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Original Research

Prevalence of vitamin B12 deficiency in *Helicobacter pylori* infected patients in Jordan

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Abstract

Objective: The purpose of this study is to assess the frequency of vitamin B 12 deficiency in patients with *Helicobacter pylori (H. pylori*) infection to determine whether low serum vitamin B12 levels correlate with serological evidence of *H. pylori* infection.

Background: An association between *H. pylori* infection and vitamin B12 deficiency has been recently reported.

Subjects: From 200 people blood was collected. For each subject serum vitamin B12, folic acid, complete blood count and *H. pylori* IgG antibodies were measured. 150 subjects have *H. pylori* infection and 50 subjects healthy control group. The following cases were excluded, history of *H. pylori* eradication or antacid take, liver disease, chronic alcoholism inflammatory bowel disease, previous gastrointestinal surgery and a vegetarian diet or multivitamin supplementation.

Results: Vitamin B12 deficiency was found in 87(58%) patients with seropositive *H. pylori* IgG antibodies (HP+) versus 9 (18%) patients with seronegative *H. pylori* IgG antibodies (HP). The mean±SD of serum vitamin B12 level in overall subjects (males and females) was 345.717± 215.214 and 271.254±35.234 ($p < 0.01$) respectively. The mean±SD of serum vitamin B12 level in *H. pylori* infected subjects was 295,574±71,482 versus 411,973±368,241in healthy group ($p < 0.02$). The mean±SD of serum vitamin B 12 level in overall *H. pylori* infected subjects with vitamin B12 deficiency (male and female) was 142±24.423 and 144.322±18.736 ($p < 0.02$) where it was 565.197±149.488 and 615.33±139.981 with normal serum vitamin B12 level in the same group.

Hemoglobin and hematocrit levels were significantly lower in *H. pylori* positive subjects, hemoglobin (g/dL) was 13.5±0.15 mean±SD versus 14.2±0.25, $p < 0.05$ and hematocrit (%) 40.1±0.35 versus 42 ±0.7 $p < 0.05$, Mean Corpuscular Volume (MCV) (μm^3) was 95±2 versus 85±2.

Folic acid levels (normal range 4.6-12.7 ng/ml) were significantly lower in *H. pylori* seropositive subjects (5.65±0.2 versus 9.1±0.5 $p < 0.05$).

Conclusion: Collectible results suggest a causal relationship between *H. pylori* infection and vitamin B12 deficiency in Jordanians.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, spirally shaped microaerophilic bacterium exclusively lives in the mucosa of the stomach and duodenum; the bacterium has a unique way of adapting in the hostile environment of the stomach [1, 2].

Infection with *H. pylori* has been recognized as a public health problem worldwide and more prevalent in developing than developed countries. It likely related to socioeconomic factors rather than ethnic factors [3, 4].

Vitamin B12 deficiency is frequently seen in clinical practice in Jordan with high rates. Vitamin B12 deficiency often goes undetected, with manifestations that range from asymptomatic to wide spectrum of hematologic and/or neuropsychiatric features. It is

worth stating that the stomach plays a major role in both absorption of vitamin B12 and the pathogenesis of cobalamin deficiency [5].

Food cobalamin malabsorption has been linked to *H. pylori* infection. Serological evidence of *H. pylori* infection has been shown to be present in a significantly larger percentage of subjects with food cobalamin malabsorption, when compared between subjects with normal absorption and eradication of *H. pylori* infection results show an increasing of vitamin B12 levels after eradication completed [6]. *H. pylori* also a known contributor in gastritis, ulcers and it can prevent the stomach from being able to absorb the vitamin B12 which consumed which leads to deficiency of vitamin B12 [7].

The primary goal of the present study to observe if *H. pylori* infection is responsible for vitamin B12 deficiency because early detection and eradication of *H. pylori* can prevent development of complications as megaloblastic anemia, the second goal is to assess the prevalence of vitamin B12 deficiency in *H. pylori* infected patients in our country.

