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Megakaryocytic Alterations in Thrombocytopenia: A Bone Marrow Aspiration Study

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Abstract: ***Background: Thrombocytopenia is defined as platelet count less than 150,000 /mm^3 (150x10^9 /litre) and is commonly encountered in various haematological disorders. It can either be isolated or seen in association with bicytopenia or pancytopenia at most instances it requires bone marrow study which can be helpful in differentiating the hypoproliferative causes from peripheral destruction of platelets. Dysplastic changes in Megakaryocytes are well studied in thrombocytopenia cases associated with MDS. Many non-myelodysplastic haematological conditions can also cause thrombocytopenia and they too can cause dyspastic changes in megakaryocytes. Objective: This study is undertaken for better understanding of dysplastic megakaryocytic alterations and their contribution to thrombocytopenia in non-myelodysplastic haematological conditions so as to increase diagnostic accuracy. Materials and Methods: A prospective study. Total of 79 cases were studied. Patients presenting with platelet count <150,000/mm3 from June 2018 to July 2019 at S nijalingappa medical college, Bagalkot, Karnataka were included in the study. Results: The most common cause of thrombocytopenia was megaloblastic anemia followed by acute leukemia, chronic leukemia, systemic metastasis and ITP. Both dysplastic and nondysplastic features were observed in the above-mentioned conditions. The most common dysplastic feature was nuclear segmentation followed by micromegakaryocytes and hypogranular forms. Among nondysplastic features, the most common were immature forms, bare nuclei, and hypolobation. Emperipolesis and cytoplasmic vacuoles were less common. Conclusion: Dysplastic megakaryocytes are common in non-MDS-related thrombocytopenia and their mere presence should not lead to the diagnosis of MDS. Hence, proper study of megakaryocyte morphology along with patients clinical and haematological details can improve the diagnostic accuracy in cases of thrombocytopenia.***

Keywords: Megakaryocyte, Bone marrow aspiration, dysplastic features, nonmyelodysplastic syndrome, thrombocytopenia.

1. Introduction

Thrombocytopenia is defined as platelet count less than 150,000/ mm^3. It is a very common hematological presentation; bone marrow aspiration study is often required to find out the underlying cause in these cases. Patients usually present with features of bleeding tendencies such as petechiae, ecchymosis, epistaxis and gum bleeding. The cause can be either hematological or nonhematological in origin. The most common hematological conditions for thrombocytopenia are megaloblastic anemia, aplastic anemia, ITP, leukemia, multiple myeloma, bone marrow metastasis, etc. [1]

Dysmegakaryocytopoiesis is characterized by various megakaryocytic alterations in bone marrow aspirates and include both dysplastic and non-dysplastic features. Dysplastic changes are well known in thrombocytopenia associated with MDS. However these changes are also observed in non myelodysplastic conditions as well. [2]

This study was conducted for better understanding of the dysplastic megakaryocytic changes and their contributions to the thrombocytopenia in non myelodysplastic conditions and so as to increase the diagnostic accuracy.

2. Materials and methods

This was a prospective study. Total of 79 cases were studied. Patients presenting with platelet count <150,000/mm3 from June 2018 to July 2019 at S nijalingappa medical college, Bagalkot, Karnataka were included in the study. Written informed consent was obtained from all the patients. Relevant clinical information was obtained. Patients platelet count was obtained initially by automated analyzer(Pentra ES 60- Horiba) and it is later confirmed manually by preparing a peripheral smear. The smears were prepared for all cases to look for any evidence of pseudothrombocytopenia such as platelet agglutination, satellitism, and phagocytosis of the platelets by other cells. [1]

Cases with proven pseudothrombocytopenia on peripheral smear and cases with inadequate bone marrow aspiration material were excluded from the study. Bone marrow aspiration was done under standard aseptic conditions from posterior superior iliac spine and smears were stained with Leishman's stain. [3] The BMA smears were examined (using Olympus CH20i model) as per the standard guidelines and the findings were documented and analysed.

The features that were studied were number and morphology of the megakaryocytes of the each case. The number of megakaryocytes was expressed as number per 10 low-power field (LPF) and was further categorised into absent, decreased (1/5–10 LPF), normal (1/1–3 LPF), and increased (>2/LPF).

