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The Value of Duodenal Biopsies in the Evaluation of Megaloblastic Anemia

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Abstract

Introduction   Megaloblastic anemia is one of the common causes of anemia in India. Duodenal biopsies are routinely performed in the investigation of megaloblastic anemia. The present study was undertaken to analyze the value of duodenal biopsy in megaloblastic anemia and to correlate endoscopic findings with biopsy. As a secondary aim, the study has also analyzed the hematological profile and vitamin B_{12} and folate status of these patients.

Materials and Methods   All the cases of megaloblastic anemia with bone marrow studies diagnosed at PSG Institute of Medical Sciences and Research during the two year period from January 2016 to December 2017 were retrieved. Clinical and laboratory findings (serum vitamin B_{12} and folate levels) and endoscopic findings were retrieved from hospital records of the patients. Duodenal biopsies of these patients reported in the histopathology department were retrieved and reviewed. Statistical analysis was done using SPSS software 20.0.

Results   There were 93 cases of megaloblastic anemia diagnosed on bone marrow biopsies. Tropical sprue was diagnosed in 49.5% of cases, followed by intraepithelial lymphocytosis (17.2%), peptic duodenitis (17.2%), and no significant pathology in 16% of cases. Pancytopenia was present in 54.8% of cases. Isolated vitamin B_{12} deficiency including low levels was present in 48.38% and folate deficiency was seen in 4.3% cases; 34.48% cases had both vitamin B_{12} and folate deficiency.

Conclusion   The incidence of tropical sprue in megaloblastic anemia is 49.5% in the study. Duodenal biopsy is valuable in the work up of megaloblastic anemia, irrespective of the endoscopic changes in identifying the etiology.

Keywords
► megaloblastic anemia
► tropical sprue
► vitamin B_{12}
► folate
► pancytopenia
► intraepithelial lymphocytosis

Introduction

Megaloblastic anemia is characterized by the distinctive morphological appearance of developing hematopoietic cells in the bone marrow, resulting from nuclear cytoplasmic asynchrony, occurring as a result of defective deoxyribonucleic acid (DNA) synthesis.[1] The cause is usually nutritional deficiency or malabsorption of either cobalamin (vitamin B_{12}) and/or folate. Malabsorption can result from gastrectomy, tropical and nontropical sprue, intestinal resection, and drugs. It can also occur in defects of metabolism of these vitamins.[2] While pernicious anemia is reported to be the

common cause for megaloblastic anemia in Western countries, dietary deficiency and malabsorption are the common causes in India.[3,4] Duodenal biopsies are routinely performed in patients with megaloblastic anemia to look for any evidence of malabsorption since tropical sprue (TS) is reported to be more prevalent in India. These biopsies are performed irrespective of the macroscopic endoscopic findings. The present study was undertaken to study the value of duodenal biopsy in patients of megaloblastic anemia and correlate the biopsy findings with endoscopy. As a secondary aim, the study also analyzed the hematological profile of patients with megaloblastic anemia.

Materials and Methods

Approval was obtained from the institutional human ethics committee before the onset of the study. All the cases of megaloblastic anemia with bone marrow studies diagnosed at PSG Institute of Medical Sciences and Research during the period of 2 years from January 2016 to December 2017 were retrieved. Cases with nonmegaloblastic macrocytic anemia, those with other associated neoplastic conditions like myelodysplastic syndrome, and leukemia were excluded from the study. Hematology assays were performed on a LH 780 hematology analyzer (Beckman Coulter, Inc., United States) using volume conductivity and scatter technology. Clinical and laboratory findings (serum vitamin B_{12} and folate levels) were retrieved from hospital records of the patients. Duodenal biopsies of these patients reported in the histopathology department were retrieved. Endoscopic findings of these cases were retrieved from hospital information system and tabulated. Histopathological examination was performed after standard processing and hematoxylin and eosin staining procedures. All the biopsy slides were reviewed and only those that had well-oriented villi were included. Histomorphological features like villous and crypt architecture, intraepithelial lymphocyte (IEL) count per hundred enterocytes, and 20 villous tip enterocytes in 5 villi were studied. Up to 20 IELs per 100 enterocytes was considered normal; 21 to 30 IELs per 100 enterocytes and more than 30 per 100 enterocytes were graded as borderline and severe increase, respectively. Inflammatory cell component in lamina propria—lymphocytes, plasma cells, neutrophils, eosinophils, and epithelioid histiocytes were also analyzed and the severity was graded as mild, moderate, and severe.

