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RESEARCH ARTICLE

STUDY OF CORRELATION BETWEEN SERUM VITAMIN B12 LEVEL AND CLINICOHAEMATOLOGICAL FINDINGS IN MEGALOBLASTIC ANAEMIA

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ABSTRACT

Anemias are the most important disorders of blood resulting in significant morbidity and mortality, constitute a public health problem of considerable importance

INTRODUCTION

Megaloblastic Anaemia

Anemias are the most important disorders of blood resulting in significant morbidity and mortality, constitute a public health problem of considerable importance. Anemia is generally defined as a reduction in red cell mass or blood hemoglobin concentration characterized by decreased oxygen carrying capacity of blood which results in tissue anoxia producing various signs and symptoms. Anemia is not a diagnosis in itself but merely an objective sign of presence of disease.[1]
In developing countries like India Megaloblastic anaemia is one of the most common type of nutritional anaemia. In india Vitamin B12 deficiency is seen in about 3.8% of the population.[2]

Megaloblasts are characterized by their large size and by specific alterations in the appearance of their nuclear chromatin. These cells have morphologic expression of a biochemical abnormality: Retarded DNA synthesis. RNA synthesis remains unimpaired while cell division is restricted. As a result cytoplasmic components, especially Hb are synthesized in excessive amounts during the delay between cell divisions and results in enlarged cell. Megaloblastic anemias are defined by the presence of these cells or by other evidence of defective DNA synthesis[3,4].

Megaloblastic anemia (MA) is a distinct type of anemia characterized by macrocytic RBCs and typical morphological changes in RBC precursors. In megaloblastic anaemia RBC precursors are larger than the normal cells and exhibit disparity in nuclear-cytoplasmic maturation. Basic underlying pathogenic mechanism in MA is deficiency of folic acid (FA) and/or Vitamin B12 at the cellular level with resultant impairment of DNA synthesis. In developing countries, most cases of MA result from nutritional deficiency of these micronutrients[5].

Many features of megaloblastic anemia may be masked when megaloblastic anemia is combined with a microcytic anemia. The anemia can be normocytic or even microcytic, whereas the blood film may show both microcytes and macroovalocytes (a "dimorphic anemia") or microcytes alone if the microcytic component is sufficiently severe[5].

Besides haematological investigations biochemical investigatios like Serum-Vitamin B12 and folic acid are also important in diagnosis and deciding further therapeutic management of Megaloblastic anaemia[6].

MATERIAL AND METHODS

The present prospective study was carried out, from october 2013 to October 2015. 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 megaloblastic anaemia 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 following criteria -

Inclusion Criteria

Case selection was based on clinical feature or laboratory evidence of megaloblastic anemia.

1. Peripheral blood smear and Bone marrow findings with features of megaloblastic anemia.
2. Presence of Hemoglobin < 10 g/dl and Raised MCV >95.

Exclusion Criteria

Diagnosed cases of leukemia under treatment.

Complete medical history and clinical details were obtained for each patient.
Serum Vitamin B12 estimation was done in all cases.

Observations and Results

Present study was conducted during the period of October 2013 to October 2015 in the department of pathology. Total hundred cases were studied including fifty controls and fifty patients who presented with features of megaloblastic anaemia. Detailed clinical history taken and examination was done. 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 Haemoglobin(MCH), Mean corpuscular haemoglobin concentration(MCHC) on cell counter. Peripheral smears were done and examined in detail. Bone marrow aspiration yielded adequate material in all cases and examined. Also serum Vitamin B12 estimation was done by chemiluminescence method.

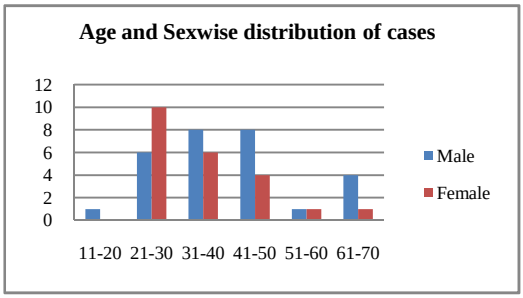
Table No.1 Distribution of cases

Sr. No.	Type of anaemia	Cases
1.	Controls	50
2.	Megaloblastic anaemia.	50
	Total	**100**

Total 100 cases were studied out of which 50 cases were Normal healthy controls and 50 cases with features of megaloblastic anaemia were studied.

