

***Helicobacter Pylori* Infection Can Be Linked to Low Levels of Serum Cobalamins**

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Abstract: Background and aims: This study aims at assessing whether infection by *Helicobacter pylori* (*H. pylori*) can be associated with low concentrations of serum cobalamins in a Jordanian population as it has been suggested else where. Material and method: The study sample comprised 215 patients, complaining of upper gastrointestinal problems who underwent an upper gastrointestinal endoscopy examination, and 104 healthy subjects. Results: One hundred and sixty two patients (75.3%) were found to be *H. pylori* positive and 53 patients (24.7%) were *H. pylori* negative. Vitamin B12 was measured in all patients as well as 104 healthy controls. Low level of cobalamin was found in 44 out of 162 *H. pylori* positive patients (27.2%) compared to only 9 out of 53 *H. pylori* negative patients (17%) and only 15 healthy controls out of 104 (14.4%). The mean value of vitamin B12 was lower in *H. pylori* positive (267 pg/ml) than that seen in *H. pylori* negative patients (320.87 pg/ml). Furthermore, the mean difference in vitamin B12 values between *H. pylori* positive patients and healthy control was highly significant in similar age groups (267 pg/ml vs 368 pg/ml). Conclusion: These findings support the idea that *H. pylori* infection is associated with low or low-normal concentrations of serum cobalamins and that the infection may predisposes to vitamin B12 deficiency.

Key words: *Helicobacter pylori*, Vitamin B12, Cobalamin, Gastritis.

INTRODUCTION

Vitamin B12, also known as cobalamin (Cbl), is a water-soluble vitamin needed for DNA replication, production of S-adenosyl-L-methionine, and normal nerve cell activity. Vitamin B12 acts with folic acid to control homocysteine levels. An excess of homocysteine is associated with an increased risk of heart disease, stroke, and potentially other diseases such as osteoporosis and Alzheimer's disease (Clarke, *et al.*, 2007; Shane and Stokstad, 19852). The human body usually stores a large amount of Cbl (2-5 mg) relative to daily requirements (0.5-2 mg/day). Therefore, even individuals who abstain from all animal proteins, such as strict vegetarians, will not deplete their body stores for 2 to 5 years. It is well recognized that food-cobalamin malabsorption is a major cause of cobalamin deficiency. Cobalamin malabsorption could be due to several factors including pernicious anemia, aging, gastroctomy, histamine-2 antagonists, proton pump inhibitors, and as it has been suggested *H. pylori* infection (Bishop, *et al.*, 1996; Del corral and Carmel, 1990; Carmel, *et al.*, 1994). *H. pylori* colonization is associated with over 90% of cases of chronic gastritis and duodenal ulceration, and more than 50% of gastric ulcers (Del corral and Carmel, 1990). There are a variety of ways in which *H.*

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pylori may directly damage the surface epithelial layer (Bodger and Crabtree, 1994), leading to a decrease in gastric acid and Pepsin production. Both of these two compounds are critical to the splitting of the Cbl from food binders. Furthermore, *H. pylori* induced damage may interfere with the secretion of the intrinsic factor in the stomach, which plays a major role in cobalamin absorption (Bodger and Crabtree, 1994). This suggests a possible role of *H. pylori* infection in many cases of food-cobalamin malabsorption (Carmel *et al.*, 1994; Carmel *et al.*, 2001). This has led to the postulation that chronic *H. pylori* gastritis may be a frequent cause of cobalamin deficiency. This idea was supported by two different studies evaluating the effect of eradication treatment of *H. pylori* infection on the improvement of vitamin B12 deficiency in patient groups with atrophic (Kaptan, *et al.*, 2000) and non-atrophic gastric mucosa (Serin *et al.*, 2002). Nonetheless, it may be also speculated that the association of cobalamin deficiency and *H. pylori* infection is coincidental. This led to the present study aiming to evaluate a possible correlation between *H. pylori* infection and vitamin B12 deficiency in a Jordanian population.

MATERIAL AND METHOD

The study sample included 215 subjects (100 male, 115 female) aged 7-91 years (mean, 39.9 years). All 215 participants had gastritis diagnosed by upper gastrointestinal endoscopic examination. Gastritis symptoms seen in diseased subjects included epigastric pain (100%), (Mean pain duration 34.6 month, range 1-240 months), nausea in 116 (52.3%), heartburn in 149 (67.1%), and vomiting in 71 (32.0%). The population studied was derived from patients attending the Princess Basma Teaching Hospital, Irbid-Jordan. One hundred and four volunteers were also included as controls (48 male, 56 female) aged 13-83 (mean, 43.7 years). None of the control group had history of *H. pylori* infection or had previously undergone investigations for gastritis or any problem in the upper gastrointestinal tract. Informed consent was obtained from all patients and healthy subjects.

