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Research Article

CLINICO-HAEMATOLOGICAL AND BIOCHEMICAL EVALUATION OF MACROCYTIC ANAEMIA: A PROSPECTIVE CROSS SECTIONAL STUDY

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A B S T R A C T

Introduction: Macrocytic anemia is not a specific disease but rather an indicator of diverse underlying diseases and demands further clinical and laboratory assessment. We went to evaluate the clinico-haematological and biochemical parameters of macrocytic anaemia in this study.

Materials and Methods: The study was cross-sectional conducted for 2 years on 99 patients of anaemia and macrocytosis. Macrocytic anaemia was identified on complete blood count (CBC) with haemoglobin (Hb) <10 g/dl and mean corpuscular volume (MCV) >100 fl and/or macrocytosis on peripheral smear. Bone marrow aspiration, reticulocyte count and serum vitamin B12, folic acid, serum lactate dehydrogenase (LDH), serum bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) were evaluated.

Results: The most common cause of macrocytic anaemia was megaloblastic anaemia (97.9%) while 2 (2.02%) cases had non-megaloblastic macrocytosis. The megaloblastic anaemia was due to either vitamin B12 deficiency (56.6%) or folate deficiency (23.2%), while combined deficiency observed in 18.2% of cases. Among 97 patients of megaloblastic anaemia 74.2% cases showed MCV >100 fl and 43.3% cases had pancytopenia. 51.5% cases had very high serum LDH levels >3000 U/L.

Conclusions: Vitamin B12 deficiency was most common cause of macrocytic anaemia, in vegetarians followed by folic acid and combined deficiency of folate and vitamin B12. Estimation of vitamin B12, folic acid and LDH levels were sufficient to diagnose megaloblastic anaemia.

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INTRODUCTION

Macrocytosis is defined as a mean corpuscular volume greater than 100 fl and is relatively common finding in the era of automated blood cell counters with 1.7% - 3.6% macrocytes being seen. [1, 2, 3]

Macrocytic anemia is not a specific disease but rather an indicator of diverse underlying diseases and demands further clinical and laboratory assessment. Macrocytic anaemia can usually be divided into two categories, based on the examination of the bone marrow 1) megaloblastic 2) non-megaloblastic[1,2] The spectrum of etiologies with a megaloblastic change is observed in deficiency of folic acid and vitamin B12, myelodysplastic syndromes, haemolytic anaemias, drugs like Methotrexate, Zidovudine, Hydroxyurea, Trimethoprim, Nucleotide analogs, Nitrous oxide (N_2O). Non-megaloblastic bone marrow is observed in aplastic anemia, pure red cell aplasia, congenital dyserythropoietic anaemias, hypothyroidism and chronic liver disease [2, 3, 4] Majority of cases of macrocytosis are due to megaloblastic anaemia due to deficiency of either vitamin B12 or folic acid [3, 5] Vitamin B12 and folic acid are essential components of DNA synthesis in red cell precursors. Folic acid is directly involved and vitamin B12 participates as a cofactor[2, 3]

Haematological parameters like automated complete blood count (CBC), peripheral smear, bone marrow examination are commonly used parameters to diagnose megaloblastic anaemia. Beside hematological investigations certain biochemical parameters like lactate dehydrogenase (LDH), bilirubin and estimation of serum vitamin B12 and folic acid levels confirm the diagnosis of megaloblastic anaemia[6]

We cater large number of cases of macrocytic anaemia in our institution and there is little literature of this condition in central India and it's been a long time that this anaemia has been studied. Hence keeping this in mind we carried out the present study with the following aims and objectives.

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Aims and objectives

1. To study clinical, haematological and biochemical parameters and etiology of macrocytic anaemia.
2. To evaluate automated complete blood count (CBC) indices, morphological features on peripheral smear and bone marrow of macrocytic anaemia.
3. To correlate biochemical parameters like serum vitamin B12, folic acid, lactate dehydrogenase (LDH), bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of macrocytic anaemia.
4. To evaluate the proper set of investigations for diagnosis of macrocytic anaemia.

MATERIALS AND METHODS

The study was carried out in department of Pathology of a tertiary care hospital and referral center during the period of October 2013 to September 2015 after obtaining permission from Institutional Ethical Committee. This was a cross-sectional descriptive study carried over a period of 2 years on 99 patients of macrocytic anaemia. Patients admitted with anaemia were selected with following criteria.

