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STUDY OF MEGAKARYOCYTES IN BONE MARROW ASPIRATION SMEARS IN PATIENTS WITH THROMBOCYTOPENIA

Pathology

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ABSTRACT

Context: Thrombocytopenia can result in insufficient clot formation and an elevated risk of bleeding. Bone marrow aspiration is frequently done for this purpose. Thrombocytopenia related to myelodysplastic diseases is known to be accompanied by dysplastic changes in megakaryocytes (MDS). Nevertheless, in non-myelodysplastic conditions, they are seen in megakaryocytes. **Aim:** The purpose of this study was to determine the incidence of various diseases related with thrombocytopenia and to document megakaryocytic changes in various cases of thrombocytopenia. **Settings and Designs:** An Observational hospital-based study **Materials and Methods:** 100 bone marrow aspirations were performed in a prospective series in a tertiary care facility in central India that serves both urban and rural residents. **Results:** Acute leukaemia (28/100) predominated among the 100 cases of thrombocytopenia included in this study, which also included cases of dimorphic anaemia, megaloblastic anaemia, hypocellular marrow, infection, and idiopathic thrombocytopenic purpura. In 35% of patients, dysplastic alterations to the megakaryocytes were found. Multiple seperated nuclei, a characteristic of ITP, were the most prevalent dysplastic trait seen (77.77% cases of ITP). Megakaryocytes showed non-dysplastic alterations in 65% of the cases. **Conclusion:** In the era of molecular diagnosis, understanding the morphological changes of megakaryocytes in bone marrow aspirates is equally important and can improve diagnostic accuracy for wide range of haematological disorders, and also allowing for appropriate therapeutic interventions.

KEYWORDS

Bone-marrow aspiration, Dysplastic changes, Megakaryocytes, Non-dysplastic changes, Thrombocytopenia

INTRODUCTION-

Megakaryocytes are rare myeloid cells (constituting less than 1%) that reside in the bone marrow primarily. Additionally, they are located in the blood and lungs. During early development, megakaryopoiesis occurs within the foetal liver and yolk sac before marrow cavities grown sufficiently to sustain blood cell growth. Function of megakaryocytes is solely to produce and release platelets into the circulation[1].

Mature megakaryocytes give rise to circulating platelets by the acquisition of the cytoplasmic structural and functional characteristics necessary for platelet action [2,3], reaching cell sizes <50-100 microns in diameter. Hallmarks of megakaryocytes maturation are endoreduplication and expansion of cytoplasmic mass. The cytoplasm of the megakaryocyte undergoes a significant remodelling during the last stages of development to form proplatelets, which are beaded extensions of the cytoplasm. Megakaryocytes produce platelets through a succession of remodelling processes that cause the release of thousands of platelets from a single megakaryocyte. It has been estimated that each megakaryocyte can give rise to 1000 to 5000 platelets[4]. Any abnormality in this process can result in significant disorders clinically. A diversity of factors can contribute to anomalous platelets 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 [5]. It is a common indication for bone marrow aspiration. Dysplastic alterations are well documented in megakaryocytes in thrombocytopenia associated with myelodysplastic syndromes (MDS). However, in non-myelodysplastic disorders, they are also seen in the megakaryocytes.

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-cytoplasmic ratio) and hypo-granular forms (with little or almost no granules). Non dysplastic features of megakaryocytes include immature forms (with basophilic cytoplasm, high nuclear- cytoplasmic ratio and no nuclear lobation), emperipolesis (intact hematopoietic cells within cytoplasm), cytoplasmic budding, vacuolization and bare nuclei without cytoplasm [6].

In order to improve diagnostic precision, this study aims to better understand dysplastic megakaryocytic change and how it contributes to thrombocytopenia in non-myelodysplastic hematological disorders. Because MDS was not present during the study period, it was not possible to examine various megakaryocytic changes between MDS and non MDS disorders. This study was therefore limited to examining the frequency of different megakaryocytic changes in thrombocytopenia not caused by MDS.

MATERIALS AND METHODS-

The study was a hospital based prospective observational study during January 2019 to June 2020. All the cases of thrombocytopenia which were which were diagnosed on haematology analyser (platelet counts <1,50,000/µL); confirmed subsequently by peripheral smear with/without bleeding manifestations due to thrombocytopenia were taken up for the study while patients with bleeding manifestations other than thrombocytopenia, those patients with thrombocytopenia where bone marrow aspiration is not indicated and non-co-operative patients were excluded from this study. For each case, a signed consent was obtained. All necessary clinical information was gathered. Then, for every case, a bone marrow aspiration was carried out using all aseptic techniques. The bone marrow aspiration smears were then stained with Leishman stain.