Exclusion criteria included: Type I diabetes, Impaired renal failure, Liver diseases, Thyroid disorders, Auto-immune diseases, Pernicious anemia, inflammatory bowel disease, previous GI surgery, Folate deficiency, Strict vegetarian, Pregnancy, and patients under Medications (Parenteral cyanocobalamin treatment, Peptic ulcer treatment, *H. pylori* eradication therapy).

Blood samples were withdrawn immediately before endoscopy. The sera were divided into 5 different aliquots and stored at -80°C until assayed. Among those 215 patients, 96 had gastric biopsy specimens, which were formalin fixed, paraffin embedded, sectioned at 4 μm and stained with Hematoxylin and Eosin and Giemsa. Slides were assessed for *H. pylori* infection according to Sydney score system. The remaining 119 patients were assessed for *H. pylori* infection using IgG and IgA ELISA kits for *H. pylori* (Virion-Serion Immunodiagnostica, GmbH, Germany). Manufacturer's instructions were applied in all tests. *H. pylori* colonized-patients were defined as patients who were positive by either direct examination of biopsy specimens or by *H. pylori* IgG and / or IgA using ELISA technique.

Serum vitamin B₁₂ levels were measured in all study subjects, using IMX automation (Abbott Laboratories, U.S.A.). Individuals with serum vitamin B₁₂ level less than 179 pg/ml were considered to be vitamin B₁₂ deficient. Determination of anti-intrinsic factor antibodies using ELISA kit (Genesis Diagnostics Ltd.), and anti-parietal cell antibodies using indirect immunofluorescence technique (Medical Diagnostic California) were performed on *H. pylori* positive patients who have vitamin B₁₂ deficiency. Antibody concentrations in the patient's sera were determined from optical density (OD) automatically by user-friendly software SERION easy base distributed by SERION IMMUNDIAGNOSTICA GmbH. Samples with optical density (OD) greater than cut-off were considered positive.

Statistical analysis:

Study groups were compared using the statistical Package for Social Sciences (SPSS for windows version 9.0). A two-tailed student's t test and Chi-square test were used as appropriate. Results are expressed as mean \pm SD. For correlations, Pearson's correlation (r) coefficient was used. The level of significance was set at $p(0.05)$.

RESULTS AND DISCUSSION

H. pylori were detected in 76 out of the 96 patients (79.2%) by histological examination and in 86 out of the 119 patients (72.3%) by ELISA technique. Therefore, the total number of infected patients was 162/215 (75.3%). The level of serum cobalamin was found to be low in 44 (27.2%) out of 162 *H. pylori* positive

patients compared to only 9 (17%) out of 53 *H. pylori* negative patients. The mean value of serum vitamin B₁₂ level was significantly lower in infected patients than in noninfected patients (267 pg/ml vs. 320.87 pg/ml, $t = -2.386$, $p = 0.018$) in similar age groups ($p = 0.267$) (Table 1).

Cobalamin deficiency occurs more frequently in older people than in adults (Gumurdulu *et al.*, 2003), *H. pylori* positive subjects were divided into two different age groups (<50 and ≥50), and the mean values of vitamin B₁₂ were compared to exclude any effect for age variations. We could not relate the decreased mean values of vitamin B₁₂ to older age ($p = .351$), and in addition, both sexes were affected equally as shown in table 2 ($p = .81$) (Table 2).

Vitamin B₁₂ concentration in colonized patients and Healthy controls A comparative study between vitamin B₁₂ values in the patients with *H. pylori* infection and their age and sex matched by healthy control was carried out. The prevalence of low cobalamin levels in healthy control was only 15 out of 104 cases (14.4%) compared to 44 (27.2%) out of 162 *H. pylori* positive cases, (Chi-square = 5.953, $p = .015$) (Table 3). Moreover, the mean difference in vitamin B₁₂ values between infected patients and healthy control was highly significant in similar age groups (267 pg/ml vs. 368 pg/ml, $t = -4.972$, $p < .005$) (Table 4).

Table 1: Vitamin B12 levels (mean ± SD) in infected and uninfected patients

Patients	<i>Helicobacter pylori</i> status		t- test	p-value
	Positive (n=162)	Negative (n=53)		
Age (year)	40.57 ± 15.63	37.74 ± 17.41	1.113	0.267
Vitamin B ₁₂ (pg/ml)	267 ± 138.5	320.87 ± 154.86	-2.386	0.018

Table 2: Vitamin B12 values (mean ± SD) in adults and older people and in both sexes in *H. pylori* infected patients.

