Original Article

Helicobacter pylori, a causative agent of vitamin B_{12} deficiency

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Abstract

Background: *Helicobacter pylori* is one of the most common causes of peptic ulcer disease worldwide and a major cause of chronic superficial gastritis leading to atrophy of gastric glands.

Methodology: A total of 60 patients suffering from gastric disease due to *H. pylori* infection were evaluated. Endoscopy was performed and gastric biopsies were obtained for histopathology and urease test. Blood was simultaneously collected for the determination of the levels of vitamin B12 and the MCV. Vitamin B12 levels were determined by chemiluminescent assay.

Results: Our results indicate that the mean vitamin B12 level ± SEM for the total population, the *H.pylori* infected and non-infected patients were 264.5±22.9, 207.7±21.9 and 419.7±39.8 respectively. *H. pylori* was found in 71.7% (43/60) of the patients tested. The level of vitamin B12 was lower than 200pg/ml (deficient) in 67.4% (29/43) of patients tested positive for *H. pylori*.

Conclusion: *H. pylori* appears to be implicated in causing vitamin B12 deficiency.

Key Words: *Helicobacter pylori*, Vitamin B12, Gastritis, Urease, MCV

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Introduction

Helicobacter pylori is a fastidious gram negative microorganism with a spiral/helical shape and 5 to 7 sheathed uni-polar flagella. It requires high humidity, a microaerophilic environment, and an incubation temperature of 37°C for growth. Although *H. pylori* colonies may appear after 3 to 5 days, the primary growth may take up to 7 days to appear and the colony morphology is described as translucent, small pinpoint colonies [1]. *H. pylori* is considered to be the most common human pathogen colonizing the human gastric mucosa [1]. The prevalence of *H. pylori* in the developed countries is 25% to 40% and is almost 100% in developing countries [2].

Studies have shown that *H. pylori* IgG antibodies increase with age. It is present in 40% of those aged 1 to 14 years, and has been reported in up to 60% of 18- to 24-year-old Mexicans and in 100% of 60 to 69-year-olds in Turkey [3]. *H. pylori* causes chronic gastritis [4] and peptic ulcer disease [5] and it has been classified as a type I (definite) carcinogen since 1994. This organism has also been implicated in increasing the risk of developing gastric cancer, which is the second most frequent cause of cancer-related death [3,6]. Furthermore, *H. pylori* infection has also been associated with an increased risk of B-cell lymphoma of gastric mucosa-associated-lymphoid-tissue (MALT – lymphoma) [7,8].

Vitamin B_{12}, which is a water soluble vitamin, is a complex molecule that cannot be synthesized in the human body and must be supplied in a diet containing meat and dairy products. Upon digestion, B12 is released from food and complexes with gastric intrinsic factor (IF). The B_{12}-IF complex binds to specific receptors in the ileum where it is absorbed. It has important functions in DNA replication, in the synthesis of red blood, and in maintaining the myelin sheath that surrounds nerve cells. Vitamin B_{12} deficiency causes pernicious anemia due to lack of production of intrinsic factor by epithelial cells in the stomach. Although many factors can contribute to the development of Vitamin B_{12} deficiency, pernicious anemia is mostly attributable to conditions associated with chronic atrophic gastritis [9].

H. pylori has been suggested as an important agent in the aetiology of vitamin B_{12} deficiency and pernicious anemia [10]. A result of *H. pylori*-induced gastritis and ulcers is destruction of the parietal cells which are important for the production of intrinsic factor which is essential for vitamin B_{12} absorption. *H.*

pylori were isolated in 56% of patients with pernicious anemia and eradication of the infection has been shown to result in improved blood levels of vitamin B_{12} in 40% patients [11]. However, *H. pylori*-induced vitamin B_{12} deficiency has been shown to occur in the absence of atrophic gastric mucosa [12]. Thus the aim of this study was to further investigate the possible association between *H. pylori* infection and vitamin B_{12} deficiency.

Materials and Methods

This cross-sectional study was conducted between November 2003 and November 2004 The inclusion criteria was limited to patients diagnosed with gastritis by endoscopy and not taking any vitamin B_{12} supplements. Patients were excluded from the study if they had any underlying medical condition which may have caused vitamin B_{12} deficiency, if they had previous administration of *H. pylori* eradication therapy, or if they had renal failure or liver disease. Pregnant women were also excluded.

For each patient enrolled, information about age, sex, smoking and symptoms suffered was recorded. Blood was collected aseptically in EDTA and plain vacutainer tubes for laboratory investigations. Complete Blood Count (CBC) was performed using Sysmex Analyzer (Sysmex, Japan) to determine the MCV. Vitamin B_{12} levels was determined by a competitive chemiluminescent immunoassay using an IMMULITE automated analyzer (DPC, CA, USA).

