Research Article

Association of *Helicobacter pylori* infection with megaloblastic anemia: a single centre experience

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Abstract

Background: Histopathological changes due to *Helicobacter pylori* infection are well characterized but clinical and pathological outcome resulting from the infection are incompletely described and many studies are available from western countries but few data are available from Indian population.

Objective: The study has been conducted to observe whether H. *pylori* infection is associated with gastric atrophy leading to the development of megaloblastic anemia.

Material and methods: It was a cross-sectional study with one year duration. Total 30 patients who were suspected to have megaloblastic anemia underwent upper endoscopy, with simultaneous test by Urease test to screen for H. *pylori* infection. Biopsies were taken, one from antrum and body and another from fundus. These biopsies were studied for H. pylori infection, as well as the level of gastritis and gastric atrophy. Statistical analysis was done by using percentage proportion.

Result: Out of 30 patients, 25 were male and 5 were female. Mean age of the study was 33.03±5.85 years. Age group 51–60 years had maximum i.e. 30% of patients. Out of 30 patients, 17 patients had knuckle pigmentation, 7 patients had gray hair, 2 patients had sensory ataxia and 4 patients had normal clinical findings. All the patients of the study had macrocytosis on peripheral smear. Six patients had thrombocytopenia and 5 patients had hypersegmented neutrophils. Out of 30 patients, 10, 2, and 4 patients had mild, moderate, and severe gastritis, respectively on histopathological examination. Five patients had chronic gastritis and 3 had superficial gastritis. Four patients had normal biopsy and 2 patients had pure atrophy. Only one patient came positive for H. *pylorus* and rest 29 patients were negative for H. *pylori*.

Conclusion: Incidence of H. *pylori* in the study population of 30 megaloblastic patients was found to be 3%. H. *pylori* are not associated with gastric atrophy.

KEYWORDS: Atrophy, gastritis, gastric mucosa, *Helicobacter pylori* infection

Introduction

The megaloblastic anemias are disorders caused by impaired DNA synthesis usually due to vitamin B12 or folate deficiency.[1] Vitamin B12 or cobalamin is a complex organometallic compound which cannot be synthesized in the body and must be supplied in the food. But its absorption requires presence of gastric intrinsic factor (IF) which is secreted by the parietal cells.[1] Helicobacter are gram negative, non-invasive bacilli, living in the mucus that over lies gastric type mucosa.[2] Although Helicobacter pylori (H. *pylori*) infection is usually asymptomatic, essentially all H. pylori infection causes gastric inflammation. H. *pylori* is the foremost cause of peptic ulcer, which occurs at some point in lifetime of about 15% of infected persons in developed countries.[2]

In developing countries, 70 to 90% of the population carries H. *pylori*; almost all of these acquire the infection before the age of 10 years.[3] H. *pylori* are also one of the major causes of chronic superficial gastritis leading to atrophy of gastric glands. It has a spectrum of affection on gastric cells from simple gastritis to atrophy of gastric mucosa.

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Studies have indicated a positive correlation between H. *pylori* induced atrophic gastritis leading to cobalamin deficiency and consequent development of megaloblastic anemia.[4-9] H. *Pylori* infection is highly associated with type B chronic atrophic gastritis.[4-9] The association of H. *pylori* infection with type B chronic atrophic gastritis and cobalamin malabsorption has suggested by several investigators that H. *pylori* infection may predispose or even be causative in pernicious anemia.[7, 8]

There are two main reasons to undertake this study. First, histopathological changes due to H. *pylori* infection are well characterized but clinical and pathological outcome resulting from the infection are incompletely described. Second, many studies are available from western countries but few data are available from Indian population about prevalence of H. *pylori* infection in patients of megaloblastic anemia.

The present study was undertaken to observe whether H. *pylori* infection is associated with gastric atrophy which has led to the development of megaloblastic anemia.

Materials and Methods

It was a cross-sectional study. Duration of the study was one year (from March 2009 to March 2010). Thirty cases of megaloblastic anemia were included in the study.

Patients with Peripheral smear showing predominant macrocytes, hypersegmented neutrophils, and clinical features suspected of megaloblastic anemia; like knuckle pigmentation, early graying of hair, dyspepsia, bald tongue along with anemia, proven B12 deficiency biochemically and bone marrow proven megaloblastic anemia were included in the study.

While patients with the classical cause of cobalamin deficiency such as post-gastrectomy state, patients with evidence of renal failure or liver disease, pregnant female patients, patients who have received H. *pylori* eradication therapy and patients on NSAIDS therapy were excluded from the study.