Morphological features of megakaryocytes were studied under 100X, for dysplastic and nondysplastic changes. Megakaryocytes were said to be showing dysplastic changes if there is evidence of nuclear segmentation, micromegakaryocytes and hypogranular forms. And nondysplastic changes included Immature froms, bare nuclei, emperipolesis, platelet budding and hypolobated forms.[1] Presence of any of the above changes is considered

as Dysmegakaryocytopoiesis. Normal megakaryocytes are considered to have 4-16 nuclear lobes. Immature megakaryocytes are defined as young megakaryocytes with scant intense basophilic cytoplasm, nucleus lacking lobulation and occupying most of the cell. Dysplastic megakaryocytes are defined as those with multiple segmented nuclei. Micromegakaryocytes are identified as megakaryocytes with size that of a large lymphocyte/monocyte and which has a single or bilobed nucleus. The megakaryocytes are considered to show platelet budding if there is evidence of budding of cytoplasmic processes from their surfaces. Hypogranular form megakaryocytes are those with pale grey to water clear cytoplasm and sparse to no granules. Emperipolesis is said to be seen if there is an another undegenerated cell in cytoplasm of megakaryocyte, the type of cell was also documented. The number and morphological characteristics of the megakaryocytes in thrombocytopenia cases was documented and then assessed.

3. Results

Among the 79 cases thrombocytopenia was slightly more common in males compared to females with M:F ratio 1.7:1. Thrombocytopenia was seen in all age groups but most of the cases were between age of 40-49 yrs (26.5%) (Table 1). Most common clinical presentation was superficial bleeding (28.6%) followed by anemia(19.3%) and weakness(11.7%).

As shown in the table no.2 most common cause of the thrombocytopenia is megaloblastic anemia (29.1%) followed by acute leukemia (21.5%) and metastasis (11.3%).

Distribution of megakaryocytes in various cases was studied as shown in table no.3. Increase in the number of megakaryocyte in BMA was observed in 17 cases (21.5%) which included cases of ITP, megaloblastic anmeia , Iron deficiency anemia and in a case of myelofibrosis. 29 cases (36.7%) showed reduced in the number of megakaryocytes including acute leukemias, metastasis, chronic leukemias, aplastic anemia, MDS and myelofibrosis. Megakaryocytes were completely absent in 13(16.4%) cases and in 20 cases (25.3%) count was normal.

Table no. 4 and 5 shows both dysplastic changes and nondysplastic changes observed in various haematological disorders. Common dysplastic features seen were multiple segmented nuclei (fig 2), micromegakaryocytes and hypogranular forms. Among the three findings multiple segmented mekaryocytes were much more common and seen in 36 cases (45.5%) including 16 cases of megaloblastic anemia and 6 cases of acute leukemias. Micromegakaryocytes were encountered in 31 cases (39.2%) including 8 cases of megaloblastic anemia, 6 cases of chronic leukemias and 5 cases of acute leukemias. Hypogranular forms were seen in cases of ITP and megaloblastic anemia 6 cases each.

Non dysplastic changes were observed in all cases. Commonly encountered features were immature forms of megakaryocytes, bare nuclei, nuclear budding (fig 3)emperipolesis (fig 4) and hypolobulated froms (fig 1). Immature forms of megakaryocytes were common finding among the nondysplastic changes which was seen in 46 cases (58.2%) including megaloblastic anemia, acute leukemias, ITP and chronic leukemias. Bare nuclei was another common finding which was seen in 32 cases (40.5%) including in 9 cases of megaloblastic anemia, 7 cases of acute leukemias and in 5 cases of ITP, Emperipolesis was comparatively not a common finding it was observed among 11 cases (14.4%).

Minimum of 30 megakaryocytes were evaluated on BMA smears, and dysplastic alterations were considered significant only when 10% or more of megakaryocytes observed show the changes. [1]

4. Discussion

In present study a total of 79 cases of true thrombocytopenia were included. The most common cause of thrombocytopenia was megaloblastic anemia (29.1%) followed by acute leukemia (21.5%) and ITP (10.1%). In contrast the most common cause of thrombocytopenia in a study performed by Muhury M et al [2] was AML(18.8%) followed by ITP (13.15%) and ALL (12.5%). In our study thrombocytopenia was most commoly seen in 40-49 years (26.5%) followed by 50-59 years of age group. In contrast Muhury M et al found it most common in children less than 10 years of age followed by that in 21-30 years of age group. In our study, thrombocytopenia was more common in males (58.2%). This was similar to that observed by Muhury et al. [2] and by Parul et al. [4]

Out of 8 cases of ITP, all 8 (100%) showed increase in number of megakaryocytes in BMA, the average being 58 per 10 LPFs. Shi XD et al had also found increased megakaryocyte number in 98% of the cases of ITP.[5] According to them 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.

