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Prevalence and Associated Factors of Pylori Infection in Symptomatic Patients Admitted to A Major Hospital in Saudi Arabia

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Abstract. Helicobacter pylori infection (H. Pylori) has a worldwide distribution, especially with gastric along with duodenal ulcers. It has been associated with various disorders such as anemia, coronary heart disease, and thyroid disease. There are limited local studies available evaluating the role and association of H. Pylori to various other diseases. Therefore, the current study aims to inspect comorbidities linked with H. Pylori infection in the Kingdom of Saudi Arabia. A prospective study was conducted by enrolling outpatients from Salman Bin Abdulaziz University Hospital and National Hospital in Riyadh. A total of 323 patients presenting with dyspeptic symptoms were recruited between 2012 and 2015. A questionnaire was utilized and distributed to study the possible dyspeptic symptoms among the participants. Initial evaluation included a liver function, complete blood count, lipid and thyroid profile test. H. Pylori diagnosis was founded on "urea breath test", "H. Pylori stool ELISA test", "serology", and "upper endoscopy with gastric biopsy". Results were presented as P-values, mean +/- SD, and 95% confidence intervals using SPSS version 20. A total of 323 patients with "dyspepsia" or "symptoms suggestive of gastritis" were enrolled. Approximately, 166 (51.4%) patients were "Pylori positive". The presence of positive H. Pylori was associated with frequent symptoms such as nausea, epigastric pain, vomiting, Dyspepsia, and bloating. Dyslipidemia, Anemia, diabetes, hypothyroidism, and hyperuricemia were more recurrent among the patients with H. Pylori infection. "Multivariate analysis" demonstrated a relationship between Pylori infection and each of "baseline cholesterol level", "diabetes", "hemoglobin", and baseline "TSH levels". H. Pylori infection is considered to be common in Saudi Arabia. It is linked with dyslipidemia, diabetes, and hypothyroidism. In individuals with such disorders, H. Pylori management and testing are recommended. Prospective studies are needed in future to assess the benefit of eradication treatment as well as clinical implication of H. Pylori infection in the patients.

Keywords: Helicobacter pylori, urea breath test, serology, stool antigen test.

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INTRODUCTION

Helicobacter pylori infection has a universal geographical distribution. *H. Pylori* infection is linked to several gastric disorders for example, peptic ulcer disease, chronic gastritis, and gastric cancer [1]. Multiple studies revealed increased occurrence of *H pylori* infection among those with adenocarcinoma, gastric lymphoma or lymphoid tissue lymphomas [2, 3]. Some extra gastric have been related with *H. Pylori* infection such "gastro esophageal reflux disease" (GERD) [4, 5], "coronary heart disease" [6] and "deficiency anemia" [7]. These associations are quiet not fully explained, and the causal relationship needs to be further investigated.

H. Pylori is well thought-out as one of the most frequent 'bacterial infections' with high rate of prevalence [8, 9]. The prevalence rates vary according to the standards of public health since *H pylori* infections are additionally prevalent in "developing countries" as compared to "developed countries". *H pylori* possess different strains that are related to diverse presentations of the infection [8, 9]. The infection can be diagnosed by different diagnostic procedures such as endoscopy including, culture, histology, urea breath test (UBT), serology, and 'stool antigen (HpSA)' test [10, 11].

H. Pylori has been indicated to be linked to some extra gastric disorders such Coronary Heart Disease, Purpura, Dermatological disorders, migraine, Iron Deficiency Anemia, Raynaud's phenomenon, plus Guillain-Barre´ syndrome. It is not clear if these observations are mere associations or causal relation [12]. Several studies demonstrated that patients with Idiopathic Thrombocytopenic Purpura had higher *H. Pylori* infection rates in addition to eradication therapy that improved the platelet counts. The relation of *Pylori* positivity and other diseases has not been studied in the KSA or Kingdom of Saudi Arabia. Therefore, the present study investigated the association of different disorders with H.*Pylori* Infection in a cohort of patients presenting with manifestations of ulcer or non-ulcer dyspepsia.

