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MEGAKARYOCYTIC ALTERATIONS IN THROMBOCYTOPENIA IN BONE MARROW ASPIRATION AND BONE MARROW BIOPSY: A NOVEL INSIGHT EXPERIENCE IN NORTH INDIA

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ABSTRACT

Introduction: Thrombocytopenia is a common clinical entity that can be seen in any patient irrespective of age and have varied differential diagnosis and presents in various clinical diseases. Bone marrow study helps to differentiate and achieve a definite diagnosis with reasonable accuracy using minimally invasive technique. **Aims and objective:** a) To find out various conditions associated with thrombocytopenia and associated megakaryocytic changes. b) To study the megakaryocytic alterations in thrombocytopenia in the bone marrow aspiration and bone marrow biopsy and their relationship with underlying etiology. **Material and methods:** The study was conducted at our department of pathology at SMS Medical college from period between Jan 2018 to feb 2020. **Observation and results:** In this study of 74 cases, most common cause of thrombocytopenia found was MDS (33%) followed by MA (20%) and ITP (16%). 25 cases (40%) show dysplastic changes of megakaryocytes, majority of them seen in myelodysplastic syndrome (MDS). Hypolobated form were seen in 65% cases of thrombocytopenia which were mostly seen in MDS (77.7%). Among non dysplastic alterations immature form seen in 17% (11 cases),dwarf form were seen in 20% (13 cases), bare form were seen in 6% (4 cases). 4.5 % (3 cases) show budding. **Conclusion:** This study may be helpful in improving diagnostic accuracy in various hematopoietic disorder. As dysplastic megakaryocytes are equaly common in non MDS related thrombocytopenia so mere presence of dysplastic changes only should not be directly given diagnosis of myelodysplastic syndrome, other morphological findings, hematological parameters and complete clinical history is equally important to correlate.

KEYWORDS: MDS, Bone marrow study, Megakaryocyte, Thrombocytopenia, Dysplastic Changes.

INTRODUCTION

Megakaryocytes (MK) are the largest hematopoietic cell in the bone marrow with size ranging from 40 to 100µm. They are the precursor cells of anuclaeted platelets and are derived from haematopoietic stem cells (HSCs), evolving from the multipotential haemangioblast. The production of platelets by megakaryocytes requires an intricate series of remodelling events that result in the release of thousands of platelets from a single megakaryocyte.[1] Various stages of maturation are megakaryoblast, promegakaryocytes, granular megakaryocytes, mature megakaryocytes and release of platelets. The process of Megakaryopoiesis is regulated both by cell autonomous process, such as transcriptional regulation and by extracellular cues dominated by thrombopoietin (TPO) that binds to its receptor. Thrombopoietin is the primary signal for megakaryocyte production.[2] Abnormality in any of these stages of megakaryocytopoiesis may result in thrombocytopenia. Thrombocytopenia (platelet counts less than 150,000/µl) can lead to inadequate clot formation and increased risk of bleeding.[3] When the platelet counts are less than 30, 000/µl, patient suffers from spontaneous bruising and purpura or with continuous/relatively long-lasting bleeding from injuries and wounds. Clinically significant spontaneous bleeding does not usually occur until the platelet count is less than 10, 000/µl,. Reasons for thrombocytopenia may be categorised into following subheadings: a) Decreased production of platelets b) Increased destruction of platelets and c) Changing of distribution of platelets.[4] Immune thrombocytopenic purpura (ITP), megaloblastic anemia, aplastic anemia and leukemia are common causes of thrombocytopenia.[5]

Thrombocytopenia may also be a presenting feature in various hematological disorders like acute myeloid leukemia, chronic lymphocytic leukemia, aplastic anemia, myelodysplastic syndrome, multiple myeloma. There are various megakaryocytic alterations in bone

marrow aspirates known as dysmegakaryocytopoiesis and it includes both dysplastic and non-dysplastic features. Dysplastic features includes multiple separated nuclei, micromegakaryocytes and hypogranular forms. Non dysplastic features are immature forms, emperipolesis, budding (blebbing of cytoplasm on their surface), cytoplasmic vacuolization and bare nuclei.[1] There are many studies highlighting the dysplastic morphology of megakaryocytes in thrombocytopenia associated with myelodysplastic syndromes (MDS) and non MDS conditions.[6,7]

Examination of Bone marrow helps to differentiate cases due to decreased platelet production from cases due to peripheral platelet destruction, it also helps to differentiate the number, size and maturity of the megakaryocytes. Aim of bone marrow examination is to achieve a definite diagnosis with reasonable accuracy using a minimally invasive technique.

