

Original article

Hematological parameters, serum iron and vitamin B₁₂ levels in hospitalized Palestinian adult patients infected with *Helicobacter pylori*: a case–control study



Saleh Nazmy Mwafy*, Wesam Mohammad Afana

Department of Biology, Faculty of Science, Al-Azhar University of Gaza, Gaza Strip, Palestine

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ABSTRACT

Objective: This study aimed to investigate the relationship between hematological parameters, serum iron, and vitamin B₁₂ levels in adult hospitalized Palestinian patients infected with *Helicobacter pylori*.

Methods: This case–control study included 150 adult (18–50 years old) patients infected with *H. pylori* and 150 healthy adults. A complete blood count was performed, and serum iron and vitamin B₁₂ levels of the patients were measured, statistically analyzed and compared with the control group. All parameters in cases were reassessed after the triple treatment of omeprazole 20 mg b.i.d., amoxicillin 1 g b.i.d., and clarithromycin 500 mg b.i.d. for 14 successive days. The triple treatment was the same for males and females.

Results: The results revealed that the mean levels of hemoglobin, red cell count, white cell count and hematocrit were significantly lower and the red blood cell distribution width significantly higher in cases compared to controls, while no significant differences were found for mean corpuscular volume, mean corpuscular hemoglobin and platelet count. Serum vitamin B₁₂ and iron levels were significantly lower in cases compared to controls (262.5 ± 100.0 vs. 378.2 ± 160.6 pg/mL and 71.6 ± 24.8 vs. 80.1 ± 20.7 µg/dL, respectively). Vitamin B₁₂ and serum iron increased significantly and was restored to close to normal levels after medical treatment.

Conclusions: *H. pylori* infection appears to cause decreases in vitamin B₁₂, iron levels and some hematological parameters. However, these were almost normalized after treatment with omeprazole, amoxicillin and clarithromycin. *H. pylori* is associated with vitamin B₁₂ and iron deficiency, thus, this may be a useful marker and a possible therapeutic agent of anemic patients with gastritis.

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* Corresponding author at: Department of Biology, Faculty of Science, Al-Azhar University of Gaza, P.O. Box 1277, Gaza Strip, Palestine.

E-mail address: smwafy@hotmail.com (S.N. Mwafy).

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Introduction

Helicobacter pylori is a spiral, flagellated, Gram-negative bacteria, specially adapted to survive in the gastric lumen¹ and considered the most successful human pathogen infecting about 50% of the global population.² It is a common and potentially curable cause of dyspepsia and peptic ulcer disease.³ One of the factors leading to iron deficiency anemia (IDA) is *H. pylori* infection, which has a high prevalence in developing countries however, this can be corrected by a *H. pylori* eradication regime.

Local and regional estimates show a considerable prevalence of *H. pylori* infection. The prevalence of *H. pylori* infection in the Gaza Strip was 72.2%,⁴ while in northern Jordan it was 82%.⁵ *H. pylori* infection can lead to many complications and its role is well established in peptic ulcer and gastrointestinal malignancy.⁶ Although *H. pylori* is associated to peptic ulcers and malignancies that can cause bleeding resulting in IDA, most patients infected with *H. pylori* do not have ulcers or malignancies. Infected subjects usually have chronic gastritis that is not associated with gastrointestinal bleeding.⁷ *H. pylori* infection could lead to decreases in vitamin B₁₂ absorption leading to its deficiency.⁸ However, there are few published reports from the Gaza Strip focused on vitamin B₁₂ deficiency and iron status associated with *H. pylori*. This study aimed to explore the relationship between hematological parameters, serum iron, and vitamin B₁₂ levels in adult hospitalized Palestinian patients infected with *H. pylori*, and to assess whether the medical treatment for *H. pylori* infection could improve vitamin B₁₂ and iron status or not.

