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Original Research Article

A prospective study on morphological alteration of megakaryocytes amongst megaloblastic anemia cases along with their clinic-haematological manifestations

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ABSTRACT

Background: Megaloblastic anemias are hematologic disorders in which abnormal DNA synthesis causes blood and bone marrow disorders. The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in some studies, whereas ineffective thrombopoeisis has been proposed in other. Objective was to study spectrum of clinic-hematological features in megaloblastic anemia and comparative bone marrow aspiration study of thrombocytopenia secondary to megaloblastic anemia, hypoproduction and hyper-destruction. This study was done to understand the various megakaryocytic alterations in hematological disorders presenting with thrombocytopenia due to different mechanisms.
Methods: Total 85 cases of thrombocytopenia included in the study. Bone marrow finding in 33 cases of thrombocytopenia of megaloblastic etiology were compared with 34 cases of marrow proven hypo productive thrombocytopenia (aplastic anemia, acute leukemia) and 19 cases of hyper destructive thrombocytopenia (immune thrombocytopenia).
Results: Most common age group presenting megaloblastic anemia is 11-20 year, with male to female ratio is1.2:1, most common complaint were generalized weakness and fever. In megaloblastic anemia 24.33%, 60% and 15.67% of the cases shows increase, decrease and normal megakaryocytes respectively. Dysplastic megakaryocytes were observed in 24.3%, 27% and 20.5% of the cases of megaloblastic anemia, acute leukaemia and immune thrombocytopenic purpura respectively.
Conclusions: Both hypoproduction and ineffective thrombopoiesis are the underlying path mechanisms in megaloblastic thrombocytopenia as evidenced by the marrow findings. We hereby infer that megaloblastic thrombocytopenia is to be included as a separate category apart from hypo proliferative and hyper destructive groups. The presence of dysplastic megakaryocyte should not prompt an interpretation of myelodysplastic syndromes and should always be correlated with patient's clinico-hematological parameter.

Keywords: Hypo productive, Hyper destructive thrombocytopenia, Megaloblastic anemia

INTRODUCTION

Megaloblastic anemia is one of the important causes of anaemias. It is a frequent entity in poor socioeconomic condition. This condition has protean manifestations in childhood, sometimes mimicking a hematological malignancy like leukemia. Diagnosing this disease assumes great clinical importance since it responds exceedingly well to treatment.[1] Megaloblastic anemias are one of the causes of pancytopenia, a group of

hematologic disorders in which blood and bone marrow disorders are caused by abnormal DNA synthesis.[2] This type of anemia occurs as a result of folic acid deficiency or impaired absorption of vitamin B12 (pernicious anemia) caused by severe Addison's anemia, complete or partial resection of stomach, resection of the ileum and the overgrowth of bacteria in the intestines and Crohn's disease.[3] Red blood cells may be macrocytic, normocytic or microcytic. Sometimes Megaloblasts are seen in the blood smear; the sign of definitive diagnosis. The number of white blood cells can be decreased, increased or normal, but leukopenia is common.

Blood bilirubin may increase. The definitive diagnosis is confirmed by bone marrow changes (with the simultaneous presence of megaloblasts and normoblasts). Almost all of megaloblastic anemias result from reduced absorption of vitamin B12 or folic acid deficiency. This type of anemia causes curable anemia and pancytopenia; as a result its diagnosis and treatment are cost effective for patients and the society.[4] Typically, these diseases are diagnosed by low plasma folic acid levels (the first biochemical sign), hyper pigmentation of neutrophils (the first morphologic sign), significant increased mean corpuscular volume (MCV), megaloblastic erythropoiesis in the bone marrow; although, there may be a slight anemia or vitamin B12 deficiency in pernicious anemia with neurological disturbances with Normal MCV.[5]

Thrombocytopenia is common, but has variable Ranges. The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in some studies, whereas ineffective thrombopoeisis has been proposed as the mechanism in others.[1]

Distinction between these categories is made by bone marrow examination. 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.[6]

The present study evaluates the varying clinico hematological Manifestations in 33 patients diagnosed as megaloblastic anemia and comparison to hyper destructive and hypo productive thrombocytopenia over a two-years period.

METHODS

A prospective study carried out in the department of pathology, over a period of two years, starting from august 2011 till April 2013. The study involved 85 cases of thrombocytopenia satisfying the inclusion criteria, based on the etiology 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: macrocytic blood picture with or without MCV values greater than 100 fl. Biochemically pure vitamin B12 deficiency and were diagnosed when serum levels were below 200 pg/ml.

