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Original Research Article

Comparative Analysis of Vitamin B12 Levels, Peripheral Smear Findings and Bone Marrow Findings in Megaloblastic Anaemia

Priti Toppo[1], Vibha Khare[2], Ranjan Yadav[3], Robin Lakra[4], R. K. Nigam[5]

[1]Senior Resident Department of Pathology MGM Medical College Indore
[2]Senior Resident Department of Biochemistry MGM Medical College Indore
[3]Assistant Professor Government Medical College Mandsaur
[4]Assistant Professor Department of Medicine RSDKS Government Medical College Ambikapur
[5]Professor Department of Pathology Gandhi Medical College Bhopal

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Corresponding author: Dr. Robin Lakra
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Abstract

Introduction: Haematological disorders constitute a broad spectrum in which patients can present with various clinical presentations. Megaloblastic anemia is deficiency anemia, the reason is that DNA synthesis is inhibited in the red blood cell production process. The disease state of megaloblastosis is characterized by the presence of many large, immature, and dysfunctional red blood cells in the bone marrow with excessive division of neutrophils.

Material & Methods: Total 60 patients with indications of bone marrow evaluation referred to Department of Pathology, Gandhi Medical College Bhopal, were included in the study. Only patients with Known serum Vitamin B12 levels, PS findings and bone marrow aspiration were included. Pregnant females, patients with blood transfusion, medication or any bleeding disorders were excluded. Serum vitamin levels, PS and marrow findings were studied and compared.

Observation & Results: Total 60 patients were included. It was found that mean hemoglobin level (6.2 gm%) WBCs count (3400/cumm) and platelet count (0.80 lakh/cumm) was least in elderly age group. Mean serum Vitamin level was least in elderly age group i.e. 96pg/ml. Vitamin B12 level was found borderline low in younger age group (148 pg/ml). Peripheral examination and morphology typing shown pancytopenia in 19 patients followed by anemia with thrombocytopenia in16, bicytopenia 13 and dimorphic anemia 12 patients.

Conclusion: Megaloblastic anemia is a common anemia most commonly caused by Vitamin B12 deficiency. Prevalence of this anemia is higher in our society due to strict vegetarian diet in various communities of our country. Marrow erythroid shows erythroid hyperplasia and megaloblastosis pointing towards the increased erythroid activity of bone marrow. Prompt diagnosis and timely treatment can prevent the serious neurological effects of Vitamin B12 deficiency.

Keywords: Megaloblastic Anemia, Vitamin B12, Bone marrow aspiration etc.

Introduction

Haematological disorders constitute a broad spectrum in which patients can present with various clinical presentations. Megaloblastic anemia is deficiency anemia, the reason is that DNA synthesis is inhibited in the red blood cell production process [1]. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth phase to the mitotic (M) phase.

Megaloblastic anemia has a slower onset, especially compared to other anaemias. The most common cause of defects in red blood cell DNA synthesis is vitamin deficiency, especially vitamin B12 deficiency [2]. Bone marrow cellularity and morphology varies in relation to the underlying pathology. The correct diagnosis can be made by a combination of several clinical, laboratory and morphological variables. To establish a diagnosis in various haematological disorders bone marrow examination continues to be the cornerstone along with other clinical and laboratory parameters [3].

The disease state of megaloblastosis is characterized by the presence of many large, immature, and dysfunctional red blood cells (megaloblasts) in the bone marrow, as well as excessive division of neutrophils (hyper segmented neutrophils defined as neutrophil with more than five lobes or at least >5% neutrophils with five lobes). These hypersegmented neutrophils can be

detected in peripheral blood [4]. Also on peripheral smear we find basophilic stippling, Cabot rings etc which suggest possibility of megaloblastic anemia. Clinically, in a mild deficiency a person may feel tired and the number of red blood cells decreases. A moderate deficiency can cause inflammation of the tongue and the onset of neurological problems, including abnormal sensations such as a tingling sensation, while a severe deficiency can include decreased heart function and more serious neurological problems. Vitamin B12 deficiency is more common in the elderly, because atrophic gastritis leads to a lack of gastric factors necessary for the absorption of vitamins. Megaloblastic anemia in pregnant women with folic acid deficiency is more likely to deliver low birth weight premature babies and babies with neural tube defects [5].

The gold standard for diagnosing vitamin B12 deficiency is low levels of vitamin B12 in the blood. Low levels of vitamin B12 in the blood are a finding and can and should usually be treated with injections, supplements or diet or lifestyle recommendations, but this is not diagnostic [6]. Vitamin B12 deficiency can be caused by a variety of mechanisms. In order to determine the cause of anemia, further medical history, lab testing and empirical treatment may be required clinically.

Pancytopenia is most common finding seen in megaloblastic anemia. Pancytopenia is the feature of many diseases which includes easily treatable to lethal conditions. Pancytopenia is described as the deficiency of all three cellular elements of blood, resulting in anemia, leucopenia and thrombocytopenia [7]. Also the count of reticulocytes decreases due to the destruction of abnormal and fragile megaloblast precursors [8].

