SERUM B_{12} AND FOLATE LEVELS IN PATIENTS WITH MEGALOBLASTIC CHANGE IN THE BONE MARROW

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ABSTRACT

Introduction: Vitamin B_{12} and folic acid are essential components of DNA synthesis in red cell precursors. Folic acid is directly involved and Vitamin B_{12} (methyl cobalamine) participates as a co-factor. A deficiency of Vitamin B_{12} causes the same symptoms as folic acid deficiency.

Objective: The study was carried out to find the cause of megaloblastic anemia.

Study design and settings: In this descriptive study, we evaluated clinical and morphological features of 80 consecutive patients with a megaloblastic change in bone marrow from 2008-2010. The study was carried out in the Hematology Laboratory, Services Institute of Medical Sciences, Lahore.

Results: Eighty patients with a megaloblastic change in bone marrow were studied. There were 32 males (40%) and 48 females (60%). The most common clinical presentation was pallor and fatigue (67 patients, 84%). Out of the 80 patients, 50 (62.5%) were deficient in folic acid and 24 patients (30%) were Vitamin B_{12} deficient. 6 patients (7.5%) were Coomb's positive, indicating Immune-mediated Hemolytic Anemia as the cause of megaloblastic anemia.

Conclusion: Folic acid deficiency was the most common cause of megaloblastic anemia (62.5%) in the given population. Vitamin B_{12} deficiency was the next most common cause (30%). 6 patients (7.5%) had normal levels of Vitamin B_{12} and Folic acid and were Coomb's positive showing that Immune – mediated hemolytic anemia can also be a cause of megaloblastic change in the bone marrow.

INTRODUCTION

Vitamin B_{12} and folic acid are essential components of DNA synthesis in red cell precursors. Folic acid is directly involved and Vitamin B_{12} (methyl cobalamine) participates as a co-factor. A deficiency of Vitamin B_{12} causes the same symptoms as folic acid deficiency. Lack of either factor disrupts the maturation process of cells and causes a megaloblastic change in precursors.[1]

Megaloblastic anaemia is characterized by macrocytic red blood cells (RBCs) and typical morphological changes in the hematopoeitic precursors. Precursors are larger than the cells of the same stage and there is disparity in nuclear – cytoplasmic maturation (Hoffbrand). Macrocytosis is a relatively common finding in the era of automated blood cell counters with 1.7% – 3.6% macrocytes being seen. In routine blood count, physiological macrocytosis is seen in infants and pregnancy.[2-4]

Macrocytosis with anaemia or macrocytic anaemia may be with a megaloblastic change in the bone marrow or with a non-megaloblastic change in the bone marrow. Macrocytosis with non-megaloblastic bone marrow is observed in aplastic anaemia, pure red cell aplasia, dyserythropoeitic anaemia, hypothyroidism and chronic liver disease[5]. Anisocytosis is found to be much higher in megaloblastic anaemia as compared to non-megaloblastic anaemia.[6]

Macrocytosis with a megaloblastic change in the bone marrow is observed in neoplastic conditions like myelodysplastic syndrome while the most common cause is deficiency of Folic acid and Vitamin B_{12}.[4-6]

Vitamin B_{12} deficiency may also result if there is surgical removal of the stomach or ileum, malabsorption disorders of intestine or worm infestation. Vitamin B_{12} deficiency is far more common in vegetarians than in non-vegetarians.[7] Children born to mothers with B_{12} deficiency are more prone to develop B_{12} deficiency as they are born with depleted stores.[5-7]

Pernicious anaemia is a peculiar type of megaloblastic anaemia which results from deficiency of Intrinsic Factor.[10]

The other cause for megaloblastic change is folic acid deficiency which is the most common vitamin deficiency. It occurs in malabsorptive syndrome (Chron's disease, and adult celiac disease). Folic acid deficiency is seen more commonly in the elderly, pregnancy, growing children and in people with haemolytic anaemias.[8-9] In chronic immune mediated haemolytic anaemia there is erythroid dyspla-

sia and it can result in conditioned folate deficiency.[12] Clinical features of megaloblastic anaemia like anorexia, irritability and easy fatiguability are common and are attributed to anemia. Those peculiar to megaloblastic anaemia are hypopigmentation, enlargement of liver and spleen and sore tongue.[11,12]