The bone marrow aspiration sample and blood sample were collected in EDTA vacutainer whereas, the bone marrow biopsy was collected in formalin and decalcified. The marrow aspiration smears were stained with Leishman's stain and the trephine biopsy sections were stained with Hematoxylin and Eosin. 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).[7]

Total 33 cases of marrow proven thrombocytopenia of megaloblastic etiology with low serum vitamin B12/folate levels (<200pg/ml) and platelet count of <1.5 lakh/cumm were selected, 34 cases of marrow proven aplastic anemia and acute leukemia with platelet count of <1.5 lakh/cumm constituted hypo productive thrombocytopenia, whereas remaining 19 cases of immune thrombocytopenia with platelet count of <1.5 lakh/cumm included in the category of hyper destructive thrombocytopenia.

RESULTS

Out of Total 85 cases of thrombocytopenia 34 cases labelled as hypo productive thrombocytopenia, 19 cases were hyper productive and 33 cases were megaloblastic anemia.

Table 1: Age and sex distribution of megaloblastic anemia (no. 33 cases).

Age groups	M	F	Total
1-10	3	2	5
11-20	4	6	10
21-30	2	4	6
31-40	1	1	2
41-50	5	1	6
51-60	1	0	1
61-70	2	1	3

Out of 33 cases most common age group presented with megaloblastic anemia was 11-20yreas (33%) (Table 1).

Male to female ratio in present study in hypo productive, hyper destructive and megaloblastic groups was 1:1.31, 1:1.45 and 1.2:1 respectively.

Generalized weakness (100%) and fever (100%)were two most common complaint presents in all cases followed by bleeding manifestation (24%), although pallor was found in all 33 cases. On examination hepatomegaly (36%) and splenomegaly were found 36% and 48% respectively. Whereas 30% cases were presented with both (Table 2).

Table 2: Clinical finding, frequency and percentage of megaloblastic anemia (no. 33 Cases).

Pallor	33
Weakness and fever	33
Bleeding	8
Hepatomegaly	12
Splenomegaly	16
Hepatosplenomegaly	10

The mean values of platelet count in hypo productive, hyper destructive and megaloblastic groups were 46,070/cumm, 54,440/cumm and 54,100/cumm respectively (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 megaloblastic anemia	
Hypoproduction	46.07X109/L
Hyperproduction	54.44 X109/L
Megaloblastic anemia	54.10 X109/L

In megaloblastic anemia 30% of cases shows Mean corpuscular volume of RBC >100 fl and reticulocyte count was increased in 48.48% of cases, decreased in 12.12% of cases and 39.4% cases were with normal reticulocyte count.

Bone marrow trephine biopsy in all the acute leukemia and aplastic anemia cases of hypo productive group showed decreased megakaryocytes. Increased megakaryocytes were a common finding in all the cases of immune thrombocytopenia in hyper destructive group. While, the megaloblastic group had mixture of cases with normal, increased and decreased megakaryocytes (Table 4). Alteration in morphology of megakaryocytes among all three conditions were seen differently in Table 5.

Table 4: Number of megakaryocyte in three study group (no. 85 cases).

Categories	No of cases	Megakaryocyte		
		Decrease	Increase	Normal
Hypoproduction	34	34	0	0
Hyperproduction	19	0	19	0
Megaloblastic anemia	33	20	08	05
Total	86	54	27	05

Table 5: Alteration in morphology of megakaryocyte in three study group (no. 85 cases).

	Hypo lobulation	Hyper nucleation	Immature form	Dysplastic form	Bare	Emperipolesis	Budding	Cytoplasmic vacuolization	Micro megakaryocyte
Hypoproduction	16	11	10	9	11	9	1	3	14
Hyperproduction	9	6	15	4	9	10	1	0	9
Megaloblastic anemia	19	18	13	8	5	14	1	5	10

There was an increase in the number of megakaryocytes in all hyper productive group and immature megakaryocytes were seen in 15 cases dysplastic forms were seen in 4 cases, bare megakaryocytic nuclei in 9 cases and micro megakaryocytes in 9 cases of hyper productive group. Emperipolesis was seen in 10 cases with lymphocytes in six cases and lymphocytes and nucleated red blood cells in four cases.

There was a decrease in the number of megakaryocytes in 20 cases of megaloblastic anaemia and hypo lobulation and hyper nucleation seen in 19 and 18 cases

respectively, dysplastic forms were seen in 8 cases followed by hypo granular forms and cytoplasmic vacuolization in 3 and 5 cases respectively. Emperipolesis was seen in 14 cases.

Hypo productive group showing 16 cases of hypo lobulation, 11 cases shows hyper nucleation and bare nuclei, and micro megakaryocyte were seen in 14 cases.