Gastric biopsies from the antrum and duodenal biopsies were obtained during endoscopy. The rapid urease which is a qualitative test for the detection of the urease enzyme produced by *H. pylori* was conducted using the CLO-test (Biomer'ieux, France). *H. pylori* hydrolyzes the urea rapidly and the color change of the indicator within 20 minutes to one hour was indicative of a positive test. If negative after one hour, the test was read again after 24 hours.

Poly Stat *Helicobacter pylori* test kit (Polymedco Inc. USA) was used for the qualitative detection of anti-*Helicobacter pylori* IgG in the human serum. Histopathology was performed on formalin fixed paraffin embedded biopsies. Sections were cut at 5 microns thick and stained with eosin and methylene blue. The sections were then evaluated for the presence of *H. pylori* by a pathologist.

Statistical analysis was conducted using SPSS version 12 (SPSS inc., Chicago). The presence of association between the different parameters was assessed by by Chi-Square and correlation coefficients was also determined ($p<0.05$ was taken as statistically siginificant).

Results

Sixty patients (29 females and 31 males) who fulfilled the inclusion and exclusion criteria were enrolled in the study. *H. pylori* was detected in 97.7% (42/43) of the patients diagnosed with gastritis. Histopathology was used to demonstrate the presence of the characteristic curved or S-shaped *H. pylori* and to provide information about the nature of the patient's gastritis. CLO test and serology revealed an infection rate of *H. pylori* in 71.7% (43/60) of the study population. Among those positive for *H. pylori*, the rate of infection was higher in males, 55.8% (24/43) versus 44.2% (19/43) in females. The age range of the patients was between 18 and 82 years (mean 43.4 $\pm$17.3 years). With respect to age, the population was divided into three groups: group I (18 – 39 years), group II (40-59 years) and group III (> 60 years). Our results indicated that *H. pylori* was present in 56.7% of group I and 21.7% in groups II and III (Table 1). With respect to smoking, 30.2% (11/43) of the *H. pylori* positive patients were smokers.

Table 1. General characteristics of the parameters monitored.

Characteristics	Frequency	Percentage
Age		
18-39 (%)	34	56.7
40-59 (%)	13	21.7
60+ (%)	13	21.7
Gender		
Males (%)	24	55.8
Females (%)	19	44.2
Smoking		
Smokers	13	30.2
Non-smokers	30	69.8
MCV		
Elevated (90+)	29	67.4
Normal (<90)	14	32.6
Vitamin B_{12}		
Deficient (<250)	29	67.4
Normal (>=250)	14	32.6
***H. pylori* Pathology**		
Present	43	71.7
Not present	17	28.3
CLO Test and Serology		
Positive	43	71.7
Negative	17	28.3

The lower limit of normal serum vitamin B_{12} level is set at 200 pg/ml [13] and the reference level used at the Mayo Clinic is 200 to 650 pg/ml [14]. The mean vitamin B_{12} level (± SEM) was 264.5 ± 22.9 pg/ml in the total patient population (n=56). Four values were excluded from the mean because the levels were extremely elevated (1088, 1103, 1185, 1200). The cut-off level to determine vitamin B_{12} deficiency was taken as <200 pg/ml, and based on this measure, 67.4% (29/43) of the *H. pylori* infected patients had Vitamin B_{12} deficiency. The mean vitamin B_{12} level (± SEM) for the *H. pylori* infected patients was 207.7±21.9 as compared to 419.7±39.8 for the *H. pylori* negative patients. There was significant difference by the independent t-test (p= 0.00) between the means of vitamin B_{12} levels of *H. pylori* negative and *H. pylori* positive patients at 0.05 level of significance. MCV was elevated (>90 fl) in 67.4% of the *H pylori* infected patients (29/43) (Table 2). There was significant association (p<0.05) between presence of *H. pylori* infection and vitamin B_{12} deficiency.

Table 2. Association of vitamin B_{12} and MCV in *H. pylori* infected cases.

	Vitamin B_{12}	
	Deficient (<200 pg/ml)	
MCV	Number	Percent
Elevated (>90 fl)	29	67.4
Not elevated (<90 fl)	3	7.0
	Not deficient (>200 pg/ml)	
MCV	Number	Percent
Elevated (>90 fl)	1	2.3
Not elevated (<90 fl)	10	23.3

Total number of cases positive for *H. pylori* = 43
Coefficient of correlation between MCV and Vitamin B_{12} = - 0.678.