Methods

Study population and experiment design

This study used a case-control design performed on randomly selected subjects from the four main hospitals of the governorates of the Gaza Strip, Palestine. The population of the study was 76 male and 74 female patients suffering from *H. pylori* infection aged 18–50 years old who were referred to the general hospitals of Gaza Strip for medical treatment. Additionally, 150 apparently healthy individuals of the same population matched for age were used as a control group for baseline comparisons. The study was conducted in the main general hospitals of the Gaza Strip: Al Shifa hospital in Gaza, Nasser hospital and European Gaza hospital in Khanyounis, and Najjar hospital in Rafah during the period from March 2015 to October 2016. Exclusion criteria were patients who received steroids or *H. pylori* eradication therapy, immunosuppressive or chemotherapeutic drugs, pregnant female patients and under 18-year-old and over 50-year-old patients complaining of *H. pylori* infections. All parameters in cases were reassessed after the triple treatment (OAC) that included omeprazole 20 mg b.i.d., amoxicillin 1 g b.i.d. and clarithromycin 500 mg b.i.d. for 14 successive days. The OAC treatment was the same for men and women and was performed under the supervision of gastroenterologists at the different clinical facilities included in the study. For ethical considerations, the necessary official approval to conduct this study was obtained from the Ethics Committee at the Palestinian Health Research

Council under approval number PHRC/HC/33/15. All participants signed the informed consent form of the study and freely participated in the study.

Specimen collection and testing

All stool samples were collected in plastic containers and sent to the laboratory within 2 h. About 5 mL of venous blood were collected from each subject (cases and controls) and divided equally (2.5 mL) in one tube containing K3-ethylenediaminetetraacetic acid (K3-EDTA) to perform a complete blood count (CBC) using a Cell Dyne 1800 electronic counter (Sequoia-Turner Corporation, California, USA) and in a serum tube to determine serum iron using the DiaSys reagent kit.⁹ The serum vitamin B₁₂ concentration was determined quantitatively using a solid phase, competitive chemiluminescent enzyme immunoassay (Immulite/Immulite 1000).¹⁰ *H. pylori* was determined by colored chromatographic immunoassay using the immunochromatographic test reagent kits (CerTest *H. pylori*).¹¹

Data analysis

Data were tabulated, encoded and statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software version 21.0 for windows (IBM Inc., Chicago, IL). Means were compared by independent-samples t-test, and percentage changes were calculated. *p*-values <0.05 were considered statistically significant.

Results

Sociodemographic characteristics of the study population

Table 1 shows the gender distribution of case and control groups as well as the sociodemographic characteristics of the study population with no significant differences between males and females in either group according to chi-square tests. About 31.3% of cases and 26.0% of controls were aged between 18 and 28 years, 40.0% vs. 50.0%, respectively were aged between 29 and 39 years, and 28.7% vs. 24.0%, respectively were aged between 40 and 50 years (*p*-value = 0.220).

Hematological parameters

Table 2 illustrates the hematological parameters of *H. pylori* patients compared to controls. The mean levels of hemoglobin (Hb – 11.4 ± 2.8 g/dL vs. 13.3 ± 2.7 g/dL), red blood cell count (RBC – 3.9 ± 0.9 × 10⁹/μL vs. 4.4 ± 0.9 × 10⁹/μL), white blood cell count (WBC – 0.7 ± 1.86 × 10⁹/L vs. 7.2 ± 1.9 × 10⁹/L) and hematocrit (Hct – 35.2 ± 7.2% vs. 40.4 ± 7.0%) were significantly lower among cases compared to controls with differences of –13.9%, –12.6%, –6.2% and –12.7%, respectively (*p*-value <0.001). The red blood cell distribution width (RDW) was significantly higher in cases compared to controls (14.0 ± 2.4% vs. 12.9 ± 1.9%; *t* value = 4.338; *p*-value <0.001). On the other hand, no significant differences were found for mean corpus-

Table 1 – Sociodemographic characteristics of the study population.