RESEARCH SETTINGS AND METHODOLOGY

A total of 323 outpatients complaining of dyspepsia, epigastric pain, nausea, vomiting or bloating were enrolled in this study from November 2012 to January 2015. At the beginning; no participants had received previous therapy for *H. pylor*i infection.

Participants with a 'history' of recent antibiotic use, bismuth or proton pump inhibitors within 1-month period prior to enrollment was excluded from participating in the study. All patients enrolled were examined clinically and were examined for eligibility to upper gastrointestinal endoscopy. 'Informed consent' was obtained before participating in the study. The Institutional Review Board (IRB) of "Prince Sattam Bin Abdulaziz University" approved this study (IRB number: GI-2012-71/11). The study was directed in accordance with the principles of the "1974 Declaration of Helsinki."

Stool Antigen Assay:

H. pylori stool antigen assay was performed using the "Antigen, EIA, Stool Test" (Quest Diagnostics, New Jersey, and USA) in accordance with the company's guidelines. Concisely, stool samples were collected and stored at $-20\ ^{o}C$ at the laboratory and extraction of DNA was undertaken. The stool was liquefied for "stool antigen assay," plus stored again at $-20^{o}C$ for retesting if required. "The assay was completed by utilizing a single monoclonal antibody against *H. Pylori* flagellin antigens."

"A container was used to add 1 g fecal sample to a vial containing 1 mL buffer. After mild vortexing, the fecal sample was emulsified. Up to four drops of emulsified sample were placed in the sample port of the test container. After 15 min, the test was interpreted at room temperature. The presence of a red line in the reading window indicates a positive result, with the presence of a positive control band also demonstrating red color. Detection of antigen (positive result) is indicative of *H pylori* infection while absence of antigen (negative result) indicates lack of H pylori or may be the level below the test limit of detection. This test has a high specificity and sensitivity for detecting H pylori infection. False-negative results may be observed if patients take medications within the last 2 weeks prior to collection (like antimicrobials, proton pump inhibitors, and bismuth)."

Helicobacter Pylori Serology:

"*H. Pylori* antibodies were performed by means of an enzyme-linked immunosorbent assay (IgG and IgA). Briefly, dilute serum is added to boreholes covered with purified antigen. IgG specific antibody, if present, will bind to this antigen. All extra ingredients are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex. The extra enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The degree of color produced is proportionate to the quantity of IgG specific antibody in the sample collected."

Urea-Breath Test:

H. Pylori status was measured using the "^{13}C urea breath test (UBT) kit". A first breath sample was obtained after a 4-h fast. The baseline breath samples were taken before the test. Subsequently, the patients were given 100mg of 13C-urea dissolved in 100ml water; they washed out the mouth with water. After 15 min, all subjects were asked to deeply inhale and hold breath for 5 seconds, then exhale slowly into a bag until it is fully inflated. "About $13CO_2$ was measured in the breath samples using isotope-selective non-dispersive infrared spectrometry."

Upper Endoscopy:

"Eligible patients were referred to upper endoscopy at King Khaled Hospital, Al Kharj, KSA. Upper endoscopy was performed for 45 patients. Detailed endoscopic examination was performed and several biopsies were taken for histological examination. Biopsy specimens were prepared for histological examination by staining with hematoxylin-eosin and Warthin-Starry silver stains; diagnoses were made using the Sydney classification [17]. At least one biopsy specimen had been obtained from the antrum, with additional specimens obtained from other sites. The grade of *H. pylori* presence was recorded as follows: mild, few focal areas of bacteria; moderate, bacteria in several areas; and severe, abundant bacteria in most glands. The presence of atrophic gastritis, intestinal metaplasia, lymphoid aggregates and/or follicles, gastric ulcers, and ulcer scars were also recorded.

