



Clinical, Endoscopic, Pathological and Serological Findings of *Helicobacter pylori* Infection in Saudi Patients with Upper Gastrointestinal Diseases

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors have made substantial contributions to the intellectual content of the paper. Author MAA planned and designed the study, wrote the protocol, participated in the interpretation of the results and analysis, drafted and critically revised the manuscript for important intellectual content and approval of the version to be published. Author TAE participated in planning and designing the study, sample collection and drafting of the manuscript. Author MSA collected the data and performed the practical laboratory activities. All authors also read and approved the final manuscript.

Research Article

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ABSTRACT

Background: The prevalence of *Helicobacter pylori* is typically higher in developing countries like Saudi Arabia.

Aims: To estimate the prevalence of *H. pylori* among patients suffering from upper gastrointestinal (GI) symptoms, know the main risk factors leading to infection, and detect the clinical, endoscopic and pathological changes of the gastric mucosa of patients infected with *H. pylori*.

Place and Duration of Study: This is a five-months prospective study conducted in King Fahd Hospital, Al-Madinah, Saudi Arabia.

Methodology: *H. pylori* status in patients was determined by two biopsy based tests (histology and rapid urease test) as invasive tests and by a serological testing (ELISA

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IgG) as non-invasive test.

Results: The age of the selected 68 patients ranged from 18-70 years with a mean age of 37.9 ± 14.15 years. *H. pylori* was more prevalent among males than females (70.5% vs 29.5%, $p=0.020$) and among age group (18-< 30) years. No significant difference was estimated between positive and negative HP cases in residency, work status, marital status, smoking, socioeconomic level and aspirin intake. The most common complaints in the selected patients having endoscopy were: epigastric pain, heart burn, and dyspepsia. A significant correlation was detected between *H. pylori* and each of age, sex and Diabetes Mellitus. The most prevalent endoscopic findings of the positive *H. pylori* cases were mild gastritis (84.1%). There was a significant difference in gastritis ($p=0.003$) and duodenitis ($p=0.031$) between positive and negative cases for *H. pylori* histopathology. The prevalence of *H. pylori* was 60.29% by rapid urease test, 64.7% by histopathological examination and 67.6% by serology among the studied cases.

Conclusion: *H. pylori*-infected patients with upper GI symptoms were found to have more abnormal endoscopic and pathological findings than those without *H. pylori* infection.

Keywords: *Helicobacter pylori*; histopathology; serology; IgG; upper gastrointestinal symptoms; endoscopy.

1. INTRODUCTION

Helicobacter pylori (HP) are gram negative spiral bacteria that colonize the human stomach [1]. HP is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors [2].

The incidence of HP infection in the developing world is higher than developed countries, and occurs at younger age [2,3]. Infection once established can persist for life if left untreated. Only 30% of those infected are clinically symptomatic and have active inflammation in the gastric mucosa [4,5]. Infection also can cause chronic gastritis that may lead to gastric atrophy and intestinal metaplasia, peptic ulcer, mucosa-associated lymphoid tissue lymphoma, or adenocarcinoma [6,7].

HP infection has been reported to be hyperendemic in Saudi Arabia and markedly increased with age [8,9].

Several techniques, both invasive and noninvasive, have been developed to diagnose HP infection. Endoscopic diagnostic tests for HP include histological examination of the gastric mucosa, culture of gastric mucosal biopsy cells and the biopsy urease test. However, these procedures are invasive and expensive [10]. Non-invasive testing as serology for HP infection is recommended for dyspeptic patients in primary care, in particular if they are aged <55 years and have no alarm symptoms [11].

The association of HP with upper gastrointestinal diseases is widely reported, but it remains unclear whether HP infection actually causes symptoms or is just an associated finding. If HP was significantly associated with upper gastrointestinal diseases and associated symptoms, eradication of the organism could be expected to lead to clinical improvement. Rapid and correct diagnosis of HP is thus critical for treatment and to prevent potential complications.

The study was conducted to estimate the prevalence of HP among patients suffering from upper gastrointestinal (GI) symptoms in King Fahd hospital, know the main risk factors leading to infection, and detect the clinical, endoscopic and pathological changes of the gastric mucosa of HP infected patients.