Variable	Casen (%)	Controln (%)	Chi-square	p-value ^a
Gender				
Male	76 (50.7)	92 (61.3)	3.463	0.063
Female	74 (49.3)	58 (38.7)		
Age (years)				
18–28	47 (31.3)	39 (26.0)	3.031	0.220
29–39	60 (40.0)	75 (50.0)		
40–50	43 (28.7)	36 (24.0)		
Place of residency				
City	77 (51.3)	87 (58.0)	1.697	0.428
Camp	51 (34.0)	41 (27.3)		
Village	22 (14.7)	22 (14.7)		
Governorate				
Gaza	75 (50.0)	75 (50.0)	1.330	0.514
Khanyounis	29 (19.3)	36 (24.0)		
Rafah	46 (30.7)	39 (26.0)		

^a One-way Analysis of variance (ANOVA) test.

cular volume (MCV), mean corpuscular hemoglobin (MCH) and platelet count between cases and controls.

Biochemical parameters

On comparing cases with controls, the mean levels of serum vitamin B₁₂ (262.5 ± 100.0 pg/mL vs. 378.2 ± 160.6 pg/mL, respectively) and iron (71.6 ± 24.8 µg/dL vs. 80.1 ± 20.7 µg/dL, respectively) were significantly lower in the patients with differences of -30.6% and -10.6%, respectively (*p*-value <0.001). In addition, the result showed that 32 (21.3%) patients and 9 (6.0%) controls had low levels of vitamin B₁₂ and 35 (23.3%) patients and 12 (8.0%) controls had low levels of serum iron (*p*-value <0.001). *H. pylori*-positive subjects are at 4.25 times (Odds ratio: 4.249; 95% confidence interval: 1.950–9.258) and 3.5 times (Odds ratio: 3.500; 95% confidence interval: 1.737–7.054) higher risk of having low levels of serum vitamin B₁₂ and iron, respectively compared to healthy individuals (Tables 3 and 4). As indicated in Table 5, the mean

levels of vitamin B₁₂ increased significantly with OAC treatment (before treatment: 137.5 ± 19.5 pg/mL; after treatment: 317.28 ± 65.26 pg/mL) with a 230.8% increase (*p*-value <0.001). Similarly, the levels of serum iron were significantly higher after treatment (82.4 ± 11.0 µg/dL; 152.3% increase; *p*-value <0.001).

Analyses using the Pearson correlation coefficient revealed significant correlations between vitamin B₁₂, iron levels with other hematological and biochemical parameters as shown in Table 6. Among these important correlations are positive correlations between vitamin B₁₂ with iron, Hb, RBC and Hct (*r* = 0.5, 0.7, 0.7 and 0.6, respectively; *p*-value <0.001) and a negative correlation with RDW. Statistically significant positive correlations were found between serum iron levels with Hb, RBC Hct and MCV (*r* = 0.7, 0.7, 0.8 and 0.5, respectively; *p*-value <0.001) and a negative correlation with RDW (Figure 1).

Discussion

H. pylori infection is one of the commonest health problems of the stomach leading to the development of gastritis especially in developing countries. Clinico-epidemiologic studies suggest that *H. pylori* is a causative agent for IDA although the mechanisms remain unclear,¹² and the malabsorption of vitamin B₁₂ that is observed in gastritis due to excessive *H. pylori* in the stomach that could result in hypochlorhydria. Thus, the lack of an intrinsic factor may play a role in causing malabsorption of vitamin B₁₂ in most patients with atrophic gastritis.¹³ Previous studies have reported that *H. pylori* is associated with IDA as colonization in the gastric mucosa may disturb some functions of the mucosa, leading to a drop in iron absorption and increases in iron loss.¹⁴

The mode of transmission of *H. pylori* remains poorly understood; no single transmission pathway has been identified. The rate of *H. pylori* acquisition is higher in developing countries than in developed countries.¹⁵ In the Gaza Strip of Palestine, *H. pylori* is common. Our current results show that 50.7% of cases were males and 49.3% were females with no significant difference between genders. These results are in agreement with previous studies from other Arabic countries

Table 2 – Hematological parameters of the different study groups.