The bone marrow smears were examined as per standard guideline and findings were documented. Number of megakaryocyte was expressed as the number per 10 low power fields (LPFs) and were further subdivided in to absent (0/10 LPFs), decreased (1/5-10LPFs), normal (1/1-3 LPFs) and increased (>2/LPF) [7]. At least 30 megakaryocytes were evaluated for megakaryocytic alterations including both dysplastic features (multiple separate nuclei, micro-megakaryocyte and hypo-granular forms) and nondysplastic features (emperipolesis, immature, bare nuclei, cytoplasmic vacuolization and budding). Dysplastic alterations were considered significant only when 10% or more of the megakaryocyte observed showed the changes. Study was approved by ethical committee of the institution.

RESULTS-

Hematological diseases can affect both children and adults on a very broad spectrum. In order to make the final diagnosis, a bone marrow

examination is a valuable test. One of the most common and generally very safe invasive procedures performed regularly in hospitals. Even in cases of severe thrombocytopenia, an invasive surgery can be easily carried out. Commonly it is done for the evaluation of unexplained cytopenia and leukemia and diagnosing storage disorders, also at times for staging of leukemias/ other malignancies.

Acute leukaemia (28 cases/100) was the most prevalent reason for bone marrow aspiration, followed by dimorphic anaemia (15 cases/100), megaloblastic anaemia (11 cases/100), hypocellular marrow, infection, and other causes. Immune thrombocytopenic purpura 9 cases each, granulomatous disease 4 cases each, chronic myeloid leukaemia 3 cases each, acute promyelocytic leukaemia, myelofibrosis, MPD 2 cases each, Parvovirus infection, Hemophagocytic lympho-histiocytosis, Plasma cell leukaemia, Gaucher's disease, Solitary plasmacytoma, Smoldering myelom Males (57%) were more likely to have thrombocytopenia than females (43%) throughout a wide age range in the 100 cases studied.

Table I- Alteration In Number Of Megakaryocytes In Various Disorders:

Name of disease	Number of cases	Megakaryocytes number			
		Normal	Increased	Decreased	Absent
Acute leukemia	28	03	-	17	08
Dimorphic anemia	15	07	05	03	-
Megaloblastic anemia	11	08	02	01	-
Immune thrombocytopenic purpura	9	-	09	-	-
Reactive to infection	9	04	02	03	-
Hypocellular marrow	9	-	-	05	04
Granulomatous disease	4	02	-	02	-
Chronic myeloid leukemia	3	01	-	02	-
Myelofibrosis	2	-	-	02	-
MPD	2	01	01	-	-

Table I shows average number of megakaryocytes per 10 LPF's in bone marrow aspiration. 100% cases of ITP had increased number of megakaryocytes followed by 33% cases of dimorphic anemia.

Table II shows morphological alteration in megakaryocytes in various hematological disorders. Dysplastic changes in megakaryocytes were observed in 35% cases. Most common dysplastic feature observed were multiple separate nuclei (26%) commonly observed in ITP (77.77%). Multiple separated nuclei was most commonly seen in ITP followed by dimorphic anemia while micro-megakaryocytes were most commonly seen in ITP followed by dimorphic anemia and Hypogranular form was most commonly observed in infection.

Non-dysplastic changes in megakaryocytes were observed in 65% cases. Immature form was most commonly seen in ITP whereas Emperipolesis was seen in all cases of ITP. Again, Cytoplasmic vacuolation was most commonly seen in ITP followed by dimorphic anemia while smooth borders, bare nuclei and hypolobated form was most commonly seen in ITP.

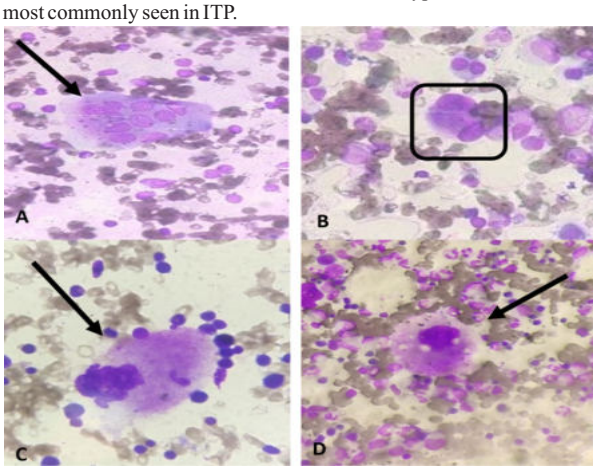

Fig I Shows Morphological Changes Of Megakaryocytes In Bone Marrow Aspirate.

