**Helicobacter pylori—Is It a Novel Causative Agent in Vitamin B₁₂ Deficiency?**

Kürşad Kaptan, MD; Cengiz Beyan, MD; Ali Uğur Ural, MD; Türker Çetin, MD; Ferit Avcu, MD; Mustafa Gülşen, MD; Rifki Finci, MD; Atilla Yalcın, MD

**Background:** Evidence for vitamin B₁₂ deficiency usually involves combinations of low serum vitamin B₁₂ levels, clinical and metabolic abnormalities, and therapeutic response. Identification of the underlying cause is important in the diagnosis of vitamin B₁₂ deficiency that is usually attributed to malabsorption. *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. It is suggested that there may be a casual relationship between *H pylori* and food-cobalamin malabsorption.

**Objectives:** To evaluate the *H pylori* incidence in patients with vitamin B₁₂ deficiency prospectively and to assess whether treatment for *H pylori* infection could correct this deficiency over time.

**Patients and Methods:** We performed a prospective cohort study involving 138 patients who had anemia and vitamin B₁₂ deficiency. An upper gastrointestinal endoscopy was performed to assess the severity of atrophic gastritis and biopsy specimens for *Campylobacter*-like organisms tests and histological examination for *H pylori* were obtained at the time of diagnosis. The diagnosis of *H pylori* prompted a combination treatment.

**Results:** *Helicobacter pylori* was detected in 77 (56%) of 138 patients with vitamin B₁₂ deficiency and eradication of *H pylori* infection successfully improved anemia and serum vitamin B₁₂ levels in 31 (40%) of 77 infected patients.

**Conclusions:** *Helicobacter pylori* seems to be a causative agent in the development of adult vitamin B₁₂ deficiency. Eradication of *H pylori* infection alone may correct vitamin B₁₂ levels and improve anemia in this subgroup of patients.

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**Megaloblastic anemia** occurs because of impaired DNA synthesis that results from deficiencies of vitamin B₁₂, folic acid, or both.¹ Abnormally low vitamin B₁₂ or folic acid levels or a combination of both is seen in 30% to 50% in hospitalized patients who have elevated mean corpuscular volume.² Vitamin B₁₂ deficiency and related anemias occur in most patients who are referred to hematology clinics and, unfortunately, life-long replacement therapy is necessary unless the cause is identified and treated.

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Food cobalamin is released as a stable complex with gastric R binder and its absorption depends on the initial release from the binders in food.² Food-cobalamin malabsorption is marked by the inability to release cobalamin from food. Therefore, cobalamin cannot be taken up by intrinsic factor for absorption. Release of cobalamin from food requires acid and pepsin, and most food-cobalamin malabsorptive states can be traced to gastric defects. However, other mechanisms may also play a role.³

By far, *Helicobacter pylori* infection is one of the most common gastric infections worldwide. It is estimated that more than half of the adult population in developed countries and 90% of those in the developing countries is infected with this bacterium.⁴⁵ Although the vast majority of infected individuals are asymptomatic, it is well known that *H pylori* is involved in gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.⁷¹¹ Numerous studies suggest that *H pylori* infection is highly associated with atrophic gastritis.¹⁰¹³ It has been pro-
Bone marrow morphologic features were characterized for megaloblastic anemia and serum vitamin B₁₂ levels lower than 147 pmol/L. The selection criteria for enrollment in the study were (1) the patient does not have any classic cause of cobalamin deficiency, such as pernicious anemia or the postgastrectomy state, (2) the patient had no evidence of renal failure or liver disease, (3) a female patient was not pregnant, and (4) the patient had not received prior *H pylori* eradication therapy. All of these patients were interviewed to exclude known medical problems that could affect cobalamin status and to determine that they had not received cyanoocobalamin treatment parenterally. All patients agreed to participate and gave written informed consent.