The present study was undertaken to calculate the prevalence of various hematopathological conditions causing thrombocytopenia and to evaluate the megakaryocytic alterations in various cases of thrombocytopenia taking in account findings noted in both BMA as well as biopsy (BMB).

AIM AND OBJECTIVE

To evaluate the megakaryocytic alterations in the bone marrow aspiration and biopsy in cases of thrombocytopenia and their relationship with underlying etiology and to find out the proportion of various conditions associated with thrombocytopenia and associated megakaryocytic changes.

MATERIAL AND METHOD

This was a cross sectional hospital based prospective study done in SMS medical college, Jaipur from January 2018 to feb 2020. A Total of 74 patients were evaluated those presenting with thrombocytopenia, written informed consent was obtained from all patients, relevant clinical data were obtained using a pro form then further workup was done with bone marrow aspirate and bone marrow biopsy. All patient who fulfilled inclusion criteria for diagnosis of thrombocytopenia with platelet count less than 150000/µl were included for study. The exclusion criteria were Cases of pseudo thrombocytopenia (platelet aggregation), Bleeding disorder other than thrombocytopenia, Inadequate material on bone marrow aspiration and bone marrow biopsy, patient who refused for bone marrow aspiration and bone marrow biopsy. Bone marrow aspiration were performed using Salah needle and Bone marrow biopsy were performed using Jamshadi needle. Both bone marrow aspiration and Bone marrow biopsy were done from posterior superior iliac spine under standard aseptic conditions. The bone marrow smears were stained with May Grunwald Giemsa (MGG) and Leishman's stain and examined. The core biopsy obtained was overnight fixed with 10 % buffered formalin then decalcified in 15 % EDTA solution. Then afterwards taken up for processing; 2-3 micron sections and were stained by hematoxylin and eosin and other special stains wherever required.

The number and morphology of the megakaryocytes were studied. The numbering was expressed as number per 10 low‑power field (LPF) and was further subdivided into absent, decreased (1/5–10 LPF), normal (1/1–3 LPF), and increased (>2/LPF).[6] Morphological alterations that were studied include both dysplastic and nondysplastic features, dysplastic features included multiple separated nuclei, micro megakaryocytes (size similar to that of large lymphocytes or monocytes and have single or bilobed nuclei), and hypogranular forms(a clear cytoplasm with no or sparse granules). Nondysplastic features included immature forms (young megakaryocytes lacking nuclear lobulation and have scant basophilic cytoplasm), emperipolesis, platelet budding, cytoplasmic vacuolization, and bare megakaryocyte nuclei.[8]

OBSERVATIONS AND RESULTS

Of the total of 74 cases studied of thrombocytopenia, it was found more common in males with a male:female ratio of 2.5:1 with 53 Male (71.6%) and 21 females (28.4%)[Graph 1]. The age group found most common was of age group of 11–20 years (21.6%, 16 cases) and least number of cases were in the age group of 51–60 years(8%, 6 cases)[Graph2].

Various causes of thrombocytopenia with frequency of occurence are shown in [Table 1].

The most common cause for thrombocytopenia in our study was Myelodysplastic syndrome(MDS) (32.4%) followed by megaloblastic anemia (21.6%) and ITP (14.86%). [Table 2] shows average number of megakaryocytes per 10 LPFs in bone marrow aspiration. There was an increase in number of megakaryocytes in BMA smears in cases of idiopathic thrombocytopenic purpura(ITP) (66.67%) [Figure 1]. Decreased numbers of megakaryocytes were seen in Megaloblastic anemia (40%) followed by myelodysplastic syndrome and Dimorphic anemia (30% each). Megakaryocytes were absent in Acute leukemia, acute and chronic lymphocytic leukemia.