2. MATERIALS AND METHODS

2.1 Study Location and Patients

This five-months prospective study was conducted in King Fahd Hospital, Al-Madinah, Saudi Arabia, in the period between 1, March to 30, July, 2011.

All the patients were subjected to a questionnaire to collect some Socio-Demographic data (age, gender, weight, height, marital, status, highest educational level and occupation), current complaint and medical history.

Education, income, and occupational status were the most commonly used variables to measure Socioeconomic Status (SES). Each SES measure had its own strengths and limitations when the relationship between SES and health outcomes was studied. Many researchers suggested that education is the most stable and robust indicator of SES. Thus, we selected education level of the subjects as the primary measure of SES similar to Zhang and Wang [12]. Education is more comparable across time than income or occupation; and had little missing data, compared with income. We coded the education level as follows: low education (less than high school, meaning ninth grade or less), medium education (high school, meaning 10th to 12th grades), and high education (college or higher) to indicate low, medium, and high SES.

Patients were excluded according to criteria designed by Westbrook et al. [13] if they (1) were inpatients, (2) did not have one or more of 10 upper GI symptoms (epigastric pain, vomiting, nausea, heartburn, acid regurgitation, anorexia, early satiety, dysphagia, bloating or moderate to severe burping/belching), (3) had a GI bleed, (4) had a previous history of peptic ulcer disease or cancer, (5) had undergone a previous endoscopy or gastric surgery, (6) under 16 years of age, (7) experienced a developmental disability or (8) had gallstones.

In addition to the above exclusion criteria, those who had received a previous anti-helicobacter therapy, non-steroidal anti-inflammatory drugs or anti-secretory drugs (including H₂-receptor antagonists or proton pump inhibitors) in the preceding 4 weeks, were also excluded.

This study thus excluded many patients after clinical examination, leaving a sample of 68 patients (41 males and 27 females). Their Nationalities were different (52 Saudi, 2 Egyptian, 4 Yemeni, 5 Pakistani, 1 Lebanese, 1 Syrian, 1 Jordanian, 1 Omani and 1 Indian). Fifteen healthy population (10 males and 5 females) were also included in this study to detect *H. pylori* IgG among them.

2.2 Collection of Samples and HP Screening Tests

Upper gastrointestinal endoscopy was performed to all the selected cases by the second author at the endoscopy unit, King Fahd Hospital using an Olympus, GIF-SP 240

Gastroscope. For all cases, a total of three biopsy specimens were taken from the antral mucosa.

HP status in patients was determined by two biopsy based tests (histology and rapid urease test) as invasive tests and by a serological testing (ELISA IgG) as non-invasive test.

A rapid urease test (CLO test, Ballard Medical Products, UTAH, USA) was performed on the first antral biopsy. The gastric biopsy specimen was immediately embedded into the gel in the kit. The kit was placed in the room temperature for 24 h. The test was considered positive if the color changed into red or magenta. The remaining specimens were sent for histopathological evaluation.

Immediately after collection, gastric biopsy specimens were fixed in 10% buffered formaldehyde and embedded in paraffin for sectioning using automated tissue processor (Thermo Scientific, Germany). The slides were stained by hematoxylin and eosin stain, using auto stainer (Thermo Scientific, Germany). The slide of biopsy specimen was then examined for the presence of inflammation, HP, atrophy, and intestinal metaplasia.

Serological detection of HP in serum of patients and healthy population was done by measuring anti-*H. pylori* IgG antibodies using Enzywell *H. pylori* IgG kits (Diesse diagnostic senese-Italy Diesse).

2.3 Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences SPSS (version 13). Frequencies, percentages and arithmetic mean were calculated. Chi-square test (with Yates Correction) and t-test were used accordingly. Bivariate correlation (spearman Correlation) was used. P value <0.05 was considered significant.

3. RESULTS

Socio-Demographic description of the studied patients are presented in Table 1. Their ages ranged from 18 to 70 years with a mean of 37.9 ± 14.15 years. The age groups were as the following: (18 - < 30 years, 47.7%), (30 - < 45 years, 20.5%), (45 - 60 years, 27.3%) and (> 60 years, 4.5%) with no significant difference among them ($p=0.172$). In addition, Males had higher HP positivity (31 cases, 70.5%) than females (13, 29.5%).