Fig I- A. Bone marrow aspirate shows multiple separated nuclei of megakaryocytes.

B. Micromegakaryocyte seen in bone marrow aspirate.

C. Emperipolesis observed in bone marrow aspirate

D. Bone marrow aspirate shows cytoplasmic vacuolation.

DISCUSSION-

In our study, Megakaryocytes are most commonly increased in ITP (100% cases) which is similar to the study of Muhury et al[6] (94.87% cases), Bhasin et al[7] (100% cases), Choudhary PK et al[8] (91%) ,

Table II- Prevalence of megakaryocytic changes in various hematological disorders:

Name of disease	No. of cases	Multiple separated nuclei	Micro-megakaryocytes	Hypo-granular from	Immature form	Emperipolesis	Cytoplasmic vacuolation	Smooth borders	Bare nuclei	Dysmorphic form	Hypo-lobated from
Acute leukemia	28	2	-	-	-	-	-	-	-	-	-
Dimorphic anemia	15	8	4	1	2	3	6	4	3	1	7
Megaloblastic anemia	11	3	3	1	-	3	1	2	3	1	6
Immune thrombocytopenic purpura	9	6	4	2	5	9	7	4	4	2	6
Reactive to infection	9	2	2	3	-	2	1	2	-	-	1
Hypocellular marrow	9	-	1	-	-	1	-	1	1	-	-
Granulomatous disease	4	1	-	-	-	1	-	-	-	-	-
Chronic myeloid leukemia	3	-	1	-	-	1	-	-	1	-	-
Myelofibrosis	2	-	2	-	2	-	-	2	-	2	2
MPD	2	1	1	1	-	-	-	-	-	-	-

Gupta et al[9] (82.3%), Vinayakmurthy et al[10] (80%), Sandeep et al[11] (100%). This could be due to stimulation of the marrow megakaryocytes to synthesize platelets at an increased rate as there is immune mediated platelet destruction in the spleen and other reticulo-endothelial tissues [8]. Decreased megakaryocytes were most commonly seen in our study in acute leukemia (25 cases, 89.3%) followed by hypocellular marrow (9 cases, 55.5%) which was similar to Muhury et al[6], Bhasin et al[7], Choudhary PK et al[8], Pokheral et al[12] and Sandeep et al[11] while in contrast to our study, Gupta et al[9] found megaloblastic anemia to be the common cause of decreased megakaryocytes.

The occurrence of decreased megakaryocytes in acute leukemia may be due to infiltration of bone marrow by leukemic cells affecting their production or chemotherapy/radiotherapy/immune-mediated destruction of megakaryocytes and the decreased/absent megakaryocytes in cases of aplastic anemia may be due to BM suppression/ nucleic acid synthesis defects[8] .

Dysplastic megakaryocytes were characterised as those with single/ multiple separate nuclei. Megakaryocytes that were smaller than a large lymphocyte or monocyte and had a single or bilobed nucleus were referred to as micro-megakaryocytes. The megakaryocytes were considered to demonstrate platelet budding if there was budding of cytoplasmic processes from their surfaces. Megakaryocytes with scant or no granules and pale grey or water clear cytoplasm were referred to as hypo-granular types. There is also evidence of the type of cell observed within the megakaryocyte during emperipolesis. In dysplastic changes, multiple separated nuclei was most commonly shown in ITP (6 cases, 66.67%) followed by dimorphic anemia (8 cases, 53.33%) which was similar to Murphy et al[6], and Vinayakmurthy et al[10]. Choudhary et al[8] found megaloblastic anemia to be the most common cause of multiple separated nuclei and attribute the cause of multiple separated nuclei to diminished and ineffective DNA synthesis leading to nuclear maturation defect. Most common cause of micro-megakaryocytes was ITP (44.45%) which was similar to Vinayakmurthy et al[10] while in contrast to our study Muhury et al[6] and Bhasin et al[7] found MDS to be the main cause of micro-megakaryocytes. Interestingly Lee EJ et al found no patient with micro-megakaryocyte associated with good prognosis chromosomal abnormalities[13]. In a study done by Jinnai et al, dysplastic megakaryocyte (micromegakaryocyte and multiple separate nuclei) was found in 10% of the cases of AML. They also found significantly lower response to chemotherapy in AML cases with dysplastic megakaryocytes[14].

