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Original Research Article

Clinico-Hematological Study of Megaloblastic Anemia and Associated Megakaryocytic Alterations

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Abstract

Context: Megaloblastic anemias are hematological disorders in which nuclear maturation lags behind the cytoplasmic maturation. Thrombocytopenia in megaloblastic anemia is because of ineffective thrombopoiesis in some studies and hypoproduction in some studies. Objective was to study the spectrum of clinico-hematological features in megaloblastic anemia & comparative bone marrow aspiration study ofthrombocytopenia secondary to megaloblastic anemia due to hyper-destruction or hypoproduction.

Aims: To understand the clinico-hematological spectrum of Megaloblastic anemia and associated megakaryocytic alterations.

Methods and Material: 100 cases of thrombocytopenia were included in the study. Bone marrow findings in 35 cases ofthrombocytopenia of megaloblastic etiology was compared with 40 cases of marrow proven hypo productive thrombocytopeniaand 25 cases of hyper destructive thrombocytopenia.

Results: Most common age group presenting with megaloblastic anemia was 20-30 years,with M:F ratio 1.4:1. Most common complaints were fatigue and generalised weakness. 14.28% of patients had shown normal in number of megakaryocytes, 57.14% of patients had shown increased number 28.57% had shown decrease in number of megakaryocytes. Dysplastic megakaryocytes were seen in 43.75%, 26.25% & 30% of cases of megaloblastic anemia, acute leukaemia and immune thrombocytopenic purpura respectively.

Conclusions: Both hypoproduction and ineffective thrombopoiesis are the underlyingmechanisms in megaloblastic thrombocytopenia as evidenced by marrow findings. We conclude that megaloblastic thrombocytopenia is to be included as separate category apart from hypo proliferative and hyper destructive groups. The presence of dysplastic megakaryocyte should not prompt interpretation of myelodysplastic syndromes and should always be correlated with patient's clinico hematological parameter.

Keywords: Hypo-Production; Hyper-Destruction; Megaloblastic Anemia.

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Introduction

Megaloblastic anemia belongs to a group of hematologic disorders in which blood and bone marrow disorders are caused by abnormal DNA synthesis. It is one of the main cause of anemia. It is frequently seen in people of low socioeconomical status. It occurs as a result of folic acid deficiency or impaired absorption of vitamin B12, complete or partial resection of stomach, resection of the ileum, overgrowth of bacteria in the intestines and Crohn's disease. Diagnosing this disease assumes great clinical importance since it responds exceedingly well to treatment [1]. Bone marrow examination also gives explanation for unexplained cytopenia and leukemia. It gives a more complete picture of the reaction of the hemopoietic tissue to anemia than can be gained from peripheral blood smear (PBS) alone. ed blood cells may be macrocytic and macro-ovalocytic. Leukopenia is commonly seen, though sometimes normal count and leucocytosis may be observed [2]. Thrombocytopenia is common, but has variable ranges. The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in some studies, whereas ineffective thrombopoiesis has been proposed as the mechanism in others. Distinction between these categories is made by bone marrow examination. [3] Hyper destructive thrombocytopenia is a result of extramedullary platelet destruction with normal or increased bone marrow production, e.g., Immune Thrombocytopenic Purpura (ITP), secondary ITP and Disseminated Intravascular Coagulation. Hypo productive thrombocytopenia are caused by decreased bone marrow production because of primary or secondary bone marrow diseases such as aplastic anemia, acute leukemia, myelodysplastic syndrome and post chemotherapy. The present study evaluates the varying clinico-hematological manifestations in 35 patients diagnosed as megaloblastic anemia and comparison with hyper destructive and hypo productive thrombocytopenia over one and half year period.