Table 1. Socio-demographic description of the studied patients

	Negative histopathology for HP		Positive histopathology for HP		Total		Test of significance p-value
	N=24	%	N=44	%	N=68	%	
Age in years							
Mean \pm SD	42.8 \pm 14.97		35.2 \pm 13.10		37.9 \pm 14.15		0.034*
Sex							
Female	14	58.3	13	29.5	27	39.7	0.020*
Male	10	41.7	31	70.5	41	60.3	
Nationality							
Saudi	16	66.7	36	81.8	52	76.5	0.159
Non Saudi	8	33.3	8	18.2	16	23.5	
Residence							
Outside Medinah	4	16.7	2	4.5	6	8.8	0.092
Medinah City	20	83.3	42	95.5	62	91.2	
Work status							
Non working	13	54.2	16	36.4	29	42.6	0.156
Working	11	45.8	28	63.6	39	57.4	
Current marital status							
Single	5	20.8	16	36.4	21	30.9	0.185
Married	19	79.2	28	63.6	47	69.1	
Active smoking							
Non smoker	18	75.0	36	81.8	54	79.4	0.506
Smoker	6	25.0	8	18.2	14	20.6	
Socioeconomic level							
Low	4	16.7	3	6.8	7	10.3	0.202
Medium & high	20	83.3	41	93.2	61	89.7	

*p-value is significant at <0.05 level

The most common complaints among the studied patients were: epigastric pain (61 cases, 89.7%), heart burn (46 cases, 67.6%), dyspepsia (45 cases, 66.2%), diarrhea (12 cases, 17.6%) (Not presented in a Table).

There was a significant difference in age, sex dyspepsia, diarrhea and Diabetes Mellitus (DM) between positive and negative HP cases ($p=0.034$, $p=0.020$, $p=0.027$, $p=0.012$ and $p=0.026$, respectively).

Interestingly, all DM cases (8/8, 100%) in our setting had HP, while only 60% of the non diabetic patients (36/60) had HP.

Regarding associated medical conditions of the studied cases (Not presented in a table), 8 cases (11.8%) had DM, while 10 cases (14.7%) had hypertension. Most of our patients were non hypertensive and 67.2% (39/58) of them had HP while only 50% of hypertensive patients (5/10) had HP.

Diastolic blood pressure was higher in HP positive cases (94.5 \pm 17.62) than negative cases (76.4 \pm 7.53) but with no significant difference ($p=0.061$). On the other hand, there was a

significant difference ($p=0.000$) in the pulse rate between positive HP cases (80.4 ± 13.15) and negative cases (80.5 ± 9.00). Box blots for blood pressure and pulse rate for positive and negative HP cases are drawn in Figs. 1 and 2, respectively.

Endoscopic findings of our patients (Table 2), revealed that positive HP cases had mild gastritis in 84.1% of them, severe gastritis in 15.9%, duodenitis in 11.4% and reflux oesphagitis in 6.8%. In addition, there was a significant difference in gastritis ($p=0.003$) and duodenitis ($p=0.031$) between positive and negative cases for HP histopathology. Normal duodenum was present in 88.6% of positive HP cases, while there was no patient with normal gastric mucosa among them.

Table 2. Endoscopic, histopathological and HP serological findings of the studied patients

	Negative histopathology for HP		Positive histopathology for HP		Total		Test of significance p-value
	N=24	%	N=44	%	N=68	%	
Endoscopic findings							
Reflux oesphagitis							
No	24	100.0	41	93.2	65	95.6	0.191
Yes	0	0.0	3	6.8	3	4.4	
Gastritis							
Normal	5	20.8	0	0.0	5	7.4	0.003*
Mild	12	50.0	37	84.1	49	72.1	
Moderate	1	4.2	0	0.0	1	1.5	
Severe	6	25.0	7	15.9	13	19	
Duodenitis							
Normal	17	70.8	39	88.6	56	82.4	0.031*
Mild	7	29.2	3	6.8	10	14.7	
Severe	0	0.0	2	4.6	2	2.9	
Histopathological and HP serological data							
Gastritis							
Mild	17	70.8	25	56.8	42	61.8	0.027*
Moderate	5	20.8	7	15.9	12	17.6	
Severe	0	0.0	10	22.7	10	14.7	
Atrophic	0	0.0	2	4.5	2	2.9	
Specific	2	8.3	0	0.0	2	2.9	
Metaplasia							
Absent	23	95.8	44	100.0	67	98.5	0.173
Present	1	4.2	0	0.0	1	1.5	
IgG							
Negative	5	20.8	17	38.6	22	32.4	0.134
Positive	19	79.2	27	61.4	46	67.6	