Subjects		Vitamin B ₁₂ (pg/ml)	t-test	p-value
Gender	Male (n=81)	264 ± 140	0.241	0.81
	Female (n=81)	270 ± 137		
Age groups	< 50 (n=124)	261 ± 130	-0.936	0.351
	≥ 50 (n=38)	285 ± 163		

Table 3: Frequency of low vitamin B₁₂ values in *H. pylori* positive patients and healthy subjects.

	Vitamin B12		Chi-square	p-value
	Low	Normal		
<i>H. pylori</i> positive	44	118	5.953	0.015
Healthy subjects	15	89		

Table 4: Mean values of vitamin B₁₂ in *H. pylori* positive patients and control subjects (mean ± SD).

	<i>H. pylori</i> positive	Healthy control	t-test	p-value
Age (years)	40.57 ± 15.63	43.16 ± 17.13	-1.273	0.204
Vitamin B ₁₂ (pg /ml)	267 ± 138.47	368.21 ± 193.11	-4.972	< 0.005

Discussion:

Patients with food-cobalamin malabsorption cannot absorb food-bound or protein-bound cobalamin even if they absorb free cobalamin normally and this results in low serum cobalamin levels. It is reported that this phenomenon is associated with gastric dysfunction (Carmel *et al.*, 1988). *H. pylori* infection is one of the most common problems in the stomach that leads to development of gastritis especially in developing countries (Sobala *et al.*, 1991). Clinical and histological signs of chronic gastritis almost always accompany colonization of the stomach by *H. pylori* (Dixon, 1991). However, in comparison with *H. pylori*-associated gastritis, gastritis in noninfected mucosa has a milder histological grade and without acute inflammatory infiltrates (Elta *et al.*, 1987).

In the present study, the prevalence of vitamin B12 deficiency in patients with *H. pylori*-associated gastritis was compared to that in patients with gastritis caused by other factors. The results show higher prevalence of vitamin B12 deficiency in infected (27.2%) than in uninfected patients (17%). Although this did not reach a statistical significance, nonetheless the mean value of vitamin B12 in colonized participants was significantly lower than that seen in noncolonized patients (267 vs. 320.87, $t = -2.386$, $p = .018$) (Table 1). Furthermore, sex and age had no significant effect on the mean values of vitamin B₁₂ among *H. pylori* positive patients (Table 2). The results also demonstrate the difference between prevalence of cobalamin in colonized patients and healthy control. The prevalence of low cobalamin levels in control subjects was about 50% less than in patients (14.4% vs. 27.2%). Moreover, the mean value of vitamin B12 was very significantly lower in patients compared with the control group (267 vs. 368, $t = -4.972$, $p < .005$).

The incidence of *H. pylori* differs from country to another according to socioeconomic status. The prevalence of *H. pylori* infection (in the present study) was 75.3%, which is higher than that reported for developed countries. In Finnish population, the prevalence was greater than 90% in cases of superficial gastritis (Siurala, *et al.*, 1988; Malaty *et al.*, 1996). have reported that the rate of acquisition of *H. pylori* infection increases with age to reach 80% at 59 years. On the other hand, the prevalence of infection in older Dutch subjects was 56% (Van Asselt *et al.*, 1998) which is similar to that reported for Japan (Replogle *et al.*, 1996) and Germany (Breuer *et al.*, 1996). In Norwegian population-based study, Bernersen *et al.*, 1992. found that the prevalence of *H. pylori* in dyspeptic subjects was 53%. The present study further support the notion that *H. pylori* infection is higher in the developing countries (Mendall *et al.*, 1992).

Our findings that infection by *H. pylori* is significantly associated with low cobalamin levels is in harmony with the observations of Carmel, *et al.*, 1994. They reported that gastritis induced by *H. pylori* predispose to a more severe form of food-cobalamin malabsorption, but they did not show the rate of decrease in vitamin B₁₂ levels in their patients Carmel, *et al.*, 1994.

Conclusion:

In conclusion, there is a strong association between *H. pylori* infection and vitamin B₁₂ deficiency that is probably caused by cobalamin malabsorption. There may be a clinical value to evaluate the serum vitamin B₁₂ in every patient with *H. pylori* infection. Furthermore, the prevalence of *H. pylori* infection in patients complaining of persisting dyspepsia and subjected for upper GI endoscopy for various gastrointestinal problems in northern Jordan is 75.3%.

