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STUDY OF CORRELATION BETWEEN SERUM VITAMIN B12 LEVEL AND CLINICOHEMATOLOGICAL FINDINGS IN MEGALOBLASTIC ANEMIA

Pathology

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ABSTRACT

Introduction- Anemia is not a diagnosis in itself but merely an objective sign of presence of disease. In developing countries like India Megaloblastic anemia (MA) is one of the most common type of nutritional anemia. Importance of this study is in planning the diagnostic and therapeutic approach in patients with Megaloblastic anemia. **Materials and Methods-** Total 100 cases were studied out of which 50 were Normal healthy controls and 50 were suspected and diagnosed cases of MA. complete blood hemogram with differential count, platelet count along with blood indices and peripheral smears, bone marrow and serum Vitamin B12 estimation was done were done and examined in detail. **Results:** Total 100 cases were studied out of which 28 (56%) controls were male while 22 (44%) were female and 28 (56%) cases of MA were male while 22 (44%) cases were female. Most common presenting complaints were generalised weakness/Fatigability in 46 (92%) cases while pallor was most common presenting sign in all 50 (100%) cases. 40 cases and 6 controls were vitamin B12 deficient. There was statistically significant association between serum vitamin B12 levels and megaloblastic anemia. **Conclusion:** To conclude estimation of serum Vitamin B12 deficiency is valuable in diagnosis and treating the Megaloblastic anemia and helps in where bone marrow aspiration facility is not available or patient is reluctant due to invasive procedure. Cobalamine deficiency appears to be emerging as significant contributor to nutritional megaloblastic anemia.

KEYWORDS

Megaloblastic anemia, Vitamin B12, Bone marrow aspiration.

INTRODUCTION

Anemia are the most important disorders of blood resulting in significant morbidity and mortality, constitute a public health problem of considerable importance. Anemia is not a diagnosis in itself but merely an objective sign of presence of disease.[1]

In developing countries like India Megaloblastic anemia (MA) is one of the most common type of nutritional anemia. In India Vitamin B12 deficiency is seen in about 3.8% of the population.[2] The classic blood count findings are anemia, high MCV and MCH and in more advanced cases, thrombocytopenia and neutropenia. Decline in red cell counts precedes that of hemoglobin and packed cell volume levels. Vitamin B12 is necessary for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function. Demyelination of the cervical and thoracic dorsal and lateral columns of the spinal cord, occasional demyelination of cranial and peripheral nerves, and demyelination of white matter in the brain (subacute combined degeneration) can occur with Vitamin B12 deficiency.[3] Thereby, importance of this study is in planning the diagnostic and therapeutic approach in patients with Megaloblastic anemia.

OBJECTIVES

1. To evaluate utility of serum Vitamin B12 estimation in diagnosis to study clinicohematological features of megaloblastic anemia.
2. To determine percentage of Vitamin B12 deficiency in megaloblastic anemia.

MATERIALAND METHODS

The study is an observational study performed for period of 2 years. Total 100 cases were studied out of which 50 were Normal healthy controls from blood donation camp, OPD and 50 were suspected and diagnosed cases of MA referred to hematology section of pathology department for hematological investigations, peripheral blood smear and bone marrow examination studied. Fifty cases were studied based on presence of Hemoglobin < 10 g/dl and Raised MCV >95. Diagnosed cases of leukemia under treatment are excluded. Complete medical history and clinical details were obtained for each patient. Primary hematological procedures were carried out, which included complete blood hemogram with differential count, platelet count along with blood indices including- Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) on cell counter. Peripheral smears were done and examined in detail. Bone marrow aspiration yielded adequate material in all cases and examined. Serum Vitamin B12 estimation was done by automated Chemiluminescence assay analyser in all cases.