Table No.2 Age and Sexwise distribution of cases studied

Sr. No.	Age group	Male	Female	Total no. Of cases.	Percentage
1	11-20	1	0	1	2
2	21-30	6	10	16	32
3	31-40	8	6	14	28
4	41-50	8	4	12	24
5	51-60	1	1	2	4
6	61-70	4	1	5	10
	Total	28	22	50	100



Graph No.1

- The majority of cases (16) 32% of megaloblastic anaemia were among the 21-30year age group followed by 28%(14) cases of 31-40 yrs. age group. Thus megaloblastic anaemia was more common during reproductive age group due to increased demand of vitamins and less intake.
- Cases of megaloblastic anaemia in age group 11-20 yrs. were (1)2%, thus megaloblastic anaemia is less common in this age group.
- Cases of megaloblastic anaemia in age group 41-50yrs. were (12)24%.
- Cases of megaloblastic anaemia in age group 51-60yrs. were (2)4%.
- Cases of megaloblastic anaemia in age group 61-70 yrs.were (5)10%.
- The mean age of presentation of megaloblastic anaemia was 38 years.

Table No.3 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

- The commonest mode of presentation was generalised weakness, which was present in 46(92%) patients. The other main symptoms were dyspnoea (64%), Anorexia and gastritis related symptoms in 24(48%) patients, Fever (42%) and glossitis was in (27.14%) of cases.
- Also 8(16%) cases complained of palpitations, Tingling and numbness was present in 6 (12%) of cases.
- Hyperpigmentation of skin was complained in 4(8%) of cases.

Table No.4 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	6	12
7	Neurological signs	4	8

- Pallor was most common presenting sign reported in all 50(100%) patients.
- Splenomegaly was seen in 11(22%) of patients followed by hepatomegaly in 10(20%) patients.
- Edema was seen in 11(22%) of patients followed by Icterus in 10(20%) of cases.
- Skin changes like hyperpigmentation was seen in 6(12%) of cases.
- 4(8%) of patients were having neurological signs.

Table No.5 Haematological parameters in cases of megaloblastic anaemia

Sr. No.	Investigations	Mean±S.D.	Range
1	Haemoglobin(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

- Among total 50 cases of megaloblastic anaemia studied Mean Haemoglobin was 5.6±1.5gm% with range of 2.5-8.5 gm%.
- In total 50 cases of megaloblastic anaemia studied Mean MCV was 108.9±9.3 fl with range of 97-134 fl.
- In total 50 cases of megaloblastic anaemia studied Mean Total leucocyte count was 4332±195/cmm with range of 1800-8400/cmm.
- In total 50 cases of megaloblastic anaemia studied Mean Platelet count was 1.37±0.8 Lac/cmm with range of 0.05-3.5 Lac/cmm.
- In total 50 cases of megaloblastic anaemia studied Mean Serum Vitamin B12 level was 169.52±69.78 pg/ml with range of 74pg/ml to 327 pg/ml.

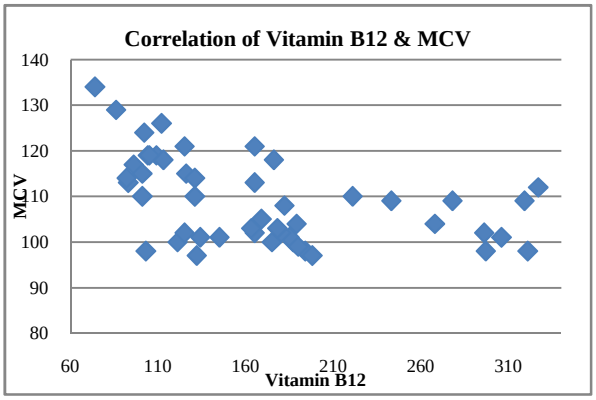
Table No.6 Table showing haematological presentation.

