Original Article

Relative Prevalence of Vitamin B12 and Folic Acid in Megaloblastic Anemia and Its Clinical – etiological Profile in a Tertiary Care Center

Praphull Deepankar[1], Rakesh Roshan[2], Harish Kumar Gupta[3], Gurdeep Buxi[4]

[1]Assistant Professor, Department of General Medicine, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, [2]Senior Resident, Department of General Medicine, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, [3]Consultant and Head of Unit, Department of General Medicine, PGIMER & Dr. Ram Manohar Lohia Hospital, N. Delhi, India, [4]Professor, Department of Pathology, PGIMER & Dr. Ram Manohar Lohia Hospital, N. Delhi, India

Abstract

Background: Megaloblastic anemia (MA) causes substantial morbidity in patients with anemia and increasing number of MA is seen in clinical practice over the past 10–15 years.

Objectives: The objectives of the study were to study the relative prevalence of Vitamin B_{12} and folic acid (FA) in MA and its clinical–etiological profile.

Methods: This is a cross-sectional observational study, conducted in the Department of Medicine, PGIMER and Dr. Ram Manohar Lohia Hospital, New Delhi, with patients of MA in whom detailed history; physical examination and nutritional assessment were recorded. Cobalamin and FA assays were done. Diagnosis of MA was confirmed by bone marrow aspiration and patients were further investigated to find out its cause.

Results: Majority of studied patients (46%) were in age group 22–40 years with male: female ratio of 2.6:1. Majority cases (95%) were from Hindu religion compared to Muslims (5%). The symptoms corresponding to anemia were the most common presentation of the patient. Fatigueness was present in 92%, exertional dyspnea and palpitation were the presenting complaints in 59% and 19% cases, respectively. Decrease appetite (80%), weight loss (54.6%), diarrhea (16%), mouth ulcer (14%), and paresthesias (11%) were the other common presenting symptoms. Pallor (96%) was most common finding in the study group. Skin hyperpigmentation was present in 48% and oral ulcer in 26% of patients. 54% of the participants had cobalamin deficiency, and 26% had FA deficiency, and 21% had both. 37% of cases had the nutritional background. Among non-nutritional etiology most frequently associated factors were alcohol and alcoholic liver disease (27%), and drugs (13%) followed by chronic infections and malabsorption.

Conclusion: The pathological conditions associated with the MA are much diverse. Proper diagnostic workup is essential before the use of hematinics and blood transfusion in all anemic patients.

Key words: Anemia, Megaloblastic anemia, Pancytopenia, Serum B12, Serum folic acid

INTRODUCTION

Megaloblastic anemia (MA) is a distinct type of anemia characterized by macrocytic red blood cells (RBCs) and typical morphological changes in RBC precursors with the disparity in nuclear-cytoplasmic maturation. Basic underlying pathogenetic mechanism in MA is a deficiency of folic acid (FA) and/or Vitamin B_{12} resulting in impairment of DNA synthesis.[1]

However, pernicious anemia is a common cause of Vitamin B12 deficiency especially in persons of European or African descent, but dietary Vitamin B12 deficiency is a leading problem in the Indian subcontinent, Mexico, Central and South America, and selected areas in Africa.[2,3]

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Corresponding Author: Dr. Praphull Deepankar, 1A, Vrindavan Garden Apartment, Ashiana – Digha Main Road, Patna – 800 025, Bihar, India. Phone:+91-9990215432. E-mail: drprafsjh@gmail.com

In India and other developing countries, most cases of MA are caused by a nutritional deficiency of FA, B12, or both. Pregnancy and lactation, alcohol, medications, infections, pernicious anemia due to intrinsic factor deficiency, and malabsorption are the other emerging causes for MA.[2,4]

FA deficiency was reported to be more common than Vitamin B12 deficiency to cause MA in earlier studies; however, recent studies from India and other countries have shown Vitamin B12 deficiency is more common cause of MA than FA deficiency.[5,6]

In this study, our aim is to study the relative prevalence of Vitamin B_{12} and FA in MA and its clinical–etiological profile.