Neurological features seen in Vitamin B_{12} deficiency are parasthesia in fingers and feet, memory loss, poor gait, loss of position sense, psychiatric disturbances, blindness and optic atrophy. Neurological symptoms are not seen in folic acid deficiency.[13]

Macrocytosis with increased MCV is seen in chronic immune mediated haemolytic anaemia. It is due to an increase in reticulocyte count. Bone marrow undergoes a megaloblastic change due to an increased demand of folic acid for hyperplastic erythropoeisis. In short, megaloblastic change is most commonly observed due to deficiency of Vitamin B_{12} and folic acid.[10-12]

Laboratory investigations reveal macrocytic normochromic RBCs. MCV is increased and RBC count is decreased. RDW is increased and the value varies proportionate to the degree of anaemia. WBC series show morphological changes like hyper-segmented neutrophils. Total leukocyte count is decreased. Platelet count is also decreased and a peripheral picture of pancytopaenia is observed.[14-16]

Characteristic bone marrow findings are seen: erythroid precursors are large (megaloblasts); nuclear maturation lags behind cytoplasmic maturation. Howell – jolly bodies and nuclear fragmentation is seen.[17,18] Myeloid precursors show giant myelocytes and metamyelocytes and hyper – pigmented neutrophils. Megakaryotcytes also show dyspoeitic features. Overall, a picture of ineffective erythropoeisis is seen which is responsible for pancytopenia.[17-18] Serum bilirubin (un-conjugated) and LDH are increased. Recently, brittleness of bone due to decrease in bone – marrow density have been described.[19]

MATERIALS AND METHODS

This study was conducted at the department of Haematology, Services Institute of Medical Sciences, Lahore. In this descriptive study, we evaluated clinical and morphological features of 80 consecutive patients with a megaloblastic bone marrow picture.

The patients were clinically evaluated in detail by history, relevant physical examination and laboratory investigations. Symptoms due to anemia including pallor and fatigue, shortness of breath and palpitations were noticed with their severity and duration. Moreover, relevant previous medical record was reviewed for evidence of fever, weight loss, infections, bleeding and gastrointestinal symptoms. On clinical examination, pallor, jaundice, hepatomegaly and splenomegaly were observed.

Routine investigations including complete blood count (CBC), erythrocyte sedimentation rate (ESR), red cell indices, platelet count and reticulocyte count were carried out on all subjects. Results of Coomb's test, serum folate and Vitamin B_{12} were also noticed. Serum folate and Vitamin B_{12} were

Table 1.0: *Age Distribution of the Patients.*

Age (years)	*Number of Patients*	*Percentage (%)*
1 – 15	18	22.5
15 – 20	24	30
21 – 30	8	10
31 – 40	6	7.5
41 – 50	5	6.3
51 – 60	9	11.3
61 – 70	10	12.5

Table 1.1: *Gender distribution of the patients.*

Gender	*Number of Patients*	*Percentage (%)*
Male	32	40
Female	48	60

Table 2.0: *Cause of Megaloblastic Anemia.*

	Number of patients	*Percentage (%)*
Total	80	-
Deficient in Folic Acid	50	62.5
Deficient in Vitamin B_{12}	24	30
Normal levels of FA and Vit. B_{12} (Coomb's positive)	06	7.5

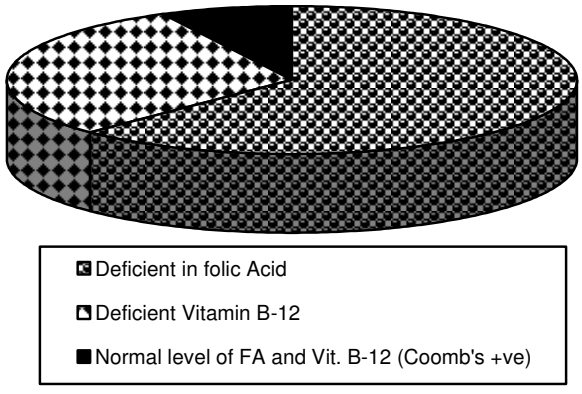

Figure 1.0: *Underlying cause of Megaloblastic Anemia.*

measured by chemiluminescent technique in Vitros immunodiagnostic system using Vitros B_{12} and folate reagent pack and calibrators (Ortho-clinical diagnosis; Johnson & Johnson Company). The morphological findings were observed after bone marrow examination.