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

- In total 50 cases studied all of the cases were anaemic. All the cases were having Hb <10.0 gm%.
- Out of 50 cases of megaloblastic anaemia studied 27 (54%) cases had leucopenia (Total leucocyte count <4000/cmm.).
- Out of 50 cases of megaloblastic anaemia studied 29 (58%) cases had Thrombocytopenia (Platelet count < 1.5 lacs/cmm.).
- Out of 50 cases of megaloblastic anaemia studied 22 (44%) cases had pancytopenia. All the parameters including Haemoglobin, TLC and platelet were below the normal level.
- Out of 50 cases of megaloblastic anaemia studied 12 (24%) cases had bicytopenia. These patients were having anemia with either leucopenia or thrombocytopenia.
- Thus cases of megaloblastic anaemia can present with pancytopenia or bicytopenia along with anaemia.
- Total 28 (56%) cases were with haemoglobin concentration ≤6 and average MCV of 114.1fl.
- Total 22 (44%) cases were with haemoglobin concentration> 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 megaloblastic anaemia patients.

Table No.7 Comparison of Vitamin B12 and haematological 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



Graph No.2

- Out of 50 patients diagnosed of megaloblastic anaemia 40(80%) patients had deficiency of Vitamin B12 (serum Vitamin B12 <200 pg/ml).
- 10(20%) patients had normal level of Vitamin B12 (serum Vitamin B12 >200 pg/ml).
- Average Vitamin B12 level in Vitamin B12 deficient group was 140+36.9 pg/ml.
- Average Vitamin B12 level in cases with Normal Vitamin B12 was 287.6+35.0 pg/ml.
- Average Haemoglobin level in Vitamin B12 deficient group was 5.5+1.5 gm%.
- Average Haemoglobin level in cases with Normal Vitamin B12 level was 5.9+1.4 gm%.
- Average MCV level in Vitamin B12 deficient group was 110+9.9 fl.
- Average MCV level in cases with Normal Vitamin B12 level was 105.2+5.22 fl.
- From correlation diagram in megaloblastic anaemia MCV rises as Vitamin B12 level decreases.

Table no.8 Comparison of Vitamin B12 in cases and control group

	Cases of Megaloblastic anaemia	Normal controls	P value
Vitamin B12 deficient	40	6	<0.0001
Normal level of Vitamin B12	10	44	
Total	**50**	**50**	

- Out of 50 cases of megaloblastic anaemia 40 cases and 6 controls were Vitamin B12 deficient. (Vitamin B12 <200pg/ml.)
- 10 cases of megaloblastic anaemia 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 anaemia.
- 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.

Microscopic pictures in megaloblastic anaemia

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

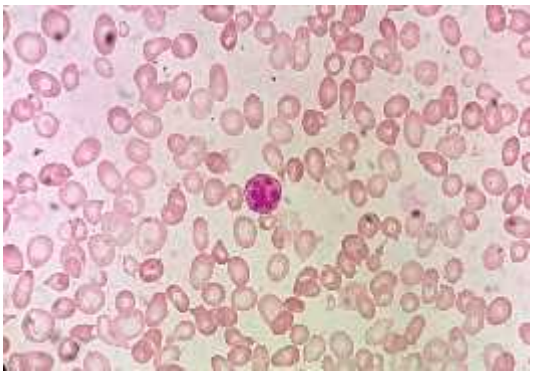


Fig.No.2 Peripheral Blood smear of Megaloblastic anaemia showing Hypersegmented Neutrophil

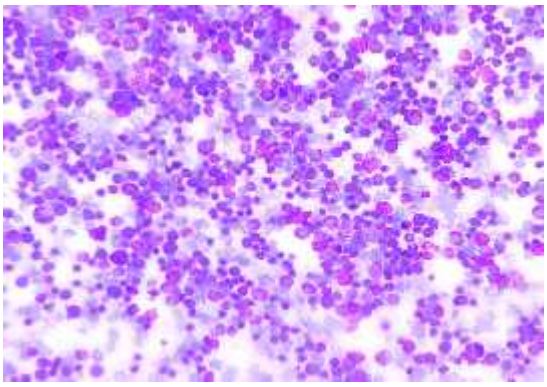


Fig.No.3 Bone marrow Aspiration of Megaloblastic anaemia showing Erythroid Hyperplasia

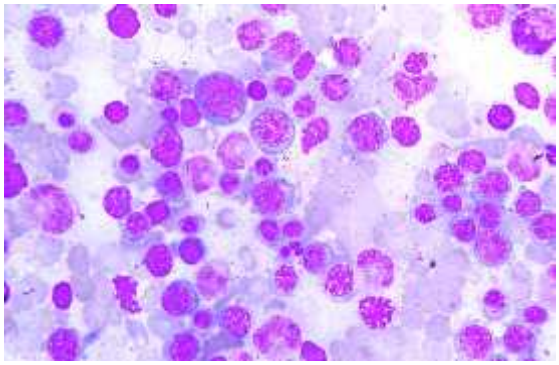


Fig.No.4 Bone marrow Aspiration of Megaloblastic anaemia showing megaloblasts with open Sieve like chromatin.