MATERIALS AND METHODS

This study was conducted at the Departments of Medicine, PGIMER and Dr. Ram Manohar Lohia Hospital, New Delhi. This study was conducted on 97 enrolled patients who attended the medical clinics and medical ward.

Design of Study
This is a cross-sectional study.

Inclusion Criteria
The following criteria were included in this study:
- Hemoglobin (Hb) <10 g/dL in female and Hb <12 g/dL in male with pancytopenia (neutropenia and thrombocytopenia defined as absolute neutrophil count <1500 per mm^3 and platelet count <150,000 mm^3).
- Peripheral smear showing a megaloblastic picture (anisopoikilocytosis, macrocytosis, hypersegmented neutrophils, macrocytes, and presence of nucleated red cells).
- Bone marrow aspiration examination suggesting megaloblastic reactions.

Exclusion Criteria
The following criteria were excluded from the study:
- Age <14 years
- Previously diagnosed and on treatment (taking vitamin tabs/blood transfusion)
- Congenital disorders
- Pregnancy in 3rd trimester.

Following inclusion, diagnosis of the MA was established by bone marrow aspiration study. A detailed history including marital status, past or current comorbidities, and alcohol intake, depression, clinical and nutritional assessment was recorded on predesigned pro forma. A clinical examination and the laboratory profile of the subjects were also recorded. Nutritional Assessment was assessed by Mini Nutritional Assessment-Short Form (MNA® -SF) questionnaire. The MNA® -SF screening maximum score is 14. Scores ≥12 indicate satisfactory nutritional status. A MNA® screening score ≤11 suggests malnutrition.

Hematological Assessment
All the subjects were undergone the following investigations:
- Complete hemogram
- Peripheral smear
- Red cells indices.

Biochemical Parameters
Following parameters were investigated further:
- Liver function test
- Serum cholesterol
- Serum triglyceride
- Serum lactate dehydrogenase
- Serum cobalamin level
- Serum FA level
- Serum iron level
- Serum ferritin level
- Thyroid profile (if needed)
- Bone marrow aspiration.

Imagings
To find out the causative factors responsible for MA, following test was performed when needed.

- Ultrasound abdomen
- Upper gastrointestinal endoscopy with/without biopsy
- Contrast enhanced computed tomography abdomen
- Colonoscopy with/without biopsy.

Statistical Analysis
The data so collected were analyzed using SPSS version 19© SPSS Inc. Mann–Whitney *U*-test and *t*-test were used to compare the mean of continuous variables depending on sample size, and Chi-square was used for nonparametric tests.

OBSERVATION AND RESULTS

Our study comprises a total of 97 patients including 70 male and 27 female cases. The age of patients studied ranged from 15 to 95 years with mean age of 39.06 ± 8.9 years. Majority of studied patients (46%) were in age group 22–40 years while the age groups 41–50 years and 51–60 years had 13 subjects (11%) each. All age groups had more number of male patients than the female counterparts except age group "age <21 years" which had comparatively more female cases [Table 1]. Majority cases of studied population (95%) were from Hindu religion compared to Muslims (5%).

Dietary preference of study group is shown in Figure 1. Majority of population 63% (61) were non-vegetarian while 37% (36) were vegetarians. Among vegetarians, vegans (18/36) were major group followed by lacto-vegetarians (10/36) and lacto-ovo vegetarians (8/36).

The symptoms corresponding to anemia (i.e. fatigueness, exertional dyspnea, and palpitation) were the most common presentation of the patient. Fatigueness was present in 92% of the study population; exertional dyspnea and palpitation were the presenting complain in 59% and 19% cases, respectively. Decrease appetite (80%), weight loss (54.6%), nausea and vomiting (22%), diarrhea (16%), mouth ulcer (14%), and parenthesis (11%) were the other common presenting symptoms [Table 2].