Materials and Methods

A prospective study carried out in the department of Pathology S. Nijalingappa Medical College, over a period of one year, from January 2017 to December 2017. The study involved 100 cases of thrombocytopenia satisfying the inclusion criteria based on the aetiology and divided them into three categories: thrombocytopenia secondary to megaloblastic anemia, hypoproduction and hyper destructive causes. The diagnosis of megaloblastic anemia was established on the basis of megaloblastic bone marrow. Other criteria included were: macrocytic blood picture with or without MCV values greater than 100 fl. Biochemically pure vitamin B12 deficiency was diagnosed when serum levels were below 200 pg/ml. The bone marrow aspiration sample and blood sample were collected in EDTA vacutainer. The marrow aspiration smears were stained with Giemsa stain. The hematological parameters were estimated by automated analyzer Sysmex kx 21. Adequacy of megakaryocytes in bone marrow aspiration was assessed as follows:

- Normal: one megakaryocyte per one to three low-power fields.
- Decreased: one megakaryocyte per five to ten low-power fields.
- Increased: more than two megakaryocytes per low-power field (Fig. 1).

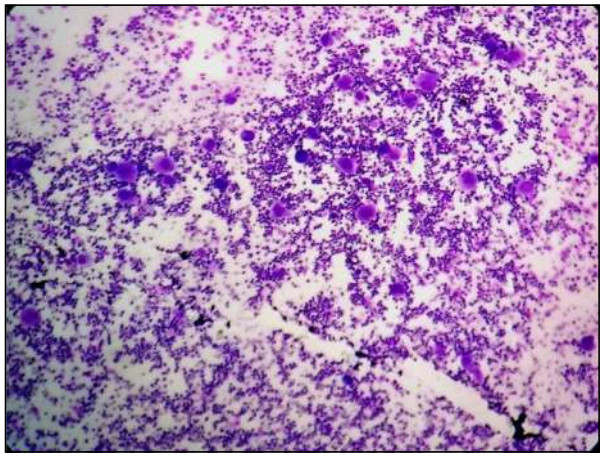

Fig. 1: Numerical alterations of Megakaryocyte

Bone marrow aspiration shows Increased number of megakaryocytes/LPF (Low power field). (Giemsa stain 10x)

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

Dysplastic features of megakaryocytes are

a. Multiple separate nuclei (Fig. 2)

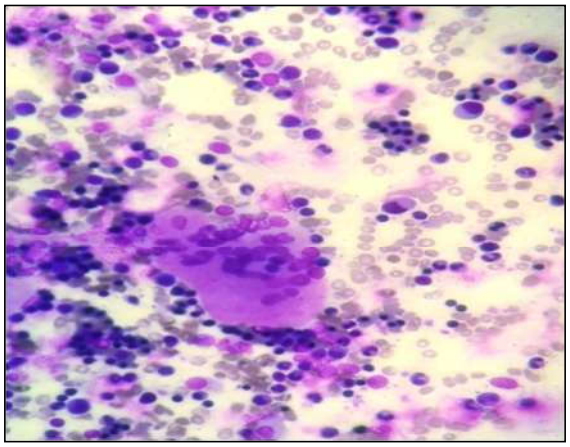

Fig. 2: Dysplastic change in Megakaryocyte- Multiple separate nuclei
Bone marrow aspiration- Megakaryocyte showing multiple separated nuclei

b. Micromegakaryocytes (size of large lymphocyte/ monocyte with single or bilobed nucleus).

c. Hypogranular forms (megakaryocyte with pale grey or clear cytoplasm and sparse or no granules) [4].

Non-dysplastic features

a. Immature forms -Immature megakaryocytes are young forms with scant bluish cytoplasm and they lack lobulation.

b. Emperipolesis -presence of other cell within the cytoplasm of megakaryocyte (Fig. 3)

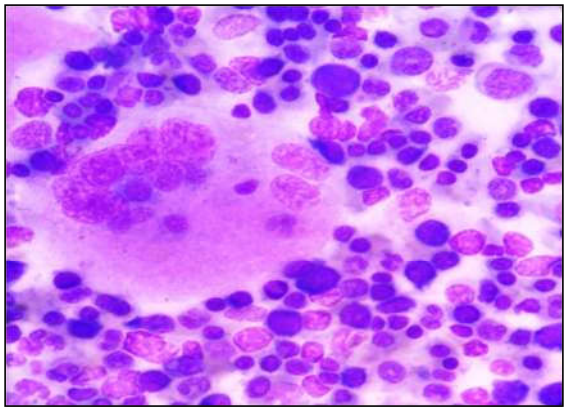

Fig. 3: Non-Dysplastic change in Megakaryocyte- Emperipolesis

Cytoplasm of the megakaryocyte was showing neutrophil. Background showing few megaloblasts and myeloid series of cells.

