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Clinico-aetiologic profile of macrocytic anemias with special reference to megaloblastic anemia

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Abstract

Purpose of study This study was conducted to study the clinical and laboratory parameters in patients with macrocytic anemia and to determine the etiology of macrocytic anemia with special reference to megaloblastic anemia.

Materials and methods This study was a cross-sectional descriptive study carried over a period of 18 months on 60 adult patients (age ≥13 years) of macrocytic anemia. Macrocytic anemia was identified when peripheral blood examination showed anemia with a mean red blood corpuscular volume of >95 fl.

Result The most common cause of macrocytic anemia was megaloblastic anemia (38.4%). The major causes of non-megaloblastic macrocytic anemia were primary bone marrow disorders (35%), liver diseases (15%) and hemolytic anemia (8.3%). There was a significant male preponderance in the study (65%). The megaloblastic anemias observed were due to either vitamin B_{12} deficiency (78.3%) or combined B_{12} and folate deficiency (21.7%). A significant proportion of non–vegetarians (73.9%) had megaloblastic anemia. Patients with an MCV of >110fl were more likely to have megaloblastic anemia (p value 0.0007). Three patients (mean age 55 years) with a megaloblastic marrow did not respond to vitamin replacement and were found to have myelodysplastic syndrome.

Conclusion Megaloblastic anemia due to Vitamin B_{12} or folate deficiency remains the most important cause of macrocytic anemia. In settings with limited laboratory facilities, a therapeutic trial of vitamins B_{12} or folic acid is useful in determining the specific vitamin deficiency.

Keywords Macrocytic anemia · Non-megaloblastic macrocytic anemia · Bone marrow disorders · Megaloblastic anemia · Myelodysplastic syndrome

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Introduction

Red cell size as related to mean corpuscular volume (MCV) has for many years been used to classify anemia [1]. The discovery, that an anemic patient's erythrocyte is abnormal in size, suggests certain diseases can be suspected or excluded on the basis of this. With the advent of electronic cell counters, the MCV has become an integral and useful feature of the red cell profile [2, 3]. The Coulter counter generates a value for the average volume of red cells, which is important for categorizing the type and cause of anemia. The normal MCV ranges from 78 to 94 fl. Anemia is classified as macrocytic if MCV exceeds 95 fl [4].

Macrocytosis is seen in 1.7–3.6% of patients seeking medical care and is a common finding in any clinical setting, often in the absence of anemia [2, 3, 5]. The disorders

that lead to macrocytic anemia comprises of a heterogeneous group that act through a variety of known and postulated mechanisms. They are generally classified as those resulting from disorders in DNA synthesis of erythrocyte precursors in the bone marrow (megaloblastic macrocytic anemia) or those caused by a variety of other mechanisms (non-megaloblastic macrocytic anemia).

Macrocytosis often precedes anemia [6–8], but is not investigated, especially if anemia is slight. Several case studies have demonstrated that vitamin B_{12} deficiency may initially produce only a mild macrocytic anemia that is maintained for long period before a rapid worsening occurs. Among the findings at routine laboratory investigations, an elevated MCV may be the only indicator of conditions like vitamin B_{12} or folate deficiency, preleukemia or alcoholism [8]. Since the clinical presentation of all types of anemia may be similar, many macrocytic anemias may go misdiagnosed as iron deficiency anemia. Only later does the non-responsiveness to iron supplementation prompt the consideration of megaloblastic anemia. Hence, a high index of suspicion during clinical examination may provide clues towards the diagnosis of macrocytic anemia. Distinct clinical features of megaloblastic anemia may help in the early recognition of cobolamin or folate deficiency.

Aims and objectives

- To study the clinical and laboratory parameters in patients with macrocytic anemia
- To determine the etiology of macrocytic anemia with special reference to that of megaloblastic anemia

Materials and methods

This study was a cross-sectional descriptive study carried over a period of 18 months on 60 adult patients (age ≥13 years) of macrocytic anemia treated in a large teaching hospital of India. Macrocytic anemia was identified when peripheral blood showed:

1. A mean red blood corpuscular volume >95 fl and
2. Anemia with a hemoglobin of
   i. <13 g/dl in male
   ii. <12 g/dl in female
   iii. <11 g/dl in pregnant female

Women in the third trimester of pregnancy were excluded from study, because of the ethical concerns about subjecting them to a bone marrow aspiration.

The history was taken in detail and a complete and thorough physical examination was carried out in all patients. Detailed information was sought on alcohol consumption, previous gastric surgery, malignant disease and drug therapy. All patients were investigated with a complete hemogram that included estimation of hemoglobin level, red cell indices, total leucocyte count, differential leucocyte count, platelet count and reticulocyte count, and peripheral smear examination and red cell distribution width estimation. A bone marrow aspiration was performed in all patients with evidence of megaloblastic anemia, as shown by presence of hypersegmented neutrophils or macro-ovalocytes in the peripheral smear, and when indicated on clinical grounds in other patients.