Dys-megakaryocytopoiesis in the form of micro-megakaryocyte, multiple separate nuclei and hypogranular forms have been known as characteristic feature of myelodysplastic syndrome and have been shown in some studies to be an independent prognostic factor. Hypo-granular form was least evident (3/9 cases) dysplastic feature which was observed in this study. Various studies proposed micro-megakaryocytes as the most diagnostic feature of myelodysplasia in MDS.

A marked shift to young, immature megakaryocytes was marked morphological feature noted in 55.5% cases of ITP cases. Similar findings were also observed by Muhury M et al[6]. Houwerzijl et al[15] attributed this to the apoptotic and para-apoptotic type of programmed cell death (PCD) of mature megakaryocytes. Inappropriate PCD of adult megakaryocytes, they claim, can compromise platelet production, resulting in apoptosis-like PCD (para-apoptosis) and thrombocytopenia in ITP. Immature megakaryocytes frequently resemble micro-megakaryocytes, which can lead to the misdiagnosis of myelodysplastic syndrome presenting with isolated thrombocytopenia and so imitating ITP.

The antiplatelet autoantibodies to glycoprotein IIb/IIIa and Ib/IX seen in ITP interfere with platelet production and release by causing megakaryocyte destruction and abnormal maturation[6].

In our study, emperipolesis was a striking feature in ITP shown by 100% cases which was similar to Muhury et al[6], Bhasin et al[7], Choudhary PK et al[8], Vinayakamurthy et al[10], while Sandeep et al[11] found megaloblastic anemia to be the main cause of emperipolesis.

Tavossoli M et al and Sabebekhitiari HA et al postulated that in the state of heightened demand for cell delivery from marrow into the circulation, some cells take a transmegakaryocytic route to enter the circulation causing the phenomenon of emperipolesis and thus suggested megakaryocytes as a component of the marrow-blood barrier[16]. They further attributed emperipolesis to blood loss and thus recommended to investigate the patient for unsuspected blood loss.

In our study, cytoplasmic vacuolization seen in 7 case (77.78%) of ITP which was similar to Muhury et al[6], Choudhary PK et al[8] and Gupta et al[9] According to Houwerzij et al[15] cytoplasmic vacuolation in megakaryocytes ultrastructurally represents mitochondrial swelling and this reflects an increased megakaryocyte turnover. Cytoplasmic vacuolization might also indicate degenerative changes such as those of apoptosis and of para-apoptosis. Another possible explanation for this according to Muhury M et al[6] is autophagy to maintain cell metabolism when there is increased metabolic demand and nutrition deficiency due to increased megakaryocytopoiesis or it might be a way of sequestration and degradation of specific pathogens. All the above features suggested dysmegakaryocytopoiesis which largely contributes to diminished platelet production.

As being a tertiary care centre and situated at the capital of the state, it covers the usual population surrounding which is usually urban, sub urban and near peripheral area, but there is a huge periphery which still remains unscreened under this study. Also, this study had a relatively smaller sample size, the results may vary with a large study group. Further morphological alteration and quanititative alterations suggested by our study should not be taken as conclusive and need to be properly validated with a large study group and follow-ups of the patients. Lastly, occurrence of the COVID-19 pandemic proved to be a hindrance as there was limited inflow of patients during the study period.

An attempt is made in this study to elucidate the numerical and morphological changes in megakaryocytes. Morphologic changes in megakaryocytes seen in MDS are also seen in various non MDS conditions which should be considered during diagnostic evaluation. Dysplastic megakaryocytes alone should never be used to diagnose myelodysplastic syndromes; instead, they should always be compared to the patient's clinical data and other haematological indicators. Additional research on the assessment of megakaryocytic change and their role to thrombocytopenia can shed light on the pathophysiology of many haematological illnesses and could reveal more clinical uses for the more recent techniques to control platelet count and function.

Leading causes of thrombocytopenia in our study found was acute leukemia which on early diagnosis can get better treatment followed by dimorphic anemia which can be treated with minimum expenditure when diagnosed correctly.

Bone marrow examination is a very important tool in diagnosis and helps in management of hematological disorders. Bone marrow aspiration turns out to be a very simple and safe investigation which could be repeated if needed and carried in outpatients as well.