DISCUSSION

Megaloblastic anemia presents with different manifestations as observed in present study. Pallor and weakness seen in all patient this is due to ineffective haematopoiesis lead to decreased life span of RBC and to premature destruction of developing megaloblasts in the marrow resulting low hemoglobin level. Bleeding most likely due to thrombocytopenia was noticed in 24.3% of patients. An earlier series documented bleeding in 20% of patients and in another study 17.2% in megaloblastic anemia.[8] Hemorrhagic emergencies like intracranial bleeding and gut bleeding though not well appreciated in this disease have been rarely seen.[8]

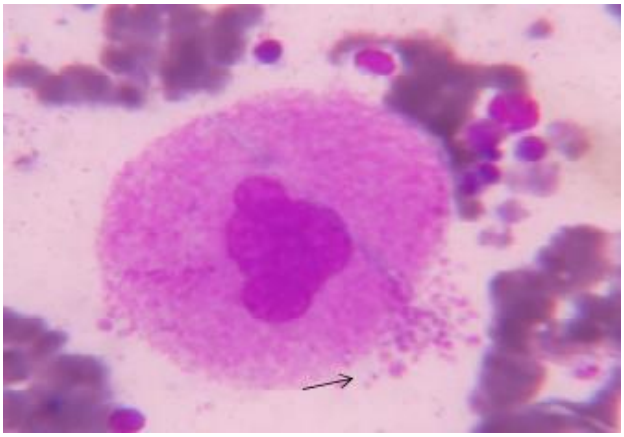

Figure 1: Normal megakaryocyte with platelet formation (Giemsa stained BMA. 1000x).

Hyperpigmentation of dorsum of hands and fingers though considered an important diagnostic sign for this disease was seen in 24.3% of the patient.[9]

In present study, low grade fever was seen in all the cases where as a study carried out by Sunil et al. mention 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]

Bicytopenia was reported in 64.8% cases and pancytopenia in 26.2% cases in present study. Megaloblastic anemia is an important cause of cytopenia (pancytopenia and bicytopenia). Study carried out by Sarode et al reported an incidence of pancytopenia in 43.8% and bicytopenia in 80.5% cases.[8]

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.[8] It is generally believed that as severity of anemia increases, thrombocytopenia develops followed by neutropenia.

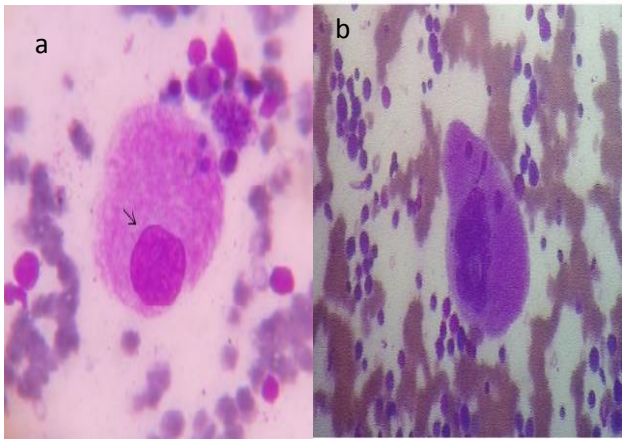

Figure 2: a) Hypo lobulation, b) Emperipolesis of lymphocyte in megakaryocyte, background shows erythroid, myeloid precursors and RBCs (Giemsa stained BMA. 400x).

Thrombocytopenia is believed to be due to impaired DNA synthesis resulting in ineffective thrombopoiesis. Platelet count below 1.5lakh/mm^3 defines thrombocytopenia but 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.[6]

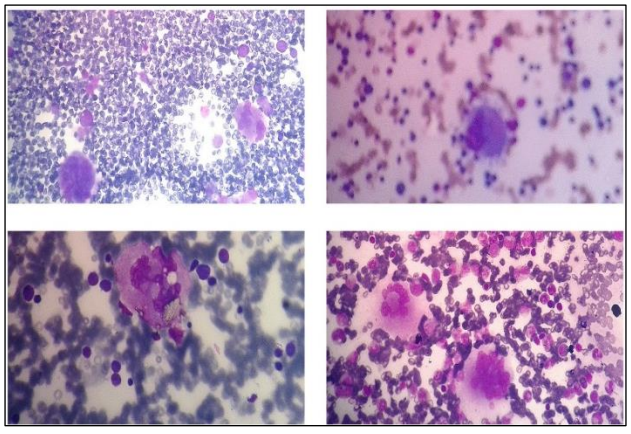

Figure 3: Dysplastic megakaryocyte, background shows erythroid, myeloid precursors and RBCs (Giemsa stained BMA. 400x).

The cause of thrombocytopenia in megaloblastic anemia has been postulated as hypoproduction in many studies where bone marrow shows decreased megakaryocytes and the platelet indices are studied including cases of megaloblastic anemia under the category of hypoproduction.[6] Whereas, ineffective thrombopoeisis

has also been proposed as a mechanism, where marrow shows normal to increased megakaryocytes. The findings of normal, increased and decreased megakaryocytes in present study support the hypothesis of both hypoproduction and ineffective thrombopoiesis in the causation of thrombocytopenia in megaloblastic anemia.