DISCUSSION

In developing countries like India Megaloblastic anaemia is one of the most common type of nutritional anaemia. In india Vitamin B12 deficiency is seen in about 3.8% of the population[2].

Megaloblastic anaemia results from abnormal maturation of haematopoietic cells due to faulty DNA synthesis. Two vitamins, cobalamin (Vitamin B12) and folic acid are essential for DNA biosynthesis. Deficiency of either vitamin results in asynchrony in the maturation of the nucleus and cytoplasm of rapidly regenerating cells. In the haematopoietic system this asynchrony results in abnormal nuclear maturation with normal cytoplasmic maturation, apoptosis, ineffective erythropoiesis, intramedullary haemolysis, pancytopenia and typical morphological abnormalities in the blood and marrow cells[6.]

Examination of the blood smear reveals the two most valuable findings for differentiating megaloblastic from nonmegaloblastic anemia: Neutrophil hypersegmentation and oval macrocytes[3].

Other changes on peripheral blood smear are anisopoikilocytosis, basophilic strippling, Howell jolly bodies and Cabots ring. It provides supportive evidence in Megaloblastic anaemia diagnosis and bone marrow examination confirms diagnosis. Besides haematological investigations biochemical investigatios like Serum-Vitamin B12 and folic acid are also important in diagnosis and deciding further therapeutic management of Megaloblastic anaemia[6.]

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 (i.e “subacute combined degeneration”) can occur with Vitamin B12 deficiency.[3,6].

Thereby, importance of this study is in planning the diagnostic and therapeutic approach in patients with Megaloblastic anaemia.

All the suspected cases and diagnosed cases of megaloblastic anaemia referred to hematology section of pathology department for hematological investigations peripheral blood smear and bone marrow examination studied. Total hundred cases were studied out which fifty controls and fifty cases selected based on the criteria already defined. Complete medical history was taken and detailed physical examination was done for each patient. All the patients selected, were then investigated, cause of Megaloblastic anaemia was ascertained and the data was analyzed on the basis of etiology, clinical and hematological findings.

Table No. 9 Comparison of most common age group & male to female ratio in various studies.

	Unnikrishnan Vet al (2008)	**Khanduri U et al (2007)**	**Shidappa G et al (2014)**	**Barik s et al (2015)**	**Present Study (2015)**
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 megaloblastic anaemia were among the 21-30 year age group followed by 28%(14) case of 31-40 yrs.age group. Thus megaloblastic anaemia is more common during reproductive age group due to increased demand of Vitamins. 28 (56%) cases of Megaloblastic anaemia were male while 22 (44%) cases were female. Thus megaloblastic anaemia is more common in male as compared to female. The incidence of megaloblastic anaemia showed slight preponderance among males. Approximately male to female ratio was 1.27:1. Khanduri U *et al* (2007)[7] studied 120 cases of megaloblastic anaemia and showed that most common age group was 11-20 years with female predominance. Unnikrishnan V *et al* (2008)[8] 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)[9] 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)[10] studied 66cases of megaloblastic anaemia 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)[8] and Shidappa G *et al* (2014)[9] and Barik S *et al* (2015)[10] but not agreed to the study of khanduri U *et al* (2007)[7] may be due to geographical variation. In our study major symptoms at presentation were Generalised weakness (92%), Dyspnoea (64%), Anorexia and gastritis (48%), Fever (42%), Glossitis (20%), palpitations(16%), Tingling and numbness (12%), Hyperpigmentation of skin(8%). Khanduri U *et al* (2007)[7] 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, diarrhoea, hyperpigmentation and early graying of hair were present in <10% of patients. Unnikrishnan V *et al* (2008)[8] had shown that common symptoms in order of their presentation were Fatigue Breathlesness, Abdominal pain, Hyperpigmentation of knuckle, glossitis. Haq S *et al* (2012)[11] studied 80 patients and found that most common presentation was pallor, fatigue, fever, dyspnea, palpitations, nausea, jaundice. Thus our study is in agreement with the study by khanduri U *et al* (2007)[7], Unnikrishnan V *et al* (2008)[8] and Haq S *et al* (2012)[11]. Major presenting signs were- Pallor (100%), Splenomegaly (22%), Hepatomegaly (20%), Edema (22%), Icterus (20%) Skin changes (12%), Neurological signs (8%). khanduri U *et al* (2007)[7] had shown that common signs were pallor (85%), glossitis (29%), mild icterus (25%) and hyperpigmentation of knuckles (18%). Gupta R *et al* (2009)[12] had shown that major symptoms were Pallor (100%) Hepatomegaly (38%), Pedaledema (24%), Icterus (14%), Hyperpigmentation (10%), Splenomegaly (10%).