1. Haemoglobin (Hb) < 10 g/dl
2. Mean corpuscular volume (MCV) > 100 fl
3. Macrocytosis on peripheral smear

Anaemic patients with pure microcytosis on CBC and peripheral smear, haemolytic anaemias and leukemias were excluded. Detailed history was taken which included symptoms of anaemia, nutritional status and alcohol consumption, previous gastric surgery, drug therapy etc. Thorough physical examination was carried out in all patients. Informed consent was taken from all patients and samples taken in EDTA and plain bulbs before any treatment given and subjected for CBC, reticulocyte count, peripheral smear, serum bilirubin, AST, ALT and lactate dehydrogenase (LDH) and bone marrow aspiration was performed in all patients with evidence of macrocytic anaemia.

Serum vitamin B12 and folic acid was estimated in all patients by Chemiluminescence immune assay. Serum ferritin was done in 9 cases to assess iron status, which showed dimorphic picture on peripheral smear and MCV < 100 fl on CBC.

Statistical analysis was done by performing independent t - test. Hematological and biochemical parameters were compared between vitamin B12 and folic acid deficient patients. p - value < 0.05 was considered as statistically significant. Statistical software STATA version 13.0 was used for data analysis.

OBSERVATIONS AND RESULTS

The mean age of patients was 36.12 ± 18.31 (15 – 79) years which consists of 62 (62.6%) males and 37 (37.4%) females. Maximum patients were in the range of 11 – 30 years (53.5%). All patients were presented with symptoms of generalized weakness and fatigability. 14 (14.1%) patients had complaint of gum bleeding and one patient presented with prolonged diarrhea.

Pallor was present in all patients and 79 (79.8%) cases showed icterus. There were 15 (15.1%) cases with hepatomegaly and spleen was palpable in 16 (16.1%) patients. 36 (36.4%) patients presented with paresthesia and 57 (57.6%) patients had knuckle pigmentation.

Most of the patients belonged to low socioeconomic status. Maximum patients i.e. 64 (64.6%) were pure vegetarian and 35 (35.4%) patients were non-vegetarian while 13 (13.1%) patients were chronic alcoholics.

Table No. 1 shows baseline haematological findings in macrocytic anaemia (n = 99) showed mean haemoglobin of 4.67 ± 1.40 g/dl and mean MCV of these patients was 105.80 ± 12.50 fl.

Table No.1 Baseline haematological and biochemical parameters in patients of macrocytic anaemia. n = 99

Haematological and biochemical parameters	Mean	SD	Minimum	Maximum
Total leucocyte count (TLC) ($x10^9$/L)	3.21	1.58	0.7	7.9
Haemoglobin (g/dl)	4.67	1.40	2.3	9.6
MCV (Femtolire) (fL)	105.80	12.50	77.9	134.8
Platelet count ($x10^{12}$/L)	1.08	0.61	0.16	3.4
Reticulocyte count (%)	0.86	0.47	0.5	2
Vitamin B12 (pg/ml)	168.65	88.06	56	543
Folic acid (ng/ml)	8.53	6.59	2.3	25
LDH (U/L)	3818.31	2684.84	324	13893

Table No. 2 shows among the 99 cases of macrocytic anaemia 97 (97.9%) cases were of megaloblastic anaemia due to either vitamin B12 deficiency (56.6%) or folate deficiency (23.2%), while combined deficiency was observed in 18.2% of cases.

Table No 2 Cause of macrocytic anaemia. n = 99

Cause		Number of patients	Percentage (%)
Megaloblastic	Deficient in vitamin B12	56	56.6%
	Deficient in folic acid	23	23.2%
	Mixed	18	18.2%
Non-megaloblastic	Liver disease	2	2.0%
Total		99	100

Two cases of non-megaloblastic anaemia were found in chronic alcoholics having normal vitamin B12 and folic acid levels with raised direct bilirubin, AST while; ALT and LDH were normal. In 97 cases of megaloblastic anaemia, 72 (74.2%) patients had MCV > 100 fl and 25 (25.7%) had MCV <100 fl while, 42 (43.2%) patients showed pancytopenia as shown in Table No 3. Out of 97 patients of megaloblastic anaemia 47 patients had LDH levels in the range of 450 – 3000 U/L and 50 patients showed LDH levels >3000 U/L while, all 97 cases had raised indirect bilirubin.