*p-value is significant at <0.05 level

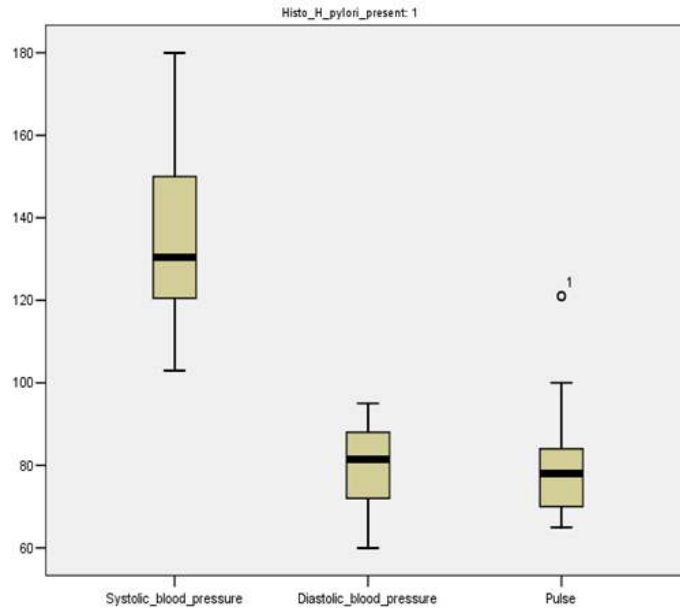


Fig. 1. Box blot for blood pressure and pulse rate for positive HP cases

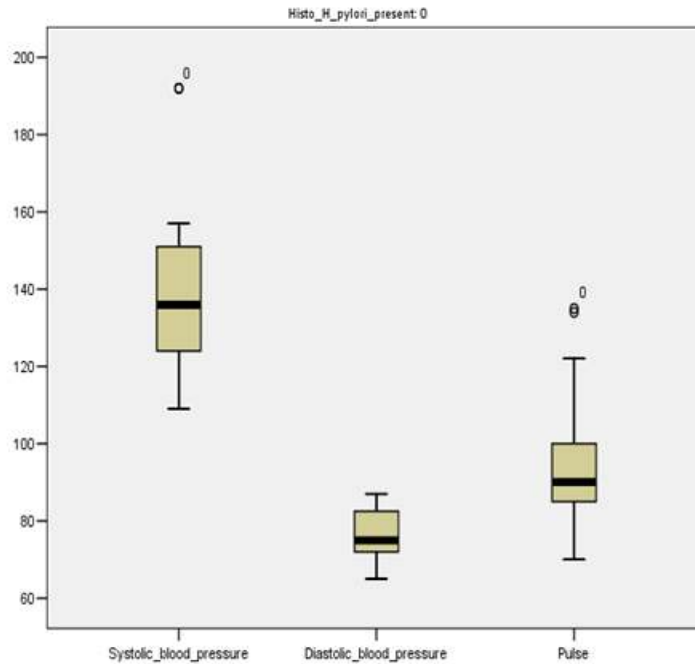


Fig. 2. Box blot for blood pressure and pulse rate for negative HP cases

Histopathological and serological findings of the studied patients are presented in Table 2. There was a significant difference in gastritis between positive and negative HP cases. The cases with the highest frequency being associated with mild gastritis.

None of our patients had dysplasia. All cases positive for HP were metaplasia and neoplasia negative. *H. pylori* IgG was positive in 61.4% of HP positive histopathology cases.

Data revealed the prevalence of HP in 64.7% (44/68 cases) of all studied patients histologically, 60.29% (41/68) by CLO and in 67.6% (46/68 cases) by serology.

The incidence of *H. pylori* IgG among healthy population was found to be 73.3% (11/15 cases), but without confirmation of HP presence as endoscopy could not be done for them.