In present study 21.7% of cases of megaloblastic anemia showed an increase in a number of megakaryocytes. This corresponds to a study done by Parul et al ., where 58.3% cases showed an increase in a number of megakaryocytes. [4] Megakaryocytes were also found to be increased in 75% cases of iron-deficiency anemia (IDA). This could be explained by the experimental study done on rat which has shown hypoxia-induced factors 1, 2 sub-unit (HIF-22) and vascular endothelial growth factor (VEGF) to be increased in rats and cultural supernatants.

The average megakaryocyte count per 10 LPFs in acute leukemia and aplastic anemia were decreased in 88.2% and 100% of the cases respectively which was also found by other studies as well. Decreased megakaryocyte number according to them is attributed to significant bone marrow suppression, inhibition of DNA synthesis and ineffective proliferation in these cases.

In our study, dysplastic megakaryocytes were appreciated in megaloblastic anemia, ITP, iron deficiency anemia and in cases of acute and chronic leukemias. The most common

dysplastic feature was multiple segmented nuclei seen in 75% cases of iron deficiency anemia, 69.5% cases of megaloblastic anemia, 35.2% cases of acute leukemias and 22.2% cases of chronic leukemias. This study corresponds to Murthy et al., where they found dysplastic features in 89.5% cases. He found multiple segmented nuclei in 15.2% cases and micro megakaryocytes in 61% cases. Wickramasinghe also found that the most common dysplastic feature was multiple separated nuclei and it is due to diminished and ineffective DNA synthesis and increased ploidy causing nuclear maturation defect. [6]

Among the nondysplastic features, immature and hypolobulated megakaryocytes were the most common and seen in 75% of cases of ITP. Similar findings were also observed by Muhury M et al.[2] Houwerzijl et al attributed this to the apoptotic and para-apoptotic type of programmed cell death (PCD) of mature megakaryocytes. According to their study inappropriate PCD of mature megakaryocytes can disrupt in platelet formation causing apoptosis-like PCD (para-apoptosis) leading to thrombocytopenia in cases of ITP. [7] The immature forms of megakaryocyte often resemble micromegakaryocyte which may mislead to an interpretation of myelodysplastic syndrome presenting with isolated thrombocytopenia, thus mimicking ITP. Das R et al therefore suggested the marker CD 61 can be used in detection and quantification of megakaryocyte including the dysplastic micromegakaryocyte.[8]

Our study also showed most of the megaloblastic anemia cases displaying dysplastic features. 69.5% of cases showed nuclear segmentation and 34.7% cases showed micromegakaryocytes and 26% cases showed hypogranular forms. This could be due to the diminished DNA synthesis leading to nuclear maturation defect. Wickramasinghe also found that the most common dysplastic feature was multiple segmented nuclei and attributed it to diminished and ineffective DNA synthesis leading to maturation defect.[6] Ashalatha et al., also found 100% cases of megaloblastic anemia with dysplastic features. [9]

Among nondysplastic features in megaloblastic anemia cases most common feature was immature forms (65.2%), bare nuclei (65.2%), followed by hypolobated forms (39.1%) and emperipolesis (26%). The reason for such nondysplastic features according to Wang et al., is due to impaired DNA synthesis and methylation caused by folate and Vitamin B12 deficiency. [10]

Dysplastic features were also documented in cases of acute leukemia. Nuclear segmentation (35.2%) was the commonest finding, followed by micromegakaryocytes (29.4%) and hypogranular forms (17.6%). Lee et al. found that 59.4% of cases of acute leukemia showed micromegakaryocytes. They also noticed that these patients were associated with bad prognosis. [11]

Nondysplastic features commonly encountered in cases of acute leukemia were immature forms (47%), bare nuclei (47%), hypolobation (41%), budding (23.5%) and emperipolesis (11.7%) Brandt et al., had found, using banding technique in cases of AML, an isochromosome 17 was found in granulopoietic, erythropoietic cells, and megakaryocytes. He also found that most megakaryocytes were diploid and the polyploidy was not normally developed. Polyploidy results in defective differentiation of the megakaryocytes and their precursors.[12]

Dyspastic and non dysplastic changes were also commonly encountered in cases of chromic leukemia. Among the dysplastic changes most common being micromegakaryocytes (66.6%) followed by multiple segmented megakaryocytes (22.7%). And among the nondysplastic changes immature forms were most common (66.6%) followed by bare nuclei (22.2%) and hypolobulated forms (22.2%).

Megakaryocytic dysplasia was also a common finding in cases of other systemic malignancies without bone marrow involvement. In present study, we had 9 cases of other systemic malignancies comprising of non-Hodgkin's lymphomas, breast malignancies, lung malignancies, and gastrointestinal malignancies.