Data Collection

A semi structured data collection system based on open and close – ended questions was de-signed to collect data about the clinical and morphological findings of the patients under study. A data entry program was developed and all the numerical data regarding the study was entered in the computer system. Final analysis was performed with the help of SPSS v 20.

RESULTS

Folic acid deficiency was observed in 50 (62.5%) patients while B_{12} deficiency was seen in 24 (30%) patients. 6 (7.4%) patients had normal levels of folic acid and Vitamin B_{12} and were Coomb's positive.

Peripheral blood picture (Table 3.0) showed that all the patients had Haemoglobin levels less than 11 g/dl while 66 patients (80%) had leucopaenia. Forty eight patients (60%) had thrombocytopaenia. Reticulocyte count was less than 2.0% in 68 patients (80%).

Fifty patients were deficient in folic acid – their levels were 1.79 ± 0.51 ng/ml (Table 3.1). The normal reference range for folic acid was *2.7→ 20 ng/ml*. 30 patients had normal levels of folic acid – 6.70 ± 2.70 ng/ml. Red cell indices in the folic acid deficient patients were: MCV = 105 ± 15 fl; MCH = 33 ± 4 pg; MCHC = 32 ± 2 g/l; PCV = 16.5 ± 6.5%; RDW = 28 ± 3 (Table 4.1).

Twenty four patients were Vitamin B12 deficient with serum B_{12} value 70.0 ± 57.4 pg/ml. The normal reference range was 239 – 931 pg/ml. A total of 56 patients who had normal B_{12} levels – 324 ± 56.7 pg/ml. Red cell indices in the Vitamin B_{12} deficient patients were: MCV = 99 ± 16 fl; MCH = 31 ± 5 pg; MCHC = 32 ± 3 g/l; PCV = 16.6 ± 5.9%; RDW = 27 ± 3 (Table 4.2).

Six patients had megaloblastic change in their bone marrow but their folic acid levels were not below reference range (6.70 ± 2.70 ng/ml). Their B_{12} levels were also within normal range 324 ± 56.7

Table 3.0: *Hematological parameters of patients with megaloblastic anemia.*

Hematological Parameter	*Number of Patients*	*Percentage of Patients (%)*
Haemoglobin less than 11 g/dl	80	100
TLC less than 4×10^9 /l	66	80
Platelet count less than 200×10^9/l	48	60
Reticulocyte count less than 2.0%	68	82

Table 4.1: *Folic Acid (FA) – Comparison of Hematological Parameters in patients with deficient and normal levels.*

	Folic Acid Deficient	*Normal Levels of Folic Acid*	*P-value*
Number of patients	50	30	
FA levels (mean ± SD) (ng/ml)	1.79 ± 0.51	6.70 ± 2.70	Sig. < 0.001
MCV (mean ± SD) (fl)	105 ± 15	101 ± 20	NS
MCH (mean ± SD) (pg)	33 ± 4	32 ± 6	NS
MCHC (mean ± SD) (g/l)	32 ± 2	33 ± 3	NS
PCV (mean ± SD) (%)	16.5 ± 6.5	15.5 ± 5.5	NS
RDW (mean ± SD)	28±3	27±3	NS

Table 4.2: *Vitamin B_{12} Comparison of Hematological Parameters in patients with deficient and normal levels.*

	Vitamin B_{12} deficient	*Normal levels of Vitamin B_{12}*	*P-value*
Number of patients	24	56	
Vit. B_{12} levels (mean ± SD) (pg/ml)	70.0 ± 57.4	324 ± 56.7	Sig. <0.001
MCV (mean ± SD) (fl)	109 ± 18	99 ± 16	NS
MCH (mean ± SD) (pg)	35 ± 6	31 ± 5	NS
MCHC (mean ± SD) (g/l)	32 ± 7	32 ± 3	NS
PCV (mean ± SD) (%)	15.2 ± 5.8	16.6 ± 5.9	NS
RDW (mean ± SD)	28 ± 3	27 ± 3	NS
Comparison p-value	NS	NS	NS

pg/dl. These patients were Coomb's positive. Red cell indices in these patients were: MCV = 97 ± 20 fl; MCH = 30 ± 5 pg; MCHC = 33 ± 3 g/l; PCV = 16 ± 6%; RDW = 27 ± 3 (Table 4.3).