Clinical findings observed in participants are tabulated in Figure 2. Pallor was most common finding in the study group and present in 96% patient. Skin hyperpigmentation was present in 48% and oral ulcer in 26% of patients. Fever was the presenting clinical finding in 34% patient. Other common clinical signs in the study were pedal edema (25%), icterus (16%), and lymphadenopathy (8%).

In this study, the majority of patients (53%) had Hb level between 6 and 10 g/dL and 36% patients had Hb <6 g/dL.

Mean Hb level of the study is 7.16 ± 2.41 with minimum and maximum value of 2.7 and 12.10 g/dL, respectively [Table 3].

Most of the patients (58%) had mean corpuscular volume (MCV) level more than 110 fL and 30% participants had MCV level between "95 and 110." Only 11% of cases had MCV <95 fL [Figure 3].

Peripheral smear findings of all 97 participants are described in Figure 4. The most common findings were the presence of macrocytes (88%), anisocytosis (75%), and macroovalocytes (68%). Hypersegmented neutrophils were present in 43% patient. Other findings such as tear drop cells, Howell–Jolly bodies, polychromatocytes, nucleated RBCs, and target cells were also reported but were less frequent.

In our study, 52 (54%) of the participants had cobalamin deficiency, and 25 (26%) had FA deficiency, and 20 (21%) participant had patient had both cobalamin and FA deficiency.

Bone marrow examinations of the studied population showed cellular bone marrow with dyserythropoiesis and erythroid hyperplasia observed in 99%, 96%, and 96%

Table 1: Composite age – sex distribution

Age group (year)	Sex		Total
	Female	Male	
<21	8	10	18
22–30	6	16	22
31–40	5	17	22
41–50	3	8	11
51–60	2	9	11
>60	3	10	13
Total (%)	27 (27.8)	70 (72.2)	97
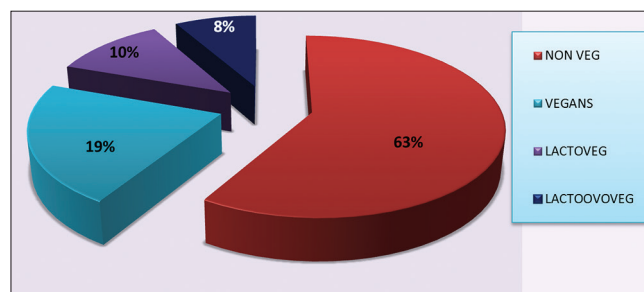
Figure 1: Dietary preference of study group

Table 2: Presenting symptoms among the study group

Symptoms	Frequency
Fatigueness	89 (92)
Exertional dyspnea	57 (59)
Palpitation	18 (18.5)
Decrease appetite	78 (80)
Vomiting	21 (21.6)
Constipation	4 (4.1)
Diarrhea	15 (15.4)
Mouth ulcer	13 (13.4)
Weight loss	53 (54.6)
Paresthesias	10 (10.2)
Poor gait	10 (10.2)
Memory loss	2 (2)
Psychiatric symptoms	2 (2)
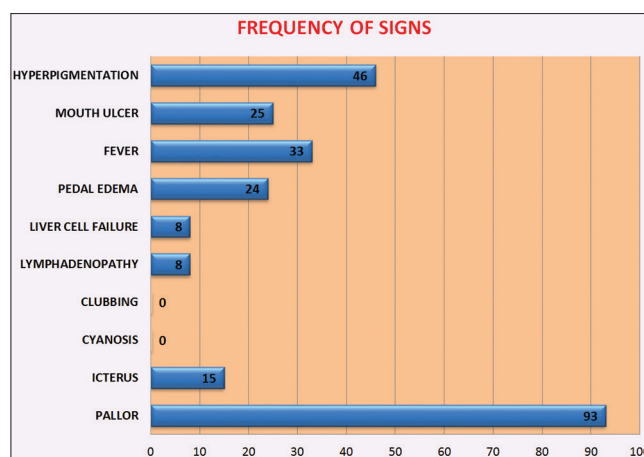
Figure 2: Clinical signs in the study group

cases, respectively. Pancytopenia was reported in 36% patient. Megaloblastic reaction was described in 78% cases while in 11% of cases bone marrow reaction was dimorphic. Bone marrow sample was inadequate in one case, and one case was diagnosed with acute lymphoblastic leukemia.