Table No 3 Pancytopenia in megaloblastic anaemia. n = 42

Deficient biochemical parameter	No. of cases (%)
Vitamin B12	22 (52.3%)
Folic acid	12 (28.5%)
Mixed	8 (19.2%)
Total	42 (100%)

In 88 patients out of 97 cases of megaloblastic anaemia, RBCs showed predominantly macrocytosis with oval macrocytes, moderate to severe anisopoikilocytosis. In severe cases morphological abnormalities including teardrop poikilocytes, fragmented RBCs, basophilic stippling, Howell - jolly bodies and Cabot's rings were seen. Megaloblasts were evident in 20 (20.6%) cases on peripheral smear. Hypersegmented neutrophils were seen in 26 (26.8%) cases. 9 patients showed dimorphic morphology of megaloblastic and

iron deficiency anaemia like, hypochromic and microcytic RBCs along with oval macrocytes, hypersegmented neutrophils and low serum ferritin. These patients showed mixed deficiency of vitamin B12, folic acid and iron.

In 88 out of 97 cases of megaloblastic anaemia, bone marrow smears were markedly hyper cellular with severe erythroid hyperplasia. Table No. 4 Myeloid erythroid ratio (M: E) was reversed, which varied from 1:1 to 1:4 and even more in some cases. Characteristic megaloblasts were seen.

Table No. 4 Bone marrow findings in macrocytic anaemia: n = 99

Bone marrow findings	Number of patients	Percentage (%)
Megaloblastic anaemia	88	88.9
Dimorphic anaemia	9	9.1
Non – megaloblastic anaemia	2	2.0
Total	99	100

Early erythroblastic precursors were more in number. These were large cells with sieve like stippled chromatin and cytoplasm matured to appropriate degree. Granulopoiesis also showed changes like giant metamyelocytes with fine nuclear chromatin. Myelocytes and promyelocytes were also increased in size. Megakaryocytes showed occasional morphological abnormality like large size with multiple nuclear lobes and paucity of cytoplasmic granules. 9 patients with dimorphic anaemia, bone marrow showed erythroid hyperplasia with normoblastic morphology and megaloblastic morphology was less prominent. But all 9 cases of dimorphic anaemia showed giant metamyelocytes indicating coexistent megaloblastic anaemia.

In 2 cases of liver disease RBCs were round macrocytic, normochromic. Total leucocyte count and platelets were normal. Bone marrow smears were normocellular with normal M:E ratio and normoblastic erythropoiesis. No definite megaloblasts and giant metamyelocytes seen.

Haematological and biochemical parameters were compared among pure vitamin B12 and folic acid deficient patients. p - Value calculated for haematological parameters was > 0.05 which was statistically not significant. p - Value calculated for vitamin B12 and folic acid levels was < 0.001 which showed high significance statistically. This significance was due to high difference in the values among vitamin B12 and folic acid levels. Table No. 5

Table No 5 Comparison of haematological and biochemical parameters in pure vitamin B12 and folic acid deficiency. n = 79

Haematological and biochemical parameters	Vitamin B12 deficient (n = 56)	Folic acid deficient (n = 23)	p - value
TLC ($x10^9$/L)	3.29 ± 1.50	2.59 ± 1.51	0.642, NS*
Haemoglobin (g/dl)	4.88 ± 1.59	4.33 ± 1.12	0.1416, NS
MCV (fl)	106 ± 12.14	103.83 ± 12.03	0.4249, NS
Platelet count ($x10^{12}$/L)	1.12 ± 0.68	0.92 ± 0.50	0.2018, NS
Reticulocyte count (%)	0.87 ± 0.53	0.78 ± 0.29	0.4774, NS
Vitamin B12 (pg/ml)	126.84 ± 42.94	287.83 ± 88.85	< 0.001, HS**
Folic acid (ng/ml)	12.09 ± 6.74	3.72 ± 0.83	< 0.001, HS
LDH (U/L)	3844.38 ± 2514.71	4066.22 ± 3104.49	0.7406, NS