c. Budding -Megakaryocytes are considered to show budding if there is blebbing of cytoplasm on their surface (Fig. 4)

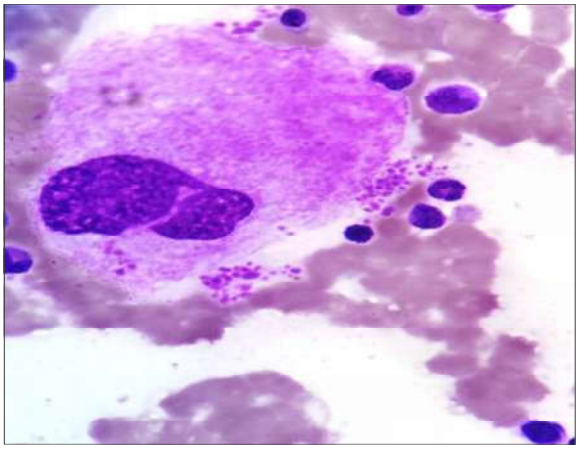

Fig. 4: Non-Dysplastic change of megakaryocyte- Budding

Bone marrow aspiration showing cytoplasmic buddings from the Megakaryocyte.

d. Cytoplasmic vacuolization (Fig. 5)

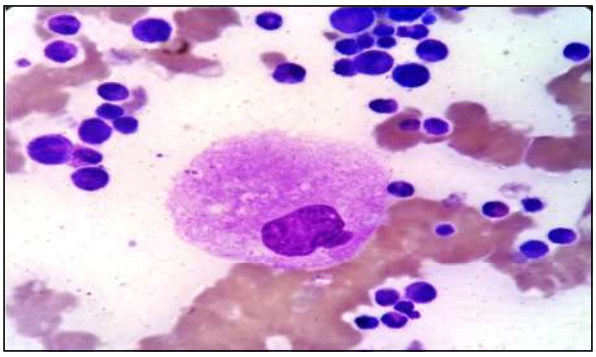

Fig. 5: Non-dysplastic change of Megakaryocyte- Cytoplasmic vacuolations.

Bone marrow aspiration- Megakaryocyte showing vacuoles in the cytoplasm; background showing erythroid and myeloid series of cells

e. Bare nuclei. [4] (Fig. 6)

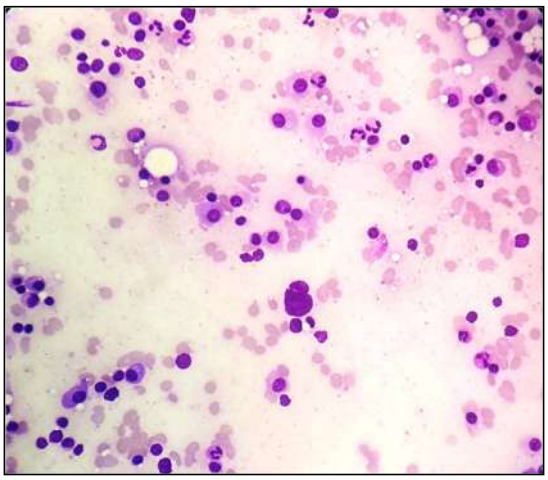

Fig. 6: Non-Dysplastic change of megakaryocyte- Bare nuclei.

f. Hypolobulated forms (Fig. 7)

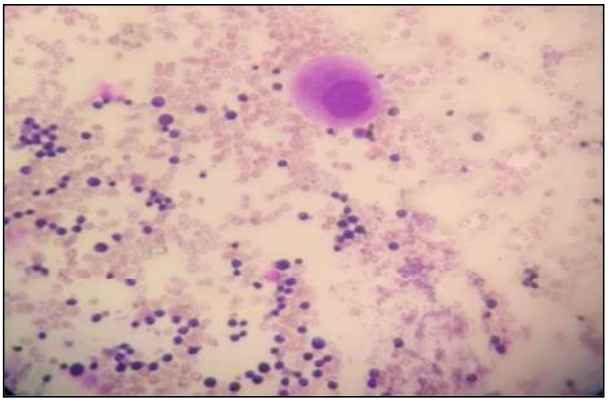

Fig. 7: Non-Dysplastic change of Megakaryocyte

Bone marrow aspiration showing hypolobulated megakaryocyte; background showing erythroid and myeloid precursors (Giemsa stain 40x)