OBSERVATIONS AND RESULTS

Total 100 cases were studied out of which 50 cases were Normal healthy controls and 50 cases with features of MA were studied. Out of total controls, 28 (56%) controls were male while 22 (44%) were female. Cases of MA in age group 11-20 yrs were (1)2%, in age group 41-50yrs were (12)24%, in age group 51-60yrs were (2)4% and in age group 61-70 yrs were (5)10%. The majority of cases (16) 32% of MA were among the 21-30year age group followed by 28%(14) cases of 31-40 yrs. age group. Thus MA was more common during reproductive age group. The mean age of presentation of MA was 38 years. Out of 50 cases, 28 (56%) cases of MA were male while 22 (44%) cases were female.

Table No.1 Major Symptoms at presentation

Sr. No.	Presenting complaints	No. of patients	Percentage
1	Generalised weakness/Fatiguability	46	92
2	Dyspnoea	32	64
3	Anorexia and gastritis	24	48
4	Fever	21	42
5	Glossitis	10	20
6	Palpitations	8	16
7	Tingling and numbness	6	12
8	Hyperpigmentation of skin	4	8

Table No.2 Major signs on presentation

Sr. No.	Presenting signs	No. of patients	Percentage
1.	Pallor	50	100
2.	Splenomegaly	11	22
3.	Hepatomegaly	10	20
4.	Edema	11	22
5.	Icterus	10	20
6.	Skin changes	06	12
7.	Neurological signs	04	08

Table No.3 Hematological parameters in cases of megaloblastic anemia

Sr.No.	Investigations	Mean±S.D.	Range
1	Hemoglobin (gm%)	5.6±1.5	2.5-8.5
2	MCV(fl)	108.9±9.3	97-134

3	TLC (cmm)	4332±195	1800-8400
4	Platelet count (lacs/cmm)	1.37±0.8	0.05-3.5
5	Serum Vitamin B12 level (pg/ml).	169.52±69.78	74-327

Table No.4 Table showing hematological presentation.

Sr.No.	Presentation	No. of cases	Percentage
1	Anemia	50	100
2	Leucopenia	27	54
3	Thrombocytopenia	29	58
4	Pancytopenia	22	44
5	Bicytopenia	12	24

Cases of MA can present with pancytopenia or bicytopenia along with anemia.

Total 28 (56%) cases were with Hb ≤6 and average MCV of 114.1fl. Total 22 (44%) cases were with Hb >6 and average MCV of 104.8 fl. Average MCV was 108.9 fl observed in total 50 cases. Thus MCV was at higher level in Severe MA patients.

Out of 50 patients 34(68%) were vegetarians with average Vitamin B12 level 127.5 pg/ml. 14(32%) patients were mixed vegetarians Vitamin B12 level 181.5pg/ml. From above findings Vitamin B12 level were low in cases with vegetarian diet as compared to cases with mixed vegetarian dietary habits. Thus there was correlation between the dietary habits and occurrence of Vitamin B12 deficiency in MA.

Table No. 5 Comparison of Vitamin B12 and hematological parameters in patients with deficient and normal level of serum Vitamin B12.

	Vitamin B12 deficient cases.	Cases with Normal Vitamin B12 level.
Number of patients	40	10
Vitamin B12 level (pg/ml) (Mean+SD)	140±36.9	287.6±35.0
Haemoglobin gm% (Mean+SD)	5.59±1.5	5.9±1.4
MCV (fl) (Mean+SD)	110±9.9	105.2±5.2

In MA, MCV rises as Vitamin B12 level decreases.

Out of 50 cases of MA 40 cases and 6 controls were Vitamin B12 deficient. (Vitamin B12 <200pg/ml). 10 cases of MA and 44 controls were having normal levels of Vitamin B12 levels. (Vitamin B12 level 200 -900pg/ml.) Chi square value was 46.54 and P value was <0.0001. So there was statistically significant association between serum Vitamin B12 levels and megaloblastic anemia.