Serum bilirubin, total protein and albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase and LDH levels were obtained on a routine basis for all patients. Prothrombin time was estimated in patients with history of alcoholism or evidence of liver disease clinically. Thyroid function tests were done in all the patients. The iron status in the patients with megaloblastic anemia was evaluated by the estimation of serum iron and bone marrow stainable iron. An upper gastrointstinal endoscopy and gastric biopsy was performed in a few selected patients with megaloblastic anemia who gave consent for the same.

Patients with megaloblastic anemia confirmed by bone marrow examination were subjected to a therapeutic trial with full replacement doses of vitamin B_{12} injections (1 mg of cyanocobalamin intramuscularly for 10 days) and, in case of a suboptimal or absent response to cyanocobalamin, followed by 1 mg of folic acid orally for 10 days to identify the cause of megaloblastosis [9]. The patients who had concomitant iron deficiency, as shown by a low serum iron levels or, more specifically, a decreased marrow iron stores – grade 3 or less – were given iron supplementation in the form of ferrous sulphate tablets 200 mg thrice a day in the intensive phase of vitamin supplementation and continued upto 3 months.

The response to treatment was assessed with a complete hemogram on days 3, 8 and 15. The response was said to be optimal if there was brisk reticulocytosis on day 3 with a rise in hemoglobin, total counts and platelet counts (in case of pancytopenia) with disappearance of hypersegmented neutrophils by day 15.

Patients who showed an optimal response to cyanocobalamin injections alone were labeled to have isolated vitamin B_{12} deficiency and were further advised cyanocobalamin injections 1 mg intramuscularly weekly for 4 weeks and then followed by 1 mg intramuscularly monthly for one year and thereafter yearly. The patients were followed up at the end of the second month for confirmation of adequacy of the response.