PATIENTS AND METHODS

Patients

The subjects in this study were selected from patients attending the outpatient specialty clinics in King Hussein Medical Center during the period from 15 January 2012 to 10 June 2012. 200 subjects were selected, 150 of them have *H. pylori* infection while 50 are healthy (113 male, 87 female) aged 23-86 years (mean 54.5 years), blood was collected from these patients for serum vitamin B12, folic acid, complete blood count and *H. pylori* IgG antibodies. All tests were done in Princess Iman Center for Researches and Laboratory Sciences in King Hussein Medical Center in Jordan.

Criteria

In this study the following cases were excluded: history of *H. pylori* eradication or antacids drug take, liver disease, chronic alcoholisms inflammatory bowel disease or previous GI surgery, a vegetarian diet or multivitamin supplementation.

Methods

For each subject the following tests were done: serum vitamin B12, serum folic acid complete blood count (CBC) and *H. pylori* IgG antibodies.

Serum vitamin B12 and serum folate were determined using a Cobas e411 analyzer (Roche Diagnosis GmbH, Manhaium, Germany) (normal range 190-945 pg/ml, 4.6-12.7 pg/ml for vitamin B12 and folate, respectively).

Complete blood count was determined using Sysmex k 1000 hematology analyzer (Tao electronics, Japan).

Circulating antibodies against *H. pylori* were detected by competitive enzyme-linked immunosorbent assay (ELISA) based on a monoclonal antibody against 64 kD *H. pylori* antigen [8].

Briefly, 100 µl of undiluted serum sample and calibrators were added to micro wells coated with an *H. pylori* solicited preparation. Then 100 µl of peroxidase conjugated HpN45 were added to all wells .After 1 hour of incubation , the plate was washed ,color was developed with a TMB solution and absorbance read at 450. Antibody concentration was expressed as units/ml. Negative values are considered < 450 U/ml. Sensitivity was 100% and specificity was 90%.

Statistical Analysis:

All statistical analysis were performed using SPSS for windows 15.0 (SPSS Inc. Headquarters, Chicago, III., USA) software program and Microsoft Excel 2007 program. $P < 0.05$ was considered to be statistically significant.

RESULTS

Prevalence of vitamin B12 deficiency

During the period of study 150 patients with seropositive for *H. pylori* were studied for vitamin B12 deficiency, of which 83 (55%) were males and 67 (45%) were females. A total of 87 (58%) seropositive *H. pylori* subjects were vitamin B12 deficient (males 55% vs. females 45%), the male gender was the predominant in *H. pylori* infected subjects with vitamin B12 deficiency (Table1).

Age of seropositive subjects did not differ from that of seronegative subjects (mean 56.2) median 50 years versus 52.6, median 48 years p = NS. Serum vitamin B12 deficiency did not correlate with age- Spearman Rank correlation r = -0.07 p = NS as shown in Table2.

Table 1. Frequency of vitamin B12 deficiency in *H. pylori* infected patients.

	Male	Female	Total	P-value
Vitamin B12				
Deficient	48(55%)	39(45%)	87(58%)	
Normal	35(66%)	28(34%)	63(42%)	0.02
Total	83(55%)	67(45%)	150(100%)	

Table 2. Mean age of subjects in relation to gender

subjects	Gender	*n*	Mean age	S.D	*p*-value
(HP+)	Female	83(55%)	58.6	18.3	0.04
	Male	67(45%)	53.8	17.2	
(HP-)	Female	21(42%)	55.8	16.9	0.04
	Male	29(58%)	49.6	14.7	

Table 3. Summary of hematologic parameters results in both groups.

subjects	Hb (g/dL) (mean ± SD)	Hct (%) (mean ± SD)	MCV (μm^3) (mean ± SD)	Folate (mean ± SD)	*P*-value
(HP+)	13.5 ±0.15	40.1±0.35	95 ±2 %	5.65± 0.2	0.04
(HP-)	14.2±0.25	42±0.7	85 ± 2	9.1±0.5	0.04