Out of 4 cases of aplastic anemia two cases showed dysplastic changes, multiple segmented nucleus was seen in 1 case (25%) and micromegakaryocyte was seen in 1 case (25%). Similar findings were seen in Muhury M et al found dysplastic change in megakaryocyte only in 12.5% of the cases. And among the non dysplastic changes 3 cases (75%) showed immature forms and one case (25%) showed hypolobulated forms.

We have come across 4 cases of iron-deficiency anemia and 75% of cases showed multiple segmented nuclei, 50% showed micromegakaryocytes. Evstatiev et al have studied the effect of iron deficiency on megakaryopoiesis in experimental animals and found that iron deficiency inhibits proliferation but increases ploidy in megakaryocytic cell lines. This may affect the normal maturation process. Among the nondysplastic features in IDA, immature forms were observed in 50% cases and hypolobated forms in 25% of cases. [13]

We have encountered 3 cases of myelofibrosis. Out of which 2 cases (66.6%) showed multiple segmented nuclei, micromegakaryocytes and hypogranular forms. Nondysplastic changes were not a common finding in these cases only changes seen are Immature forms in one case (33.3%) and budding in one case (33.3%).

Two cases of MDS were studied which showed both dysplastic and nondysplastic features such as nuclear segmentation, immature forms, hypo granular forms, and budding.

Dysplastic features represent megakaryocytes that are abnormal and have lost their ability to undergo proper differentiation and have impaired nuclear development.[14] Hence, as our study shows that dysplastic changes in megakaryocytes were also found in non-MDS-related thrombocytopenia and their presence alone does not qualify for the diagnosis of MDS. So the possibility of other hematological conditions should be considered and should be investigated for.

5. Conclusion

As our study suggests dysplastic features were observed in all the common causes of thrombocytopenia such as megaloblastic anemia, ITP, acute leukemia, chronic leukemia. Hence, we conclude that mere presence of dysplastic features should not lead to diagnosis of MDS, but other differential diagnoses should be considered.

Table 1: Age distribution

S.No	Age group	No. of cases(%)
1	0-9	3(3.7%)
2	10-19	8(10.1%)
3	20-29	9(11.3%)
4	30-39	11(13.9%)
5	40-49	21(26.5%)
6	50-59	12(15.1%)
7	60-69	8(10.1%)
8	70-79	4(5%)
9	80-89	3(3.7%)
Total		**79(100%)**

Table 2: Causes of thrombocytopenia

S.No	Bone marrow impression	No of cases (%)
1	Megaloblastic anemia	23(29.1%)
2	Acute leukemias	17(21.5%)
3	Metastasis	9(11.3%)
4	Chronic leukemias	9(11.3%)
5	ITP	8(10.1%)
6	Aplastic anemia	4(5%)
7	Iron deficiency anemia	4(5%)
8	Myelofibrosis	3(3.7%)
9	MDS	2(2.5%)
Total		**79(100%)**

Table 3: Distribution of megakaryocytes among the cases

S.No	Bonemarrow impression	No. of cases	Absent (%)	Reduced (%)	Normal (%)	Increased(%)
1	Megaloblastic anemia	23	-	05(21.7%)	13(56.5%)	05(21.7%)
2	Acute leukemia	17	06(35.2%)	09(52.9%)	02(11.1%)	-
3	Metastasis	9	03(33.3%)	05(55.5%)	1(11.1%)	-
4	Chronic leukemia	9	01(11.1%)	6(66.6%)	2(22.2%)	-
5	ITP	8	-	-	-	8(100%)
6	Aplastic anmia	4	3(75%)	1(25%)	-	-
7	Iron deficiency anemia	4	-	-	1(25%)	3(75%)
8	Myelofibrosis	3	-	1(33.3%)	1(33.3%)	1(33.3%)
9	MDS	2	-	2(100%)	-	-
Total		79	13	29	20	17

Table 4: Dysplastic features in megakaryocytes in various cases

S.No	Bonemarrow impression	No. of cases	Multiple segmented(%)	Micromegakaryocytes(%)	Hypogranular froms(%)
1	Megaloblastic anemia	23	16(69.5%)	8(34.7%)	6(26%)
2	Acute leukemia	17	6(35.2%)	5(29.4%)	3(17.6%)
3	Metastasis	9	3(33.3%)	2(22.2%)	2(22.2%)
4	Chronic leukemia	9	2(22.2%)	6(66.6%)	4(44.4%)
5	ITP	8	2(25%)	4(50%)	6(75%)
6	Aplastic anemia	4	1(25%)	1(25%)	-
7	Iron deficiency anemia	4	3(75%)	2(50%)	1(25%)
8	Myelofibrosis	3	2(66.6%)	1(33.3%)	1(33.3%)
9	MDS	2	1(50%)	2(100%)	1(50%)
Total		**79**	**36**	**31**	**24**