In the total number of 97 MA, 36 cases (37%) had nutritional background. Among non-nutritional etiology most frequently associated factors were alcohol and alcoholic liver disease (27%), and drugs (13%), and others factors are depicted in Table 4.

Table 5 elaborates the drugs associated with the MA in this study. Antiretroviral therapy (ART) was responsible for maximum cases in this category followed by metformin, pantoprazole, and antiepileptic drugs. One case of sulfasalazine used in ulcerative colitis also presented.

Moreover, the analysis shows that nutritional deficiency is entirely associated with vegetarian diet preference. Among vegetarian, it is vegans (18/36) who are affected most and followed by lacto-ovo vegetarian (10/36). There is no case

of nutritional deficiency among non-vegetarian and this distribution is statistically significant ($P < 0.0001$).

In nutritional group cobalamin deficiency was present in 31/36 cases and the combined deficiency was in 5/36 cases. In non-nutritional group 25/61 cases had isolated FA deficiency, 21/61 cases had cobalamin, and 15/61 cases had combined deficiency [Table 6].

DISCUSSION

MA causes substantial morbidity in patients with anemia. Data regarding the magnitude of the problem in different parts of India and the factors that might influence its incidence are lacking. Dietary deficiency of vitamin is well-established cause of MA. The pathological conditions associated with MA are much more diverse. In our study, investigations have revealed many causes of MA including nutritional deficiency, alcohol and alcoholic liver disease, drugs, HIV, tuberculosis, celiac disease, chronic pancreatitis, malaria, kalazar, tropical sprue, hypothyroidism, and hepatitis.

The study population comprised 97 patients, with age 39.06 ± 8.9 years (mean ± standard deviation). The peak incidence of megaloblastic anemia was in the age group of 22–40 years (45.4%), followed by 19% in an age <21 years, and 14% in the age group more than 60 years.
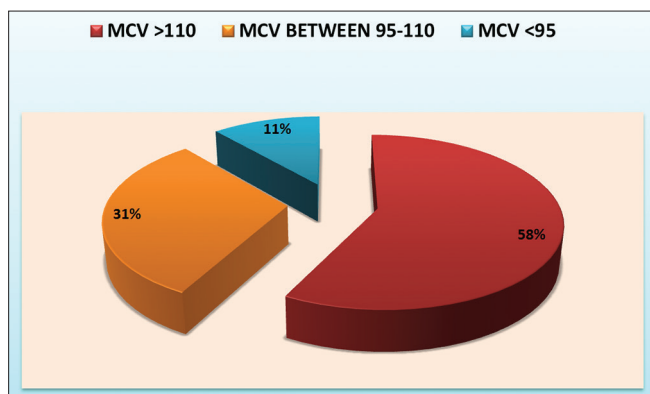

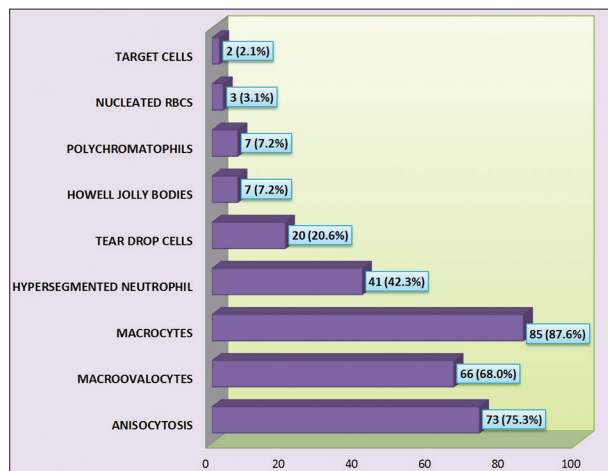**Figure 3: Stratifications of participants according to mcv level**