Serum vitamin B₁₂ and folate levels were measured by a radioimmunoassay (Dualcount Solid-Phase, No-Boil Assay for Vitamin B₁₂/Folic Acid; Diagnostic Products Corp, Los Angeles, Calif). Serum levels of ferritin were measured by an automated chemiluminescence system (Ciba Corning Automated Chemiluminescence System; Ciba Corning Diagnostic Corp, Medfield, Mass). *Helicobacter pylori* status was confirmed by rapid urease testing and histological examination of gastric biopsy specimens. An upper gastrointestinal endoscopy was performed and 4 biopsy specimens were obtained from the gastric antrum and corpus by separate sterile forceps in each site. One biopsy specimen from antrum and corpus was placed in the *Campylobacter*-like organism test gel, and biopsy specimens from the adjacent mucosa were placed in formalin solution for subsequent histological evaluation. Biopsy sections were stained with hematoxylin-eosin for histological examination and toluidine blue to identify *H pylori*. Results of *Campylobacter*-like organism tests were read after 1 and 24 hours. Biopsy specimens were evaluated for *H pylori* and histopathological changes by an attending pathologist (R.F.) who was blinded to the study design. Peripheral blood smears and bone marrow aspirates at diagnosis were evaluated in all cases.

Patients who were detected to have *H pylori* infection received eradication therapy and were followed up as outpatients. *Helicobacter pylori* eradication therapy was done using the following combination therapy: amoxicillin, 750 mg thrice daily, plus clarithromycin, 500 mg thrice daily, for 2 weeks and omeprazole sodium, 40 mg/d, for 4 weeks or amoxicillin, 750 mg thrice daily, plus metronidazole, 750 mg thrice daily, for 2 weeks and omeprazole, 40 mg/d, for 4 weeks. A second gastrointestinal endoscopy was performed 4 weeks after termination of antimicrobial therapy.

*Helicobacter pylori* status was evaluated by *Campylobacter*-like organism test as well as histopathological examination of gastric antrum and corpus biopsy specimens. Cure was defined as no evidence of *H pylori* infection 4 weeks after ending eradication therapy. Thereafter, the patients were followed up at 3-month intervals ranging from 6 months to 5 years (mean follow-up, 42.18 ± 19.48 months). None of the patients received replacement therapy with cyanocobalamin, iron, or folic acid. In cases where *H pylori* infection could not be eradicated, replacement therapy was administered.

Statistical analysis was performed using paired *t* and Wilcoxon rank sum tests; all results were given as mean ± SD. An α level of .05 was considered to be statistically significant. Statistical analysis was performed using the Minitab (Minitab Inc, State College, Pa) statistical software.

## RESULTS

One hundred thirty-eight patients were enrolled in this study. Anemia and vitamin B₁₂ deficiency were detected in all patients, and anemia was caused by vitamin B₁₂ deficiency alone. Examination of peripheral blood smears indicated that all patients had macrocytosis, but 112 (81%) of 138 patients had hypersegmentation of neutrophils. Bone marrow morphologic features were characterized by megaloblastic changes in all patients. Thrombocytopenia was observed in 24 (17%) of the 138 patients. These abnormalities improved after eradication treatment and normalization of vitamin B₁₂ levels.

*Helicobacter pylori* and associated gastric histological changes were detected in 77 patients (56%). *Helicobacter pylori* was found in biopsy specimens of both the gastric antrum and corpus from 30 patients (39%), antrum only from 40 patients (52%), and corpus only from 7 (9%) of 77 infected patients. *Helicobacter pylori* was identified by *Campylobacter*-like organism test and staining in 70 cases, and by staining only in 7 of the infected patients. Two of 77 *Helicobacter pylori*-positive patients had duodenal ulcer disease, 21 had only dyspepsia, and the others had *H pylori* gastritis. Of the 77 *H pylori*-infected patients, 1 had been previously diagnosed with gastric ulcer disease, 4 had duodenal ulcer disease, 3 had dyspepsia, and 18 had gastritis. Of the 66 patients who were not infected with *H pylori*, 1 had been previously diagnosed with gastric ulcer disease, 2 had duodenal ulcer disease, 17 had dyspepsia, and 8 had gastritis.

Fourteen patients with gastrectomy, 2 patients with malabsorption, 23 patients with atrophic gastritis, 11 pregnant patients, 14 patients with β-thalassemia minor, 2 patients with refractory anemia with excess blasts, and

**PATIENTS, MATERIALS, AND METHODS**

One hundred thirty-eight patients (101 females, 37 males; median age, 59.54 ± 15.43 years) who were examined in the Department of Hematology, Gülhane Military Medical Academy, Ankara, Turkey, between April 1, 1994, and March 31, 1999, were enrolled in this study. All patients had megaloblastic anemia and serum vitamin B₁₂ levels lower than 147 pmol/L. The selection criteria for enrollment in the study were (1) the patient does not have any classic cause of cobalamin deficiency, such as pernicious anemia or the postgastrectomy state, (2) the patient had no evidence of renal failure or liver disease, (3) a female patient was not pregnant, and (4) the patient had not received prior *H pylori* eradication therapy. All of these patients were interviewed to exclude known medical problems that could affect cobalamin status and to determine that they had not received cyanoocobalamin treatment parenterally. All patients agreed to participate and gave written informed consent.