In 43(86%) cases the peripheral blood smear was predominantly macrocytic with presence of macro-ovalocytes. while in 7(14%) cases it was dimorphic consisting of predominantly macro-ovalocytes as well as microcytic RBCs with hypochromia. Hypersegmented neutrophill with 5 or more lobes was seen in 34(68%) cases in peripheral blood smear. In all 50 cases the bone marrow shows Megaloblastic maturation consisting of large megaloblastic erythroblasts with open sieve like chromatin, Nuclear fragmentation, paranuclear halo, Howell-jolly bodies, Giant metamyelocytes and band forms.

Bone marrow aspirations in present study of MA showed two distinct types of cellularity, 41(82%) hypercellularity with predominantly Erythroid hyperplasia with Megaloblastic maturation and In 9(18%) cases bone marrow was Normocellular with predominantly erythroid hyperplasia with megaloblastic maturation.

MICROSCOPIC PICTURES IN MEGALOBLASTIC ANEMIA

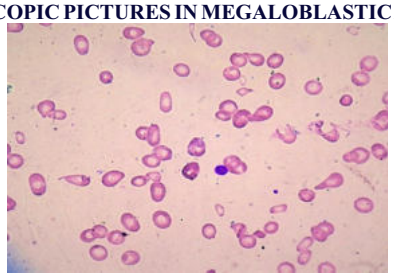


Fig.No.1 Peripheral Blood smear of Megaloblastic anemia showing Macro- ovalocytes.

Fig.No.2 Peripheral Blood smear of Megaloblastic anemia showing Hypersegmented Neutrophil.

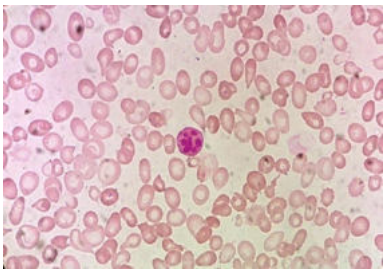


Fig.No.3 Bone marrow Aspiration of Megaloblastic anemia showing Erythroid Hyperplasia.

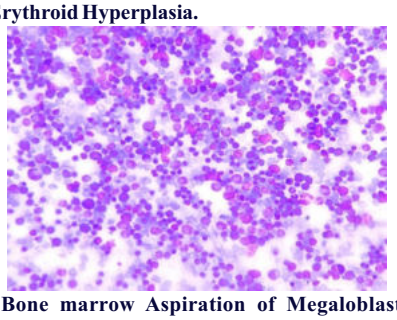
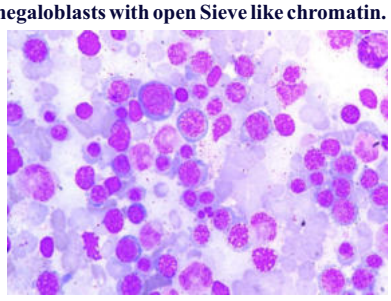


Fig.No.4 Bone marrow Aspiration of Megaloblastic anemia showing megaloblasts with open Sieve like chromatin.



DISCUSSION

Table No. 6 Comparison of most common age group & male to female ratio in various studies.

	Khanduri U *et al* (2007)	Unnikrishnan V*et al* (2008)	Shidappa G *et al* (2014)	Barik s *et al*(2015)	Present Study
No. of Cases	120	60	34	66	50
Commonest Age Group	11 to 20 Years	21 to 30 Years	21-40 years	21 to 35 years	21 to 30 years
Male to female ratio	1 : 1.4	1.8 : 1	3.2:1	1.56 : 1	1.27 : 1