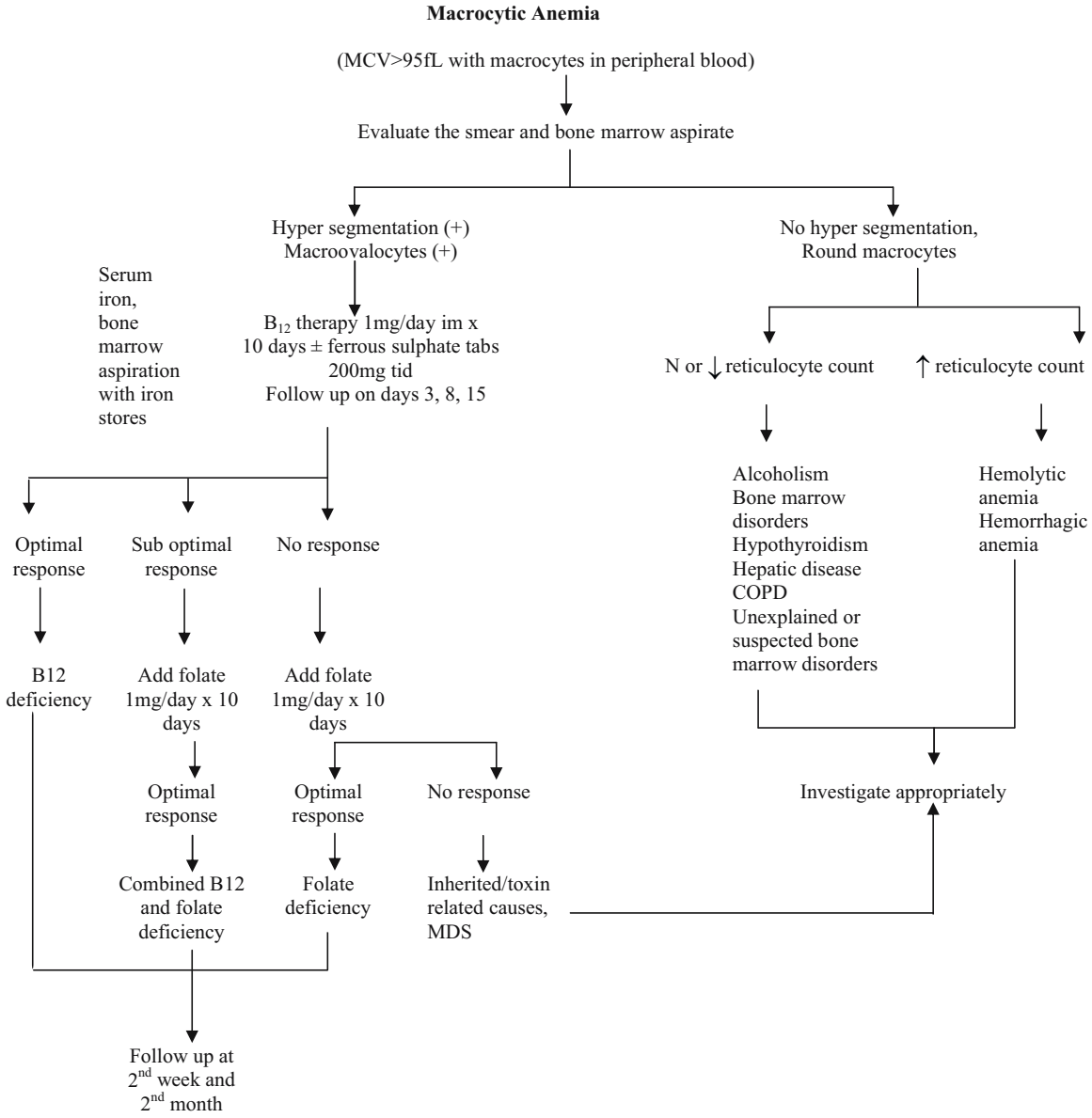

Chart Algorithmic approach to the study

The response was classified as suboptimal if the patients responded to vitamin B_{12} replacement with an initial brisk reticulocyte response on day 3 with a minimal rise in hemoglobin and leukocyte and platelet counts without disappearance of hyper-segmented neutrophils or macro-ovalocytes by day 15 (vide chart). The response was taken as nil if there was no reticulocytosis on day 3 or a rise in hemoglobin or counts by day 8. If the response at day 15 was not optimal with vitamin B_{12} replacement alone, folic acid was supplemented at a dose of 1 mg per day for a further period of 10 days and then response was assessed with hemograms on day 3, 8 and 15. Patients who showed optimal response to folic acid replacement alone were labeled to have isolated folate deficiency. These patients were advised to continue folic acid 1 mg per day for 3 months and were followed up at the end of second month with a complete hemogram.

An intermediate group of patients who had earlier suboptimal response to cyanocobalamin and now showed an optimal response on addition of folic acid were diagnosed to have combined B_{12} and folate deficiency, and were followed up at the end of second month for assessment of complete response following cyanocobalamin injections and oral folic acid. The therapeutic tests were necessitated since there was no facility for vitamin B_{12} or folic acid estimation in the hospital.

The patients whose anemia did not respond to replacement of both vitamins were labeled as having refractory anemia and repeat bone marrow aspiration was performed to rule out any primary bone marrow disorder.

Statistical analysis was performed using students' *t* test

Result

The mean age of the sixty adult patients was 38.96 ± 16.4 years. They comprised of 39 males and 21 females. Most of them belonged to the low socioeconomic group. There was a bimodal distribution in the age with a higher proportion of patients in the 21–30 and 51–60 year groups.

The major symptoms at presentation were predominantly that due to anemia; however, 8.3% of patients did not have the typical symptoms of anemia. They had predominantly various other symptoms like abdominal pain and swelling of feet (Table 1). Twenty-two patients had various bleeding manifestations, while 15 patients had presented with history of jaundice. One patient had noticed jaundice for the past one year, and had been investigated outside with no apparent cause having been made out.

On clinical examination, pallor was universally present, ranging from mild to severe. Icterus was observed in 21 patients, 19 patients had splenomegaly, 17 had hepatomegaly. Other signs included edema, neurological manifestations and mucocutaneous changes (Table 2). Six patients had presented with altered sensorium due to hepatic failure. A 37-year old lady was brought with history of delusions of infidelity and persecution, with poor food intake and consequent malnutrition.

The hematological study at baseline showed mean hemoglobin of 5.6 ± 2.12 g/dl (Table 3). Thirty-eight patients had severe anemia (<6g/dl) at presentation. The mean MCV of these patients was 106.5 ± 9.59 fl. Maximum number of patients had MCV in the 95–105 fl range. No correlation was found between the severity of anemia and the degree of elevation of the MCV (p = 0.239) (Table 4). The average MCH was 33.25 ± 3.6 pg, while the MCHC was 34.12 ± 4%.

The peripheral smear examination showed neutrophilic hypersegmantation and /or macro-ovalocytosis in 26 patients suggesting megaloblastic erythropoiesis (Table 5). These patients were subjected to a bone marrow examination and all of them were found to have megaloblastic erythropoiesis in the bone marrow. Morphologic abnormalities in the peripheral blood which included hyper-segmented neutrophils, anisocytosis, macro-ovalocytosis, teardrop cells and nucleated red cells, were strongly associated with megaloblastic hematopoiesis. Twenty-three of these patients had megaloblastic anemia and three turned out to have myelodysplastic syndrome (MDS). Among the 34 patients who did not have either hypersegmented neutrophils or macroovalocytes in the peripheral smear, 21 were found to have primary bone marrow disorder leading to macrocytosis, nine had liver disease and five had hemolytic anemia. A 24-year-old woman who had been on oral cyclophosphamide for steroid-resistant nephrotic syndrome had hemorrhagic cystitis with macrocytic anemia (Table 6).