Figure 4: Findings on peripheral smears

Table 3: Stratifications of participants based on HB level

Hemoglobin level	Frequency (%)	Cumulative percentage
Low HB (6.0–10.0 g)	51 (52.6)	52.6
Very low HB (<6.0 g)	35 (36.1)	88.7
HB (>10.1 g)	11 (11.3)	100.0
Total	97 (100.0)	

Table 4: Etiological factors of megaloblastic anemia

Etiology	Frequency (%)	Cumulative percent
Nutritional	36 (37.1)	37.1
Alcohol and ALC. liver DS	25 (26.8)	63.9
Drugs	13 (12.4)	76.3
Tuberculosis	6 (5.2)	81.4
HIV	4 (4.1)	85.6
Celiac disease	3 (3.1)	88.7
Chronic pancreatitis	2 (2.1)	90.7
Kala azar	2 (2.1)	92.8
Malaria	2 (2.1)	94.8
ALL	1 (1.0)	95.9
Hepatitis B	1 (1.0)	96.9
Hypothyroidism	1 (1.0)	97.9
Tropical sprue	1 (1.0)	99.0
Total	97 (100.0)	

The peak incidence in the study by Khanduri *et al.* was seen in the age group of 10–30 years (48% of patients), and there was a preponderance of women (71%).[7] Similar age distribution with a preponderance of younger patient is also observed by Unnikrishnan *et al.* from Pondicherry, India, in their study (mean 35.7 ± 16.1).[8] Iqbal *et al.* from Pakistan studied MA and described similar age mean in their study.[9] Mussarrat *et al.* observed, of 349 patients, 210 (60.17%) were males, and 139 (39.82%) were females in their study (male-female ratio - 1.5:1).[10] Chandra *et al.* reported sex ratio of 1.33:1 in their study.[11]

In the study, 95% participants belonged to Hindu community and only 5% patients were from Muslim community. Unnikrishnan *et al.* found 96% patient (25/26) of MA were from Hindu community and 4% (1/26) from Muslim.[8]

Hindu Indians are usually strict vegetarian and are more prone to develop nutritional anemia. Matthews and Wood had studied the incidence of MA in Asians and found 95% patients from Hindu community and 5% from Muslim community, similar to our study.[12]

In the study, nutritional MA was present in the only vegetarian group. 60% participants with cobalamin deficiency were vegetarians and 40% are non-vegetarian. In combined deficiency, 25% participants were vegetarian and 75% were non-vegetarian. In isolated FA deficiency group, all are non-vegetarian.

Britt *et al.* studied 25 Indian patients with MA and found 68% (17/25) participants were vegetarians. 96% of patients with cobalamin deficiency, 60% with combined deficiency, and 100% with FA deficiency were reported vegetarians.[13]

Khanduri *et al.* described 87% of patients with cobalamin deficiency were vegetarian, and 71% of patients in combined deficiency were vegetarians.[7] Chanarin *et al.* studied 138 Indian patients with MA in the year 1885 and their study all were vegetarians.[14]

Clinical Profile

In our study, most frequent clinical symptoms associated with MA were fatigueness, decrease appetite, exertional

Table 5: Drugs associated with MA

Drugs	Frequency (%)
ART (Virocom-N)	5 (5.2)
Metformin	2 (2.1)
Pantoprazole (40)	3 (2.1)
Epitoin (300)	2 (2.0)
Sulfasalazine	1 (1.0)
Total	13