Table 5: Nondysplastic features in megakaryocytes in various cases

S.no	Bonemarrow Impression	No. of cases	Immature forms	Bare nuclei	Budding	Emeripolesis	Hypolobulation
1	Megaloblastic anemia	23	15(65.2%)	15(62.5%)	11(47.8%)	6(26%)	9(39.1%)
2	Acute leukemia	17	8(47%)	8(47%)	4(23.5%)	2(11.7%)	7(41.1%)
3	Metastasis	9	4(44.4%)	4(44.4%)	5(55.5%)	1(11.1%)	5(55.5%)
4	Chronic leukemia	9	6(66.6%)	2(22.2%)	1(11.1%)	-	2(22.2%)
5	ITP	8	6(75%)	3(37.5%)	6(75%)	2(25%)	5(62.5%)
6	Aplastic anemia	4	3(75%)	-	-	-	1(25%)
7	Iron deficiency anemia	4	2(50%)	-	1(25%)	-	1(25%)
8	Myelofibrosis	3	1(33.3%)	-	1(33.3%)	-	-
9	MDS	2	1(50%)	-	1(50%)	-	1(50%)
Total		**79**	**46**	**32**	**30**	**11**	**31**

References

[1] Choudhary PK, Singh SK, Basnet RB. Study of megakaryocytes in bone marrow aspiration smears in patients with thrombocytopenia. J Pathol Nepal 2013;3:476-81.
[2] 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;52:490-4
[3] Lewis SM, Bain BJ, Bates I. Bone marrow biopsy. In: Practical Hematology. 10th ed. Philadelphia: Churchill Livingstone; 2006. p. 116-20.
[4] Parul G, Alpeshpuri G, Jitendra C, Nutanbala G, Shaila S. Study of megakaryocytes in bone marrow aspiration

smears in patients with thrombocytopenia. IOSR JDMS 2015;14:30-3.
[5] Shi XD HT, Feng YL, Liu R, Li JH, Chen J, Wang TY. A study on micromegakaryocytes in children with idiopathic thrombocytopenic purpura. Chin J Paediatr 2004;42:192-5.
[6] Wickramasinghe SN. Morphology, biology and biochemistry of cobalamin-and folate-deficient bone marrow cells. Baillieres Clin Haematol 1995;8:441-59.
[7] Houwerzijl EJ, Blom NR, van der Want JJ, Esselink MT, Koornstra JJ, Smit JW, et al. Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. Blood 2004;103:500-6.
[8] Das R, Hayer J, Dey P, Garewal G. Comparative study of myelodysplastic syndromes and normal bone marrow biopsies with conventional staining and immunocytochemistry. Anal Quant Cytol Histol 2005;27:152-6.
[9] Ashalatha N, Netravathi P, Ragupathi A, Nagarajappa A. Hemogram and bone marrow morphology in cases of pancytopenia. . The Internet Journal of Laboratory Medicine. 2010 4(2) [cited 25 June 2013]. Available from :http://archive.ispub.com/journal/the-internetjournal-of-laboratory-medicine/volume-4-number-2/hemogram-and bone-marrow-morphology-in-cases-of-pancytopenia.html#sthash. SMuztQJe.dpbs. [Last cited 2013 Jun 25].
[10] Wang C, Ran JY, Liu L. Ultra structural study of megakaryocytes in megaloblastic anaemia. Chin J Internal Med 1990;29:158-60.
[11] 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.
[12] Brandt L, Levan G, Mitelman F, Sjogren U. Defective differentiation of megakaryocytes in acute myeloid leukemia. Acta Med Scand 1974;196:227-30.
[13] Evstatiev R, Bukaty A, Jimenez K, Kulnigg-Dabsch S, Surman L, Schmid W, et al. Iron deficiency alters megakaryopoiesis and platelet phenotype independent of thrombopoietin. Am J Hematol 2014;89:524-9.
[14] Houwerzijl EJ, Blom NR, van der Want JJ, Esselink MT, Koornstra JJ, Smit JW, et al. Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. Blood 2004;103:500-6.