Variables	Case	Controls	Difference (%)	t value	p-value ^a
Hb (g/dL)	11.4 ± 2.8	13.3 ± 2.7	-13.9	-5.797	0.001
RBC (×10 ⁹ /µL)	3.9 ± 0.9	4.4 ± 0.9	-12.6	-5.265	0.001
WBC (×10 ⁹ /L)	6.7 ± 1.8	7.2 ± 1.9	-6.2	-2.061	0.040
Hct (%)	35.2 ± 7.2	40.4 ± 7.0	-12.7	-6.259	0.001
MCV (fL)	85.5 ± 12.6	84.9 ± 8.0	0.65	0.453	0.651
MCH (pg)	29.4 ± 3.4	29.3 ± 2.3	0.46	0.398	0.691
MCHC (g/dL)	33.5 ± 2.0	34.035 ± 1.358	-1.6	-2.780	0.006
RDW (%)	14.0 ± 2.4	12.9 ± 1.9	8.4	4.338	0.001
PLT (×10 ⁹ /L)	276.7 ± 106.6	261.6 ± 84.5	5.7	1.354	0.177

WBC: white blood cell count; RBC: red blood cell count; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: mean red cell distribution width; PLT: platelet count; SD: standard deviation.

p < 0.05: significant.

Values are expressed as means ± standard deviation (SD) of 150 subjects.

^a One-way Analysis of variance (ANOVA) test.

Table 3 – Serum vitamin B₁₂ and iron levels of the different study groups.

Variables	Case	Control	Difference (%)	t value	p-value ^a
Vitamin B ₁₂ (pg/mL)	262.5 ± 100.0	378.2 ± 160.6	–30.6	–7.496	0.001
Iron (μg/dL)	71.6 ± 24.8	80.1 ± 20.7	–10.6	–3.206	0.001

Vitamin B₁₂ – low level: <174 pg/mL; normal: 174–878 pg/mL; high: >878 pg/mL.
 Serum iron in men – low <35 μg/dL, normal 35–168 μg/dL, high >168 μg/dL, in women: low <23 μg/dL, normal 23–134 μg/dL, high >134 μg/dL.
 Values are expressed as means ± Standard deviation (SD).
^a One-way Analysis of variance (ANOVA) test.

Table 4 – Association between *H. pylori*, vitamin B₁₂ and iron of the different study groups.

	Category	Case n (%)	Control n (%)	95% confidence interval	Chi-square	p-value ^a
Vitamin B ₁₂	Low	32 (21.3)	9 (6.0)	4.249	14.9	0.001
	Normal	118 (78.7)	141 (94.0)	(1.950–9.258)		
Iron	Low	35 (23.3)	12 (8.0)	3.500	13.3	0.001
	Normal	115 (76.7)	138 (92.0)	(1.737–7.054)		

Vitamin B₁₂ – low level: <174 pg/mL, normal: 174–878 pg/mL, high: >878 pg/mL.
 Serum iron in men: low <35 μg/dL, normal 35–168 μg/dL, high >168 μg/dL, in women: low <23 μg/dL, normal 23–134 μg/dL, high >134 μg/dL.
 Values are expressed as means ± Standard deviation (SD).
^a One-way Analysis of variance (ANOVA) test.

Table 5 – Vitamin B₁₂ and iron levels before and after omeprazole, amoxicillin and clarithromycin treatment.

Parameter	Before	After	% change	t value	p-value ^a
Vitamin B ₁₂ (pg/mL)	137.5 ± 19.5	317.3 ± 65.3	230.8	15.619	0.001
Iron (μg/dL)	32.7 ± 3.3	82.4 ± 11.0	152.3	24.786	0.001

Values are expressed as means ± Standard deviation (SD).
^a One-way Analysis of variance (ANOVA) test.

Table 6 – Correlation of vitamin B₁₂ and iron levels with the study parameters.