Table 1. Major symptoms at presentation

Symptoms	Number of patients n=60	Percentage %
Related to anemia (Breathlessness, easy fatiguability, malaise)	55	91.7
Abdominal pain	26	43.3
Bleeding manifestations	22	36.7
Swelling of feet	16	26.7
Jaundice	15	25
Pruritus	6	10
Neurologic	6	10
Chronic diarrhea	1	1.7

Table 2. Major signs at presentation

Signs	Number of patients n=60	%
Icterus	21	35
Splenomegaly	19	31.7
Hepatomegaly	17	28.3
Skin changes	14	23.3
Edema	14	23.3
Glossitis	12	20
Neurological manifestations	6	10
Clubbing	2	3.3

Table 3. Hematological parameters at baseline in patients with macrocytic anemia (n = 60)

Parameter	Mean ± SD
Hemoglobin (g%)	5.61 ± 2.12
PCV (%)	16.8 ± 3.47
Red cell count (× 10^{12} cells/L)	1.8 ± 0.73
MCV (fL)	106.5 ± 9.59
MCH (pg)	33.25 ± 3.6
MCHC %	34.2 ± 1.4
TLC (× 10^3/μl)	10.43 ± 28.13
Platelet count (× 10^5/μl)	1.09 ± 1.1
Red cell distribution width	20.7 ± 6.06

Primary bone marrow disorders

Among the patients with primary bone marrow disorders, nine had aplastic anemia of varying etiology; eight had leukemia that included acute as well as chronic, and myeloid as well as lymphoid types. A 50-year-old woman who had megaloblastic changes in the marrow responded to neither B_{12} nor folate and was found to be suffering from acute erythroleukemia secondary to myelodysplastic syndrome (MDS). MDS without transformation into leukemia was found in two patients. One patient had myelofibrosis with macrocytic anemia (Fig. 1). The mean hemoglobin in this category was 4.94 ± 2.22g/dl and MCV was 102.92 ± 7.11 fl. Of these, three patients of chronic myeloid leukemia were on hydroxyurea.

Hepatobiliary disease including alcoholism

Nine patients had evidence of liver disease, all of them were chronic alcoholics and had clinical and laboratory evidence of alcoholic liver disease. All of them were males. Two of them had alcoholic hepatitis and the rest had cirrhosis of liver with hepatic failure. Target cells were present in four of these patients.

Hemolytic anemia

Five patients had hemolytic anemia. Of these four had autoimmune hemolytic anemia and a fifth had G6PD deficient hemolysis. All of them had icterus and three had mild splenomegaly. Their mean reticulocyte count was 14.3 ± 11.7%. The patients in this group had mean hemoglobin of 5.76 ± 0.99 g/dl with MCV of 115.1 ± 9.14 fl.

No case of hypothyroidism was documented as a cause of macrocytic anemia in our study.

Megaloblastic group

Out of 26 patients in this group, 23 patients had megaloblastic anemia with a mean age of 35.7 ± 16.1 years. There were 13 males and 10 females. There was a preponderance of younger patients in this group.

All the patients had symptoms of anemia. In addition, bleeding manifestations were seen in 26 % of patients in

Table 4. Relation between the severity of anemia and the degree of macrocytosis

Hemoglobin (g%)	Number of Patients n=60	Percentage	Average MCV Mean ± SD	P Value
< 6.0	38	63.3	107.7 ± 8.64	0.239
≥ 6.0	22	36.7	104.5 ± 10.97	

Table 5. Peripheral smear in Patients with Macrocytic Anemia

Peripheral smear	Number of Patients n=60	Percentage
Macrocytic, no neutrophil hyper segmentation or macroovalocytes	34	56.7
Macrocytic, with neutrophil hyper segmentation or macroovalocytes	26	43.3

Table 6. Etiology of macrocytic anemia

Etiology	Number n=60	Percentage
Megaloblastic anemia	23	38.4
Primary bone marrow disorders	21	35
Liver disease	9	15
Hemolytic anemia	5	8.3
Other	2	3.3

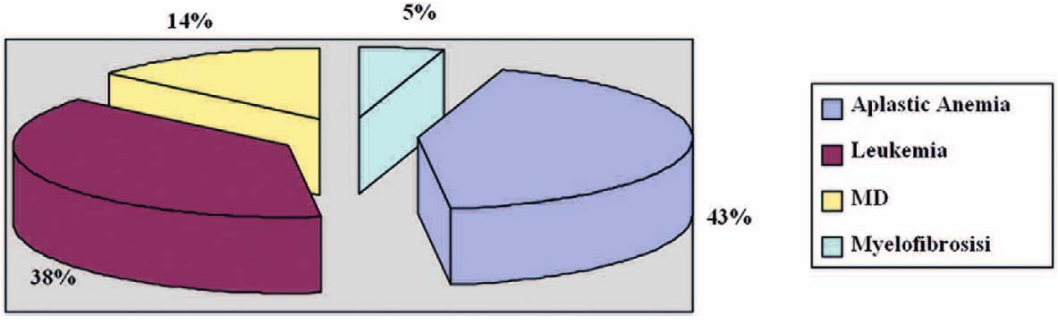

Fig. 1 Primary Bone marrow disorders with Macrocytic Anemia (n=21)

the form of epistaxis, gum bleed or melena. All of them had thrombocytopenia. Mean platelet count was 64,166 ± 67,930/µl. Jaundice was a predominant feature in six patients. The mean total bilirubin in these patients was 3.15 ± 1.86mg/dl, the direct fraction being 1.47 ± 1.12 mg/dl. Six patients had presenting complaints of numbness, paresthesia and imbalance of gait (Table 7). One patient gave history of chronic watery diarrhea for one year. He did not give history of steatorrhoea.