Figures

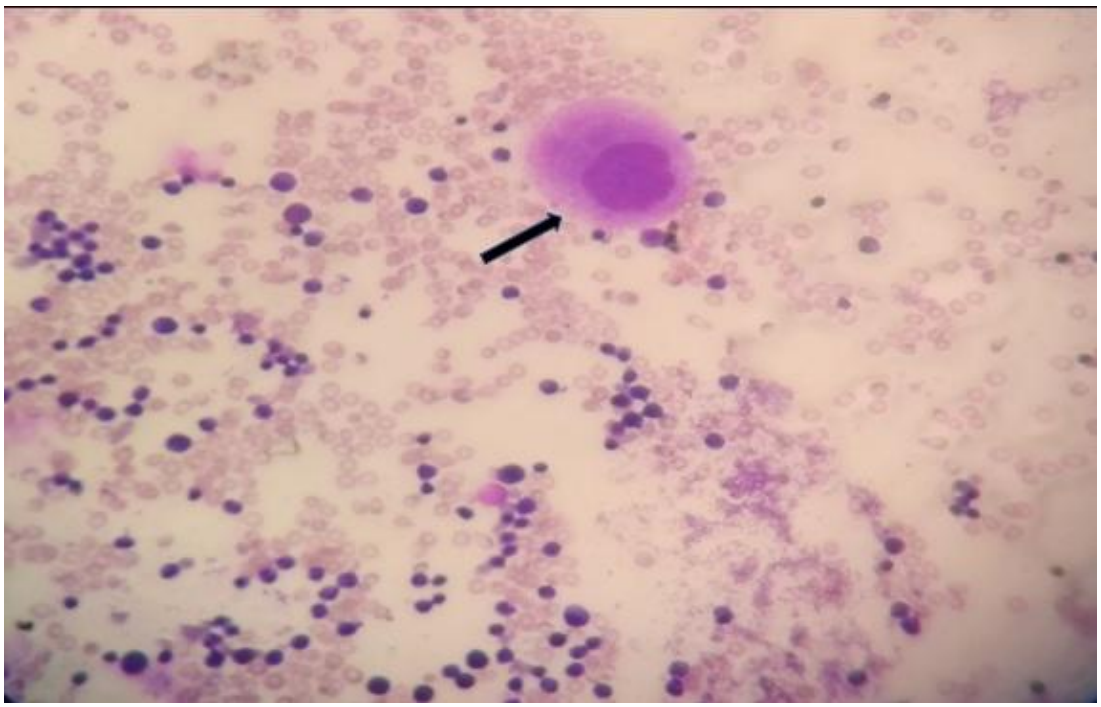

Figure 1: BMA showing Hypolobulated Megakaryocyte; background showing erythroid and myeloid precursors (Giemsa, 100x).