Only those with unequivocal partial or complete villous atrophy, intraepithelial lymphocytosis, and increase in inflammatory cell content were diagnosed as TS. Patients with increase in IELs and no abnormality of villous architecture (Marsh I, lesion) were categorized as intraepithelial lymphocytosis. Data were analyzed using SPSS software for windows, version 20.0, IBM Corporation, NY, United States.

Results

There were 93 cases of megaloblastic anemia diagnosed on bone marrow biopsies during the study period. There were 68 (73.2%) males and 25 (26.8%) females. Age ranged from 16 to 85 years, the median age being 42 years. Out of 93 cases, vitamin B_{12} assay was done in all whereas folate levels were available in 73.

According to the World Health Organization (WHO), vitamin B_{12} status in adults is defined by the serum levels of the micronutrient with the following cutoffs and definitions: greater than 221 pmol/L (> 163 pg/mL) is vitamin "B_{12} adequacy"; between 148 and 221 pmol/L (109–163 pg/mL) is "low B_{12}"; and lower than 148 pmol/L (< 109 pg/mL) is "B_{12} deficiency."[5]

In our series we found 59 (63.4%) out of 93 patients to be vitamin B_{12} deficient. 18 (19.3%) had low vitamin B_{12} levels and 16 (17.2%) were categorized as to have adequate B_{12}. Out of the 16 patients with adequate B_{12}, 4 had folate deficiency. Five cases had vitamin B_{12} levels more than 2,000 pg/mL.

Isolated vitamin B_{12} deficiency including low levels was present in 45 (48.38%) patients and folate deficiency (4 ng/mL, reference range being 4.6–34.8 ng/mL) was seen in 4 (4.3%) patients; 32 (34.48%) patients had both vitamin B_{12} and folate deficiency. Twelve (12.9%) patients had normal serum vitamin B_{12} and folate levels.

Considering the hematological parameters, mean hemoglobin and mean corpuscular volume (MCV) of the patients were 6.68 g/dL and 103.9 fL, respectively. MCV was less than 90 fL in five patients (5%). These five patients had coexisting iron deficiency anemia (►**Table 1**).

Fifty-one patients (54.8%) had pancytopenia at presentation (defined by hemoglobin less than 11 g/dL, total WBC count less than 4×10^9/L and platelet count less than 150×10^9/L), 48 of whom had vitamin B_{12} levels < 30 pg/mL. Forty patients had bicytopenia and two had anemia. Peripheral smear and bone marrow morphology are highlighted in ►**Figs. 1** and **2**.

Duodenal biopsies were available for all 93 patients. Out of these, 46 (49.46%) patients who had villous blunting of any degree (mild, moderate, or severe) and intraepithelial lymphocytosis in a decrescendo pattern (more IELs in the crypts) and nucleomegaly were diagnosed as TS (►**Figs. 3** and **4**). Five other cases of TS diagnosed previously did not show unequivocal villous blunting and hence were categorized as intraepithelial lymphocytosis. Sixteen (17.2%) patients had only intraepithelial lymphocytosis, 16 (17.2%) had peptic duodenitis, and 15 (16.12%) had no significant pathology (►**Fig. 5**).

TS was found to be more frequent in the three groups (isolated vitamin B_{12} deficiency, isolated folate deficiency, and combined B_{12} and folate deficiency). TS was less frequent in the patients who had normal vitamin B_{12} and folate levels (►**Table 2**).