The majority of cases (16) 32% of MA were among the 21-30 year age group followed by 28%(14) case of 31-40 yrs age group. Thus MA is more common during reproductive age group due to increased demand of Vitamins in our study. 28 (56%) cases of MA were male while 22 (44%) cases were female. Thus MA is more common in male as compared to female in our study. The incidence of MA showed slight preponderance among males. Approximately male to female ratio was 1.27:1. Khanduri U *et al* (2007)[4] studied 120 cases of MA and showed that most common age group was 11-20 years with female predominance. Unnikrishnan V *et al* (2008)[5] had shown that commonest age group was 21-30 yrs. and male to female ratio as 1.8:1 with male predominance. Shidappa G *et al* (2014)[6] had shown that commonest age group was 21-40 yrs. and male to female ratio as 3.2:1 with male predominance. Barik S *et al* (2014)[7] studied 66cases of MA and found that commonest age group was 21-35 yrs. and male to female ratio as 1.56:1 with male predominance. Thus our study is in agreement with the study by Unnikrishnan V *et al* (2008)[5] and Shidappa G *et al* (2014)[6] and Barik S *et al* (2015)[7] but not agreed to the study of khanduri U *et al* (2007)[4] may be due to geographical variation. From table no.1 as compared to our study, Khanduri U *et al* (2007)[4] had shown that common symptoms in order of their presentation were Fatigue (70%),anorexia and gastritis low grade fever (50%), cardiovascular (shortness of breath, palpitations and syncope) (30%)

and yellow discoloration of eyes (20%). Paraesthesias, diarrhea, hyperpigmentation and early graying of hair were present in <10% of patients. Unnikrishnan V *et al* (2008)[5] had shown that common symptoms in order of their presentation were Fatigue Breathlessness, Abdominal pain, Hyperpigmentation of knuckle, glossitis. Haq S *et al* (2012)[8] studied 80 patients and found that most common presentation was pallor, fatigue, fever, dyspnea, palpitations, nausea, jaundice.

From table No.2 as compared to our study, khanduri U *et al* (2007)[4] had shown that common signs were pallor (85%), glossitis (29%), mild icterus (25%) and hyperpigmentation of knuckles (18%). Gupta R *et al* (2009)[1] had shown that major symptoms were Pallor (100%) Hepatomegaly (38%), Pedal edema (24%), Icterus (14%), Hyperpigmentation (10%), Splenomegaly (10%). Barik S *et al* (2015)[7] studied 66 cases and found that major signs on presentation were Pallor, Splenomegaly and Hepatomegaly.

Table No.7 Comparison of Hematological parameters in different studies.

	Unnikrishnan V et al (2008)	Veda P. (2013)	Present study
Total no. of cases	23	38	50
Haemoglobin (gm%)	4.96	8.8	5.6
MCV(fl)	111.18	111.8	108.9
TLC(cmm)	4.30	5.13	4.33
Platelet count(cmm)	0.98	1.92	1.37

From table no.3 and 7 In our study we studied 50 cases with Hb ranging from 2.5 gm% to 8.5 gm% and found average Hb 5.6gm%, MCV ranging from 97-134 fl and average MCV was 108.9 fl, TLC ranging from 1800-8400/cmm and average TLC was 4332/cmm, Platelet count ranging 0.05 -3.5 lacs/cmm and average platelet count of 1.37 lacs/cmm. Unnikrishnan V *et al* (2008)[5] studied 23 cases and found average Hb 4.96 gm%, MCV 111.18 fl ,TLC 4300 /cmm, Platelet count of 0.98 lacs/cmm. Veda P. (2013)[9] studied 38 cases and found average HB 8.8 gm%, MCV 111.8 fl, TLC 5013 /cmm, Platelet count of 1.92 lacs/cmm. Thus in MA we found that majority of cases showed increase in MCV and decrease in TLC and Platelet count. Thus our study is in agreement with the study Unnikrishnan V *et al* (2008)[5] and by Veda P. (2013)[9].

From table no.4 as compared to our study, Khanduri U *et al* (2007)[4] in their study found that pancytopenia was present in 62% of patients. Chandra J *et al* (2010)[10] had shown that leucopenia in 17-49% cases and thrombocytopenia in 44-80% cases and MA manifest with pancytopenia and MA is most common cause of pancytopenia. Haq S *et al* (2012)[8] in their study found that 80% patients had leucopenia and 60% patients had thrombocytopenia. Siddiqui B *et al* (2015)[11] found that 26.6% patients showed pancytopenia. Thus MA can be presented with pancytopenia or bicytopenia.

