



## Evaluation of the Relation of *Helicobacter pylori* Infection and Levels of Serum Iron among Peptic Ulcer Patients in Ibb City Yemen

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### Abstract

**Background:** *Helicobacter pylori* (*H. pylori*) is a Gram-negative, spiral-shaped bacterium, it infects the stomach and may inhibit the absorption of iron from fortified foods. More than 4.4 billion people worldwide are infected by *H. pylori*. Whereas in Yemen, the prevalence of *H. pylori* infection among dyspeptic patients was 82. 2%. The primary **objective** of this study to assess the association between *H. pylori* infection and serum iron levels in peptic ulcer patient.

**Methods:** This case-control study is conducted in the gastroenterology ward of Al-Manar Hospital and AL-Shifa Hospital, in Ibb city, Yemen, from December 2021 to September 2023. This study includes 150 participants divided into two groups. Group (I) comprised 75 patients infected with *H. pylori*. Group (II) consisted of 75 controls. *H. pylori* was confirmed or ruled out using the rapid urease test conducted biopsy specimens. Additionally, hemoglobin levels and serum concentrations of iron and ferritin were evaluated.

**Results:** Out of the total 150 study participants, (52%) were females while (48%) were males. The most frequented age group in this study was age group (18-30 yrs), (34.7%) followed by age group (31- 40 yrs) (25.3%). A significant decrease in iron and ferritin levels was found among peptic ulcer patients,  $p$ -value  $<0.001$  versus controls. It was also found that, a significant decrease in hemoglobin among patients versus controls. The mean of serum levels of ferritin, iron and Hb were significantly decrease in patients ( $90.4\pm 4$ ,  $73.9\pm 40.59$ ,  $11.9\pm 1.92$ ) when compared with controls ( $113.5\pm 40.7$ ,  $99.2\pm 37.8$ ,  $12.7\pm 1.9$ ) respectively,  $p < 0.05$ .

**Conclusion:** This study confirmed the strong effect of *H. pylori* on serum iron, ferritin and hemoglobin with peptic ulcer patients.

**Keywords** *H. pylori*, Iron, Ferritin, Peptic Ulcer, Yemen, RUT

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## Introduction

*H. pylori* is a Gram-negative spiral-shaped bacterium, known to infect the stomach [1]. It is the only microorganism that is known to be able habit in the stomach's severely acidic environment [2].

More than 4.4 billion people worldwide are infected by *H. pylori* [3]. Whereas in Yemen, the prevalence of *H. pylori* infection among dyspeptic patients in (2018) was 82.2% [4]. Other researches in Yemen confirmed that the prevalence was 68% 65.0, 18.5% and 27.99% [5] [6] [7] [8] respectively. About 90% of the population in developing nations may be infected with *H. pylori* [9]. The prevalence rate ranged from 50.8% (95% CI: 46.8-54.7) in developing nations compared with 34.7% (95% CI: 30.2-39.3) in developed nations [10]. Furthermore, it is the main risk factor for peptic ulcers and stomach cancer [11]. The estimated prevalence of *H. pylori* infection worldwide was 48.5%, and reports from continents included South America (69.4%), North America (37.1%), Oceania (24.4%), Asia (54.6%), Europe (47.0%), and Africa (79.1%). Geographical location, age, ethnicity, socioeconomic status, and eradication therapeutic techniques have all been linked to this discrepancy [12, 13].

The majority of *H. pylori* carriers do not exhibit any symptoms, however when it comes to human health, it can cause gastritis, stomach ulcers, and duodenal ulcers (DU) [14]. *H. pylori* infection has been linked to extra gastric illnesses, neurological, dermatological, hematological, ophthalmic, cardiovascular, metabolic, allergic, and hepatobiliary diseases, according to recent research reports [15]. The proven relationship between *H. pylori* infection and iron deficiency anemia (IDA) has been demonstrated by numerous reviews and meta-analyses [16]. Recently, the role of *H. pylori* in idiopathic thrombocytopenic purpura and IDA is demonstrated [17]. IDA is a general nutritional problem that causes almost half of the world's anemia cases; the most of IDA patients living in developing countries [18]. By causing, the gastric mucosa to become chronically inflamed, *H. pylori* further modifies stomach physiology [19]. It also produces the enzymes phospholipase A2 and C and glycosulfatase, which are critical for elevating the pH of the stomach and rupturing the stomach mucosal barrier, which may affect the absorption of drugs and nutrients [19]. One of the primary reasons of IDA during infection is bleeding (sometimes referred to as ulcer hemorrhage) in individuals with *H. pylori* infection [20]. As a micronutrient, iron is needed by almost all living