Both dysplastic and nondysplastic morphological features of megakaryocytes were studied as shown in [Tables 3,4 and Figure 2]. The most common dysplastic features observed were multiple seprated nuclei and micromegakaryocytes. Nuclear segmentation was observed in 18 cases of MDS and 5 cases of megaloblastic anemia followed by 2 cases of ITP Micromegakaryocytes were seen in 11 cases of MDS, 3 cases of ITP and 1 case of Megaloblastic anemia. Hypogranular forms were seen in 2 cases of ITP and 1 each in dimorphic anemia and MDS.

Among nondysplastic features, the most common were hypolobation, dwarf form, immature forms, emperipolesis, cytoplasmic budding, cytoplasmic vacuolation and bare nuclei. Immature megakaryocytes were seen in 6 cases of ITP, 2 cases of megaloblastic anemia and MDS each. Cytoplasmic budding on the surface of megakaryocytes was noted in 3 cases of MDS and 1 case of ITP. Dwarf form were seen in 6 cases of Megaloblastic anemia, 5 cases of MDS and 2 cases of ITP. Bare nuclei were another feature encountered in total of 5 cases of thrombocytopenia with 2 case each of ITP and MDS. Hypolobated forms were seen in 19 cases of MDS, 9 cases of megaloblastic anemia, 8 cases of ITP, 3 cases of Dimorphic anemia, 1 case each of AML, Chronic lymphoproliferative neoplasm and plasma cell dyscrasia. Emperipolesis was observed in only 1 case of MDS. Also cytoplasmic vacuolation was seen in 1 case of ITP.

Table 1: Various causes of Thrombocytopenia in the study.

Sr. No.	Disease	No. of cases	Percentage (%)
1.	Myelodysplastic syndrome	24	32.4
2.	Megaloblastic anemia	16	21.6
3.	Idiopathic thrombocytopenic purpura	11	14.86
4.	Dimorphic anemia	9	12.2
5.	Acute leukemia	6	8.1
6.	Acute myeloid leukemia	3	4
7.	Acute lymphocytic leukemia	2	2.7
8.	Chronic lymphocytic leukemia	1	1.35
9.	Chronic Lympho proliferative neoplasm	1	1.35
10.	Plasma cell dyscrasia	1	1.35
11.	Total	74	100

Table: 2 Distributions of no. of megakaryocytes in various hematological conditions with thrombocytopenia in present study (Number per low power field).

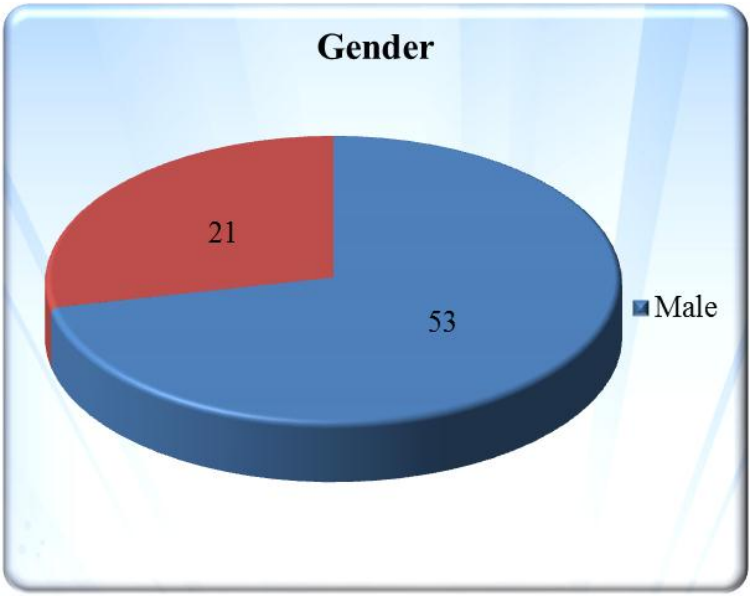
Sr. No.	Etiology of thrombocytopenia	No. of cases	Normal	Increased	Decreased	Absent
1.	Myelodysplastic syndrome	24	19	2	3	0
2.	Megaloblastic anemia	16	10	2	4	0
3.	Idiopathic thrombocytopenic purpura	11	3	8	0	0
4.	Dimorphic anemia	9	5	0	3	1
5.	Acute leukemia	6	0	0	0	6
6.	Acute myeloid leukemia	3	3	0	0	0
7.	Acute lymphocytic leukemia	2	0	0	0	2
8.	Chronic lymphocytic leukemia	1	0	0	0	1
9.	Chronic Lympho-proliferative neoplasm	1	0	0	1	0
10.	Plasma cell dyscrasia	1	1	0	0	0
11.	Total	74				

Table 3: Dysplastic changes in Megakaryocyte in various causes of thrombocytopenia.