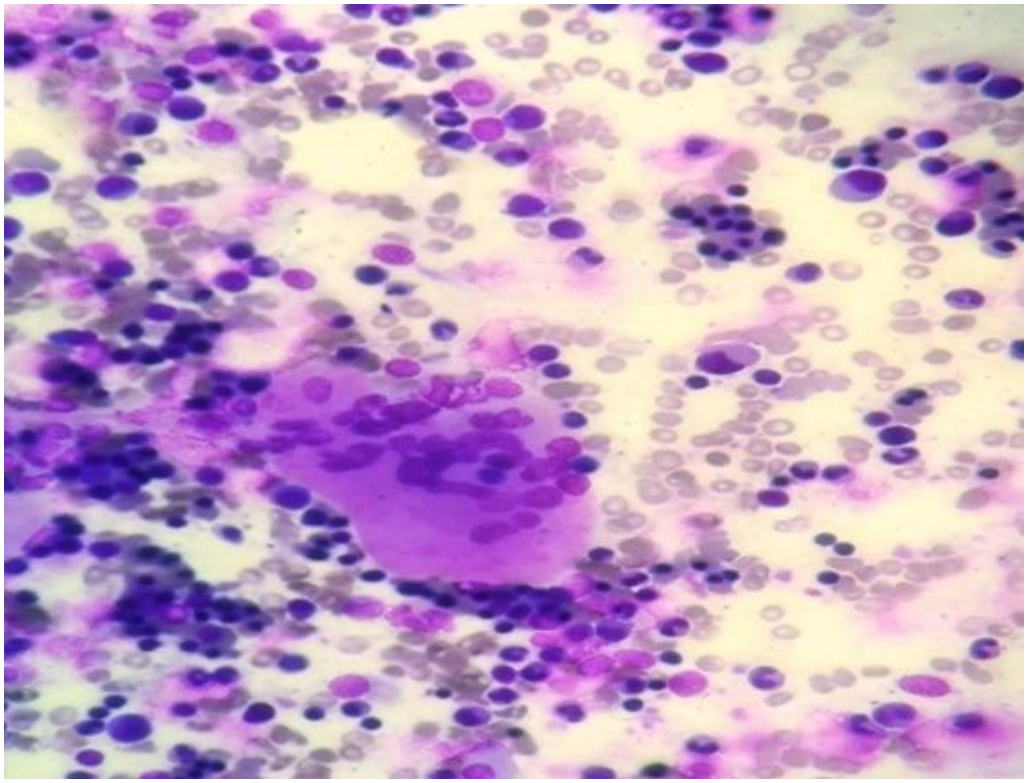

Figure 2: BMA showing dysplastic change-Multiple separate nuclei. (Giemsa, 100x)

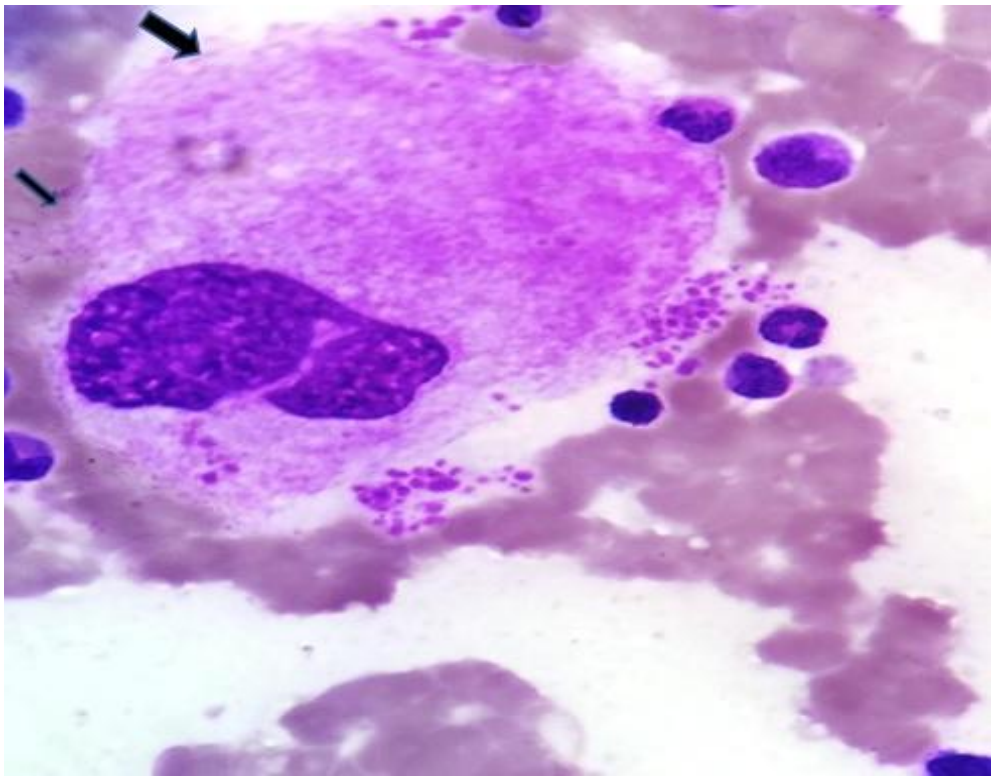

Figure 3: BMA-Megakaryocyte showing cytoplasmic budding (Giemsa, 100x)