Fig. 3 is photomicrograph of gastric biopsy specimen from one clinical case.

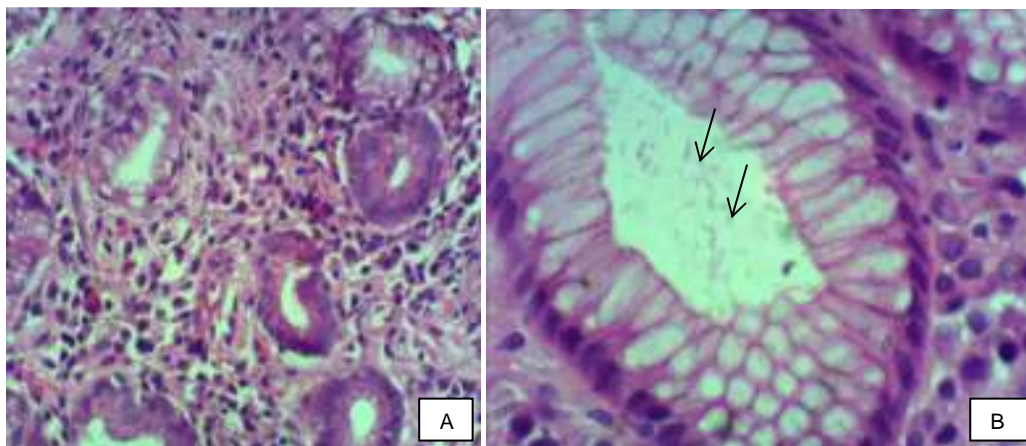


Fig. 3. Photomicrograph of gastric biopsy specimen of the antrum from patient no 36 (A) Moderate active gastritis, with no intestinal metaplasia or neoplasia. (B) Enlarged photomicrograph A, the arrows are pointing to HP.

All patients positive for HP were treated for 2 weeks with the triple therapy using a proton pump inhibitor (PPI) (esomeprazole 20 mg twice daily), amoxicillin (1 g twice daily), and clarithromycin (500 mg twice daily).

Only 40 patients were seen at follow up after 2 weeks. All but five reported improvement in their presenting symptoms during their follow up visits. The other five patients continued to have abdominal pain in spite of good compliance with the treatment and were subjected for further evaluation.

3.1 Discussion

Histological study is still considered by many to be the “gold standard” for HP diagnosis. Not only does it reliably identify infection, but it also can provide information on underlying gastric pathology [14]. We had three biopsies from each case because HP is not evenly distributed throughout the gastric mucosa, and, at least two biopsies need to be performed to sensitively diagnose infection [15]. A significant correlation between age and HP ($r=0.257$, $p=0.034$) was detected in this setting, whereas other studies [16,17] found no significant correlation between HP infection and age.

In the present study, HP incidence was much higher (47.7 %) in the younger age group (18 - < 30 years) meaning that it is acquired in a younger age. This is in accordance to the result

of Iman et al. [18] who reported a much higher incidence below 40 years than above 40 years. Morad et al. [19] revealed high frequencies of HP infection during the second, third, and fourth decades of life. Bakka et al. [20] detected the prevalence of *H. pylori* of 82% among patients < 45. On the other hand, Akbar and Eltahawy [16] observed a higher prevalence of positive HP infection (71%) among individuals between 35-75 years of age, when compared with frequency in other age groups.

HP was detected in 64.7% of gastric biopsies from our 68 patients with upper GI symptoms. Different HP detection rates in gastric biopsies (54.9%, [21]; 65%, [18]; 73.98%, [22]; 75.4%, [23]) were previously reported.

In the present work, *H. pylori* was significantly more prevalent in males than females regarding histopathology (Table 1). Several studies also reported that males were affected more than females [24-27]. Furthermore, de Martel and Parsonnet [28] showed a significant and homogeneous elevated risk of infection among adult men concordant with their elevated risk for HP related diseases.

Other studies reported a higher HP prevalence in females than males [16,18,29]; while no gender-related significant difference in the prevalence of HP infection in another studies [8,17,19,30-32].