The diagnosis of *H. pylori* infection is confirmed by the agreement 2 or more positive test results from the 3 tests performed for example, urea breath test, histology, HP stool antigen or HP ELISA). Positive histology result and either of the other tests demonstrated a positive result; probable positive results by stool antigen assay and HP ELISA; and negative, all 3 tests are negative."

Statistical Analysis

"The Fisher's test or chi-square test was used to measure the frequencies and to compare if the expected frequency for any cell was five or lower. Student's t -test or the Mann–Whitney U test, when appropriate, were used for comparing continuous variables. Results were expressed as mean +/- standard deviation (SD), P-values and 95% confidence intervals (C.I.) or median and IQR as appropriate. The proportions between groups was done using Z test. Statistical analysis was done using Statistical Analysis Software version 20."

RESULTS

"Among the 323 enrolled patients, 166 (51%) (72 males and 94 females) patients were positive for *H. pylori* infection. The mean age of the patients was 33.45±12.95 (table 1). The incidence of *H. Pylori* infection in patients less than 40 and ≥ 40 years was 33.3 and 55.2, respectively (p=0.04). As shown in fig. 1, dyspepsia, nausea, abdominal pain, heartburn is the most common presenting symptoms.

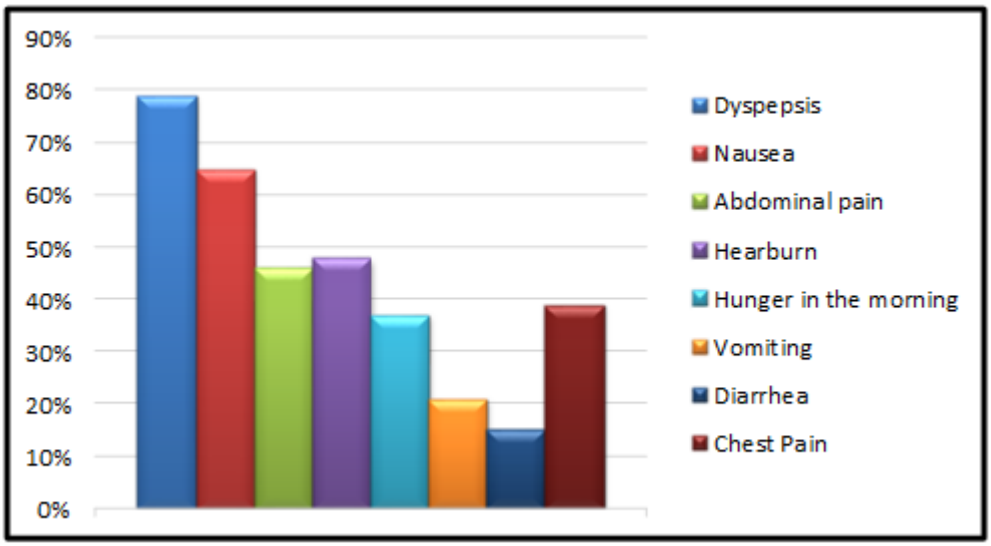

Figure 1: Frequency of Symptoms in Enrolled Patients

Table 1: Demographics and clinical characteristics of study groups

Parameter	**Group A** H. Pylori positive N=166	**Group B** H. Pylori negative N=157	**P (95% CI)**
Age (years) means S.D.	33.45±12.95	34.29±10.52	P = 0.577865
Gender Male: Female	72: 94	102: 55	P<0.0001*****
Nationality			
Saudi	88(53.01)	98 (62.4)	
Non-Saudi			
Egyptian	45 (27.2)	40 (25.47)	P = 0.3358
Jordanian	12 (7.2)	6 (3. 8)	(-
Yemeni	11 (6.62)	6 (3.8)	10.738 % to 37.045
Sudanese	10 (6.1)	4 (2.5)	%)
Nepali	6 (3.6)	0	
Pakistani	6 (3.6)	3 (1.9)	