Table No.10 Comparison of Haematological parameters in different studies

	Unnikrishnan V *et al* (2008)	**Veda P. (2013)**	**Present study (2015)**
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

Barik S *et al* (2015)[10] studied 66 cases and found that major signs on presentation were Pallor, Splenomegaly and Hepatomegaly. Thus our study is in agreement with the study of khanduri U *et al* (2007)[7] Gupta R *et al* (2009)[12] and Barik S *et al* (2015) [10].

In our study we studied 50 cases with Haemoglobin ranging from 2.5 gm% to 8.5 gm% and found average Hamoglobin 5.6gm%, MCV ranging from 97-134 fl and average MCV was 108.9 fl ,Total leucocyte count ranging from 1800-8400 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)[12] studied 23 cases and found average Hamoglobin 4.96 gm%, MCV 111.18 fl, Total leucocyte count 4300 /cmm, Platelet count of 0.98 lacs/cmm. Veda P. (2013)[13] studied 38 cases and found average Hamoglobin 8.8 gm%, MCV 111.8 fl, Total leucocyte count 5013/cmm, Platelet count of 1.92 lacs/cmm. Thus in megaloblastic anaemia we found that majority of cases showed increase in MCV and decrease in Total leucocyte count and Platelet count. Thus our study is in agreement with the study Unnikrishnan V *et al* (2008)[69] and by Veda P. (2013)[13].

In our study all 50 patients were anaemic and we found that 27 (54%) patients had leucopenia (TLC <4000/cmm) Also 29 (58%) patients had Thrombocytopenia. (Platelet count <1.5 lacs/cmm) .In our study 22 (44%) patients presented with pancytopenia and 12 (24%) patients presented with Bicytopenia, they were presented with either leucopenia or thrombocytopenia along with anaemia. Leucopenia and thrombocytopenia were due to ineffective production of cells by abnormal precursor cells in bone marrow. Khanduri U *et al* (2007)[7] in their study found that pancytopenia was present in 62% of patients. Chandra J *et al* (2010)[14] had shown that leucopenia in 17-49% cases and thrombocytopenia in 44-80% cases and megaloblastic anaemia manifest with pancytopenia and megaloblastic anaemia is most common cause of pancytopenia. Haq S *et al* (2012)[11] in their study found that 80% patients had leucopenia and 60% patients had thrombocytopenia. Siddiqui B *et al* (2015)[15] found that 26.6% patients showed panctopenia. Thus Megaloblastic anaemia can be presented with pancytopenia or bicytopenia.Thus our study is in agreement with the study of khanduri U *et al* (2007)[7], Chandra J *et al* (2010)[14], Haq S *et al* (2012)[11] and siddiqui B *et al* (2015)[15] .