*NS - Not significant, **HS – Highly significant

DISCUSSION

In present study majority of patients presented with generalized weakness and fatigue (100%) followed by dyspnea (83.8%) and palpitations (49.5%). Bleeding gums (14.1%) and diarrhea (1%) were other complaints. Pallor was present in all patients, icterus was seen in 79 (79.8%) cases, splenomegaly and hepatomegaly was seen in 16 (16.1%) and 15 (15.1%) patients respectively. Knuckle pigmentation (57.6%) and paraesthesia (36.4%) were prominent findings. In the study done by Haq *et al* (2012) icterus and hepatomegaly was found in 28% cases while, splenomegaly was present in 48% cases.[2] Our findings were in accordance with Chan *et al* (1998), Unnikrishnan *et al* (2008), Khanduri *et al* (2007) and Haq *et al* (2012) [2,5,7,8]

In present study pancytopenia was seen in 42 cases (43.2%) of megaloblastic anaemia which correlated with other studies. Chan *et al* (1998), Maktouf *et al* (2006), Khanduri *et al* (2007) and Haq *et al* (2012) observed pancytopenia in 23.1%, 39.5%, 62% and 40% of cases respectively. [2,5,7,9]

Maktouf *et al* (2006) studied 478 patients of megaloblastic anaemia and found 33.5% of cases had MCV ≥ 120 fl; 55.1% cases had MCV between 100 to 120 fl and 11.4% cases of patients had MCV <100 fl.[9] In present study 72 (74.2%) cases had MCV >100 fl and 25(25.7%) cases had MCV <100 fl. However, Jain *et al* (2012) studied serum vitamin B12 and MCV in general population and concluded that every third person was vitamin B12 deficient in their region, but there was no correlation between vitamin B12 levels and MCV in majority of cases.[11] This might be due to that, they had not considered haemoglobin as parameter and pateints with normal haemoglobin might be included in their study. This suggests that raised MCV should not be the only criterion to diagnose megaloblastic anaemia.

In present study majority 56 (56.6%) of patients were deficient in vitamin B12 with mean B12 value of 126.84 ± 42.94 pg/ml (56 – 207 pg/ml). 23 (23.2%) patients were folic acid deficient with mean value of 3.72 ± 0.83 ng/ml (2.3 – 4.9 ng/ml) and 18.2% patients showed combined deficiency, while 9 cases had dimorphic anaemia showing iron deficiency in combination with megaloblastic anaemia. Two patients of non – megaloblastic macrocytic anaemia had normal B12 and folic acid levels and elevated direct bilirubin, AST levels. The Present study showed similar findings regarding the predominance of vitamin B12 deficiency as the major cause of megaloblastic anaemia as in the studies by Chan *et al* (1998), Khanduri *et al* (2007) and Unnikrishnan *et al* (2008). However, one study from Lahore by Haq *et al* (2012) showed folic acid deficiency as the main cause of megaloblastic anaemia[2,5,7,8] This might be exlpained by the fact that most of their cases were non-vegeterians.

Zimran *et al* (1983) found B12 deficiency due to pernicious anaemia (69%) as the most common cause of megaloblastic anaemia followed by gastrointestinal disease (12%), nutritional deficiency (9%) and selective vitamin B12 malabsorption with albuminuria (7%)[10] Chan *et al* (1989) and Maktouf *et al* (2006) also found pernicious anaemia as most common cause of megaloblastic anaemia [5,9] These findings might be due to affluent society, where nutrition was fairly good as compared to our population having low socioeconomic status and customs of strict vegetarianism. In

present study, due to economic constraint, evaluation of pernicious anaemia was not done.

Jaswal *et al* (2000) studied LDH in 75 patients of macrocytic anaemia. They categorized the patients on bone marrow examination into megaloblastic and non – megaloblastic to evaluate the efficacy of total serum LDH level and isoenzyme pattern in the diagnosis of megaloblastic anaemia. In their study, total serum LDH levels were elevated in 100% of patients which had macrocytic blood picture with megaloblastic erythropoiesis and presence of stainable iron. Serum LDH was > 3000 U/L in 44% cases with mean value of 3280 ± 2636 U/L[6]. In present study mean serum LDH level of megaloblastic anaemia cases was 3890.13 ± 2664.69 U/L. Out of 97 patients of megaloblastic anaemia, 47 (48.5%) cases had serum LDH levels between 450 – 3000 U/L and 50 (51.5%) patients had serum LDH levels >3000 U/L, which was in concordance with that of Jaswal *et al* (2000).