35 cases of bone marrow proven thrombocytopenia of Megaloblastic aetiology with low serum vitamin B12/folate levels (<200pg/ml) and platelet count of <1.5 lakh/cumm were selected, 40 cases of marrow proven aplastic anemia and acute leukemia with platelet count of <1.5 lakh/cumm constituted hypo productive thrombocytopenia whereas remaining 35 cases of immune thrombocytopenia with platelet count of <1.5 lakh/cumm were included in the category of hyper destructive thrombocytopenia.

Results

Out of 100 cases of thrombocytopenia, 35 cases were of Megaloblastic anemia, 40 cases were of hypo productive thrombocytopenia and 25 cases were of hyper destructive thrombocytopenia. Out of 35 cases of megaloblastic anemia, the most common age group observed was between 21-30 years (31.42%). Male to female ratio in the present study in megaloblastic groups, hypo productive and hyper productive groups was found to be 1.3:1, 1.6:1 and 1.5:1 respectively. (Table 1).

Table 1: Age and sex distribution of Megaloblastic Anemia

Age Groups (years)	Male	Female	Total
1-10	1	1	2
11-20	4	3	7
21-30	6	5	11
31-40	5	3	8
41-50	2	1	3
51-60	1	1	2
61-70	1	1	2

Generalise weakness (100%), fatigue (100%) and pallor (100%) were observed in all the cases followed by fever (71.42%), beefy tongue (28.57%) and bleeding (28.57%). On examination, splenomegaly and hepatomegaly were observed in 22.85% and 20% respectively where as 5.88% cases presented with hepatosplenomegaly. Knuckle pigmentation was observed in 17.14% of cases (Table 2).

Table 2: Clinical findings in Megaloblastic Anemia

Clinical Feature	No of cases	Percentage
Generalised weakness	35	100%
Fever	25	71.42%
Fatigue	35	100%
Pallor	35	100%
Beefy tongue	10	28.57%
Hepatomegaly	7	20%
Splenomegaly	8	22.85%
Hepatosplenomegaly	5	5.88%
Bleeding	10	28.57%
Knuckle hyperpigmentation	6	17.14%

The average platelet count in megaloblastic groups were 50.44X109/L, hypoproduction and hyper destruction groups were 66.44 X109/L and 70.07 X109/L respectively. In megaloblastic anemia, 71.42 % of cases had shown mean corpuscular volume (MCV) >110 femtoliters and remaining 28.58% of cases had shown MCV between 100-110 femtolitres. Reticulocyte count was increased in 55.55% of cases, decreased in 20.25% of cases and normal in 24.2% of cases. (Table 3).

Table 3: Average number of platelet count in megaloblastic anemia compared to hyper and hypoproduction of platelet.

Average number of platelet count in various groups	
Megaloblastic anemia	50.44X10[9]/L
Hypoproduction	66.44 X10[9]/L
Hyper destruction	70.07 X10[9]/L

Bone marrow aspiration in all the megaloblastic group had mixture of cases with normal, increased and decreased number of megakaryocytes. Acute leukemia and hypoplastic anemia cases of hypo productive group showed decreased number of megakaryocytes. Increased number of megakaryocytes was a common finding in all the cases of immune thrombocytopenia in hyper destructive group (Table 4).

Table 4: Number of Megakaryocytes in three study groups

Study group	No of cases	Megakaryocytes		
		Decrease	Increase	Normal
Megaloblastic anemia	35	10	20	5
Hypoproduction	40	40	0	0

Hyper destruction	25	0	25	0
Total cases	100	50	45	05

Morphological alterations of megakaryocytes in megaloblastic anemia cases were multiple separate nuclei (9 cases), hypolobulated forms (6 cases), hypogranular forms (4 cases), immature forms (5 cases), emperipolesis (2 cases), budding (3 cases), cytoplasmic vacuolations (2 cases), bare nuclei (3 cases) and micro megakaryocyte in one case (Table 5).