To the exact cause of anemia complete 2 haematological workup along with good clinical correlation is needed. Bone marrow aspiration is an essential adjunct to the study of hematopoietic disorders, can be performed easily and repeated with safety [9]. The correct diagnosis can be made by a combination of several clinical, laboratory and morphological variables. To establish a diagnosis in various haematological disorders bone marrow examination continues to be the cornerstone along with other clinical and laboratory parameters [10].

We undertook this study with aim to find the correlation between serum Vitamin B12 levels, blood count, morphological findings of peripheral smear and bone marrow aspirate.

Material and Methods:

Patients presented with indications of bone marrow evaluation referred to Department of Pathology, Gandhi Medical College Bhopal, during the period of January 2021 and June 2022, are included in the study. Total 260 cases of anemia were seen during study time, of them 60 cases were included in study.

Inclusion Criteria: Cases suspected of megaloblastic anemia. All the cases in whom bone marrow examination was done by bone marrow aspiration and peripheral smears findings & Vitamin B12 levels were known.

Exclusion Criteria: Pregnant females. Patients of bleeding disorders or coagulopathies. Those patients whose Vitamin B12 levels, bone marrow aspiration or peripheral smear findings were not known. Those patients who have received blood transfusion or medical treatment for deficiency anemia and patients not willing to give consent.

Pregnant females were excluded because combined folic acid deficiency is more common and folic acid supplementation is usually given to all pregnant females.

After complete clinical examination and history taking, total CBC count and peripheral smear examination was done. Anemia typing done on the basis of morphology. Patients suspected with Vitamin B12 deficiency were advised for serum Vitamin B12 assay. Further patients were also advised for bone marrow examination. Bone marrow aspiration was done under aseptic conditions. Before aspiration, past clinical history of the patient was obtained, any allergies and co-morbidities documented and any premedications explained. Informed consent obtained from the patient. The preferred anatomic site for BM aspiration was the posterior superior iliac spine.

After complete clinical history of patient serum Vitamin B12 levels, peripheral smear findings and bone marrow smear findings were studied and compared. To avoid observer bias morphology was reported by single observer.

Observation and Results:

Total 60 patients were included in our study, of them males were 27 and females were 33. Patients of all age groups were included, youngest patient is of age 11 years and eldest one is 66 years of age. Maximum patients were in elder age group of age >50 years and least patients in our study were from third decade of life. Mean hemoglobin level, mean WBCs count and mean platelet counts were noted. It was found that mean hemoglobin level was least in elderly age group (6.2 gm%). Similarly least WBCs count (3400/cumm) and platelet count (0.80 lakh/cumm) was also seen in elderly age group patients.

Mean serum Vitamin level was least in elderly age group i.e. 96pg/ml. Vitamin B12 level was found borderline low in younger age group (148 pg/ml).

Peripheral examination and morphology typing shown pancytopenia in 19 patients followed by anemia with thrombocytopenia [16], bicytopenia [13] and dimorphic anemia [12]. PS finding of megaloblastic anemia shows macrocytes, macroovalocytes nucleated RBCs with MCV>100fl. Basophilic stippling, cabots ring, howell-jolly bodies and hypersegmented neutrophils. Bone marrow examination shows hypercellular marrow in 60% cases followed by normocellular marrow (30%) and hypocellular marrow (10%). All marrow examined shown features of erythroid hyperplasia and all stages of erythroid precursors. Megaloblasts in the marrow are seen even before the appearance of macrocytes in the PS. They are characterised by the increase in size and open sieve like nuclear chromatin. Early megaloblasts are more than late megaloblast. Myeloid Erythroid ratio reversal was seen. Myeloid series show giant myelocytes and metamyelocytes.

Table 1: Age wise distribution with mean Vit B12 levels, Mean Hb levels, Mean WBCs count and Mean platelet count

Age group	No of Patients	Mean Vitamin B12 level (pg/ml)	Mean Hb (gm%)	Mean WBCs (10^3/cumm)	Mean Platelets (lakhs/cumm)
<20	12	148	8.2	4.60	1.45
21-30	10	145	8.5	5.10	1.60
31-40	09	122	7.7	4.65	1.35
41-50	13	108	6.8	3.85	1.20
>50	16	96	6.2	3.40	0.80
Total	60				

Table 2: Distribution of patients on the basis of peripheral smear finding

PS finding	No. of patients	%
Pancytopenia	19	31.7
Bicytopenia	13	21.6
Dimorphic Anemia	12	20.0
Anemia with Thrombocytopenia	16	26.7
Total	60	

Table 3: Bone marrow aspiration finding:

Bone marrow finding	Number of patients (%)	Myeloid : Erythroid ratio (mean)
Hypercellular	36 (60%)	1:3
Normocellular	18 (30%)	1:2
Hypocellular	06 (10%)	1:1
Total	60	

Discussion:

In our study majority of patients belonged to more than 50 years of age and slight female predominance was noted in our study, with male: female ratio of 1:1.14. Similar findings were observed by Gilotra M et al [11] with 54.6% males (M:F=1.2:1) and50 to 60 years as most common age group. With serum Vitamin B12 assay and marrow morphology we correctly diagnosed all cases of megaloblastic anemia. Verma N et al (12) documented the accuracy of 94% for BMA for diagnosis of haematological disorders.