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Fourteen patients with gastrectomy, 2 patients with malabsorption, 23 patients with atrophic gastritis, 11 pregnant patients, 14 patients with β-thalassemia minor, 2 patients with refractory anemia with excess blasts, and
2 patients with gastric carcinoma were excluded from the study. Twenty-six H pylori–positive patients had previous surgery (10 appendectomy, 9 cholecystectomy, and 7 tonsillectomy). Thyroid disorders (6 euthyroid diffuse goiter, 2 nodular goiter, 7 thyroid surgery, 2 hyperthyroidism, 2 hyperthyroidism, and 2 thyroiditis) were present in 21 cases. Diabetes mellitus and related disorders were found in 6 patients. However, none of the patients with coexisting disorders differed for the presence of H pylori.

There was no neurologic dysfunction in our patients except 1 who had tingling sensation in the hands.

Eradication therapy as assessed by a second gastrointestinal endoscopy and biopsy was successful in 31 (40%) of 77 patients who were confirmed to be infected with H pylori. The hematologic parameters and serum vitamin B₁₂ levels improved without receiving cyanocobalamin replacement therapy in all of these 31 patients. The complete blood cell counts and serum vitamin B₁₂ levels in the others (46 of 77 patients) were not improved and cyanocobalamin replacement therapy was given to these patients.

Although improvement in hematologic parameters was detectable 4 weeks after termination of eradication therapy, full improvement in hematologic status and serum vitamin B₁₂ levels was detected at the second or third follow-up visit (3 or 6 months after the first follow-up visit). Of the 31 patients who responded without cyanocobalamin replacement therapy, 3 patients had a recurrence of H pylori infection and a decrease of serum vitamin B₁₂ level without deterioration in hematologic status on the 9th, 9th, and 11th month, respectively. The condition of all 3 patients improved with a second eradication treatment, which differed from the first combination, without receiving cyanocobalamin replacement. In 5 patients, recurrence of H pylori infection and a decrease in vitamin B₁₂ level were associated with hematologic deterioration after eradication treatment at the 8th, 11th, 11th, 15th, and 17th month. A second eradication treatment that differed from the first one was successful in 4 of these 5 patients. The duration of hematologic remission in the others (23 of 31 patients) ranged from 10 months to 5 years (mean hematologic remission range, 31.61±13.40 months).

Lactate dehydrogenase levels, mean corpuscular volume, hematocrit, and serum vitamin B₁₂ levels reached normal levels after eradication therapy (Table).

**COMMENT**

Vitamin B₁₂ deficiency is the most common cause of megaloblastic anemia. The determination of serum vitamin B₁₂ levels is the standard test used for the diagnosis of vitamin B₁₂ deficiency. It is necessary to establish the cause of this deficiency. The causative mechanism in patients with pernicious anemia is evident. The Schilling test or detection of anti-intrinsic factor antibody is used to document pernicious anemia. Endoscopy combined with gastric biopsy is indicated in patients with pernicious anemia to exclude gastric atrophy or malignancy.

Upper gastrointestinal endoscopy documented H pylori infection in 77 (56%) of 138 patients; however, no data concerning pernicious anemia could be provided. Appropriate laboratory techniques (Schilling test, antibody detection and others) might have been useful in ruling out pernicious anemia; however, these tests were unavailable to us at the time of this study.

These findings, together with the results of our study, ie, restoration of anemia and the vitamin B₁₂ deficient state following eradication of H pylori infection, may help eliminate the existence of pernicious anemia in infected subjects.
clinical and histologic signs of chronic gastritis associated with both local and systemic immune response. Resolution of gastritis, mucosal immune response to H pylori, and normal appearance of gastric epithelium is demonstrated following eradication of the infection with antibiotic therapy. The inflammation disappears completely within 2 to 3 years after bacterial eradication treatment. However, vitamin B12 levels in serum were restored to normal in less than 2 years in our study. This suggests that normalization of vitamin B12 levels and anemia may be more directly correlated to bacterial eradication rather than improvement of inflammation.