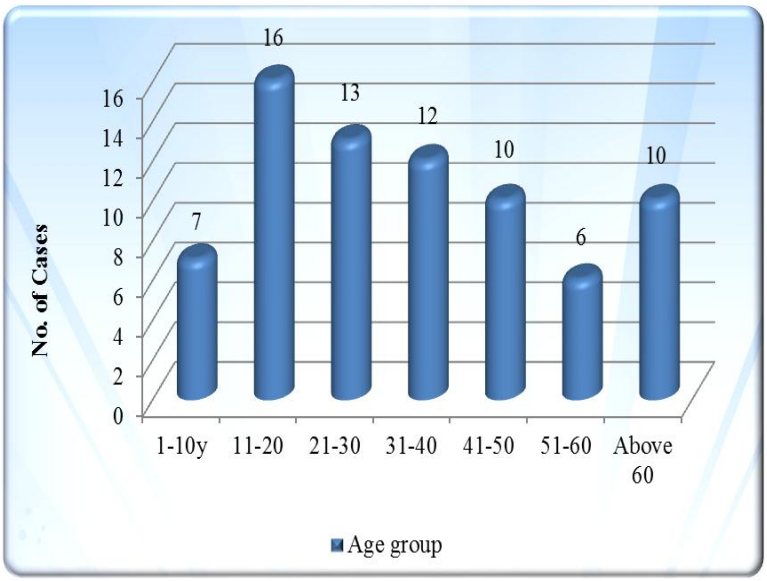
Sr. No.	Disease	No. of cases	Micromega.	Multiple separate nuclei	Hypergranular Form	Hypogranular Form
1.	Myelodysplastic syndrome	24	11	18	1	1
2.	Megaloblastic anemia	16	1	5	0	0
3.	Idiopathic thrombocytopenic purpura	11	3	2	1	1
4.	Dimorphic anemia	9	0	0	1	2
5.	Acute leukemia	6	0	0	0	0
6.	Acute myeloid leukemia	3	0	0	0	0
7.	Acute lymphocytic leukemia	2	0	0	0	0
8.	Chronic lymphocytic leukemia	1	0	0	0	0
9.	Chronic Lympho-proliferative neoplasm	1	0	0	0	0
10.	Plasma cell dyscrasia	1	0	0	0	0
11.	Total	74	15	25	3	4

Table 4: Non dysplastic changes in]Megakaryocyte in various causes of thrombocytopenia.

Sr. No.	Disease	No. of cases	Hypolobated	Dwarf Form	Bare Form	Budding	Emperipolesis	Cytoplasmic Vacuolation	Immature Form
1.	Myelodysplastic syndrome	24	19	5	2	3	1	0	2
2.	Megaloblastic anemia	16	9	6	1	0	0	0	2
3.	Idiopathic thrombocytopenic purpura	11	8	2	2	1	0	1	6
4.	Dimorphic anemia	9	3	0	0	0	0	0	0
5.	Acute leukemia	6	0	0	0	0	0	0	0
6.	Acute myeloid leukemia	3	1	0	0	0	0	0	1
7.	Acute lymphocytic leukemia	2	0	0	0	0	0	0	0
8.	Chronic lymphocytic leukemia	1	0	0	0	0	0	0	0
9.	Chronic Lympho-proliferative neoplasm	1	1	0	0	0	0	0	1
10.	Plasma cell dyscrasia	1	1	0	0	0	0	0	0
11.	Total	74	42	13	5	4	1	1	12



Graph 1: Gender Distribution.



Graph 2: Age group distribution in present study.