Parameter	Pearson correlation (r)	p-value
Vitamin B₁₂		
Iron (μg/dL)	0.469	0.001
Hb (g/dL)	0.724	0.001
RBC (×10 ⁶ /μL)	0.683	0.001
Hct (%)	0.645	0.001
RDW (%)	–0.371	0.001
Iron		
Hb (g/dL)	0.752	0.001
RBC (×10 ⁶ /μL)	0.723	0.001
Hct (%)	0.770	0.001
MCV (fL)	0.501	0.001
RDW (%)	–0.803	0.001

Hb, Hemoglobin; RBC, Red blood cell count; Hct, hematocrit; RDW, Red blood cell distribution width; MCV, Mean corpuscular volume.

including Jordan and Saudi Arabia.^{16,17} There was no significant correlation between *H. pylori* infection and age. These results are in agreement with Khan who concluded that *H. pylori* infection is acquired early in life and there is no rise in the incidence with advancing age. The Hb, RBC, Hct and MCHC levels were significantly lower in cases compared to controls at the beginning of the study. Similar observations were reported elsewhere.¹⁸ Ciacci et al., in 2004, suggested a possible pathogenic mechanism of anemia and explained

it by blood loss secondary to chronic erosive gastritis and decreased iron absorption secondary to chronic gastritis and hypochlorhydria.¹⁹

Before starting the treatment regimen with OAC, serum iron was significantly lower among cases compared to controls. The results also reflected statistically significant associations between *H. pylori* and serum iron and that individuals who are positive for *H. pylori* are at 3.5 times higher risk of developing IDA compared to healthy subjects.²⁰ The possible mechanism that may explain the development of IDA in *H. pylori*-infected patients was addressed by Annibale et al. in 2000 and might be a result of the pattern of gastritis and related to the effects on gastric physiology affecting the normal process of iron absorption; increases in gastric pH lead to decreases in iron solubility. *H. pylori* binding proteins lead to iron protein complex in the bacterium with decreases in vitamin C secretion in gastric juices and decreases the bioavailability of vitamin B₁₂ and folate.²¹

The vitamin B₁₂ level was significantly lower among cases compared to controls, which reflects an association between *H. pylori* and B₁₂ deficiency; individuals who are positive for *H. pylori* are at a 4.2-times higher risk of having low levels of vitamin B₁₂ compared to healthy individuals. This finding is in agreement with previous studies that showed a statistically significant relationship between *H. pylori* infection and serum vitamin B₁₂ levels with the prevalence of vitamin B₁₂ deficiency being 28% and 11% in *H. pylori*-positive and *H. pylori*-negative groups, respectively.^{22,23} The mechanisms of vitamin