* significance p. value ≤ 0.05

Of the 166 pylori positive patients, 88 (53 %) were Saudis and 77(47%) were non Saudis (Table 1). *H. pylori* infection was associated with various disorders particularly irritable bowel disease in 53.6%, anemia in 45.9%, dyslipidemia in 41.6%, non-alcoholic liver disease in 40.96%, and diabetes mellitus in 31.32 %, (Table 2). Clinical manifestations related to *H. Pylori* were more pronounced in patients with other disorders compared to individual with sole pylori disease. A significant difference was observed between patients with or without *H. Pylori* infections in the prevalence of anemia, diabetes, dyslipidemia, hypertension, hyperuricemia, and IBS (Table 2).

Table 2: Co-morbidities associated with Helicobacter pylori infection

Parameter	**Group A** H. Pylori positive N=166	**Group B** H. Pylori negative N=157	**P value**
Anemia	74	33	P =0.000007 *
Diabetes	52	36	P = 0.034990 *
Dyslipidemia	67	38	P = 0.001997 *
Hypertension	31	21	P = 0.181008
Hypothyroid	56	31	P = 0.011495 *
Hyperuriceamia	38	19	P = 0.013345 *
Recurrent UTI	45	32	P = 0.193493
IBS	89	51	P = 0.000424 *
NAFLD	68	52	P = 0.133685

* significance p. value ≤ 0.05

Anemia was frequently observed in patients presenting with dyspepsia and positive *H. pylori* infection. These patients presented with fatigue, recurrent headaches, and nausea. A significant difference was observed in hemoglobin levels between patients with H. pylori infection and dyspeptic patients negative to pylori (Fig 2 and 3).

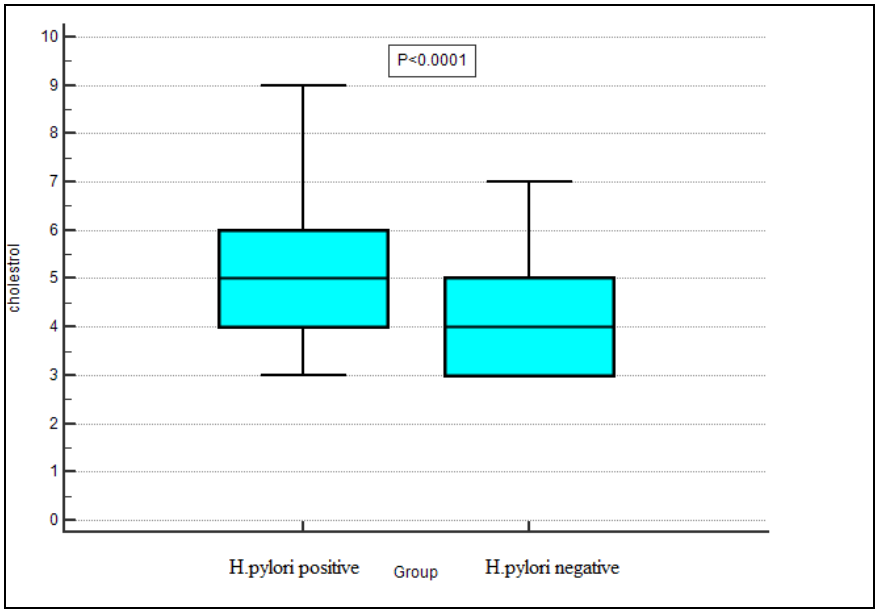

Figure 2: Anemia (Hemoglobin levels) in patients with and without H. pylori infection