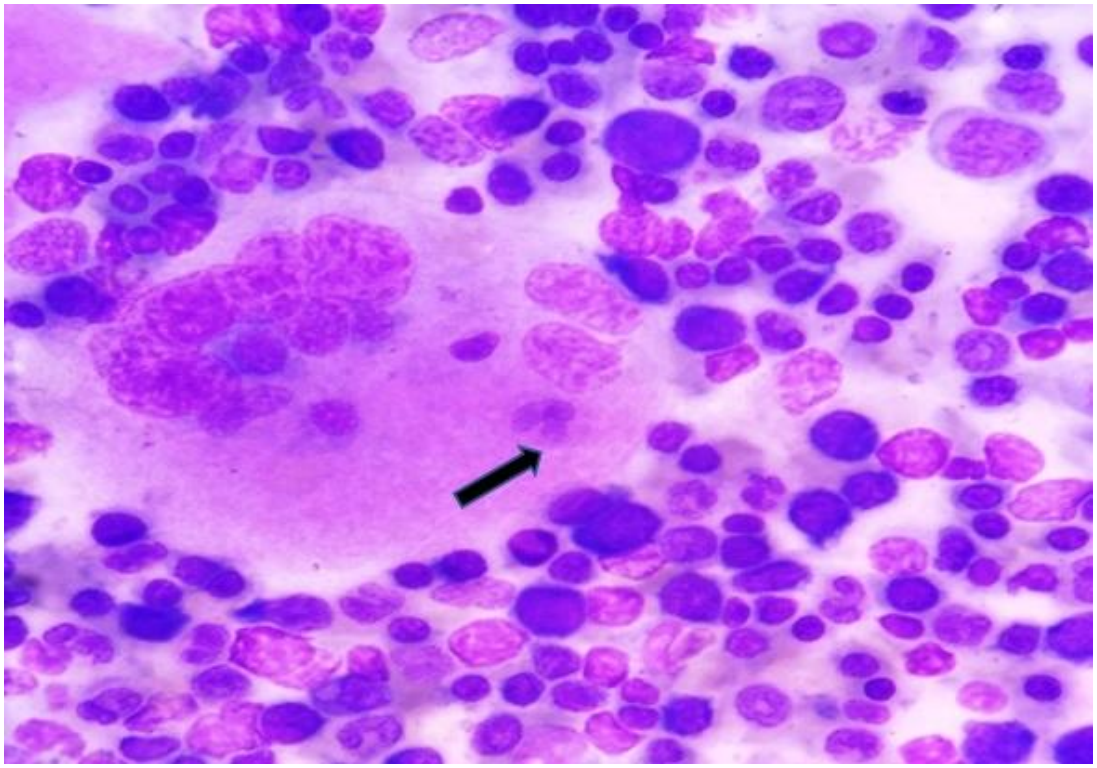

Figure 4: BMA-Megakaryocyte showing Emperipolesis. Background showing erythroid and myeloid series of cells (Giemsa, 100x).

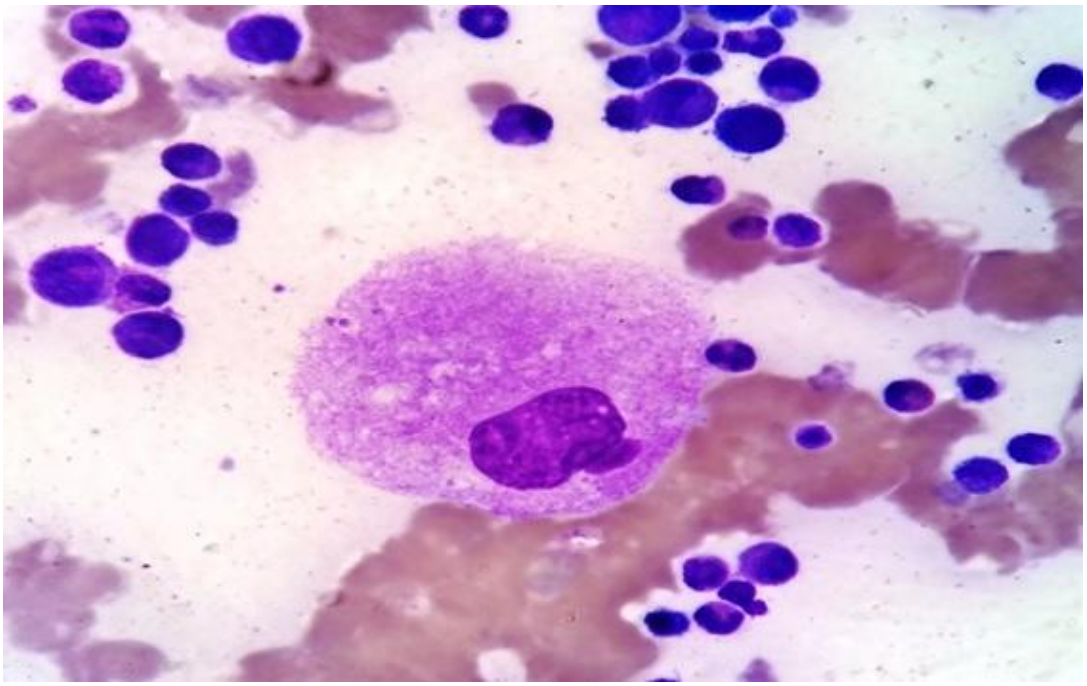

Figure 5: BMA-Megakaryocyte showing vacuoles in the cytoplasm; background showing erythroid and myeloid series of cells (Giemsa, 100x).