There was decrease in the number of megakaryocytes in all the cases of hypo productive group. Hypolobulated forms were seen predominantly in this group. Immature forms were seen in 5 cases, multiple separate nuclei in 7 cases, hypogranular forms in 5 cases, emperipolesis in 3 cases, emperipolesis and cytoplasmic vacuolations were seen in 2 cases each, budding and bare nuclei were seen in 3 cases each (Table 6).

There was increase in the number of megakaryocytes in all the cases of hyper productive group. Predominantly observed morphological alteration in megakaryocytes were multiple separate nuclei (6 cases) followed by cytoplasmic vacuolations (4 cases). Immature forms, budding and emperipolesis were seen in 3 cases each. Hypolobulated, hypogranular and bare nuclei were seen in 2 cases each.

Discussion

Megaloblastic anemia patients present with various manifestations as observed in the present study. Generalised weakness and pallor was seen in all the patients. This is due to ineffective haematopoiesis which lead to decreased life span of RBC's and eventually premature destruction of developing megaloblasts in the marrow resulting

Table 5: Morphological alteration of megakaryocytes in three study groups

Study groups	Multiples paratenucei (MSN)	Hypolobulatedforms (HL)	Hypogranular (HPG)	Immatureform (IMF)	Emperipolesis (EMP)	Budding (BD)	Cytoplasmicvacuolations (CYV)	Bare nuclei (BN)	Micromegakaryocytes (m MGK)
Megaloblastic anemia	9	6	4	5	2	3	2	3	1
Hypo production	7	10	5	5	3	2	2	4	2
Hyper Destruction	6	2	2	3	3	3	4	2	0

Table 6: Number of Megakaryocytes per LPF in various studies.

	Number of Megakaryocytes per LPF				
	Normal	Increased	Decreased	Absent	Total cases
Bhasin et al. [3] (2013)	1	1	1	-	3
Choudary et al. [11] (2013)	7	21	1	18	47
Guptha P et al. [4] (2015)	1	7	4	-	12
Pokharel et al. [16] (2016)	1	-	-	-	1
Tirumalasetti et al. [13] (2016)	11	21	2	-	34
Deepika et al. [14] (2017)	20	3	10	-	33
Vinayaka murthy et al. [15] (2017)	4	9	2	-	15
Our study (2017)	16	20	10	-	46

low haemoglobin level. Bleeding manifestation was most likely due to thrombocytopenia which was observed in 28.57% of patients. An earlier study documented bleeding in 17.2% and in another study, 20% of patients had bleeding manifestations. [8] Knuckle pigmentation was another important diagnostic sign for this disease which was seen in 17.14% of patients.

In the present study, 71.42% of patients showed low grade fever. In a study done by Sunil et al, 65.5% of patients presented with low grade fever. Fever was significantly the commonest cause for infection to which the individual is much more susceptible due to impaired intracellular killing of ingested bacteria by neutrophils and macrophages [10].

In the present study, bicytopenia was reported in 44.44% and pancytopenia in 36.66% of cases. Cytopenia's (bicytopenia and pancytopenia) are most commonly seen in Megaloblastic anemia. A Study done by Sarode et al mentioned the incidence of pancytopenia in 43.8% and bicytopenia in 80.5% cases. [12] The varying results in the two studies could be due to the difference in the duration of anemia which is proportional to the development of cytopenia. It is generally believed that as severity of anemia increases, thrombocytopenia develops which is followed by neutropenia.

Thrombocytopenia is defined as platelet count less than 1 lakh/mm [3] but it does not alone explain the underlying pathomechanism unless a bone marrow examination is done which shows decreased production of megakaryocytes, ineffective thrombopoiesis or increased peripheral destruction. [11] Thrombocytopenia is believed to be due to ineffective thrombopoiesis because of impaired DNA synthesis.

In many studies, thrombocytopenia in Megaloblastic anemia has been postulated as hypoproduction where bone marrow shows decreased number of megakaryocytes and the platelet indices are studied including cases of megaloblastic anemia under the category of hypoproduction [11] whereas, ineffective thrombopoiesis has also been proposed as a mechanism where marrow shows normal to increased megakaryocytes.