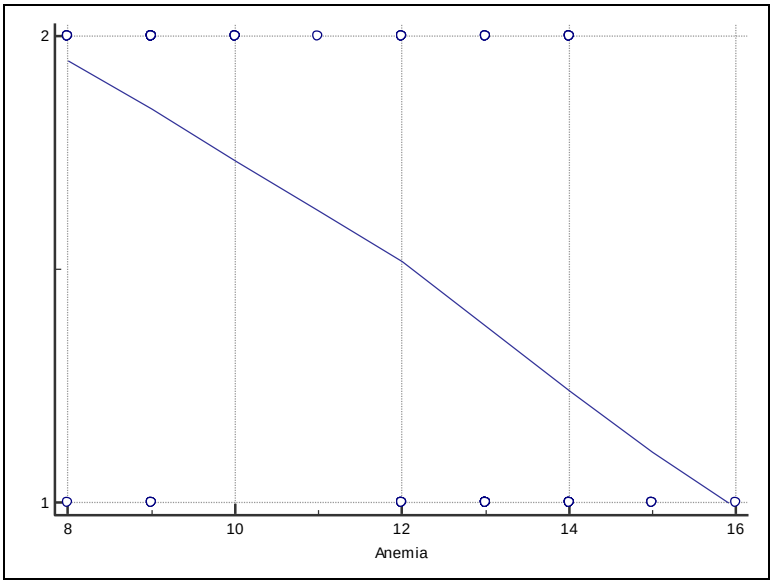

Figure 3: Relation between anemia and H. pylori status

Table 3 shows that the likelihood *of H. Pylori* infection increased with lower hemoglobin levels.

Table 3: Likelihood ratio of *Pylori* positivity in anemia

+	Positive	Negative	Likelihood ratio	95% CI
≤ 7	9	1	6.545	0.854 to 50.195
8	7	3	1.697	0.459 to 6.277
9	10	1	7.273	0.958 to 55.189
10	13	4	2.364	0.814 to 6.867
11	10	2	3.636	0.829 to 15.953
12	20	16	0.909	0.519 to 1.592
≥ 13	8	29	0.201	0.0993 to 0.405

As shown in table 4, 40% of patients with *H. pyl*ori had hemoglobin levels < 10 gm/dl. Anemia was particularly frequent among diabetic patients with H. Pylori infection. Most patients with proven *H. Pylori* were initially diagnosed as having irritable bowel disease based on clinical presentation. In patients with pylori and IBS, the main presentations include nausea, dyspepsia, vomiting, abdominal distension with gases and alternating bowel habits. Another frequent association was observed between *H. Pylori* and elevated cholesterol levels as well as between pylori and hypothyroidism (Table 2 and Figure 4). The symptoms are more severe among diabetics as well as patients with hypothyroidisms.

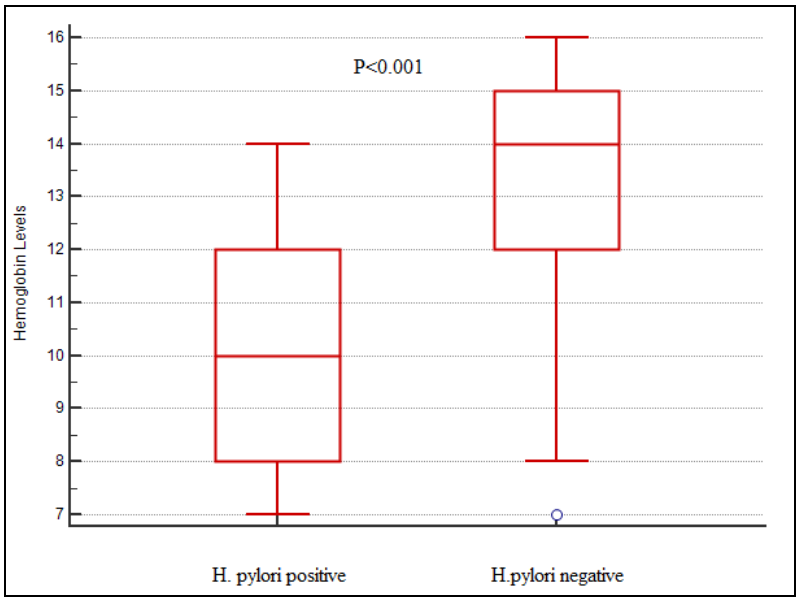

Figure 4: Cholesterol levels in patients with and without H. pylori infection

Table 4: Likelihood ratio of Pylori positivity in diabetes

DM	Likelihood ratio	95% CI
No DM	1.316	0.134 to 27.2845
Mild DM (FBS 110-130)	2.540	0.216 to 29.427
Moderate DM (FBS 130-160)	5.961	0.729 to 37.529
Uncontrolled DM (FBS>160)	7.153	0.947 to 418.516