Figure legend 1.

a) Bone Marrow aspiration showing megakaryocytic clusters in case of idiopathic thrombocytopenic purpura. (10x40)

b) Bone Marrow biopsy showing increased number of megakaryocytes and megakaryocytic clustering in case of idiopathic thrombocytopenic purpura. (10x40)

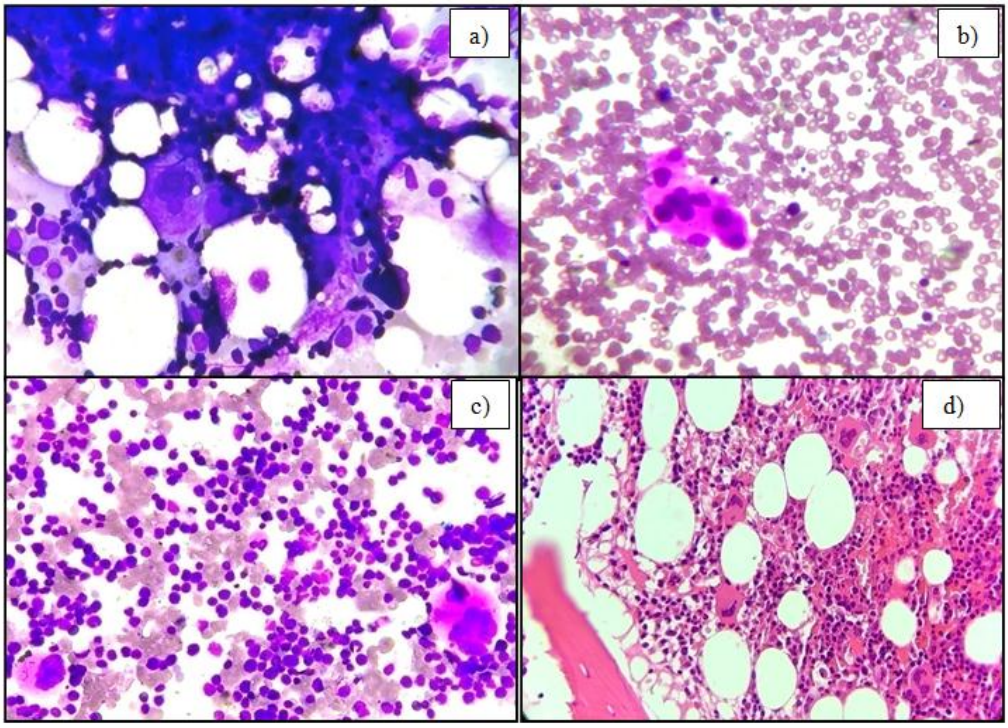


Figure legend 2.

a) Bone Marrow aspiration showing nuclear budding in megakaryocytes in case of idiopathic thrombocytopenic purpura. (10x40)

b) Bone Marrow aspiration showing megakaryocytes with multiple separate nuclei in case of myelodysplastic syndrome. (10x40)

c) Bone Marrow aspiration showing hypolobated megakaryocytes in background of erythroid and cells of myeloid series in megaloblastic anemia. (10x40)

d) Bone Marrow biopsy showing hypolobated and Dysplastic Megakaryocytes in case of myelodysplastic syndrome. (10x10)

DISCUSSION

Thrombocytopenia is the most common hematological disorder with varied differential diagnosis for which bone marrow aspiration is indicated. This may be an isolated finding or associated with pancytopenia refractory to treatment.[9] Thrombocytopenia is commonly seen in various hematological disorders including myelodysplastic syndromes (MDS) as well as various non-myelodysplastic hematological conditions.[6] Dysplastic changes are well known in megakaryocytes in thrombocytopenia associated with MDS. However, they may also be observed in megakaryocytes in non-myelodysplastic hematological conditions.

In present study, total of 74 cases of thrombocytopenia were included in which 53 cases (71.6%) were observed in male and 21 cases (28.4%) were female with M:F ratio of 2.5:1 and male predominance similarly in the studies done by Vinayakamurthy et al[10] and Choudhary et al[8] with male predominance. In this study most common age group studied was of 11-20 years (21.6%; 16 cases) and least common age group 51-60years (8%, 6 cases). In contrast a study by Vinayakamurthy et al[10] found age group 30-39 years as most common. In a study by Muhuryet al[6], less than 10 year was most common age group. Less than 20 years age group were found in study by Pokharelet al.[9]

In both study by Muhury[6] et al and Vinayakamurthyet al[10], which included only non-MDS cases MDS cases in thrombocytopenia were excluded in their study.