REFERENCES

- Clarke, R., S. Lewington, P. Sherliker, J. Armitage, 2007. Effects of B-vitamins on plasma homocysteine concentrations and on risk of cardiovascular disease and dementia. *Curr Opin Clin Nutr Metab Care.*, 10(1): 32-9.
- Shane, B., E.L.R. Stokstad, 1985. Vitamin B12-Folate Interrelationships. *Annu Rev Nutr.*, 5: 115-141.
- Bishop, M., J. Duden-Engelkirk, E. Fody, 1996. *Clinical Chemistry: principles, procedures, correlations.* In: Miller, SM, editors. *Vitamins.* JB. Lippincott Co., pp: 581-635.
- Del corral, A., R. Carmel, 1990. Transfer of cobalamin from the cobalamin-binding protein of egg yolk to R-binder of human saliva and gastric juice. *Gastroenterology*; 98: 1460-1466.
- Carmel, R., G.I. Perez-Perez, M.J. Blaser, 1994. *Helicobacter pylori* infection and food-cobalamin malabsorption. *Dig Dis and Sci.*, 39: 309-314.
- Bodger, K., J.E. Crabtree, 1994. *Helicobacter pylori* and gastric inflammation. *Brit Med Bull.*, 54(1): 139-150.
- Carmel, R., G.I. Perez-Perez, M.J. Blaser, 1994. *Helicobacter pylori* infection and food-cobalamin malabsorption. *Dig Dis Sci.*, 39: 309-14.
- Carmel, R., I. Aurangzeb, D. Qian, 2001. Associations of food-cobalamin malabsorption with ethnic origin, age, *Helicobacter pylori* infection, and serum markers of gastritis. *Am J Gastroenterol.*, 96: 63-70.
- Kaptan, K., C. Beyan, A.U. Ural, *al et*, 2000. *Helicobacter pylori*-Is it a novel causative agent in vitamin B12 deficiency? *Arch Intern Med.*, 160(9): 1349-53.
- Serin, E., Y. Gümürdülü, B. Özer, *al et*, 2002. Impact of *Helicobacter pylori* on the development of vitamin B12 deficiency in the absence of gastric atrophy. *Helicobacter.*, 7(6): 337-41.
- Gumurdulu, Y., E. Serin, B. Ozer, *al et*, 2003. Predictors of vitamin B12 deficiency: age and *Helicobacter pylori* load of antral mucosa. *Turk J Gastroenterol.*, 14(1):44-9.
- Carmel, R., R.M. Sinow, M.E. Siegel, M. Samloff, 1988. Food-cobalamin malabsorption occurs frequently in patients with unexplained low serum cobalamin levels. *Arch Intern Med.*, 148: 1715-1719.
- Sobala, G.M., J.E. Crabtree, M.F. Dixon, *al et*, 1991. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. *Gut.*, 32: 1415-1418.
- Dixon, M.J., 1991. *Helicobacter pylori* and peptic ulceration: histopathological aspects. *J Gastroenterol and Hepatol.*, 6: 125-130.
- Elta, G., S. Kern, T. Nostrant, H. Appelman, 1987. *Campylobacter*- like organisms in erosive gastroenteritis. *Gastroenterology*, 92: 1382-1386.
- Siurala, M., P. Sipponen, M. Kekki, 1988. *Campylobacter pylori* in a sample of Finnish population: relations to morphology and functions of the gastric mucosa. *Gut.*, 29: 909-915.

Malaty, H.M., J.G. Kim, S.D. Kim, D.Y. Graham, 1996. Prevalence of *Helicobacter pylori* infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. *Am J Epidemiol.*, 143: 257-262.

Van Asselt, D.Z., de L.C. Groot, van W.A. Staveren, *al et*, 1998. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. *Am J Clin Nutr.*, 68: 328-334.

Replogle, M.L., W. Kasumi, K.B. Ishikawi, 1996. Increased risk of *Helicobacter pylori* associated with birth in wartime and post-war Japan. *Internat J Epidemiol.*, 25: 211-214.

Breuer, T., T. Sudhop, J. Hoch, *al et*, 1996. Prevalence of and risk factors for *Helicobacter pylori* infection in western part of Germany. *Europ J Gastroenterol and Hepatol.*, 8: 47-52.

Bernersen, B., R. Johnsen, L. Bostad, *al et*, 1992. Is *Helicobacter pylori* the cause of dyspepsia? *Brit Med J.*, 304: 1276-1279.

Mendall, M.A., P.M. Goggin, N. Molineaux, *al et*, 1992. Association between childhood living conditions and *Helicobacter pylori* seropositivity in adult life. *Lancet.*, 1: 896-897.