Table 4. Vitamin B12 levels and frequency in both groups.

subjects	Vitamin B12 (pg/ml) (Mean ±SD)	Deficient (%)	Normal (%)	P-value
(HP+)	295,574± 71,482	87(58%)	63(42%)	0.01
(HP-)	411,973± 368,241in	9(18%)	41(92%)	0.01

Hematological Parameters:

Hemoglobin (Hb) and hematocrit (Hct) levels were significantly lower in *H. pylori* positive subjects, Hb (g/dL) 13.5±0.15 mean±SD versus 14.2±0.25, $p < 0.05$ and Hct (%) 40.1±0.35 versus 42 ±0.7 $p < 0.05$. Mean Corpuscular Volume (MCV) (μm^3) also significantly higher in *H. pylori* infected subjects 95±2% versus 85±2.

Folic acid levels (normal range 4.6-12.7 ng/ml) were significantly lower in *H. pylori* seropositive subjects (5.65±0.2 versus 9.1±0.5 $p < 0.05$) as shown in table3.

Vitamin B12 levels

The mean±SD of serum vitamin B12 level in overall subjects (males and females) was 345.717±215.214 and 271.254±35.234 ($p < 0.01$), respectively. The mean±SD serum vitamin B12 level in *H. pylori* infected subjects was 295,574±71,482 versus 411,973±368,241in healthy group ($p < 0.02$) (Table 4). The mean±SD of serum vitamin B12 level in *H. pylori* infected subjects with vitamin B12 deficiency (male and female) was 142±24.423 and 144.322 ±18.736 ($p < 0.02$) where it was 565.197±149.488 and 615.33±139.981 with normal serum vitamin B12 level in the same group.

DISCUSSION

H. pylori infection is probably the most common infection in the world. It affects approximately 40% of the world population, especially from disadvantaged background. The mode of transmission of *H. pylori*

remains uncertain .Indeed, the bacterium was isolated from the feces, saliva and dental plaques, suggesting that transmission is possible by oral-oral or fecal-oral route. It is clear that *H. pylori* have a role in etiopathogenesis of chronic gastritis, peptic ulcer, gastric cancer and gastric lymphoma [9, 10].

H. pylori infection causing immediate developments of persistent gastritis, colonization of the stomach by *H. pylori* is almost always accompanied by clinical and histological signs of chronic gastritis associated with both the local and systemic immune response [8].

The association between *H. pylori* and vitamin B12 deficiency remains even more argumentative. Previous studies by Karnes et al [11] and Varis et al [12] had showed that the most patients with atrophic gastritis of the stomach body have been infected with *H. pylori* and had suggested that *H. pylori* is involved in initiating an irreversible process leading to vitamin B12 deficiency.

H. pylori have been determined as an etiologic factor in vitamin B12 deficiency [11]. In population with high prevalence of *H. pylori* infection, the prevalence of vitamin B12 deficiency expected to be high. In the present study the borderline for low B12 status (< 190 pg/ml) was used and markedly prevalence (58%) was found which is higher than that reported by Gumurdulu et al [12], Tucker et al [13] and Bikha et al [14].

In this study we used serum vitamin B12 to determine vitamin B12 levels because the assessment of serum vitamin B12 levels is the standard test used for the diagnosis of vitamin B12 deficiency and it necessary to establish the causal agent of the deficiency [15].

The current study confirmed the strong correlation between vitamin B12 deficiency and MCV that has been already noted in previous studies, it was significantly higher in *H. pylori* infected subjects [5, 14]; also, seropositive *H. pylori* subjects have significantly lower hemoglobin and hematocrit levels in comparison with seronegative subjects. Interestingly, folic acid levels were significantly lower in the seropositive subjects.

In our study the prevalence of vitamin B12 deficiency in seropositive *H. pylori* was 58%, the rate is close to that reported in Italy 60% [16] and was significantly higher than that reported in other countries: 11% in United States [17] and 21.4% in U.K. [7]. The prevalence of vitamin B12 deficiency among our subjects studied did not correlate with age; it was notable in previous studies [14, 18].