In present study, patients who were suspected to have megaloblastic anemia clinically were investigated for the same. When severe anemia was present patients were initially transfused till hemoglobin was built up, so that patients were fit for endoscopy. A proper informed consent was taken for endoscopy. All patients underwent upper endoscopy, with simultaneous test by Urease test to screen for H. *pylori* infection. Two biopsies, one was taken from antrum and body, and one was taken from fundus. These biopsies were studied for H. *pylori* infection, as well as the level of gastritis, gastric atrophy. If there was any H. *pylori* infection which was significant then it was treated according to the standard protocol till infection was eradicated. Simultaneous B12 level were monitored during the treatment duration. When there was no H. *pylori* organism found, patients were treated for B12 deficiency with injectable preparation of B12 along with folic acid preparation till patients were clinically cured.

Statistical analysis

Statistical analysis was done by using percentage proportion. Results were obtained in form of *p* (probability) value. *p* value of <0.05 was considered significant.

Ethical consideration

The detailed plan of this study was submitted to ethical committee of the institute and after approval of this committee study was started.

Result

Total number of patients was 30. The number of male patients 25 (83.33%) outnumbered the female 5 (17.66%) and male to female ratio was 4.8:1.

Table 1 shows that the mean age of the study was 33.03 ± 5.85 years. Age group 51–60 years had maximum number of patients and included 30% patients. In this age group there were 8 (32%) males and 1 (20%) female. Age group 40–50 years had 3 (10%) patients only and all of them were male. Remaining age groups were having 6 (10%) patients each.

Table 2 shows that out of 30 patients, clinically 17 (56.66%) were having knuckle pigmentation, 7 (23.33%) patients were having gray hair and only 2 (6.66%) patients were having neurological involvement in the form of sensory ataxia. Remaining 4 patients did not have significant clinical findings on examination.

Table 3 show that all the patients of the study were having macrocytosis on peripheral smear. Six (20%) out of 30 were

Table 1: Age-wise distribution of study population

Age group (years)	Number of patients		Total no. of patients (*n* = 30)
	Males (*n* = 25)	Females (*n* = 5)	
15 – 20	4 (16%)	2 (40%)	6 (20%)
21 – 30	4 (16%)	2 (40%)	6 (20%)
31 – 40	6 (24%)	Nil	6 (20%)
41 – 50	3 (12%)	Nil	3 (10%)
51 – 60	8 (32%)	1 (20%)	9 (30%)

Table 2: Clinical presentation of megaloblastic anemia

Clinical presentation	No. of patients *n* = 30
Knukle pigmentation	17 (56.66%)
Gray hair	7 (23.33%)
Sensory ataxia	2 (6.66%)
No significant clinical finding	4 (13.33%)

Table 3: Hematological parameters: peripheral smear examination

Peripheral Smear findings	No of patients (*n* = 30)
Macrocytosis	30 (100%)
Thrombocytopenia	6 (20%)
Hyper segmented neutrophils	5 (16.66%)

Table 4: Histopathology-wise distribution of study population

Histopathology	No. of patients
Normal	4 (13.33%)
Mild gastritis	10 (33.33%)
Mod gastritis	2 (6.66%)
Severe gastritis	4 (13.33%)
Chronic gastritis	5 (16.66%)
Superficial gastritis	3 (10%)
Pure atrophy	2 (6.66%)

having thrombocytopenia and 5 (16.66%) patients were found to have hypersegmented neutrophils.

Table 4 shows that out of 30 patients 10 patients were having mild gastritis, 2 were having moderate gastritis, and 4 were having severe gastritis. Five were having chronic gastritis and 3 were having superficial gastritis on histopathological examination. Four patients were having normal biopsy without any pathology and 2 patients were having pure atrophy.

Only one (3.33%) patient came positive for H. *pylorus*, who was a male patient. And rest of patients 29 (96.66%) were negative for H. *pylori*. There was no statistical significant association of gender and H. *pylori* positivity ($p > 0.05$).

Discussion

The present study was conducted to study the relation of H. *pylori* infection with gastric atrophy and megaloblastic anemia. Total 30 patients were studied this included 25 males and 5 females.

The age range in the study was 15–60 years. Maximum number of patients were from age group 51–60 years i.e. 10 (30%) and only 3 (10%) in age group 41–50 years. Mean age of the study was 33.03 ± 5.85. Flejau et al.[8] studied 86 patients and found to have median age of the patients was 58±6 years. Kaptan et al.[4] studied 138 patients and found to have median age of the patients was 59.54 ± 15.43 years.

In present study total number of patients was 30 out of which male patients were 25 (83.33%) and female 5 (16.66). Flejau et al.[8] studied total 86 patients out of which 39 (45.34%) were male and 47 (54.66%) were female. Kaptan et al.[4] studied total 138 patients out of which female were predominant 101 (73.19%) and only 37 (26.81%) were male.