Endoscopy was performed in 43 h. Pylori positive patients, endoscopy showed gastroesophageal reflux disease (GERD) in 12(27.9%) patients, reflux esophagitis in 10 (23.25%) patient, atrophic gastritis in 19 (44.2%) patients; erythematous gastritis in 8 (18.6%) patients; combination of atrophic and erosive gastritis, 26 (60.5%) patients atrophic gastritis in 32% and Barrett's esophagus in (2%) patients, it has been observed that diabetic patients have more severe gastritis. (Figure 5)

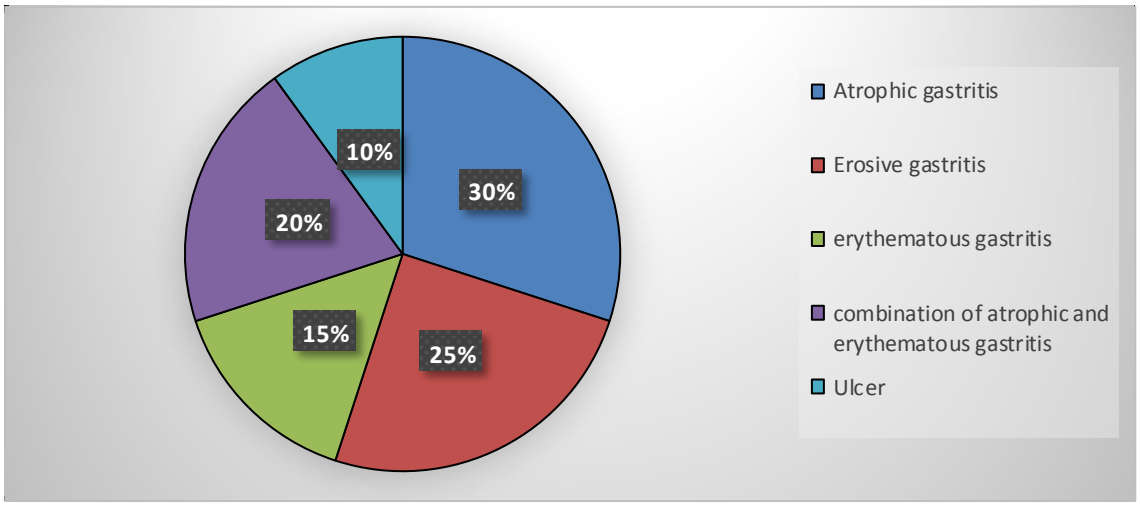

Figure 5: Endoscopy results in a subset of patients undergoing upper GI endoscopy (n=31)

Multivariate analysis indicated that older age, male gender, diabetes mellitus, anemia and higher cholesterol levels were independent factors for *H. Pylori* infection, (Table 5)."

Table 5: Multivariate analysis of the association between chronic diseases and *Helicobacter pylori* Infections

Parameter	OR	95%CI	P
Baseline age (older Vs. younger)	6.2262	0.7563 to 11.9881	0.0408 *
Gender Male (reference) Female	0.791	0.599 to 1.044	0.092
Hemoglobin (lower than 10 mg/dl vs. higher	5.7658	1.4733 to 9.2393	<0.001 *
Baseline Cholesterol level (Lower than 6 vs higher)	3.6379	0. 892 to 12.04554.	0.003 *
Triglycerides	0.6519	0.9431 to 14.6603	0.2713
Hypertension	0.3730	08.8898 to 9.9515	0.6201
Diabetes	9.6016	5.4877 to 36.6355	< 0.0001 *
Hypothyroidism	3.5129	2.2336 to 5.525	0.02 *
Urinary tract infections	0.7924	0.2241 to 1.6872	0.0871