The findings of decreased, normal and increased number of megakaryocytes in present study support the hypothesis of both hypoproduction and ineffective thrombopoiesis in the causation of thrombocytopenia in megaloblastic anemia.

Since bone marrow findings in cases of thrombocytopenia give a definite diagnosis of the underlying pathomechanism, bone marrow study is frequently asked in cases of thrombocytopenia. The finding of decreased megakaryocytes in aplastic anemia and leukemia and increase in the number of megakaryocytes in immune thrombocytopenia were consistent with other studies [2,3,11].

The number of megakaryocytes in Megaloblastic anemia was decreased in 28.57%, normal in 14.28% and increased (Figure-1) in 57.14% of cases. Choudhary et al, found absent, decreased, normal and increased megakaryocytes in 2.4% 16.0%, 47.4% and 34.2% of cases respectively. [11] Similar study done by Gupta et al, showed normal, increased and decreased megakaryocytes in 8.3%, 58.3%, 33.3 % of cases respectively. [4] Rajashekar RB et al [5], found normal, increased and decreased in megakaryocytes in 25%, 43.7% and 31.2% of cases [5] (Table 7).

Table 7: Comparison of morphological alterations of megakaryocytes in various studies

S. No	Condition	MSN	mMGK	HPG	IMF	EMP	BD	CYV	BN	HL
1	Bhasin et al. [3] (2013)	-	00	02	07	01	02	03	05	02
2	Choudary et al. [11] (2013)	-	-	-	01	-	-	-	-	
3.	Guptha P et al. [4] (2015)	06	07	-	-	-	-	01	-	
4	Pokharel et al. [6] (2016)	-	-	01	-	-	-	01	-	
5	Tirumalasetti et al. [13] (2016)	09	10	01	16	-	-	02	03	02
6	Deepika et al. [14] (2017)	-	08	02	01	-	-	-	04	-
7	Vinayaka murthy et al. [15] (2017)	12	03	02	11	04	06	03	14	-
8	Our study (2017)	09	01	04	05	02	03	02	03	06

The most common dysplastic feature observed in megaloblastic anemia in our study was multiple separate nuclei (Fig. 7) which was similar to the study done by Tirumalasetti et al. [13] In the study done by Vinayakamurthy et al. [15], 12 out of 15 cases showed multiple separate nuclei which was similar to our study.

In the study done by Wong et al, the ultrastructure of the megakaryocytes in the bone marrow from 14 cases of megaloblastic anemia was studied. The most common change observed was the development of the nucleus which lagged behind that of the cytoplasm. There were some evidences supporting the theory of ineffective thrombocytopoiesis which was nuclear separation and occurrence of nuclear fragments which was due to decreased DNA synthesis. This led to nuclear maturation defects.

A shift to young, immature, less polypoid megakaryocytes and fewer mature platelet-producing megakaryocytes were the outstanding morphological feature noted in almost all the cases of ITP in the present study. Similar findings were observed by Houwerzijl et al. [6] who observed that Megakaryocytes in cases of ITP had a higher nuclear/cytoplasmic ratio ($p = 0.021$), lower nuclear roundness factor ($p = 0.04$) and lower nuclear contour ratio ($p = 0.027$).16 Cellular circularity and compactness were significantly different in ITP as compared to non-ITP cases, indicating that the megakaryocytes were less round in ITP.

In the present study, decreased or absent of megakaryocytes were observed in aplastic anemia which was similar to the study done by Shadduck [17]. They attributed this to bone marrow suppression and significant inhibition of nucleic acid synthesis in the megakaryocytes.

Conclusion

Based on our clinical features, peripheral blood smear and bone marrow findings, mixed mechanisms are operative in the causation of thrombocytopenia in megaloblastic anemia. We hereby infer that megaloblastic thrombocytopenia is to be included as a separate category apart from hypo proliferative and hyper destructive groups. Bone marrow examination remains the gold standard for discriminating hypo productive thrombocytopenia from the hyper destructive causes.

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(If present, give more details): Nil

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