In present study, the most common cause of thrombocytopenia found was MDS (32.4%) followed by MA (21.6%) and ITP (14.86%). In contrast the most common cause of thrombocytopenia was megaloblastic anemia found in other studies by Choudhary et al[8], Das et al[11] and Deepika et al.[12] Bhasin et al[13] found, dimorphic anemia as most common cause of thrombocytopenia. ITP was most common cause found

in a study done by Gupta et al.[1] Acute leukemia was second leading cause of thrombocytopenia in a study by Muhury et al.[6] In the present study, Megaloblastic anemia was second common cause of thrombocytopenia similar in the study done by Gupta et al[1] and Tirumalasetti et al.[7] In present study, cellularity of bone marrow was normal in 65% cases followed by hypercellularity in 27% and hypocellularity in 8% in contrast to 9%, 79% and 12%, respectively in a study by Deepika et al.[12] In current study, increased numbers of megakaryocytes were seen in 12 cases (16.2%) out of 74 cases majority seen in ITP, comparable to a study by Muhuryet al[6] and Deepika et al[12] where percent increase was 31 % and 16% respectively. Decreased numbers of megakaryocytes were seen in 13.5 % in contrast to a study by Muhuryet al[6] and Deepika et al[12] which was 25 % and 31 % respectively. Megakaryocytes were absent in 13.5 % of cases of thrombocytopenia in present study, majority in acute leukemia reason may be due to infiltration of bone marrow by leukemic cells leading to decreased production, also Chemotherapy, radiotherapy, and immune-mediated destruction of megakaryocytes also result in decreased megakaryocytes. This in contrast to other studies where this percentage was 14% and 24% as by Deepika et al[12] and Muhury et al[6] respectively.

Dysplastic changes in megakaryocytes were appreciated in MDS, megaloblastic anemia, ITP and Dimorphic anemia. The most common dysplastic change observed was multiple separate nuclei observed in 25 cases (33.7%) followed by micro megakaryocyte in 15 cases (20.3%). Hypo granular forms were seen in 4 cases (5.4%). Similarly, Tirumalasetti et al[7] observed micro megakaryocytes in 25 cases (32.0%), multiple separate nuclei in 17 cases (21.7%) and hypo granular form was the least observed dysplastic feature seen in 7 cases (8.9%). Hypolobated form, Dwarf form and Immature forms were the most common nondysplastic features seen in 42(56.7%) majority in MDS, 13 cases (17.5%) and 12 cases (16.2%) respectively. Bare form were seen in 6.7% (5 cases), budding was seen in 5.4 % (4 cases). Emperipolesis was seen in only one case of thrombocytopenia caused by MDS. The finding of Cytoplasmic vacuolization was seen in one case of ITP. Study done by Muhury M et al[6] observed bare nuclei as the most common nondysplastic finding which was seen in 67 cases (46.5%). This was followed by immature form in 58 cases (40.2%), emperipolesis in 45 cases (31.2%), cytoplasmic vacuolization in 21 cases (14.6%) and platelet budding in 17 cases (12.2%). Therefore, this study shows that dysplastic changes of megakaryocytes are found both in MDS as well as non-MDS related thrombocytopenia. Hence, presence of dysplastic changes in megakaryocytes alone should not lead to diagnosis of MDS and other hematological conditions should also be considered in differential diagnosis. All the findings noted in bone marrow aspiration were corroborated with finding in bone marrow biopsy.

CONCLUSION

There were many similarities in morphological changes of megakaryocytes observed in various hematological conditions. Hence diagnostic accuracy for different causes of thrombocytopenia can be increased by understanding the these morphological changes and correlating the findings with patient's clinical findings and other hematological parameters. This helps further improve therapeutic interventions. Also further studies on the evaluation of megakaryocytic alteration and their contribution to thrombocytopenia can provide growing knowledge to the pathogenesis of numerous hematopoietic disorders.

LIMITATIONS

This study was done in limited number of cases and with limited time duration, each case should be correlated with patient's clinical findings and other hematological parameters which aids in making diagnosis, Further, cytogenetics and molecular studies whenever needed can help in evaluation of patients in regard to prognosis and determination of clonality.

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