things, including *H. pylori*, which competes with the host for iron absorption [21]. Numerous nations have identified a link between *H. pylori* infection and trace elements [22-25]. Whereas in Yemen, we didn't find recent studies about effect of *H. pylori* infection on the levels of serum iron. Hence, this study aimed to evaluate the effect of *H. pylori* infection on the levels of serum iron among Yemeni peptic ulcer patients.



## Materials and Methods:

### Study subjects, design and population:

This case-control study was conducted on 150 participants, 51.3 % of them were females and 48.7 % were males. All participants attended Al-Manar Hospital and AL-Shifa Hospital, Ibb city, Yemen, from December 2021 to September 2023. An informed agreement was taken from every participant. A biopsy sample by endoscopy was tested for rapid urease test. A stool sample from each participant was tested for *H. pylori* antigen using immunochromatography technique. A blood sample was also collected, half of which was EDTA sampled and analyzed for hemoglobin, while the remaining half was left to clot and the serum was separated to be used for *H. pylori* antibodies testing, iron and ferritin measurement.

### *H. pylori* Diagnostic Tests:

Rapid urase test was performed to confirm the presence or absence of *H. pylori* in human gastric. It has high sensitivity and specificity. In this test, urea hydrolysis by urease to produce ammonia and bicarbonate that increase the pH to alkaline and lead to change the phenol color from yellow to pink-red color [26]. The procedure of this test based on a CLO test of manufactures instructions.

*H. pylori* antigen test is a qualitative immunochromatographic assay which used to investigate the presence of *H. pylori* antigens in stool sample. A small piece of stool sample was prepared for testing by dissolving it in a buffer solution provided by the manufacturer (Right sign) [27].

### Measurement of Hemoglobin and Serum Level of Iron and Ferritin

Hemoglobin measurements by cyanmethemoglobin method (drabkin's method). as manufacturer's instructions (normal value) men 14 - 18 g/dL= 8.7-11.2 mmol/L women 12-16g/dL= 7.5-9.9 mmol/L[28]. Iron in ionic trivalent form reacts with cromazole (CAB) and acetyl-methyl ammonium–bromide (CTAB) to give strongly coloured compound [29, 30].

Ferritin was measured by the spectrophotometric method, as manufacturer's instructions [30, 31].



### Statistical Analyses:

Data were collected, revised, coded and entered to (SPSS) version 21. Qualitative data were presented as number and percentage while quantitative data were presented as mean, standard deviation and range. Comparison between two groups with qualitative data was done using Chi-square test and/or Fisher exact test. Comparison between two independent groups with quantitative data was done by using independent t-test, while comparison between more than two independent groups with quantitative data was done by using One Way ANOVA test [32]. Association between *H. pylori* infection and iron and ferritin were assessed by chi-square. *P value* was set at  $< 0.05$  for statistical significance.

### Results:

#### Demographic Characteristics of *H. pylori* Infected Patients and Controls

Out of (150) participants included in this study seventy-five (75) as a patient and seventy-five (75) as a control, out of 150 study participants, (48%) were males while (52%) were females. The table (1) shows the demographic characteristics of *H. pylori* infected patients and controls. The most frequented age group in patients was age group (18-30 Yrs) (33.3%), as well as in controls (36%). According to residency, (56%) of patients were rural while (52%) of controls were urban as showed in the table (1).

**Table (1) Demographic characteristics of *H. pylori* patients and controls**

Variable	Patients (75)		controls (75)	
	Frequency	Percentage	Frequency	Percentage
Gender				
Male	35	46.7%	38	50.7%
Female	40	53.3%	37	49.3%
Age groups				
18-30	25	33.3%	27	36%
31-40	15	20%	23	30.7%
41-50	23	30.7%	13	17.3%
51-60	10	13.3%	8	10.7%
61-70	2	2.7%	4	5.3%
Residence				
Urban	33	44%	39	52%
Rural	42	56%	36	48%

Data assessed by cross-tabulation and represented as frequency and percent%.