Discussion

H. pylori has been found to colonize the stomach of humans worldwide. *H. pylori* causes the majority of cases of both gastritis and duodenal ulcers. The prevalence of *H. pylori* varies depending on age, race, ethnicity, geographical location, crowding and socioeconomic class. Our results indicated that age, gender and smoking are not significant in causing infections with *H. pylori.*

Pernicious anemia is a type of megalobastic anemia due to Vitamin B_{12} deficiency. In order to establish an association between *H. pylori* infection and vitamin B_{12} deficiency, we have chosen patients symptomatic for *H. pylori* infection. Our results showed that 71.7 % of the patients with gastritis were infected with *H. pylori*, and 67.4% of these patients had pernicious anemia as seen in the elevated MCV levels. In this study, selection of patients was restricted to those who had no inherent problems related to vitamin B_{12} absorption or malnutrition. *H. pylori* and related gastric pathology emerges as a major factor responsible for vitamin B_{12} deficiency. Several studies on the relationship between *H. pylori*, gastritis and vitamin B_{12} deficiency have been conducted [9,11]. Our results agree with the results obtained by these studies. The main variables in this study were *H. pylori* infection and vitamin B_{12} deficiency. Our results indicated a significant association between the two (P<0.05). In conclusion, the presence of *H.pylori* in the gastric mucosa can be considered as an important factor in causing vitamin B_{12} deficiency. In developing countries, where there is a high prevalence of *H. pylori* infections, one expects to also find vitamin B_{12} deficiency in certain members of the population. Therefore, diagnostic evaluation of vitamin B_{12} deficiency must include tests for *H. pylori.*

References

1. Winn W Jr., Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins. pp 405-406.
2. Windsor HM, Abioye-Kuteyi EA, Lebor JM, Morrow SM, Bulsara MK, Marshall BJ (2005) Prevalence of *Helicobacter pylori* in indigenous Western Australians: Comparison between urban and remote rural populations. MJA 182 (5): 210-213.
3. Kikuchi S and Dore MP (2005) Epidemiology of *Helicobacter pylori* infection. Helicobacter (10).
4. Cave DR (2001) Chronic gastritis and *Helicobacter pylori*. Semin Gastrointestinal Disease. 12: 9-12.
5. Cohen H (2000) Peptic ulcer and *Helicobacter pylori*. Gastroenterol Clin North Am 29: 29-33.
6. Replogle ML (1995) Biologic sex as a risk factor for *Helicobacter pylori* infection in healthy young adults. Am J Epidemiol 142: 146-159.
7. Fischbach W, Chan AO, Chun-Yu Wong B (2005) *Helicobacter pylori* and Gastric Malignancy. HELICOBACTER (10) Sup 1.
8. Lehours P, Dupouy S, Bergey B, Ruskone-Foumestraux A, Delchier JC, Rad R, Richy F, Tankovic J, Zerbib F, Megraud F, Menard A (2004) Identification of a genetic marker of *Helicobacter pylori* strains involved in gastric extranodal marginal zone B-cell lymphoma of the MALT-type. Gut 53: 931-7.
9. Gumurdulu Y, Sern E, Ozer B, Kayaselcuk F, Kul K, Pata C, Guclu M, Gur G, Boyacioglu S (2003) Predictors of vitamin B_{12} deficiency: Age and *helicobacter pylori* load of antral mucosa. The Turkish Journal of Gastroenterology. (14) 1: 1-6.
10. Perez-Perez GI (1997) Role of *Helicobacter pylori* infection in the development of pernicious anemia. Clin Infect Disease. 25: 1-39.
11. Kaptan K, Beyan C, Ural AU, Cetin T, Aycu F, Gulsen M, Finci R, Yalcin A (2000) *Helicobacter pylori*: is it a novel causative agent in Vitamin B_{12} deficiency? Arch Intern Med 160: 12-16.

12. Gumurdulu Y, Serin E, Ozer B, Kayaselcuk F, Kul K, Pata C, Guclu M, Gur, G, Boyacioglu G (2003) Impact of *H. pylori* on the development of vitamin B_{12} deficiency in absence of gastric atrophy. The Turkish Journal of Gastroenterology (14) 1: 44-49.
13. Snow CF (1999) Laboratory diagnosis of vitamin B_{12} and folate deficiency. ARCH INTERN MED (159).
14. Klee GG (2000) Cobalamin and Folate Evaluation: Measurement of Methylmalonic Acid and Homocysteine vs Vitamin B_{12} and Folate. Clinical Chemistry 46:8(B) 1277–1283.

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