Table 6: Cobalamin and folic acid level among different etiology of MA

Etiological Factors	Serum cobalamin level			
	Low cobalamin		Normal cobalamin	
	Serum folate level		Serum folate level	
	Low folate	Normal folate L	Low folate	Normal folate
	Count	Count	Count	Count
Nutritional	5	31	0	0
Abd. Koch's	1	0	0	0
Alc. CLD	2	0	3	0
Alcoholic	4	0	16	0
ALL	1	0	0	0
ART induced	0	5	0	0
Chronic pancreatitis	1	1	0	0
Coeliac disease	0	0	3	0
Diss. Koch's	1	1	0	0
Hepatitis B	1	0	0	0
HIV	3	1	0	0
Hypothyroidism	0	1	0	0
Ileocecal TB	0	1	0	0
Kala azar Fever	0	2	0	0
Malaria	0	2	0	0
Metformin induced	0	1	1	0
Nutritional deficiency	5	31	0	0
Pantoprazole induced	0	3	0	0
Phenytoin induced	0	0	2	0
TB pericardial effusion	0	1	0	0
TBM	0	1	0	0
Tropical sprue	0	0	1	0
Sulfasalazine	1	0	0	0

dyspnea, weight loss, and vomiting in 92%, 80%, 59%, 55%, and 22% cases, respectively. Other symptoms such as palpitations were present in 19%, diarrhea in 16%, oral ulcers in 13%, and neuropsychiatric symptoms in 24% (paresthesias - 10%, poor gait - 2%, and memory loss - 2%), cases.

In a study by Khanduri *et al.* predominant symptoms were fatigue (70%), anorexia and gastritis (60%), fever (50%), and exertional dyspnea and palpitation (30%). They reported paresthesias, diarrhea, hyperpigmentation, and early graying of hair in <10% of patients.[7]

Unnikrishnan *et al.* described all the patient (26) in their study, had symptoms of anemia (fatigue, dyspnea, and palpitation), 26% had bleeding manifestations, neurological symptoms in 26%, paresthesia in 17%, pedal edema in 26%, and diarrhea in 4% cases.[8] Niazi and Khan reported the frequency of clinical features as pallor (90.25%), fever (56.73%), weakness (40.97%), bleeding manifestations (31.80%), and diarrhea (22.92%).[15] Lindenbaum *et al.* found in their study that patients with cobalamin deficiency present with neuropsychiatric symptoms in 28% of cases, comparable to our result.[16]

All the study has almost similar clinical presentations and its frequency. However, we did not find any patient with bleeding manifestation. This may be due to the absence of severe thrombocytopenia in our study.

In this study, observed clinical signs were pallor in 96%, knuckle hyperpigmentation in 47%, fever in 34%, oral ulcer in 26%, pedal edema in 25%, and icterus were present in 16% patients. Other signs such as lymphadenopathy and signs of liver cell failure were noted in 8.5% and 8%, respectively.

Unnikrishnan *et al.* reported pallor (100%), pedal edema (70%), hyperpigmentation (48%), neurological manifestation (26%), oral ulcer (43%), and icterus (26%) in their study.[8]

Khanduri *et al.* described pallor (85%), glossitis (29%), mild icterus (25%), and hyperpigmentation of knuckles (18%) in their study.[7]

Hematological Evaluation
In the hematological study, we found mean ESR of the patient were 29.03 ± 26.68, and it was 19.63 in female and 21.48 in male. Mean hemoglobin value of study group was 7.16 ± 2.41 (female - 6.40 and male - 7.46). 88% of the participants had Hb level <10 g/dL, and 11% cases had value more than 10 g/dL, and 53% of the patients had <6 g/dL. Mean total leukocytes count (TLC) of the patient in this study was 7183.5 ± 5816.01. 37% of the patients

had leukopenia, 32% of patients with normal TLC, and 28% of population were with leukocytosis in this study. Red cell indices and other analysis showed that the mean value of packed cell volume (PCV) was 20.5 ± 7.6, mean value of mean corpuscular hemoglobin (MCH) was 38.04 ± 6.3, mean MCHC was 34.9 ± 3.28 in this study. Mean MCV value in the study was 112.44 ± 13.27 (male - 111.53 and female - 114.83). In the study, 89% of patient had high MCV with 56% of patient had MCV >110. 11% of participants had MCV <95.

Mean value of platelet count was 1.56 ± 0.92 lacs/mm³. Thrombocytopenia was present in 50% of the patients. Mean value of reticulocyte count in the study was 2.27 ± 1.67. In our study, 38% of the patient had high reticulocyte count. Studies showing similar hemogram analysis in MA are mentioned below.