## The Clinical History and Gastrointestinal Symptoms of the Patients and Controls

The clinical history and GIT symptoms of the patients and controls were presented in table (2). The data showed that, most of patients and controls did not use antacid (70.7%) and (84%) respectively. In addition, the majority (85.3%) of patients and (92%) of controls had not history of antibiotics use. The most frequented GIT symptoms were stomachache (68%) in patients and (42.7%) in controls, and peptic ulcer (37.3%) in patients (20%) in controls.

**Table (2) clinical history and gastrointestinal symptoms frequency among patients and controls**

Variable	Patients		controls	
	Frequency	Percentage	Frequency	Percentage
Stomachache	51	68%	32	42.7%
Antacid use				
Yes	22	29.3%	12	16%
No	53	70.7%	63	84%
Antibiotics use				
Yes	11	14.7%	6	8%
No	64	85.3%	69	92%
Endoscopy results				
No ulcer	47	62.7%	60	80%
Peptic Ulcer	28	37.3%	15	20%

Data represented as frequency and percent%.

## Mean Ferritin and Iron and Hb for Patients and Controls

In this study, the mean of serum levels of ferritin, iron and Hb were significantly lower in patients (90.4±44.8, 73.9±40.59, 11.9±1.92) when compared with controls (113.5±40.7, 99.2±37.8, 12.7±1.9) respectively,  $P < 0.05$ , as show in the table (3).

**Table (3) Mean ferritin and iron and Hb for patients and controls**

Variables	Patients Mean ±SD	controls Mean ±SD	P value
Ferritin	90.4±44.8	113.5±40.74	0.001*
Iron	73.9±40.6	99.16±37.81	0.0001*
Hb	11.95±1.92	12.72±1.88	0.02*

The data represented as mean ±SD and assessed by independent sample t test,  $*P \leq 0.05$  considered as significant

## Comparison of Serum Level of Iron, Ferritin and Hemoglobin among Patients and Controls based on Peptic Ulcer Detection Using Endoscopy

The association between serum levels of iron, ferritin and Hb among patients and controls according to endoscopy results was studied by using one way ANOVA test. The result revealed that, the mean levels of iron, ferritin and Hb were significantly decreased in patients with peptic ulcer when compared to patients without peptic ulcer  $P < 0.05$ . Among controls, the mean level of iron with peptic ulcer was significantly decrease ( $77.1 \pm 31.3$ ) than in controls without peptic ulcer ( $103.6 \pm 36.8$ ),  $P < 0.05$ , whereas, there was not significantly decreased with mean levels of ferritin and Hb as shown in table (4).

**Table (4) Comparison of serum level of iron, ferritin and hemoglobin among patients and controls based on ulcer detection using endoscopy**

Variable		Iron Mean±SD	Ferritin Mean±SD	Hb Mean±SD
Patients	No ulcer (47)	87.99±37.8	106.90±42	12.31±1.87
	Peptic Ulcer (28)	50.33±34.0	62.75± 33	11.37±1.8
<i>P value</i>		<0.001*	<0.001*	0.040*
Controls	No ulcer (60)	103.57±36	115.69±38	12.98±1.7
	Peptic Ulcer (15)	77.13±31.3	103.66±50	11.76±2.0
<i>P value</i>		0.011*	0.300	0.025*

The data represented as mean±SD, independent t test,  $*P \leq 0.05$  considered as significant.

### Association of *H. pylori* Infection and Risk Factors:

The table (5) showed the association of *H. pylori* infection and many risk factors involved in this study, the results showed that there was significant association between *H. pylori* infection with ulcer and education level ( $P < 0.05$ ). On the other hand, there was no statistically significant association between gender, residency, smoking and Qat chewing, ( $P > 0.05$ ).