Table 1 Hematological profile of patients with megaloblastic anemia

| Hematological parameters	Mean	Range
Hemoglobin (g/dL)	6.2 + 2.37	3.0–11.9
Mean corpuscular volume (fL)	103.9 + 15.49	51.8–144.4
Red cell distribution width (%)	19.2	16–28
Platelets (×10⁹/L)	83.8	9–315
Total leucocyte count (×10⁹/L)	3.1	1–18.1
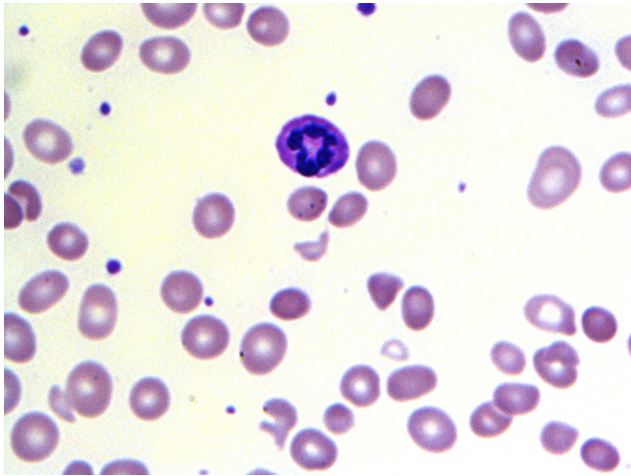
Fig. 1 Peripheral smear with hypersegmented neutrophil and macro-ovalocyte (100×).
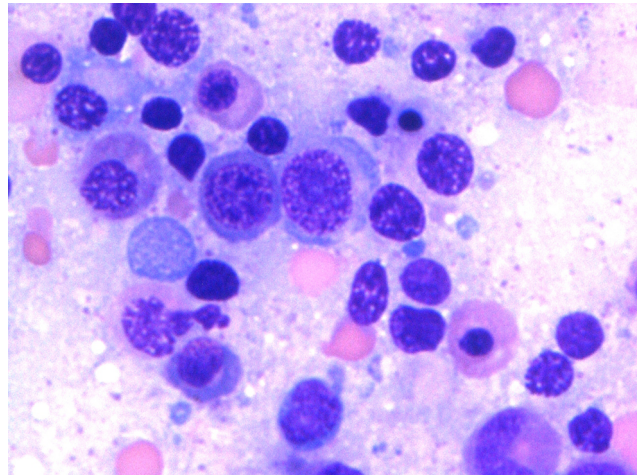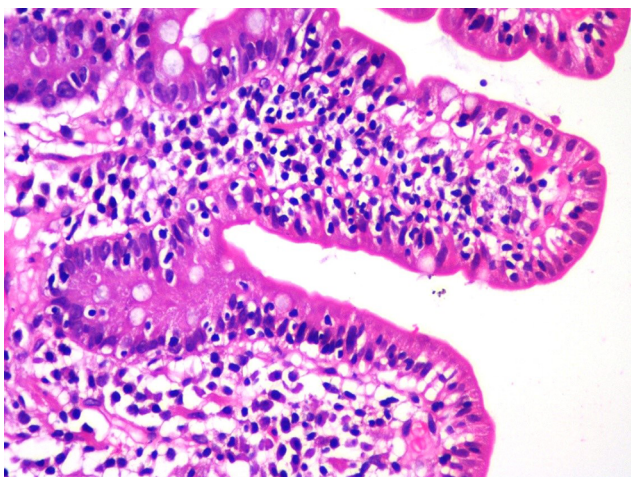

Fig. 2 Bone marrow aspirate showing megaloblastic erythroid hyperplasia (100×).
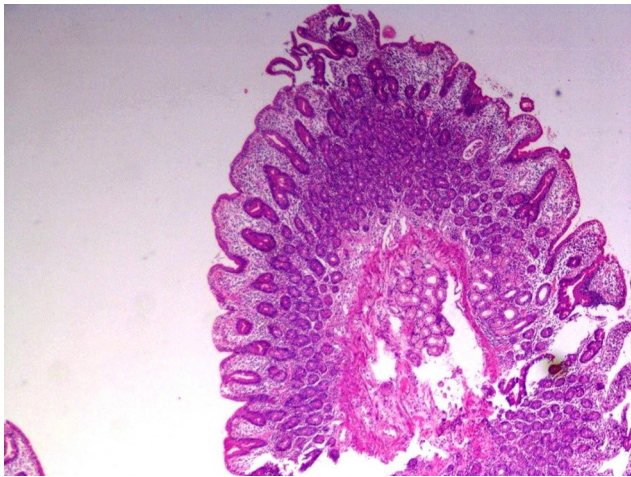
Fig. 3 Duodenal biopsy with villous blunting (4×, Hematoxylin and Eosin).
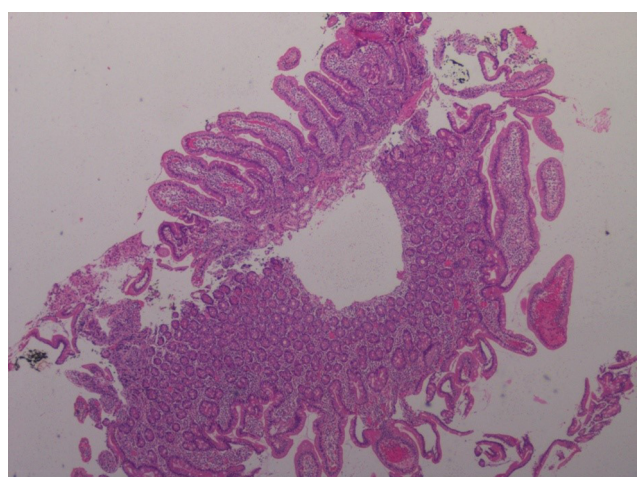
Fig. 4 Duodenal biopsy with variable villous blunting (Hematoxylin and Eosin, 4×).