Unnikrishnan *et al.* described mean of hematological parameters as Hb - 4.96 ± 1.26, PCV - 14.65 ± 3.86, reticulocyte count - 1.78 ± 0.48, MCV - 111.8 ± 9.56, MCH - 35.05 ± 4.43, MCHC - 34.2 ± 2.35, TLC - 4.30±2.25, platelet count - 0.98 ± 0.95, and reticulocyte count - 2.5 ± 0.05.[8]

Niazi and Khan in their study, described hematological profile as 21.8% of patients had hemoglobin level of <5 g/dL, 65.3% of patients had hemoglobin level of 5–10 g/dL while 12.9% of patients had hemoglobin level of >10 g/dL (i.e. 87% of patients had Hb <10 g). 43.55% of patients had leukopenia while severe leukopenia was noted in 11.89% patients. Thrombocytopenia was detected in 72.20% patients.[15]

Khanduri *et al.* in their study reported MCV ranged from 77 to 123 and reticulocyte count was found to be higher (more than 2) in 42% case.[7] Chanarin *et al.* described 60% of patient had Hb <10, 84% of patients with raised MCV level.[14]

Peripheral smear of the participants in our study showed macrocytosis in 88%, anisocytosis in 76%, and macro-ovalocytes in 68%, and hypersegmented neutrophil in 43%. Other cells present were Howell–Jolly bodies (7%), polychromatophils (7%), nucleated RBCs (3%), and target cells (2%) in small proportions.

Niazi and Khan found macrocytosis (68.5%), hypochromia (31.5%), anisopoikilocytosis (65.9%), and hypersegmentation of neutrophils (51.5%) on peripheral smear examination.[15]

Unnikrishnan *et al.* in their study found that all the 26 patients with MA had hypersegmented neutrophils and/or macro-ovalocytoes in peripheral smear.[8]

Mwanda and Dave found anisocytosis, macro-ovalocytes in 83% cases, and hypersegmented neutrophils in 51% cases in peripheral smears.[17]

Serum FA and Cobalamin Level
In our study, we found 53% (52/97) patients with isolated cobalamin deficiency and 26% (25/97) cases with isolated FA deficiency. Combined deficiency was present in 21% (20/97) patients. FA deficiency was present in 5/18 cases of vegans, but no any case in lacto-vegetarian and lacto-ovo vegetarian, and 40/61 cases of FA deficiency were present in non-vegetarians. Cobalamin deficiency was present in all vegetarian groups (36). 36/61 cases of cobalamin deficiency were present in non-vegetarians.

Khanduri *et al.* reported cobalamin deficiency in 65%, FA deficiency in 23%, and 12% cases of combined deficiency.[7] Moreover, Unnikrishnan *et al.* reported cobalamin in 69% cases and FA deficiency in 19% cases in their study.[8] Results of these studies regarding relative prevalence of cobalamin and FA deficiency are comparable to our result.

Chanarin *et al.* found 74% case with cobalamin deficiency and 26% case of FA deficiency but did not mention the combined deficiency.[14]

Maktouf *et al.* found 98% patients with low serum cobalamin level and only 2% patient with FA deficiency. This large percentage of cobalamin deficiency and low of FA deficiency as comparison to our study may be due absence of group having combined deficiency.[18]

Bone Marrow Aspiration Study
In BMA analysis, 99% smears showed cellular bone marrow. Dyserythropoiesis along with erythroid hyperplasia was noted in 96% cases. 80% of the BMA showed megaloblastic reaction and 18% dimorphic reaction. Pancytopenia was mentioned in 37% of cases. One case showed features of ALL. The incidence of pancytopenia was studied in 30.09% patients by Niazi and Khan.[15] Moreover, Unnikrishnan *et al.* reported pancytopenia in 48% of case[8] while Khanduri *et al.* found pancytopenia in 74/120 patients (62%).[7]

Etiological Factors Responsible MA
In this study, most cases (37%) of MA were caused by nutritional deficiency (36/97).