Table (5) Association of *H. pylori* infection and risk factors

Variable		Rapid Urease Test		X <sup>2</sup>	OR (95% CI)	P value
		Positive No. (%)	Negative No. (%)			
Gender	Male	35 (46.7)	38(50.7)	0.107	1.113 (0.586-2.112)	0.744
	Female	40 (53.3)	37(49.3)			
Ulcer	No ulcer	47 (62.7)	60 (80)	4.833	2.383 (1.144-4.966)	0.030*
	Peptic ulcer	28 (37.3)	15 (20)			
Residence	Rural	42 (56)	36 (48)	0.669	1.307 (0.688-2.485)	0.414
	Urban	33 (44)	39 (52)			
Education	Illiterate	31 (41.3)	28 (37.3)	23.88	0.597 (0.421-0.847)	<0.001*
	Primary	30 (40)	8 (10.7)			
	Secondary	0	10(13.3)			
	Graduate	14 (18.7)	29 (38.7)			
Smoking	Yes	5 (6.7)	3 (4)	0.108	1.268 (0.327-4.918)	0.742
	No	70 (93.3)	72(96)			
Qat chewing	Yes	43 (57.3)	41 (54.7)	0.108	1.114 (0.585-2.124)	0.742
	No	32 (42.7)	34 (45.3)			

The data represented as frequency, percent%, *Chi* square test, \* $P \leq 0.05$  considered as significant.

## Discussion

In the present study, *H. pylori* infection was detected using two diagnostic tests. The rapid urease test (RUT) was initially performed as primary diagnostic tool, followed by the *H. pylori* antigen test, which gave similar results with the RUT.

The current study compared the serum levels of iron, ferritin and hemoglobin (Hb) between patients and controls, revealing significantly decreased levels in the patient group with a statistically significant difference ( $P \leq 0.05$ ). These findings similar with previous studies [1, 24, 25, 33]. Elsaadany *et al.* found that, the mean values of hemoglobin and serum ferritin were significantly lower in *H. pylori*-positive patients compared to controls [25]. Also, similar results were reported by Elshagier *et al* but not statistically significant [24].

*H. pylori* infection was correlated with lower serum ferritin and iron, the geometric mean ferritin (GMF) for cases with and without *H. pylori* infection were 37  $\mu\text{g/L}$  and 50  $\mu\text{g/L}$ , respectively ( $p=0.04$ ), and 19/121 *H. pylori*-positive cases who had iron deficiency compared with 8/120 *H. pylori* negative ( $p=0.02$ ) [34].



Tsay, F.-W (2018) detected the correlation between *H. pylori* and iron deficiency anemia and immune thrombocytopenic purpura [17]. The bacterium may also bind iron to iron-binding proteins for its own growth, resulting in iron deficiency anemia [35].

This study showed that the mean levels of iron, ferritin and Hb were significantly lower in patients with ulcer when compared to patients without ulcer  $P < 0.05$  and this agree with Goh, K.L [36]. Many risk factors of *H. pylori* infection were investigated in this study, includes smoking, Qat chewing, education, residence and gender. The results showed that, there was a significant association between *H. pylori* infection and education level ( $P \leq 0.05$ ). Illiterate and primary education patients showed more positivity to *H. pylori* infection by RUT. In this study female had high rate of *H. pylori* infection but without statistically significant. These results disagree with the study performed in Saudia Arabia by Al-Hussaini, A.A., *et al* [37].

*H. pylori* atrophic gastritis has been shown to inhibit the secretion of stomach acid in adult populations, which in turn leads to decreased iron absorption and IDA [38]. *H. pylori* can obtain the iron from host transferrin and lactoferrin, so it binds the iron-free forms of these proteins over their the iron-loaded proteins [39, 40]. Other ferrokinetic iron mechanism issues have also been linked to *H. pylori*, including the secretion of hyatosine by hepatocytes, which causes decreased iron absorption [41]. Additional form of association is the elevated iron absorption by 19K Da iron binding protein surface receptors and as a result of mordant gastritis and decrease iron absorption secondary to the hypochlorite conditions which results in occult blood loss [41, 42].

López de Romaña, D., *et al.* demonstrated that *H. pylori*-positive patients absorb less iron from a wheat product fortified with iron than *H. pylori*-negative patients, irrespective of the type of iron salt used for fortification [43].

Possible pathogenic mechanisms for IDA in *H. pylori*-infected individuals are occult blood loss secondary to chronic gastritis, enhance iron uptake and consumption by *H. pylori* bacteria, decreased iron absorption secondary to chronic erosive gastritis and hypo/achlorhydria, and ascorbic acid shortage [44]





## Conclusion

The results of this study revealed the association between the H. pylori infection and decreased of serum iron, ferritin and hemoglobin in peptic ulcer patient. H. pylori patients had significantly decreased in serum iron levels. More than 37.3% of patients had peptic ulcer whereas 80% of controls had not ulcer. Additionally, the study concluded that major predisposing factor for the establishment of H. pylori infection among patients with peptic ulcers were found to be low level of education. The study also indicated that the results of H. pylori antigen test from stool sample and RUT were similar. For future studies larger sample sizes should be utilized to validate the results. H. pylori Patients should uptake adequate iron rich nutrition, or receive iron supplements. To decrease the transmission rate of H. pylori infection, improvements in overall sanitation, including access to clean water, proper waste disposal, and household hygienic practices, are necessary.