Three patients gave history of taking drugs, which could interfere with the metablolism of B_{12} or folate (Table 8). One person had been on phenytoin for seizure disorder for three years. A 31-year-old man had been taking omeprazole for acid peptic disease for the past 2 years. A 55-year-old woman was on oral methotrexate for 4 years for rheumatoid arthritis. Three patients were chronic alcoholics. The patients with megaloblastic anemia were predominantly non-vegetarian. Two patients with megaloblastic anemia were in the second trimester of pregnancy. Of them one person had absent iron stores, another had normal iron stores but went on to develop iron deficiency in the course of therapy. One patient responded to B_{12} therapy while the other responded to addition of folate.

On clinical examination, all the patients had moderate to severe pallor (Table 9). Skin changes in the form of hyperpigmentation or patchy alopecia were found in 11 patients and mucosal changes in ten (Table 10). Glossitis was found to be a sensitive indicator of megaloblastic anemia. Cutaneous hyper pigmentation was also a sensitive finding in megaloblastic anemia. lcterus was observed in six patients. Neurological findings were present in six patients (Table 11).

Table 7 Major symptoms at presentation

Symptoms	Number (n=23)	Percentage
Related to anemia (Breathlessness, easy fatiguability, malaise)	23	100
Abdominal pain	7	30.4
Bleeding manifestations	6	26.1
Epistaxis	2	8.7
Gum bleed	2	8.7
Melena	2	8.7
Swelling of feet	6	26.1
Jaundice	6	26.1
Neurological symptoms	6	26.1
Imbalance	4	17.4
Paresthesia	3	13.0
Numbness	1	4.3
Pruritus	2	8.7
Chronic diarrhea	1	4.3

Table 8 Other significant history

History	Number (n=23)	Percentage %
Drug intake	3	13.04
Anticonvulsant therapy	1	4.34
Chronic antacid use	1	4.34
Other	1	4.34
Alcohol abuse	3	13.04
Dietary history		
Vegetarianism	5	21.74
Veganism	1	4.34

Table 9 Major signs at presentation

Sign	Number n=23	%
Pallor	23	100
Severe	18	78.3
Moderate	5	21.7
Mile	0	0
Edema	16	69.6
Skin changes	11	47.8
Mucosal changes	10	43.8
Icterus	6	26.0
Hepatomegaly	6	26.0
Neurological manifestations	6	26.0
Splenomegaly	5	21.7

Table 10 Mucocutaneous manifestations in patients with megaloblastic anemia

Sign	Number (n=23)	%
Hyper pigmentation	11	47.8
Knuckle	11	47.8
Palmoplantar	1	4.3
Glossitis	10	43.5
Angular cheilitis	3	13.0
Alopecia	2	8.7

Table 11 Neurological manifestations in megaloblastic anemia

Sign	Number (n=23)
Impaired vibration sense	6
Impaired position sense	6
Impaired touch or pain perception	6
Ataxia	2
Romberg's sign	2
Decreased reflexes	5
Increased reflexes	1
Spasticity	1
Psychiatric disorders	1

The hematological parameters at baseline are given in Table 12. The mean corpuscular volume of this subgroup was higher than that of the whole study population while the mean hemoglobin, total leukocyte count and platelet count were lower. Eleven patients had pancytopenia (48%). There was a positive correlation between the level of MCV elevation and the incidence of megaloblastic anemia; but there was no positive correlation between the severity of anemia and the extent of MCV elevation.

Table 12. Hematological parameters at baseline in patients with megaloblastic anemia (n = 23)

Parameter	Mean ± SD
Hemoglobin (g%)	4.96 ± 1.26
PCV (%)	14.65 ± 3.83
Red cell Count (× 10^{12} cells/L)	1.78 ± 0.48
MCV (fL)	111.18 ± 9.56
MCH (pg)	35.05 ± 4.43
MCHC %	34.2 ± 2.35
TLC (× 10^3/µl)	4.30 ± 2.25
Platelet count (× 10^3/µl)	98.5 ± 95.2
Red Cell Distribution Width	22.25 ± 6.9
Reticulocyte Count (%)	2.5 ± 0.04
Serum Iron (µg/dL)	64.32 ± 37.7

Table 13. Response to therapy

Optimal response	Number of patients n=23	Percentage %
To B_{12} supplementation (Group I)	18	78.26
To B_{12} and folate supplementation (Group II)	5	21.74

The iron status of these patients was assessed by the serum iron levels and the bone marrow iron stores. The mean serum iron level was 64.32 ± 37.7 µg/dl. Four patients had grade 0 iron stores.

All 26 patients who had megaloblastic marrow were subjected to a therapeutic trial with pharmacological doses of vitamin B_{12} and followed up at days 3, 8 and 15. Iron was supplemented in four patients who had evidence of iron deficiency as per the absent iron stores.

