryocytes were normal in 4 cases of megaloblastic anemia and two cases each of multiple myeloma and hypocellular marrow. Decreased megakaryocytes were seen 13 cases of megaloblastic anemia followed by 8 cases of acute leukemia and 5 cases each of multiple myeloma and hypocellular marrow. Megakaryocytes were increased in 7 cases with mixed maturation i.e 88% and cases of immune thrombocytopenia i.e 66%.[9] Similar observation

was done by study conduected by Shi et al.[10] In 2 cases of hypersplenism, there were normal megakaryocytes. Similarly, in acute leukemia, megakaryocytes were decreased in 89% and normal in 12 % cases. In acute leukemia, megakaryocytes were decreased in 88% of cases, similar to Pokharel S et al[9] and Dameshek W et al.[11]

Different morphological alteration of megakaryocytes in the bone marrow due to myelodysplastic syndrome or non-myelodysplastic syndrome carries value and brings special interest in country like ours with limited resources as we lack facility for sophisticated megakaryocyte studies like culture, specific marker, electron microscope for ultrastructural details.

This study showed that the diagnostic accuracy for different causes of thrombocytopenia can be enhanced by correlating different alteration in megakaryocytes morphology and number that were observed in the bone marrow aspirate.

Conclusion

There are many similarities in morphological changes of megakaryocytes among different hematological diseases; never the less, the diagnostic approach will vary when detailed knowledge about morphological changes of megakaryocytes is available. In this study, increased megakaryocytes count and presence of bare megakaryocytic nuclei and hypolobated forms were found to be significant in immune thrombocytopenia. Understanding of different morphological changes of megakaryocytes in the bone marrow aspirates can improve the diagnostic accuracy for a wide range of hematological disorders there by enabling proper therapeutic interventions.

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