Macrocytosis is not always due to megaloblastic anaemia as other conditions are also associated with non-megaloblastic macrocytosis. However, the presence of macro-ovalocytes and hypersegmented neutrophils in peripheral smear are important diagnostic features of megaloblastic anaemia. Hence, the investigation parameters should be chosen in accordance to clinical presentation. Apart from pallor other clinical features like knuckle pigmentation, icterus, neurological signs and symptoms, the laboratory set of investigations like CBC, vitamin B12, folic acid and LDH are sufficient for the diagnosis and etiology of megaloblastic anaemia. In such cases invasive procedure like bone marrow examination is not required.

CONCLUSION

Megaloblastic anaemia was the most common cause of macrocytic anaemia, commonly seen among young age group. Vitamin B12 deficiency was the most common cause of megaloblastic anaemia due to poor nutrition and among strict vegetarians. Pancytopenia was common presenting feature of megaloblastic anaemia. Raised MCV should not be the only criteria, but normal MCV and macrocytosis on PS should also be considered to evaluate magaloblastic anaemia. Markedly raised serum LDH level was found to be an important diagnostic indicator of megaloblastic anaemia. Clinical, hematological and biochemical features resulting from the deficiency of vitamin B12, folic acid were similar. Estimation of vitamin B12, folic acid and LDH levels were sufficient to diagnose megaloblastic anaemia. Invasive procedure like bone marrow aspiration could be avoided. In setting of limited laboratory facilities the investigation should be chosen giving importance to clinical presentation.

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Disclosure of potential conflict of interest

We have not obtained any grant from any pharmaceutical company or person. Therefore there is no potential conflict of interest of any author of this manuscript.

Research involving human participants and consent

This research involves human participants for which we have taken written informed consent from all participants. This article does not contain any studies with animals performed by any of the authors.

Ethical approval

All procedures performed in study involving human participants were in accordance with the ethical standards of the institution.

References

1. Aslina F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. *Clin Med Res*. 2006; 4(3):236-241.
2. Haq S, Iqbal N, Fayyaz F, Tasneem T. Serum B12 and folate levels in patients with megaloblastic change in bone marrow. *Biomedica.* 2012; 28:35-39.
3. Lichtman MA, Kipps TJ, Kaushansky K, Beutler E, Seligsohn U, Prchal JT. Williams hematology 7th Ed. New York, McGraw- Hill. 2006; 477-528.
4. Tefferi A, Hanson CA, Inwards DJ. How to interpret and purue an abnormal complete blood cell count in adults. *Mayo Clinic Proceedings*. 2005; 80(7):923 - 936.
5. Chan J, Liu H, Kho B *et al*. Megaloblastic anaemia in Chinese patients: a review of 52 cases. *HKMJ*. 1998; 4(3):269-274.
6. Jaswal TS, Mehta HC, Gupta V, Singh M, Singh S. Serum lactate dehydrogenase in diagnosis of megaloblastic anemia. Indian *J. Pathol. Microbiol*. 2000; 43(3):325-329.
7. Khanduri U, Sharma A. Megaloblastic anaemia: prevalence and causative factors. *Natl Med J India*. 2007; 20(4):172-175.
8. Unnikrishnan V, Dutta TK, Badhe BA, Bobby Z, Panigrahi AK. Clinico-aetiologic profile of macrocytic anemia. *India J. Hematol*. 2008; 24(4):155-165.
9. Maktouf C, Bchir F, Louzir H *et al*. Megaloblastic anemia in North Africa. *Hematologica.* 2006;91(7):990-991.
10. Zimran A, Hershko C. The changind pattern of Megaloblastic anemia: megaloblastic anemia in Israel. *Am J Clin Nutr*. 1983; 37:855-861.
11. Jain R, Kapil M, Gupta GN. MCV should not be the only criteria to order vitamin B12 for anemia under evaluation. *OJGas.* 2012; 2:187-190.

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