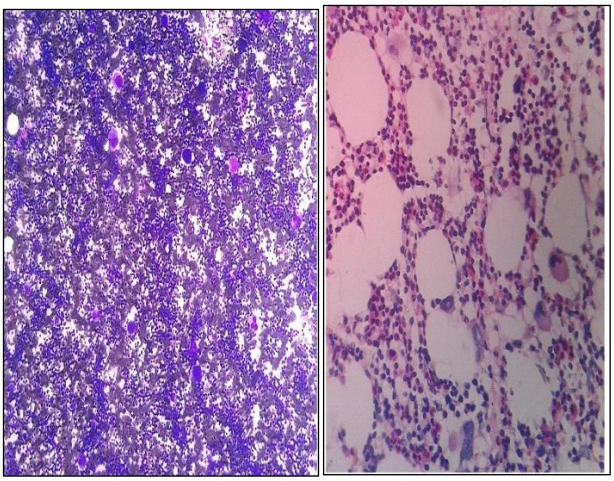

Figure 4: Case of ITP, a bone marrow aspiration and b-bone marrow biopsy, showing increase number and immature form of megakaryocyte, background shows erythroid, myeloid precursors and RBCs (Giemsa stained. 100x).

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 findings of decrease in megakaryocytes in aplastic anemia and leukemia, and increase in the number of megakaryocytes in immune thrombocytopenia were consistent with other studies.[7,11,12]

Morphological changes in megakaryocyte described as, normal megakaryocyte has four to sixteen nuclear lobes and an immature megakaryocyte is defined as a young form of megakaryocyte having scant bluish cytoplasm and lacking lobulation of the nucleus which occupies almost all of the cell.[13] Dysplastic megakaryocytes were defined as those with single/ multiple separate nuclei. Micro megakaryocytes were defined as megakaryocytes whose size was that of a large lymphocyte/monocyte and which had a single/bilobed nucleus. The megakaryocytes were considered to show platelet budding if there was budding of cytoplasmic processes from their surfaces. Hypo granular forms were defined as megakaryocytes with pale grey or water clear cytoplasm and sparse or no granules. The type of cell seen within the megakaryocyte in emperipolesis was also documented.

The number of megakaryocytes in megaloblastic anemia was normal, increased and decreased in 15%, 24.3% and 60.7% of cases. Similar results were seen in a study by Gupta et al, with normal, increased and decreased megakaryocytes in 8.3%, 58.3%, 33.3 % of cases respectively.[14] Choudhary et al, found absent, decreased normal and increased megakaryocytes in 2.4% 16.0%, 47.4% and 34.2% of cases respectively.[7] Rajashekar RB et al, found megakaryocytes in megaloblastic anemia was normal, increased and decreased in 25%, 43.7% and 31.2% of cases.[15]

Megakaryocytic proliferation and differentiation is typically abnormal in patients with myelodysplastic syndromes (MDS). But it is also seen in non MDS cases.

In megaloblastic anemia, dysplastic forms of megakaryocytes were seen in eight (24%) cases which is contrary to the findings of Tricot et al and Muhury et al. However, Wickramasinghe observed megakaryocytes with separation of nuclear lobes and nuclear fragments and attributed this to diminished DNA synthesis leading to nuclear maturation defect. The finding of emperipolesis in anemia was in agreement with the observation of Tavassoli. However, no platelet budding was observed in any of the cases.[11]

A shift to young, immature, less polypoid megakaryocytes and fewer mature platelet-producing megakaryocytes was the outstanding morphological feature noted in almost all the cases of ITP in the present study. Similar findings were observed by Houwerzijl et al, Deka L et al, observed that Megakaryocytes in cases of ITP showed 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.

Megakaryocytes were decreased or absent in aplastic anemia which was also observed by Shadduck.[17] They attributed this to bone marrow suppression and significant inhibition of nucleic acid synthesis in the megakaryocytes. The hypo lobulated forms and dysplastic forms seen were in contrast to those of Tricot et al. Where megakaryocytes were of normal morphology. Presence of stem cell defect in ITP patients can progress to overt marrow failure. Leukemia-lymphoma syndrome showed decreased or absent megakaryocytes. This may be because of the autoantibodies against glycoprotein iia-iiib complex which have been demonstrated in patients with lymphoma. According to Lim and Ifthikharuddin, along with immune-mediated platelet destruction, decreased platelet production when the marrow is involved by lymphoma, bone marrow suppression by chemother-apeutic agents and platelet sequestration in the spleen also contribute to thrombocytopenia in lymphoma.[18]

CONCLUSION

Based on our results of clinical, peripheral 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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Ethical approval: The study was approved by the Institutional Ethics Committee

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