Table 4.3: *Normal levels of Folic Acid and Vit. B_{12} Comparison of Hematological Parameters between Coomb's positive and negative patients*

	Coomb's Positive	*Coomb's Negative*	*Comparison P-value*
Number of patients	06	74	NS
Folic Acid level (mean ± SD) (ng/ml)	6.70 ± 2.70	6.70 ± 2.70	NS
Vitamin B-12 level (mean ± SD) (pg/ml)	324 ± 56.7	324 ± 56.7	NS
MCV (mean ± SD) (fl)	97 ± 20	105 ± 17	NS
MCH (mean ± SD) (pg)	30 ± 5	33 ± 5	NS
MCHC (mean ± SD) (g/l)	33 ± 3	32 ± 5	NS
PCV (mean ± SD) (%)	16 ± 6	16.1 ± 5.2	NS
RDW (mean ± SD)	27 ± 3	27 ± 3	NS

DISCUSSION AND CONCLUSION

Our study included 80 patients who were referred to the Department of Haematology with anaemia. All the patients had MCV of more than 100 fl (normal range: 76 – 96 fl) and their bone marrow showed a megaloblastic change. Work up of the patients was done to find out the cause of megaloblastic anaemia in these patients. Females (48 patients, 60%) were more than males (32 patients, 40%). The maximum number of patients was aged between 2 and 15 years (24) and the next most common age range was between 61 – 70 years (18) (Table 1.0).

The study was in accordance with Gomber (1998), Gera (2001), Khanduri (2005) who have shown similar age distribution. Mikibi et al (1992), Ali and Mannahet (1995) showed B_{12} and folic acid deficiency in all age groups.

Presenting clinical features varied with different patients. The majority of them presented with pallor and fatigue (67, 84%). Dyspnea and palpitations were the next common symptoms (63, 79%). Abdominal pain, nausea and vomiting was seen in 51 patients (64%) and splenomegaly was seen in 38 patients (48%).

Table 5.0: *Clinical Findings in patients with Megaloblastic Anemia.*

Clinical Findings	*Number of Patients*	*Percentage (%)*
Pallor and fatigue	67	84
Fever and weight loss	36	45
Dyspnea and palpitations	63	79
Abdominal pain, nausea and vomiting	51	64
Infections, bleeding	27	34
Jaundice and hepatomegaly	22	28
Splenomegaly	38	48

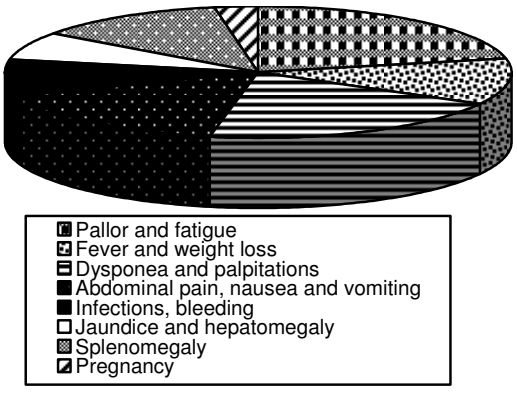

Figure 2.0: *Clinical Findings in patients with Megaloblastic Anemia.*

Our findings are in accordance with other authors (Gomber). Bleeding tendency was seen in 22 patients. Similar findings have been reported by other studies (Gupta et al) (Saxena et al).