As compared to our study, Unnikrishnan V *et al* (2008)[5] had shown that 38(63%) patients have Hb <6 gm% have average MCV 107.7fl and 22(36%) patients have Hb >6 gm% have average MCV 104fl. Thus level of MCV was higher in severe MA.

As compared to our study, Barik S *et al* (2014)[7] studied 66 cases of MA and found that 53 (82%) cases were vegetarian and 13(18%) cases were non vegetarian. Siddiqui B *et al* (2015)[11] studied 15 cases of MA and found that 12 (80%) cases were vegetarian and 3(20%) cases were non vegetarian. Mahajan S *et al* (2015)[12] studied 67 cases of MA and found that 33 (33%) cases were vegetarian and 67(67%) cases were non vegetarian. From above findings MA was more common among the vegetarians as compared to mixed vegetarian as the most dietary sources of Vitamin B12 are obtained from food of animal protein origin like kidney, liver, heart, muscle meats, fish but deficient in vegetables.

From Table No.5 there was statistically significant association between serum Vitamin B12 levels and MA. Haq S *et al* (2008)[8] had shown that in Vitamin B12 deficient patients Mean Vitamin B12 level were 70.0$\pm$57.4 pg/ml with P value <0.001(significant), Hb 5.1$\pm$1.7gm%, MCV 109$\pm$18fl and in Non Vitamin B12 deficient patients Mean Vitamin B12 level were 324$\pm$56.4 pg/ml, Hb 5.8$\pm$1.8gm% , MCV 99$\pm$16fl.

Khanduri U *et al* (2008)[4] studied 120 cases and showed that hypersegmented neutrophils in all cases. Bone marrow was performed in 22 cases of MA showed moderate to marked hypercellularity with megaloblastic maturation. Unnikrishnan V *et al* (2008)[5] studied 26 cases of MA and showed that presence of macro-ovalocytes and hypersegmented neutrophils in peripheral smear always goes with diagnosis of MA. Gupta RK *et al* (2009)[1] studied 50 cases and also showed similar findings. Haq S *et al* (2012)[8] studied 80 cases and showed hypersegmented neutrophils and macro-ovalocytes in peripheral blood smear. Bone marrow was performed in 80 cases of MA showed hypercellular marrow with megaloblastic maturation. Veda P. (2013)[9] studied 43 cases of MA and showed hypersegmented neutrophils in 32 (86%) cases and macro-ovalocytes in 27 (72%) cases. Bone marrow was performed in 17 cases of MA showed megaloblastic maturation

Thus to conclude the discussion in patients with clinical features of MA and raised MCV, P.S. examination should be carried out for presence of Hypersegmented neutrophils and macro-ovalocytes. Bone marrow examination confirms the diagnosis with presence of megaloblastic features and the Vitamin B12 estimation should be carried out before initiating the treatment. There are many other causes of MA which were not assessed in present study and it needs further studies.

CONCLUSION

To conclude estimation of serum Vitamin B12 deficiency is valuable in diagnosis and treating the Megaloblastic anemia and helps in where bone marrow aspiration facility is not available or patient is reluctant due to invasive procedure. The Vitamin B12 estimation should be carried out before treating the anemia because treatment with folic acid alone is dangerous in MA due to Vitamin B12 deficiency. As neurological lesions can progress even though the anemia is undergoing significant improvement if Vitamin B12 is not treated. Cobalamine deficiency appears to be emerging as significant contributor to nutritional megaloblastic anemia. Thereby, importance of this study is in planning the diagnostic and therapeutic approach and follow up of patients with Megaloblastic anemia.

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Authors declare and acknowledge that we have no conflict of interest.

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