Twenty-three patients responded to treatment, 18 had complete clinical and hematological response to B_{12} therapy and were followed up till the end of second month of treatment (Tables 13, 14). They were categorized as Group I patients of megaloblastic anemia. Five patients had suboptimal response to B_{12} alone, but showed optimal response after addition of folic acid at day 15 and were followed up for approximately 75 days They were categorized as Group II patients (Tables 15, 16).

The clinical improvement was noticed by most patients initially as a subjective sense of well being, as early as third day of therapy, followed by gradual improvement of other symptoms. At the end of two months, all the responders were remarkably free of all symptoms and sign. The men-

Table 14. Changes in the hematological parameters after B12 supplementation in Group I (n=18)

Parameter	Baseline	Day 3	Day 8	Day 15	End of 2nd month	p value
	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	
Hemoglobin (g%)	5.10 ± 1.06	5.99 ± 1.37	7.06 ± 1.55	8.64 ± 1.24	11.05 ± 0.84	.000
MCV (fl)1	109.72 ± 9.91	106.86 ± 8.60	102.14 ± 9.22	96.69 ± 5.69	90.64 ± 3.94	.000
TLC (× 10^3/µl)	4.41 ± 1.93	5.26 ± 1.89	6.62 ± 2.47	7.25 ± 2.55	7.23 ± 0.67	.000
Platelet count (× 10^5/µl)	.96 ± 0.92	1.72 ± 1.40	2.1 ± 1.28	2.62 ± 1.09	2.30 ± 6.16	.000
Reticulocyte count (%)	2.9 ± 0.05	4.21 ± 0.14	6.33 ± 1.56	5.28 ± 1.28	2.3 ± 0.72	

Table 15 Changes in the hematological parameters after supplementation of B12 in Group II (n = 5)

Parameter	Baseline	Day 3	Day 8	Day 15	p value
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	
Hemoglobin (g%)	4.46 ± 1.86	5.16 ± 2.88	5.72 ± 2.49	6.0 ± 2.57	.015
MCV (fl)	116.2 ± 6.90	108.54 ± 1.08	108.4 ± 1.04	106.24 ± 0.88	.014
TLC (× 10^3/µl)	5.34 ± 3.47	4.80 ± 2.81	6.10 ± 2.16	5.96 ± 1.77	0.453
Platelet count (× 10^5/µl)	1.08 ± 1.24	1.54 ± 1.46	2.70 ± 1.14	2.59 ± 1.26	.000
Reticulocyte Count (%)	1.3 ± 0.01	2.7 ± 1.04	3.2 ± 0.83	2.2 ± 0.54	

tal status of the lady, who had presented with psychotic features, had considerably improved after two months of therapy along with increased food intake, weight gain and improvement in social and family relationships. The patient with megaloblastic anemia following methotrexate therapy had a delayed response to the addition of folate.

The reticulocyte response started on Day 3 with the peak levels at Day 8 (Tables 14–16), but the extent of rise was not as high as was expected as per literature (Table 17).

Seven patients with normal bone marrow iron stores and one patient with increased bone marrow stores who were not supplemented with iron developed red blood cell hypochromia at the end of therapy, probably indicating the development of iron deficiency due to increased demand of erythropoiesis. Five of these patients were in Group II, which had undergone therapy for a longer period than Group 1. The Mean serum iron of these patients was 59.3 ± 50.4 µg/dl. After this observation, even patients with normal iron stores were supplemented with iron at the start of therapy and none of them developed hypochromia at the end of therapy.

Three patients with megaloblastic bone marrow did not respond either clinically or hematologically to vitamin supplementation and were subjected to a repeat bone marrow aspiration and were found to have myelodysplastic syndrone. The average age of these patients was 55. One of these patients was in the transformation phase to acute erythroleukemia and two had refractory anemia. The final diagnosis in the patients with megaloblastic bone marrow is depicted in figure 2.

Discussion

In 1934, Wintrobe established the value of morphologic classification of anemia. He characterized anemias as macrocytic, normocytic, simple microcytic and hypochromic microcytic [1]. Several surveys of macrocytosis have been published. Relatively small numbers of patients were described in these reports and the definition of macrocytosis varied considerably. Some investigators have used a threshold MCV value of red blood cell of 100 fl; others

Table 16 Changes in the hematological parameters in Group II after addition of Folic acid (n = 5)

Parameter	Day 3	Day 8	Day 15	End of 2nd month	p value
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	
Hemoglobin (g%)	6.70 ± 2.58	7.66 ± 2.43	9.08 ± 1.40	11.34 ± 1.37	.000
MCV (fL)	107.14 ± 7.74	103.98 ± 8.55	99.44 ± 6.28	92.22 ± 2.93	.000
TLC (× 10^3/µl)	6.54 ± 1.27	7.0 ± 1.56	7.08 ± 0.88	7.62 ± 1.28	.04
Platelet count (× 10^5/µl)	2.41 ± 1.38	2.37 ± 0.88	2.86 ± 0.98	2.09 ± 0.55	
Reticulocyte Count (%)	5.8 ± 3.49	7.0 ± 3.39	7.0 ± 3.65	2.7 ± 3.0	