* significance p. value ≤ 0.05

DISCUSSION

"Helicobacter pylori (*H. Pylori*) is a pathogenic bacterium with worldwide prevalence causing varying degrees of gastritis, peptic ulcer disease, and/or gastric malignancy [1, 2]. Since *H. Pylori* infection is particularly frequent in developing countries, this infection may be associated and may influence to be affected by several health disorders. Few studies showed that *H. Pylori* infection may represent a public health problem in the Kingdom of Saudi Arabia. However, no large studies investigated the relation of pylori infection with frequent disorders in the Saudi community. Utilizing several specific techniques to diagnose and confirm *H.Pylori* infection, pylori infection was proved in 51% of patients enrolled in the present study. This rate is in agreement with studies conducted on the prevalence of *H. Pylori* in KSA demonstrating prevalence rates ranging between 50-80% according to the location of the study and the methods of diagnosis [13-16].

Pylori infections were more frequent among Saudis. However, this finding should be cautiously interpreted since we analyzed patients attending hospital setting rather than community based survey. The main symptoms in our patients were similar to those previously reported [2, 3] namely dyspepsia, nausea, abdominal pain, and heartburn.

We studied the relation of *H. Pylori* infection with several frequent diseases in Saudi Arabia. A highly significant association was found between *H. Pylori* and diabetes mellitus. KSA is a country with very high rates of diabetes mellitus. According to the international federation of diabetes mellitus, about 4 million Saudis have diabetes in 2014 [17]. In our study, 31% of pylori positive patients were diabetics who presented with severe hearth burn, nausea and/or vomiting. Our results agree with studies that supported a positive relation [18-26] and a meta-analysis, which confirmed that the odds ratio (or) for *H. Pylori* infection was 1.33 more among patients with diabetes [27].

Our findings indicate a higher rate of association between *H. Pylori* and IBS. It is not clear whether the patients' complaints were primarily due to *H.* Pylori and there were overestimation of IBS diagnosis. This association has not been reported except by Gerards et al [28] who observed that the abdominal discomfort in patients with IBS was more severe in *H. Pylori* infected patients. Our findings along with those of Gerards et al suggest that *H. Pylori*

infection may be involved in triggering IBS. In this study, *H. Pylori* positive patients had higher cholesterol and triglyceride levels compared to *H. Pylori* negative patients in agreement with other studies [29, 30] which showed that a percentage of *H. Pylori* infected patients had metabolic syndrome. This association may play a role in the increased atherosclerosis with *H. Pylori* infection, which may induce a chronic inflammatory state that contributes to dyslipidemia to endothelial impairment and atherosclerosis [31].

The rate of anemia was high among *H. Pylori* positive patients in this study. Several other studies have shown that *H. Pylori* colonization in the gastric mucosa may interfere with iron uptake and may lead to iron deficiency anemia [32, 33] which is the type of anemia observed in our study. A correlation was detected between the degree of anemia and the grade of gastritis as shown by upper endoscopy. There have been no studies performed in patients with intact gastric mucosa. Furthermore, we found an association between hypothyroidism and *H. Pylori* infection in agreement with a study [34] that demonstrated that autoimmune thyroiditis is an extra-gastric manifestation of *H. Pylori* infection.

In conclusion, *H. Pylori* infection in Saudi Arabia is associated with several diseases such as anemia, diabetes, dyslipidemia, thyroid disease and irritable bowel disease. Patients with these disorders should be screened for *H. Pylori* and treated if positive. Furthermore, an individual needs to expand the study prospectively in order to evaluate the prevalence of the infection in larger patient's group along with observing the consequences postoperatively in order to examine the associated comorbidites and benefits of eradication treatment."

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