It was observed that 28(56%) patients had haemoglobin $\leq$6 gm% with average MCV 114.1fl and 22(44%) patients had haemoglobin >6 gm% with average MCV 104.8fl. Unnikrishnan V *et al* (2008)[8] had shown that 38(63%) patients have haemoglobin <6 gm% have average MCV 107.7fl and 22(36%) patients have haemoglobin >6 gm% have average MCV 104fl. Thus level of MCV was higher in severe megaloblastic anaemia. Thus our study is in agreement with the study of Unnikrishnan V *et al* (2008) [8]. out of 50 patients diagnosed of megaloblastic anaemia 40(80%) patients had deficiency of Vitamin B12 (serum Vitamin B12 < 200 pg/ml) and 10(20%) patients had normal level of Vitamin B12 (serum Vitamin B12 200-900 pg/ml).Mean Vitamin B12 level in Vitamin B12 deficient cases was 140$\pm$36.9 pg/ml, and in cases with Normal Vitamin B12 level was 287.6$\pm$35.0 pg/ml. Chi square value was 46.54 and P value was <0.0001. So there was statistically significant association between serum Vitamin B12 levels and megaloblastic anaemia. Thus Vitamin B12 deficiency was important cause of megaloblastic anaemia. Mean Haemoglobin level in Vitamin B12 deficient cases was 5.5$\pm$1.5 gm% and in cases with Normal Vitamin B12 level was 5.9$\pm$1.4 gm%. Mean MCV level in Vitamin B12 deficient

cases was 110±9.9 fl and in cases with Normal Vitamin B12 level was 105.2±5.22 fl. Haq S *et al* (2008)[71] had shown that in Vitamin B12 deficient patients Mean Vitamin B12 level were 70.0±57.4 pg/ml with P value <0.001(significant), Hb 5.1±1.7gm%,MCV 109±18fl and in Non Vitamin B12 deficient patients Mean Vitamin B12 level were 324±56.4 pg/ml, Hb 5.8±1.8gm% ,MCV 99±16fl. . Thus our study is in agreement with the study of Haq S *et al* (2008)[11].

we had studied 50 cases of megaloblastic anaemia and found that on peripheral blood smear examination Anisopoikilocytosis with Howell jolly bodies, Cabot rings and basophilic stippling with some nucleated red cells. Macro-ovalocytes were seen in 43(86%) cases. These were large oval /egg shaped red cells. Dimorphic RBCs on P.S. consisting of predominantly macro-ovalocytes as well as microcytic hypochromic RBCs in 7(14%) cases with Hyper segmented neutrophils consisting of >5 lobes in more than 5% of neutrophills were seen in 39(78%) cases and this was the morphological marker and first haematological abnormality to appear on P.S.

On bone marrow examination it was hypercellular with erythroid hyperplasia with reversal of M:E ratio in 41(82%) patients and Normocellular in 9(18%) patients. in all the 50 patients bone marrow showed Megaloblastic maturation consisting of large megaloblastic erythroblasts with open sieve like chromatin, Nuclear fragmentation, paranuclear halo, Howel jolly bodies. There was preponderance of early and intermediate megaloblasts as compared to late megaloblasts. In patients with dimorphic anaemia both megaloblasts and micronormoblasts were seen.In myeloid cells giant metamyelocytes and band forms seen. Megakaryocytes show hypersegmentation and fragmentation with decreased thrombocytopoiesis.

Khanduri U *et al* (2008)[7] studied 120 cases and showed that hypersegmented neutrophils in all cases .Bone marrow was performed in 22 cases of megaloblastic anaemia showed moderate to marked hypercellularity with megaloblastic maturation. Unnikrishnan V *et al* (2008)[8] studied 26 cases of megaloblastic anaemia and showed that presence of macro-ovalocytes and hypersegmented neutrophils in peripheral smear always goes with diagnosis of megaloblastic anaemia. Gupta RK *et al* (2009)[12] studied 50 cases and also showed similar findings. Haq S *et al* (2012)[11] studied 80 cases and showed hypersegmented neutrophils and macro-ovalocytes in peripheral blood smear. Bone marrow was performed in 80 cases of megaloblastic anaemia showed hypercllular marrow with megaloblastic maturation. Veda P. (2013)[13] studied 43 cases of megaloblastic anaemia and showed hypersegmented neutrophils in 32 (86%) cases and macro-ovalocytes in 27 (72%) cases.Bone marrow was performed in 17 cases of megaloblastic anaemia showed megaloblastic maturation Thus our study is in agreement with the study of Unnikrishnan V *et al* (2008)[8], khanduri U *et al* (2008)[7], Gupta R *et al* (2009)[12], Haq S *et al*(2012)[11], Veda P. (2013)[13].

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

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