Related to gender distribution in our study, the vitamin B12 deficiency was more marked in males (55%) with statistically significant difference (P = 0.01), these results were similar to that which reported by Bikka et al [12] whereas the female gender is predominant in that reported by Gumurdulu et al]13].

In conclusion the higher prevalence vitamin B12 deficiency among patients infected by *H. pylori* suggests a causal relationship between *H. pylori* infection and vitamin B12 deficiency.

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REFERENCES

1. Blaser M. The bacteria behind the ulcers. Sci Am 1996;274:92-7.
2. Logan R, Hirschl A. Epidemiology of helicobactor infection. Curr Opin Gastroenterol 1996;12:1-5.
3. Frenck R, Clemens J. Helicobacter in the developing world. Microbes Infect 2003;5:705-13.
4. Sarari AS, Farra MA, Hamodi Wessawi TA. Helicobacter pylori a causative agent of vitamin B12 deficiency. Jinfect Dev Ctries 2008;2:346-9.
5. Annibale B, Lahner E, Bordi C, Martino G, Caruana P, Grossi C. Role of helicobacter pylori infection in pernicious anaemia .Dig liver Dis 2000,32,756-62.
6. Fong TL, Doley CP, Dehesa M, Cohen H, Carmel R, Fitzgibbons PL. Helicobacter pylori infection in pernicious anemia: a prospective controlled study. Gastroenterology 199,100;328-32.
7. Oconnor HJ, Axon AT, Dixon MF. Campylobacter-like organisms unusual in type A (pernicious anaemia) gastritis. Lancet 1984;2;1091.
8. Nergini R, Zanella I, Savio A,Poiesi C, Verardi R, Ghielmi S. Serodiagnosis of Helicobacter pylori-associated gastritis with a monoclonal antibody competitive enzyme-linked immunsorbent assay. Scand J Gastroenterol 1992;27;559-65.
9. Dale A, Thomas JE, Darboe MK, Coward WA, Harding M, Weaver LT. Helicobacter pylori infection, gastric acid serection, and infant growth. J pediatr Gastroenterol Nutr 1998;26:393-7.
10. Karnes WE, Samloff IM, Siurala M. Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis Gastroenterology 1991;101:167-74.
11. Varis O, Valle J, Suirala M. Is Helicobacter pylori involved in the pathogenesis of the gastritis characteristic of pernicious anemia? Scand J Gastroenterol 1993;28:705-8.
12. Gümürdülü Y, Serin E, Ozer B, Kayaselçuk F, Kul K, Pata C, Güçlü M, Gür G, Boyacioğlu S. Predictors of vitamin B12 deficiency: age and Helicobactor load of antral mucosa .Turk .J. Gastroenterol, 2003;14(1):44-9.
13. Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, Selhub J. Plasma vitamin B-12 concentration

relate to intake source in the Framingham offspring study. Am J Clin Nutr 2000;71(2):514-22.

14. Bikka RD, Shaikh MZ, Syed ZA, Tarachand D, Raj K, Thanwar D. Helicobactor pylori: A cause of vitamin B12 deficiency world. Applied Sciences Journal 2011;12(9):1378-1381.
15. Colon-Otero G, Menke D, Hook CC. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. Med Clin North Am. 1992;76(3):581-97.
16. Federici L, Henoun Loukili N, Zimmer J, Affenberger S, Maloisel F, Andrès E. Update of clinical finding in cobalamin deficiency: personal data and review of the literature. Rev Med Interne. 2007;28(4):225-31.
17. Fong TL, Dooley CP, Dehesa M, Cohen H, Carmel R, Fitzgibbons PL. Helicobacter pylori infection in pernicious anemia: a prospective controlled study. Gastroenterology 1991;100:328-32.
18. Shuval-Sudai O, Granot E. An association between helicobacter pylori infection and serum vitamin B12 levels in healthy adults. J Clin Gastroenterol. 2003;36(2):130-3.