There is no sufficient evidence to propose a casual relationship between H pylori infection and cobalamin deficiency anemia. However, our study demonstrates that the vitamin B12 deficient state is restored to normal following eradication of H pylori infection in 55% (40%) of 138 patients. The results of the eradication therapy are awaited in some of the remaining patients, while others are given replacement therapy due to failure in eradicating the bacterium. Helicobacter pylori eradication rate (40%) in our study is lower than reported cure rates (80%-95%). Only one course of antibiotic treatment was given since multiple courses of combination treatment might have interfered with the normal vitamin B12 metabolism. Lower eradication rate than reported cure rates may be due to compliance or reacquisition of patients or resistance to treatment regimens. Since our goal was not necessarily to achieve a very high rate of H pylori elimination, we did not treat the patients further.

It may be speculated that association of vitamin B12 deficiency and H pylori infection is coincidental, but restoration of anemia and the vitamin B12 deficient state in a significant group of patients via eradication therapy is strongly suggestive of this gram-negative rod’s role in the pathogenesis. There is little information available regarding the possible association of H pylori infection with nonpernicious megaloblastic anemias. One study that investigated the association between H pylori infection and megaloblastic anemia, examined patients with food-cobalamin malabsorption. The investigators found that patients with low levels of serum cobalamin had a higher seroprevalence of H pylori infection. Low serum cobalamin levels were unrelated to pernicious anemia in this case, and the association between H pylori infection and food-cobalamin malabsorption suggests that gastritis induced by H pylori infection predisposes to a more severe form of food-cobalamin malabsorption. Moreover, a former study in 1991 has demonstrated improved protein-bound vitamin B12 absorption in 8 hypochlorhydric-achlorhydric elderly patients following antibiotic therapy. Bacterial overgrowth in the gastric mucosa was postulated as the cause of malabsorption, although H pylori was not mentioned. All these findings are consistent with our observations. Impaired ingestion of food peptides in the stomach interferes with disintegration of vitamin B12 and impairs its binding to intrinsic factor which may lead to the vitamin B12 deficient state.

Subjects with H pylori infection may have circulating IgG autoantibodies against epitopes on specialized cells in the gastric mucosa. It has been shown that the lipopolysaccharide of 80% of H pylori strains has an antigenic structure that mimics Lewis x and y blood group antigens of the host. The β-chain of the parietal proton pump has Lewis y epitopes in common with most H pylori strains. Studies suggest that autoimmunity may play a role in the development of H pylori gastritis. There may be a relationship between intrinsic factor produced by the parietal cells of stomach and antibody produced by the host against H pylori. These antibodies or H pylori may affect the parietal cells, production of intrinsic factor, function of intrinsic factor or R proteins that bind cobalamin in the stomach.

The question as to why the vast majority of infected individuals remain asymptomatic while others have more serious disease is unclear. Variations in the phenotype or genotype of the infecting H pylori strain can play a role in the severity of disease. However, individuals infected with the more virulent strains of H pylori often never develop serious disease. Host genetics may also play a significant role in H pylori–related diseases. The tendency of an individual to respond to infection with specific immune mechanisms can dramatically affect the severity of disease and possibly put an individual at increased risk of progressing to disorders such as atrophic gastritis.

It has also been reported that atrophic gastritis develops in 28% to 30% of infected individuals. The prevalence is estimated to be 1.15% to 2% per year.

In summary, H pylori thus seems to be an important factor in the process of development of atrophic gastritis; nevertheless, this process is slow. Possibly a longer follow-up period of our patients may reveal some who may go on to develop pernicious anemia. Thus, the results of previous studies and our study suggest that eradication of H pylori infection may be beneficial in amelioration of cobalamin deficiency.

A larger cohort of patients is being studied in a similar design prospectively to further establish the H pylori–vitamin B12 deficiency relationship. The preliminary results of this prospective study indicate that improvement of anemia with H pylori eradication therapy establishes a relationship between the 2 disorders, emphasizing the role of H pylori as a novel causative agent.

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Reprints: Küraş Kaptan, MD, Department of Hematology, Gülhane Military Medical Academy, 06018 Etilik, Ankara, Turkey (e-mail: kkaptan@gata.edu.tr)

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