Alcohol liver disease was the second most common cause of the MA accounting for 26% of cases (25/97). In this group alcohol as etiological factor was present in 20 cases and alcoholic liver disease was in 5 cases. Third most common cause was drugs accounting for 13% cases. In this group, ART (VIROCOM-N) was responsible in 5/13 cases,

metformin in 2/13 cases, pantoprazole in 3/13 cases, phenytoin in 2/13 cases, and one case of sulfasalazine was found. Other etiological factors were tuberculosis (6/97), HIV (4/97), celiac disease (3/97), chronic pancreatitis (2/97), malaria (2/97), kalazar (2/97), ALL (1/97), hepatitis B (1/97), hypothyroidism (1/97), and tropical sprue (1/97).

Various Study Describing Causative Factors Similar To Our Study
Chanarin *et al.* reported diagnosis in 138 Indian patients with MA as, nutritional cobalamin deficiency in 69%, nutritional FA deficiency in 2% case, pernicious anemia in 15% cases, pregnancy in 2%, anticonvulsant therapy in 2%, blind loop syndrome in 2%, gastric carcinoma in 1% and 7% case were unevaluable. They also mentioned tuberculosis as associated disorder in 12% cases.[14]

Unnikrishnan *et al.* reported nutritional deficiency in 26% cases and alcohol abuse in 13% cases as etiological factors for MA. They reported drug-induced MA in 13% and responsible drugs are phenytoin (4%), omeprazole (5%), and methotrexate (4%).[8]

Khanduri *et al.* in their study reported 25% cases (30/120) of pantoprazole and ranitidine induced MA.[7]

Britt *et al.* studied MA among Indians in Britain and found 68% cases of nutritional deficiency, 12% cases of pernicious anemia, and 20% cases of malabsorption. However, they did not mention the etiologic of malabsorption.[13]

Matthews and Wood had studied the incidence of MA in Asians and found that 81% had nutritional deficiency and 19% had true pernicious anemia.[13]

Lippi *et al.* performed a retrospective analysis to retrieve results of serum FA, B12, and TSH performed on outpatients and found 20% cases of FA deficiency and 6% cases of cobalamin deficiency in hypothyroidism.[19]

Pradhan described the MA in case of malarial fever. They mentioned it is related to nitrous oxide and cobalamin interaction. However, exact prevalence is not mentioned. In our study, we found two cases of malarial fever with MA.[20]

Nervo *et al.* suggested a prevalence of Vitamin B12 deficiency was 7% in metformin-treated diabetic patients in their study.[21] Pflipsen *et al.* found a 22% prevalence of metabolically confirmed B_{12} deficiency in the type 2 diabetic population taking metformin.[22] However, we did not find any study on prevalence of metformin among MA as etiological factor.

Due to lack of facility available in the center, we did not consider "pernicious anemia" in our study; however, it is

the most common cause of MA described in many western literatures, but in India, the exact scenario of pernicious anemia is yet to be defined.

CONCLUSION

MA emerged as an important cause of morbidity and mortality in tropical countries, and increasing number of MA is seen in clinical practice over the past 10–15 years. It is very obvious that there is "resurgence" of articles on FA -B12 deficiency/MA over the past two decades.

In our study, there is a preponderance of young people. Hindu community is most commonly affected. Vegetarians especially vegans are most susceptible to MA especially cobalamin deficiency. Lacto-ovo vegetarian diet may protect from FA deficiency but not the from cobalamin deficiency. The pathological conditions associated with the MA are much diverse. Nutritional deficiency is the most common cause of MA, followed by alcohol and alcoholic liver disease. Other common causes are drugs induced and malabsorption (i.e. chronic pancreatitis, celiac disease, and tropical sprue) and chronic infections such as tuberculosis, HIV, and Kalazar. Hence, proper diagnostic workup is essential before the use of hematinic and blood transfusion in all patients of MA.

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