In present study, clinically patients were evaluated and found to have knuckle pigmentation in 17 (56.66%) patients and gray hair in 7 (23%) patients.

Also, 2 patients were having neurological involvement in the form of sub acute combined degeneration. Baker et al.[10] studied 21 cases of megaloblastic anemia all were having skin pigmentation mostly confined to hand and fingers. Carmel[11] studied 4 cases of pernicious anemia, out of these 2 (50%) patients were having reddish gray hairs. Wadia et al.[12] studied 167 cases of megaloblastic anemia and detected neuropathy in 42 (25.14%) cases. Half of these had only sensory neuropathy. Evidence of spinal cord involvement was found in 12 (7.2%), and 7 of these had evidence of involvement of peripheral nerves, posterior columns, and pyramidal tracts.

In present study, all the patients of the study were having macrocytosis on peripheral smear. Six (20%) out of 30 were having thrombocytopenia and 5 (16.66%) patients were found to have hypersegmented neutrophils.

It was more than 100 fl in 22 (74.33%) patients and between 97–100 fl in 8 patients. Savage et al.[13] found macrocytosis in 83% of the total cases. Carmel et al.[14] found hypersegmented polymorphs in only one out of 34 cases. Olgiati and Mombelli[15] studied 30 cases of megaloblastic anemia and found thrombocytopenia in 18 patients (60%) and both thrombocytopaenia and leucopenia in 11 (36.66%) of cases.

In present study, out of 30 patients, 10 (33.33%) patients were having mild gastritis, 4 (13.33%) patients were having severe gastritis, 5 patients (16.67%) were having chronic gastritis and 2 patients (6.67%) were having pure gastric atrophy on gastric biopsy when examined histopathologically. Flejou et al.[8] had found chronic gastritis of the body in all 86 patients (100%). Djurkov et al.[16] studied 40 patients of pernicious anemia all were having chronic atrophic gastritis. Haruma et al.[7] studied 24 patients all were having severe fundic atrophic gastritis and 17 (71%) had antral atrophic gastritis. Fong et al.[17] studied 28 patients out of which 50% patients were having active chronic gastritis. Atrophic changes were found more commonly in patients with pernicious anemia. Gonzalez et al.[5] studied 36 patients and found that 20 patients (55.55%) had both antral and intestinal metaplasia; 10 patients (27.78%) were having only antral metaplasia and 6 patients (16.67%) were having intestinal metaplasia.

In the present study, patients' results showed presence of H. *pylori* in only one patient out of 30 patients of megaloblastic patients studied. This included simultaneous study of urease test and histopathological study by routine staining. This was calculated to an incidence of 3% in the study population. In comparison to the study by Flejou et al.[8] where biopsy from body was carried in all the patients, but biopsy of antrum was carried only in 44 of 86 patients. This study had only found only 3 H. *pylori* positive patients out of 86 patients. Calculated incidence in same study was 6% only. Gonzalez et al.[5] found 1 (2.7%) H. pylori positive patient out of 36 patients. Djurkov et al.[16] studied 40 patients, only 3 (7.5%) were positive for H. *pylori*. Haruma et al.[7] studied 24 patients, out of which nobody was positive for H. pylori. Fong et al.[17] had studied 28 patients out of which 3 (11%) came out to be positive for H. *pylori*. Kaptan et al.[4] studied 138 pernicious anemia patients, out of which 77 (55.80%) were H. *pylori* positive. Annibale et al.[6] studied 81 patients in which 49 (60.4%) found to be positive for H. *pylori*.

H. *pylori* like organisms were identified on microscopy in only one of 30 patients with megaloblastic anemia. It is clear that these bacteria only rarely colonize the abnormal body in megaloblastic anemia and are unlikely to be involved in the pathogenesis of the disease.

Irrespective of whether the antral gastritis in pernicious anemia is an extension of an immune process into the antrum or a superimposed pattern of hypersecretory (type B) gastritis, the frequency of H. *pylori* like organisms in the involved antrum is still well below that found in any of the study which has taken control group. Small sample size is the limitation of the present study.

Conclusion

Incidence of H. *pylori* in the study population of 30 megaloblastic patients was found to be 3%. H. *pylori* are not associated with gastric atrophy found in the patients of megaloblastic anemia.

Antral gastritis with gastric atrophy of varying degree is very common in megaloblastic patients; beginning from mild to severe gastritis and or atrophy. Normal gastric biopsy in the patients of megaloblastic patients is commoner in younger patients.

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