Table 17 Reticulocyte response in group I

Red Cell Count at baseline (Million cells/µl)	Expected Reticulocyte response to therapy at Day 8 (%)	Actual Reticulocyte response observed at Day 8 (%) (Mean ± SD)
<1.0	50–70	5.5 ± 0.34
1.0–1.49	36–47	5.3 ± 0.78
1.5–1.99	25–34	7.0 ± 0.99
2.0–2.49	15–22	7.3 ± 0.65

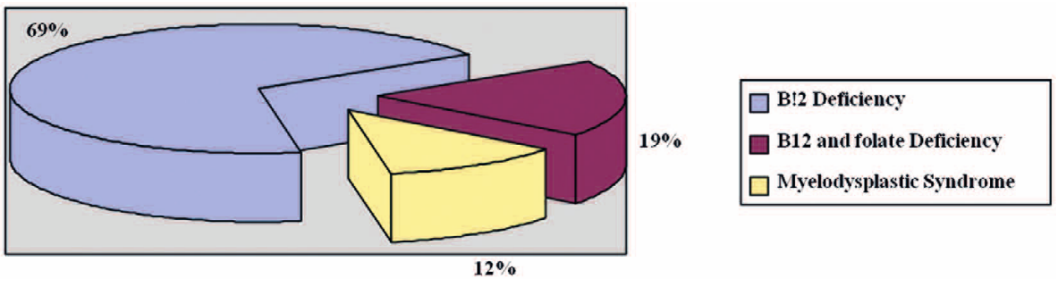

Fig. 2 Final Diagnosis in Patients with megaloblastic bone marrow (n=23)

used values ranging from 105 to 115 fl. Wintrobe (1934) and Hattersley (1964) had previously surveyed the causes of macrocytic anemia [1,10].

Etiology of macrocytic anemia:

In Wintrobe's study the most common cause of macrocytic anemia was megaloblastic anemia produced by pernicious anemia [1]. The other causes, in order of decreasing frequency, were disorders of the liver like cirrhosis, bone marrow disturbances like leukemia, myelodysplasia and aplasia, the anemia of acute blood loss and the anemia of pregnancy. Patients with pernicious anemia had extreme macrocytosis and severe anemia. Glossitis was also found to be an indicator of the severity of anemia and macrocytosis[1].

In 1964 Hattersley reported a survey of 120 patients with macrocytosis calculated from electronic cell counters and reported cirrhosis of the liver as the most common cause of macrocytosis[10]. In his series, more than 50% of the patients had an MCV of 100-104.5 fl.

Davidson, in 1971, reported that 1% of all blood samples submitted for routine hematological examination had macrocytes in the peripheral blood. McPhedran (1973), Davidson (1978) and Colon-Otero et al (1992) have variously described their findings in patients with macrocytosis [2, 3, 9]. McPhedran defined the cause of macrocytosis in the absence of anemia in 100 consecutive patients and reported that the most common cause of macrocytic anemia was megaloblastic anemia due to vitamin B_{12} and folate deficiency [2]. On the other hand, alcohol abuse was the most common cause of macrocytosis as per Colon-Otero, followed by vitamin B_{12} and folate deficiency [9]. In the large study by Savage et al in 2000, the most common cause of macrocytosis was drug therapy, followed by alcohol liver disease, and reticulocytosis [11]. Megaloblastic hematopoiesis accounted for less than 10% of his cases, with cobalamin or folate deficiency being present in only 6% of patients. MCV values >120 fl were usually caused by cobolamin deficiency in his series. Brigden also stated that folate and vitamin B_{12} deficiencies might be relatively rare causes of macrocytosis compared to alcoholism, liver disease, drugs or myelodysplasia [12].

The findings in our series parallel the results of the studies of Wintrobe and McPhedran with megaloblastic anemia being the most common cause of macrocytic anemia [1, 2], followed by primary bone marrow disorders, liver disease and hemolytic anemia.

Our study had a preponderance of male patients in contrast to that of any study on iron deficiency anemia where there is usually a female preponderance. There was a bimodal distribution of the patients when classified according to the age. The mean age of the patients was lesser than that of the series of McPhedran and Davidson [2, 3]. 63.3% of our patients had severe anemia (Hb less than 6g%). This is in contrast to Davidson's observation where none of the patients had hemoglobin less than 7g% [3]. He found that the severity of macrocytosis increases in proportion to the degree of anemia. In our study we could not find a positive correlation between the degree of macrocytosis and the severity of anemia. The likelihood of finding megaloblastosis was higher if the MCV was >110 fl.

In Wintrobe's series, it was found that the group of patients with liver disorders was, on an average, less macrocytic and less anemic than the megaloblastic group [1]. The other group, with bone marrow disorders and blood loss, had more severe anemia but only borderline macrocytosis. These are similar to the findings in our study.