Pancytopenia was seen in 33 patients while bicytopenia, i.e., reduced TLC and Hb was seen in 53 patients. Our findings are in accordance with other studies carried out.[17,18]

The majority of our patients were folic acid deficient (50 patients, 62.5%). These results are in agreement with Bhhende et al, Gracia-casal et al [23]. The next most common cause of megaloblastic anaemia was Vitamin B_{12} deficiency (24 patients, 30%). Combined deficiency was seen in 10 patients, the results are in accordance with Khanduri et al,[7] Gomber et al,[8] Khunger et al.[15]

Patients who present with a megaloblastic change in the bone marrow are usually not tested for immune mediated Haemolytic Aanemia in Pakistan.[4] This study has shown that 06 patients (7.5%) were Coomb's positive and had normal Vitamin B_{12} and Folic Acid levels. Hence, future investigations for such a bone marrow change should include a Coomb's test as part of normal diagnostic routine if Vitamin B_{12} and Folic Acid levels are reported within normal range.

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REFERENCES

1. Florence A., Joseph J., Steren H. Megaloblastic anemia and other causes of macrocytosis. Clinical medicine and Research 2008; 4 (3): 236-241.
2. Gupta PK, Saxena R, Karan AS, Choudhury VP. Red cell indices for distinguishing macrocytosis of aplastic anemia and megaloblastic anemia. Indian J of Pathol Microbiol 2003; 46: 375-377.
3. Chaudhry MW. Clinico-hematological study of nutritional anemia is young children. Thesis for MD Pediatrics, Delhi University, 2001.
4. Savage DG, Ogundipe A. Allen RH, Stabler SP, Lindenbaum J. Etiology and diagnostic evalution of macrocytosis. Am J. Med Sci 2000; 319: 343-352.
5. Marwaha RK, Singh S, Garewal G et al. Bleeding manifestations in megaloblastic anemia. Indian J Pediatr 1989; 56: 243-247.
6. Gracia – Casal MN, Osorio C, Landaeta M, Leets I, Matus P, Fazzino F, Marcos E. High prevalence of folic acid and Vitamin B_{12} deficiencies in infants, children, adolescents and pregnant women in Venezuela. Eur J. Clin Nutr 2005; 59: 1064-1070.
7. Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. Natl Med J India 2005; 18: 182-183.
8. Gomber S, Kumar S, Rusia U et al. Prevalence and etiology of nutritional anemias in early childhood in an urban slum. Indian J. Med Research 1998; 107: 269-273.
9. Cagnacci A, Cannoletta M, Baldassari F, Volpe A. Low vitamin B_{12} and bone loss: A role for folate deficiency. J. Clin Endocrinol Metabol 2004; 89: 4770-4771.
10. Gomber S, Kela K, Dhingra N. Clinico-hematological profile of megaloblastic anemia. Indian Pediatr 1998; 35: 54-57.
11. Madood-ul-Mannan, Anwar M, Saleem A el al. Study of serum Vitamin B-12 and folate levels in patients of megaloblastic anemia in northern Pakistan. J. Pak Med Assoc 1995; 45: 187-188.
12. Gehrs BC, Friedberg RC Autoimmune hemolytic anemia. Am J Hematol 2002; 69: 258-271.
13. Louwman MWJ., CVan Dussledorp M., Van de Vijver FJR et al. Signs of impaired cognitive functions in adolescents with marginal cobalamin status. Am. J. Clin. Nutr. 2000; 72: 762-769.
14. Sarode R., Garewal G., Marwala N et al. Pancytopenia in nutritional megaloblastic anemia A study from north – west India. Trop. Geog. Med. 1984; 41: 331-336.
15. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH, Pancytopenia – a clinicohematological study of 200 cases. Indian J. of Pathol Microbiol 2002; 45: 375-379.
16. Maktouf C, Bchir A, Louzir H, Mdhaffer M, Elloumi M, Ben Abid H. Megaloblastic anemia in North Africa. Haematologica 2006; 91 (7): 990–991.
17. Ward PC. Modern approaches to the investigation of Vitamin B_{12} deficiency. Clin Lab Med 2002; 22: 435-445.
18. Gera R, Singh ZN, Chaudhry P. Profile of nutritional anemia in hospitalized children over a decade. Conference Abstracts, 38th National conference of Indian academy of pediatrics Patna 2001; HO-09: pp 60.
19. Jaswal TS, Mehta HC, Gupta V, Singh M, Singh S. Serum lactate dehydrogenase in diagnosis of megaloblastic anemia. Indian J Pathol Microbiol 2000; 43: 325-329.