Though we included only megaloblastic anemia, but bone marrow studies by Gayathri BN et al [13] & Taori G et al [14] shown megaloblastic anemia as most common diagnosis followed by aplastic anemia. We did not find marrow aspiration morphology as adequate modality; Milosevic R et al documented trephine biopsy to be the most accurate tool for assessment of cellularity and diagnosis of aplastic anaemia [15]. Ebrahim H et al (2022) also confirmed the findings of present study, in which the authors reported anaemia. Leucocytosis and thrombocytopenia to be common features in patients with hematological disorders depending upon underlying causes [16]. Majority of patients presented with generalized weakness, followed by fever, bleeding manifestations and neurological signs. Basak TB et al documented that patients with hematological manifestations may present with wide variety of symptoms including pallor, weight loss, fever, bleeding manifestations, increased risk of infections etc [17].

Conclusion:

Megaloblastic anemia is a common anemia most commonly caused by Vitamin B12 deficiency. Prevalence of this anemia is higher in our society due to strict vegetarian diet in various communities of our country. Most common presentation is pancytopenia, so an elderly person of strict vegetarian diet should always be screened for megaloblastic anemia. Marrow erythroid shows

erythroid hyperplasia pointing towards the increased erythroid activity of bone marrow. Prompt diagnosis and timely treatment can prevent the serious neurological effects of Vitamin B12 deficiency. Lastly, we included limited cases in our study so more extensive and deeper studies were needed for better understanding of disease

References:

1. Megaloblastic Anemia: Overview – e Medicine Hematology. Retrieved. 2009-02-07
2. Bain, Barbara J.; Bates, Imelda; Laffan, Mike A. Dacie and Lewis Practical Haematology E-Book. Elsevier Health Sciences. ISBN. 2016:9780702069253.
3. Hashimoto M. Pathology of bone marrow. Acta Haematol (Basel), 1962;27: 193–216.
4. Hunt, A; Harrington, D; Robinson, S. Vitamin B12 deficiency (PDF). BMJ. 4 September 2014; 349: g5226.
5. Wang, H; Li, L; Qin, LL; Song, Y; Vidal-Alaball, V; Liu, TH. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. Cochrane Database of Systematic Reviews. 2018.
6. Stabler, S P; Lindenbaum, J; Allen, R H. Vitamin B-12 deficiency in the elderly: current dilemmas. The American Journal of Clinical Nutrition. 1997;66 (4): 741– 749.
7. Kishore Khodke, S Marwah, G Buxi, RB Yadav, NK Chaturvedi. Bone Marrow Examination in Cases of Pancytopenia. Journal of Indian Academy of Clinical Medicine 2001; 1: 55-59.
8. Bunn HF. New agents that stimulate erythropoiesis. Blood. 2007; 09:868-73.82.
9. Gupta V, Tripathi S, Tilak V, Bhatia BD. A study of clinicohaematological profiles of pancytopenia in children. Trop Doct. 2008; 38(4):241-243.
10. Dacie and Lewis. Bone marrow biopsy. In: Imelda Bates editors. Practical Haematology. 10th ed. Philadelphia: Elsevier publication; 2006;115- 118.
11. Gilotra M, Gupta M, Singh S, Sen R. Comparison of bone marrow aspiration cytology with bone marrow trephine biopsy histopathology: An observational study. Journal of Laboratory Physicians. 2017 Jul; 9(03):182-9.
12. Verma S, Bansal R, Sharma S, Gupta A, Gupta M, Garg S, Mishra S. Correlation between bone marrow aspiration and bone marrow biopsy with imprint smears in hematological disorders. National J Lab Med. 2016;5(3):64-9.
13. Gayathri BN, Rao KS. Pancytopenia: a clinico hematological study. J Lab Physicians. 2011 Jan; 3(1):15-20.
14. Taori G, Ukey A, Bajaj P. Comparison of Bone Marrow Aspiration Cytology, Touch Imprint Cytology and Bone Marrow Biopsy for Bone Marrow Evaluation at a Tertiary Health Care Institute. MVP Journal of Medical Sciences. 2019 Dec 1:152-7.
15. Milosević R, Janković G, Antonijević N, Jovanović V, Babić D, Colović M. Histopathologic characteristics of bone marrow in patients with aplastic anemia. Srpski Arhiv za Celokupno Lekarstvo. 2000 May 1;128(5-6):200-4.
16. Ebrahim H, Fisha T, Debash H, Bisetegn H. Patterns of Bone Marrow Confirmed Malignant and Non-Malignant Hematological Disorders in Patients with Abnormal Hematological Parameters in Northeast Ethiopia. Journal of Blood Medicine. 2022; 13:51.
17. Basak TB, Talukder SI. Etiological spectrum of pancytopenia. Dinajpur Med Col J. 2014; 7(1):21-5.