Fig. 5 Duodenal biopsy with marked intraepithelial lymphocytosis (Hematoxylin and Eosin, 40×).

Endoscopic macroscopic changes pertaining to malabsorption like scalloped duodenal and attenuated duodenal folds were identified in 26.8% ($n = 25$) of patients. Of these, the biopsy diagnosis of TS was made in 15 patients (60%). The remaining cases of TS diagnosed on biopsy showed either normal endoscopic appearance or other features like nodularity (►**Table 3**).

Discussion

Megaloblastic anemia is one of the commonest causes for pancytopenia in India. It has a characteristic peripheral smear picture (macrocytosis, ovalocytes, and hypersegmented neutrophils) and low reticulocyte count.[2] Bone marrow examination is done to rule out other neoplastic disorders which can have megaloblastosis, like myelodysplastic syndrome and leukemia. Serum vitamin B_{12} and folate levels will point toward the cause for megaloblastic anemia.[6,7] Duodenal biopsies are performed to identify if there is any evidence of malabsorption. The study reports a male predominance with a ratio of 2.7:1 which is considerably higher when compared with a vast majority of other studies.[8,9] The possible explanation could be the higher population of elderly men in the

Table 2 Duodenal biopsy findings in patients with vitamin B_{12} and folate deficiency

Number of cases (N = 93)	Tropical sprue	Intraepithelial lymphocytosis	Peptic duodenitis	No significant pathology
Vitamin B_{12} deficiency n = 45	24 (53.3%)	8 (17.7%)	5 (11.1%)	8 (17.7%)
Folate deficiency n = 4	3 (75%)	–	1 (25%)	–
Both n = 32	17 (53.1%)	6 (18.75%)	6 (18.75%)	4 (12.5%)
Normal n = 12	2 (16.6%)	2 (16.6%)	4 (33.36%)	3 (25%)

Table 3 Endoscopic findings with histological diagnosis

N = 93	Tropical sprue (n = 46)	Intraepithelial lymphocytosis (n = 16)	Peptic duodenitis (n = 16)	No significant pathology (n = 15)
Scalloped duodenal folds n = 25	15 (60%)	2 (8%)	3 (12%)	5 (20%)
Nodularity n = 25	10 (40%)	6 (24%)	3 (12%)	6 (24%)
Gastroduodenitis n = 12	6 (50%)	2 (16.6%)	1 (8.33%)	3 (25%)
No macroscopic abnormality n = 31	15 (48.5%)	6 (19.3%)	8 (25.8%)	2 (6.45%)

series in whom vitamin B_{12} deficiency due to intestinal malabsorption are reported to be common.[10]

Cobalamin and folate deficiency are predominantly due to decreased dietary intake or malabsorption in Indian population while pernicious anemia has been reported to be the common etiology in Western population.[11,12] Vitamin B_{12} deficiency has been reported to be the common cause for megaloblastic anemia in our population constituting to 63%, similar to that of Khandhuri et al (68%)[8] and Sarode et al (76%).[9] Combined vitamin B_{12} and folate deficiency was seen in 34% of cases which is slightly higher when compared with 8.8% and 12% reported by Sarode et al and Khandhuri et al, respectively. This is probably because of higher percentage of people with malabsorption in this series where combined micronutrient deficiencies are common. Folate deficiency was seen in 4.3% of patients which is similar to other studies. The cases with vitamin B_{12} levels greater than 2,000 pg/mL could represent cases treated inadvertently with vitamin B_{12} injections prior to investigation.