### **Declaration:**

This paper has no conflict of interest and is the responsibility of authors.  
Consent for publication

The authors give consent for publication to Practical Laboratory Medicine.

Availability of data and materials

All data are available in this manuscript.

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### **Authors' contributions**

KAA prepared and wrote the main manuscript, was responsible for conducting the main analysis, interpretation of the results and writing the manuscript, while AMS and RA, MAH contributed in supervision.

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### المخلص:

**الخلفية:** تُعد البكتيريا الملوية البوابية (*Helicobacter pylori* (H. pylori) بكتيريا سالبة الجرام، حلزونية الشكل، تعرف بإصابتها للمعدة وتعرف بجرثومة المعدة. تشير الدراسات الى ان أكثر من 4.4 بليون انسان مصاب بجرثومة المعدة حول العالم وفي اليمن وجد انها تنتشر في 82.2% من المرضى المصابين بعسر الهضم. تسبب هذه الجرثومة قرحة المعدة والاثني عشر وتعتبر أحد أهم العوامل المسببة لسرطان المعدة. إضافة الى مضاعفات أخرى خارج الجهاز الهضمي منها انها تثبط امتصاص الحديد في الأطعمة المتناولة.

**الهدف:** تهدف هذه الدراسة الى تقييم العلاقة بين الإصابة بجرثومة المعدة ومستويات الحديد في سيرم المرضى المصابين بالقرحة الهضمية.

**المنهجية:** أجريت هذه الدراسة على مرئادي عيادات الباطنة والجهاز الهضمي في مستشفيات المنار والشفاء في مدينة اب-اليمن خلال الفترة من ديسمبر 2021 الي سبتمبر 2023. وهي دراسة مقارنة شملت 150 شخص تم أخذ موافقتهم للمشاركة في الدراسة وتم تقسيمهم الى مجموعتين. المجموعة الأولى عبارة عن 75 شخص مصاب بجرثومة المعدة والمجموعة الثانية عبارة عن 75 شخص غير مصاب بجرثومة المعدة. وتم تشخيص الإصابة عن طريق الفحص السريع لليورايز من خلال خزعة مأخوذة من المعدة وفحوصات سيربولوجية كشفت عن وجود الاجسام المضادة في السيرم والانتيجينات في البراز. كما تم حساب نسبة الهيموجلوبين في الدم ومستويات الحديد والفريتين في السيرم.

**النتيجة:** أظهرت النتائج ان من اجمالي 150 مشاركا كان منهم 78 (52%) إناثا بينما 72 (48%) كانوا ذكورا. معظم الأشخاص تتراوح أعمارهم بين 18 و 30 عاما (52، 34.7%) وجاء بعدهم الأشخاص ذوي الاعمار من 31 الى 40 عاما (38، 25.3%). ولقد توصلت الدراسة الى وجود انخفاض بنسبة معنوية في مستويات الحديد والفريتين بين المصابين بجرثومة المعدة والقرحة الهضمية وبقيمة اقل من 0.001 وبعكس الأشخاص الغير مصابين بالقرحة الهضمية. وفي هذه الدراسة كان متوسط مستوى الفريتين والحديد والهيموجلوبين عند الأشخاص المصابين بجرثومة المعدة منخفضا وذو دلالة إحصائية (4±90.4, 40.59±73.9, 1.92±11.9) عند مقارنتها في الغير مصابين (40.7±113.5, 37.8±99.2, 1.9±12.7) على التوالي وبقيمة معنوية اقل من  $p < 0.05$ .

**الاستنتاج:** اثبتت الدراسة وجود تأثير قوي لجرثومة المعدة على الحديد والفريتين لدى المرضى المصابين بالقرحة الهضمية

**الكلمات المفتاحية:** البكتيريا الملوية البوابية، الحديد، الفريتين، القرحة الهضمية، اليمن، الفحص السريع لليورايز.