Dysplastic megakaryocytes were more frequently found in ITP, megaloblastic anaemia, and dimorphic anaemia in haematological diseases. The criterion for dysplasia in megakaryocytes should be increased from the suggested 10% due to the high prevalence of dysplastic megakaryocytes in non-MDS haematological diseases. To comprehend the importance of the presence of dysplastic megakaryocytes in thrombocytopenia unrelated to MDS, additional comparative research involving instances of MDS must be conducted. Our's is a small study group, resulting a lack of statistical power, therefore further studies with large cohort preferably multicentric, are needed along with proper follow-up to explore the role of megakaryocytes in bone marrow aspiration in patients of thrombocytopenia along with its correlation with morphological details.

CONCLUSION

Dyplastic megakaryocytes were more prevalent in hematological illnesses such as ITP, megaloblastic anemia, and dimorphic anemia. Because dysplastic megakaryocytes are so widespread in non-MDS hematological illnesses, the threshold for dysplasia in megakaryocytes should be increased from the suggested 10%. However, additional

comparative research with MDS patients is needed to understand the relevance of the presence of dysplastic megakaryocytes in non-MDS associated thrombocytopenia.

REFERENCES:

1. Patel SR, Hartwig JH & Italiano JE. The biogenesis of platelets from megakaryocyte proplatelets. J Clin Invest. 2005;115(12):3348–54.
2. Richardson JL, Shivdasani RA, Boers C, Hartwig JH, Italiano Jr JE. Mechanisms of organelle transport and capture along proplatelets during platelet production. Blood. 2005 Dec 15;106(13):4066-75.
3. Tomer A, Harker LA & Burstein SA. Purification of human megakaryocytes by fluorescence-activated cell sorting. Blood. (1987): 70, 1735–42.
4. Greer JP, Arber DA, Glader BE, List AF, Means RM, Rodgers GM. Wintrobe's clinical hematology. Lippincott Williams & Wilkins; 2018 Nov 19.
5. McKenzie SB, editor. Textbook of hematology. 2nd ed. Pennsylvania. Willaims and Wilkins; 1996.
6. Muhury M, Mathai AM, Rai S, Naik R, Pai MR, Sinha R. Megakaryocytic alterations in thrombocytopenia: a bone marrow aspiration study. Indian J Pathol Microbiol 2009;52(4):490–04.
7. Bhasin TS, Sonam S, Mridu M et al. Changes of megakaryocytes in cases of thrombocytopenia: Bone marrow aspiration & biopsy analysis. J Clin Diagn Res. Mar 2013; 7(3): 473-479.
8. Choudhary PK, Sing SK, Basnet RB: Study of megakaryocytes in patients with thrombocytopenia. J of Pathology of Nepal (2013);(3):476-481.
9. Gupta P, Goswami A, Chavda J, Goswami N, Shah S. Study of megakaryocytes in bone marrow aspiration smears in patients with thrombocytopenia. IOSR J of dental and medical sciences. June 2015; 14(6): 30-33.
10. Vinayakamurthy S, Potluri R, Shivajirao P, Singh R, Pujahari R, Maniketh I. A study of megakaryocyte morphology in bone marrow aspiration smears of cases of thrombocytopenia. Medical journal of Dr,D.Y.Patil university, 2017;(10):51-57
11. Sandeep, Veerpaneni and Dr. Radhika C Sasturkar. "Megakaryocytic Alterations in Thrombocytopenia: A Bone Marrow Aspiration Study." (2020).
12. Pokharel S, Upadhyaya P, Karki S, Paudyal P, Pradhan B, Poudal P. Megakaryocytic alterations in thrombocytopenia: A bone marrow aspiration study. Journal of pathology of Nepal, 2016;6:914-921
13. Lee EJ SC, Tomiyasu T, Testa JR. Clinical and cytogenetic correlations of abnormal megakaryocytopoiesis in patients with acute leukemia and chronic myelogenous leukemia in blast crisis. Leukemia 1990;4:350-3
14. Jinnai I, Tomonaga M, Kuriyama K et al. Dysmegakaryocytopoiesis in acute leukaemias: its predominance in myelomonocytic (M4) leukaemia and implication for poor response to chemotherapy. Br J Haematol 1987;66:467-72.
15. Houwerzijl EJ, Blom NR, van der Want J. Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. Blood. 2004;103(2):500-6.
16. Tavassoli M. Modulation of megakaryocyte emperipolesis by phlebotomy, megakaryocytes as a component of marrow blood barrier. Blood cells 1986;(12):2015-16.