Pancytopenia and bicytopenia were observed in 54.8% and 43% of the patients, respectively. There are wide variations reported for the frequency of pancytopenia ranging from 17.2 to 43.8%.[13,14] Another study done in South India has reported pancytopenia in 40% of the patients.[15] The higher incidence of pancytopenia in our population can be attributed to severe vitamin B_{12} deficiency in these patients (48 out of 51 had vitamin B_{12} levels < 30 pg/mL). Mean MCV observed in the current study was 103.9 + 15.49 fL, which is lower when compared with that reported by Chidambaram et al (110 fL).[15] MCV was found to be normal in 5.3% of the patients which is lower than another study.[14]

TS was the commonest biopsy diagnosis in the study accounting for 49.5% of patients diagnosed as having megaloblastic anemia, which is similar to most other studies.[16,17] Endoscopic finding of scalloped duodenal folds was identified in 32.6% of TS. In the remaining cases, the diagnosis was possible only with the help of biopsy.

The diagnostic yield of gastrointestinal endoscopy is low in our study. This could be because subtle endoscopic changes were not documented since duodenal biopsies were routinely done in all the patients with megaloblastic anemia. However, Sharma et al[18] in their systematic review had found normal duodenal folds to be more common in TS.

Intraepithelial lymphocytosis without villous abnormality was the second commonest biopsy finding identified in 34.7% of patients. IEL without villous abnormality had been reported in various conditions such as gluten-sensitive enteropathy, infections, autoimmune conditions, drugs, and idiopathic etiology.[19] It has also been reported to be an early morphological feature of sprue.[19,20]

TS and IEL without villous abnormality together constituted 66.6% of cases, indicating malabsorption as the etiology for vitamin B_{12} and folate deficiency in these cases. Hence, biopsy contributes in identifying the etiology in 66.6% of cases.

In the remaining cases, the etiology of megaloblastic anemia could have been dietary deficiency or intrinsic factor deficiency.

Although the guidelines for investigation of megaloblastic anemia recommends serological assays of anti-intrinsic factor antibodies (anti-IFAB)[5] the incidence of pernicious anemia is very low in India. Duodenal biopsies have become a

routine practice in the workup more based on the evidence of high number of cases of TS being reported. Anti-IFAB levels were not done in the patients in the study.

It was found in the present study that routine duodenal biopsy in cases of megaloblastic anemia yields etiologic information in majority of the cases, irrespective of endoscopy. Hence it is recommended even in the absence of macroscopic endoscopic changes.

Current spectrum of malabsorption in India has found celiac sprue to be increasing in incidence.[19] Since the clinical, endoscopic, and histologic difference between celiac and tropical sprue are not well defined, serum antibodies against tissue transglutaminase and endomysial antibodies are needed in cases where there is no response to therapy for TS.

Limitations

Since this is a retrospective study, follow-up of patients to look for response to therapy for TS is not available. Since the study was conducted in a teaching hospital, endoscopy being done by different endoscopists would have influenced the results. Serological tests for celiac sprue and anti-IFAB antibodies were not done in the study.

Conclusion

Pancytopenia was the commonest presentation of megaloblastic anemia; vitamin B_{12} deficiency and folate deficiency constituted 82% and 4.3%, respectively. The incidence of TS in megaloblastic anemia is as high as 49.5% in the study population. Duodenal biopsy is valuable in the workup of megaloblastic anemia, irrespective of the endoscopic changes in identifying the etiology.

Authors' Contribution

N.N.N. and S.S. contributed to the literature research, data acquisition, and manuscript preparation. All the authors contributed to the development of the concept, design, definition of intellectual content, and manuscript editing and review. S.S. is the guarantor for this article.

Ethical Approval

Ethical approval was obtained from the institutional human ethics committee.

Conflicts of Interest

None declared.

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