In Davenport's study, the most common form of non-megaloblastic macrocytic anemia resulted from alcoholism [13]. Davenport also states that the most common cause of macrocytosis in the UK is alcohol consumption. In our study we found more of macrocytosis due to hematological malignancy.

The morphological findings of hypersegmented neutrophils and macroovalocytes in the peripheral smear may preclude the need for a bone marrow aspiration procedure in patients with a clinical picture of megaloblastic anemia. In our study, we found that all the 26 patients with hypersegmented neutrophils and/or macro-ovalocytes in the peripheral blood had megaloblastic erythropoiesis in the marrow, making these peripheral blood findings very sensitive indicators for megalopoiesis in the marrow.

We found drug therapy to cause macrocytosis in some patients. The drugs implicated were omeprazole, methotrexate, phenytoin and hydroxyurea. Except for the patients on hydroxyurea, all others had megaloblastic anemia, which responded to parenteral vitamin supplementation. The patient who had megaloblastic anemia following methotrexate therapy for rheumatoid arthritis had a delayed but complete response to vitamin B_{12} and folate supplementation.

Clinical features

A sizable number of non-vegetarians had megaloblastic anemia in our study group. Masalha, in his study of urban Bedouin patients found that nutritional deficiency of cobalamin is common, even among non-vegetarians [14]. Matthews JH had studied the incidence of megaloblastic anemia in Asians and found that of the 27 Asians with a megaloblastic anemia, 22 (81%) had nutritional deficiency of vitamin B12, while five (19%) had true pernicious anemia [15]. All the patients were Hindu vegetarians except

for a single Muslim who had pernicious anemia. Dietary intakes of calories, protein, iron, vitamin B_{12} and folate were below recommended level in both groups. He stated that nutritional deficiency of vitamin B_{12} is the most common cause of megaloblastic anemia in Hindu vegetarians, but the incidence of true pernicious anemia is higher than previously thought and may approximate to that of the white population. Hence, in our study population, pernicious anemia may have been the cause for the megaloblastic anemia among the non-vegetarians in addition to the poor consumption of meat products due to economic reasons, but we were unable to establish the diagnosis of pernicious anemia for the want of appropriate tests.

A history of chronic diarrhea could be elicted in only one patient with megaloblastic anemia, but he was not symptomatic at presentation and barium meal and colonoscopy studies were normal. Stool microscopy was normal in all patients. Glossitis and cutaneous hyperpigmentation were found to be the sensitive markers of megaloblastic anemia. Wintrobe had described glossitis in his study as one of the commonest findings in patients with megaloblastic anemia. Bleeding manifestations are characteristically mild in the thrombocytopenia of Vitamin B_{12} or folate deficiency; but in our series, bleeding manifestations were severe enough to be the presenting complaint in six patients. Neuro-psychiatric features that showed complete response to vitamin replenishment were observed in six of our patients. This has been documented in previous studies by several authorities. Lindenbaum found in his study in 1998 that patients with cobalamin deficiency may present with neuropsychiatric disease in the absence of anemia in as many as 28% of cases, hence any patient with unexplained neuropsychiatric abnormality should be screened for cobalamin or folate deficiency, especially if they have macrocytosis [16]. Jaundice, which responded to vitamin replacement, was also documented in six patients (26%).

Hematological features

The maximum number of patients had an MCV of 95–105 fl. Thus, if the cut off level for the MCV had been kept at higher level as in some studies, a significant proportion of patients with macrocytosis would have been missed.

The presence of hyper-segmented neutrophils and /or macro-ovalocytes was found to be a sensitive indicator of megaloblastic hematopoiesis in the marrow, but it was not specific for megaloblastic anemia since three patients with this finding in peripheral smear had myelodyspastic syndrome. According to Davenport, neutrophilic hypersegmentation is one of the most sensitive and specific signs of megaloblastic anemia [13]. Pancytopenia, which responded to therapy, was documented in 11 patients (48%) with megaloblastic anemia in our group. Myelodysplastic syndrome had to be considered in older patients with megaloblastoid marrow who were unresponsive to standard therapy. None of the patients with megalobalstic anemia showed the kind of peak reticulocyte response on treatment as described in the literature.

The presence of target cells was a sensitive indicator of liver disease as was the finding of an elevated gamma glutamyl transpeptidase (GGT) as an indicator of alcoholism.

Conclusion

The pathological conditions associated with macrocytic anemia are much more diverse than is often appreciated and macrocytosis is not to be equated with megaloblastosis, since there are varied conditions associated with non-megaloblastic macrocytosis. However, the presence of macro-ovalocytes and hypersegmented neutrophils in peripheral smear almost always goes with a diagnosis of megaloblastic anemia. Megaloblastic anemia still remains the most important cause of macrocytic anemia in our setting. The diversity and complexity of factors leading to macrocytic anemia preclude a single or uniform method of investigation. The investigative pattern must be tailored to the individual patient, giving importance to the clinical presentation. In settings with limited laboratory facilities, a therapeutic trial of vitamin B_{12} or folic acid is useful in determining the specific vitamin deficiency in megaloblastic anemia.

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