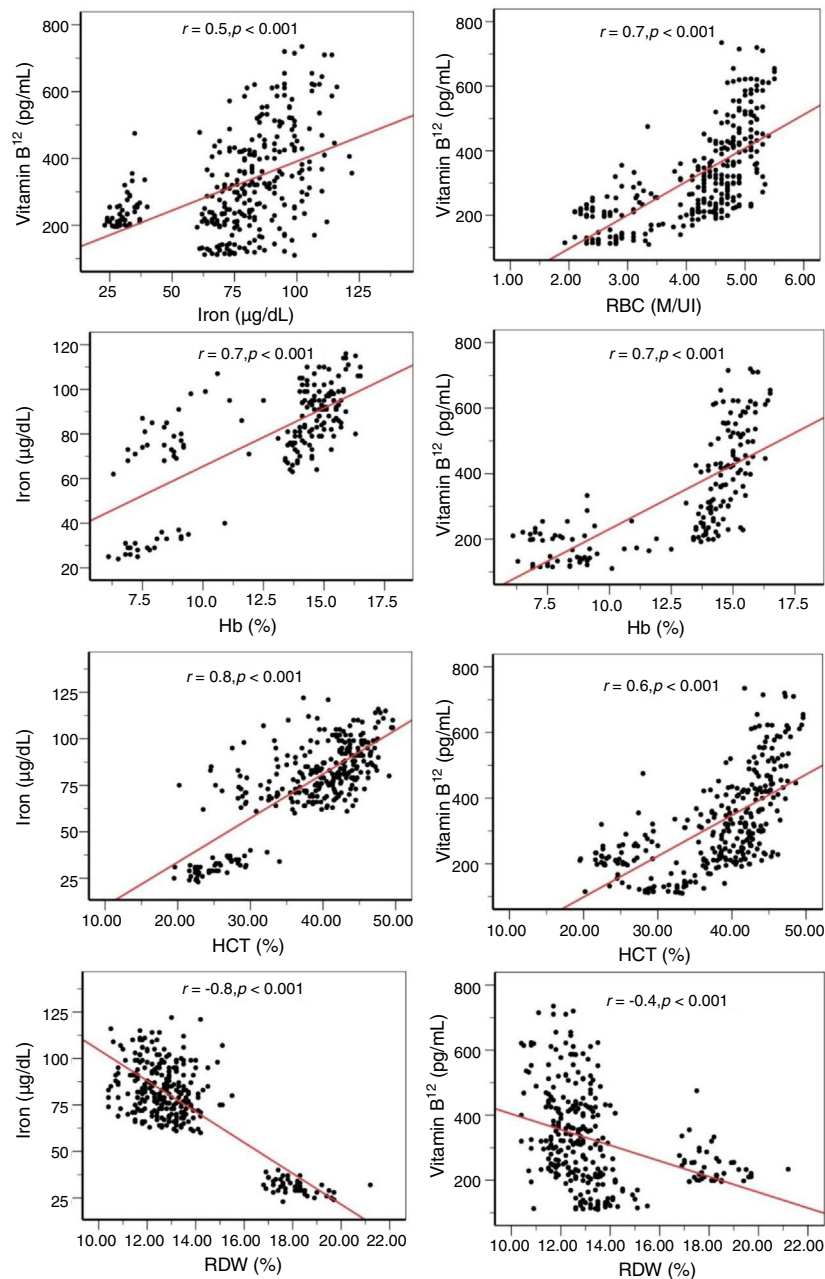


Figure 1 – Pearson correlation coefficient analysis of vitamin B₁₂ and iron levels with study parameters.

B₁₂ malabsorption caused by *H. pylori* infection are unclear with the following being some possibilities:

- (a) Diminished acid secretion in *H. pylori*-induced gastritis may lead to a failure of critical splitting of vitamin B₁₂ from food binders and its subsequent transfer to R binder in the stomach;
- (b) A secretory dysfunction of the intrinsic factor²⁴ and
- (c) Decreased secretion of ascorbic acid from the gastric mucosa and increased gastric pH.²⁵

The occurrence of chronic *H. pylori* infection in the gastric mucosa may impair the absorption of vitamin B₁₂.²⁶

H. pylori infection might cause low iron levels and vitamin B₁₂ deficiency however, it is a strong possibility that this bacterium causes other serious or moderate micronutrient deficiencies. Our findings showed that after three months of patients receiving the recommended medical treatment with omeprazole, amoxicillin, and clarithromycin (OAC), the levels of B₁₂ improved significantly. In addition, there were significant differences in levels of iron before and after OAC treatment, which means that levels of iron improved significantly after the patients received treatment. The results show that vitamin B₁₂ levels were restored in 40% of the patients following eradication of *H. pylori*, but there is a high recurrence of gastric *H. pylori* during gastroscopic evaluations.¹³ The

American College of Gastroenterology has recommended four specific drug regimens that use a combination of at least three medications to cure this medical condition.^{27–29} For *H. pylori* treatment to be effective, it is important to take the entire course of all medications.

Conclusions

H. pylori seems to be a causative agent in the development of vitamin B₁₂ and IDA. *H. pylori* patients had significant decreases in vitamin B₁₂, serum iron and hemoglobin levels. Treatment of *H. pylori*-infected patients with OAC seems to restore vitamin B₁₂, serum iron and hemoglobin levels and improve the general health of patients. Vitamin B₁₂ is strongly correlated to Hb levels and RBC counts in gastritis patients. Thus, it may be considered a useful marker for anemic patients with gastritis. Routine testing of vitamin B₁₂, iron and ferritin levels, and total iron binding capacity are recommended for *H. pylori* patients as is confirming *H. pylori* infection by gastroscopy.

Conflicts of interest

The authors declare no conflicts of interest.

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