HP infection among Saudi patients (36/52 cases, 69%) was found to be higher than non-Saudi (8/16 cases, 50%) similar to Morad et al. [19]. On the other hand, Akbar and Eltahawy [16] reported a significantly higher HP prevalence in the non-Saudi patients.

The prevalence of HP infection varies among countries and within a country [33]. The risk factors of HP infection confirmed that low socioeconomic status, crowded living conditions in childhood, low educational level of parents and unreliable well-water supply in the childhood household are associated with the infection [34].

In this work, there was no significant correlation detected between socioeconomic level and HP infection. This could be attributed to a higher admission rate of patients of medium socioeconomic level (89.7%).

Regarding other risk factors (Table 1), no significant difference was estimated between positive and negative HP cases in residency, work status, marital status, smoking, socioeconomic level and aspirin intake ($p=0.09$, $p=0.15$, $p=0.18$, $p=0.5$, $p=0.20$ and $p=0.88$ respectively). In accordance, Bakka et al. [20] observed no significant difference in the prevalence of HP among patients and marital status, residency and smoking.

A significant difference in DM between positive and negative HP cases ($p=0.026$) have been detected in this work.

The relationship between DM and HP infection is controversial. According to some studies, there is a high prevalence of HP infection in patients with either Type 1 or Type 2 DM which is correlated with the duration of DM, the presence of dyspeptic symptoms, cardiovascular autonomic neuropathy, age, gender, BMI, blood pressure, fasting glucose, and the glycated hemoglobin levels. In contrast, other studies showed that HP infection is not associated with DM, as the microvascular changes in the gastric mucosa may create an unfavorable environment for the establishment or survival of HP [35-40].

The clinical significance of HP in upper GIT disorders has been confirmed [41,42]. Salih et al. [43] reported a high HP density and the associated genotypes affect the severity of the gastric histology and the disease outcome; and that eradication of HP resulted in a histological improvement of the gastric mucosa. Moreover, Lawal et al. [29] and Kandulski et al. [44] suggested the possible role of the HP infection in dyspeptic conditions.

HP prevalence among the selected dyspeptic patients was 55.6%; which is comparable to Akbar and Eltahawy [16].

HP seems to be a health problem in the neighboring regions of Saudi Arabia. The prevalence of HP among dyspeptic patients differs among different countries. For instance, 86% in Jordan [45]; between 81% and 96.6% in Kuwait [46,47] and 82.2% in Yemen [48]. Difference in HP prevalence among dyspeptic patients was also observed among other countries. It ranged from 33%-95% [49-56].

90.9% of the studied cases were suffering from epigastric pain. HP infection was more common (71.4%) in patients suffering from epigastric pain [9,57]. Saadah [57] added that the presence of epigastric pain, especially if associated with dyspeptic symptoms is more associated with abnormal gastric pathology.

Antral mucosa is the main target for HP colonization [58]. Regarding endoscopic finding, HP infection was present in 64.7% among gastritis and 7.3% among duodenitis. The frequency of HP infection among those with endoscopic diagnosis of gastritis ranged from 60-80% [21,29,50,56,59-61].

Gastritis occurs due to the inflammation of the gastric mucosa that can be caused by HP [62,63]. Blaser [64] reported that chronic active gastritis is mainly induced by HP. We have discovered a significant difference ($p=0.027$) between positive HP histopathology and grading of gastritis was discovered similar to Akbar and Eltahawy [16]. In healthy population, HP seroprevalence ranges from 25% in developed countries up to 80% in developing countries [65]. In this setting, it was 73.3%. This result is comparable to an earlier study by Al-Moagel et al. [8], while lower than study of Khan and Ghazi [66]. Other studies from Libya [20], Nigeria [67], Tunis [68] and Egypt [69] detected anti-*H. pylori* antibodies in 76%, 80%, 83% and 87.6% of asymptomatic subjects, respectively. We must mention that our small control number (15 healthy population) should be reported as a limitation in this study. In addition, the HP seroprevalence among those individuals was mostly an indication of past HP exposure not an active infection.

Various diagnostic tests for HP infection may have false negative results, and the use of multiple tests may help to provide a more accurate diagnosis of HP infection [70]. That is the reason for using three different diagnostic HP tests. The prevalence of HP in patients included in the present study was 64.7% by histology and 67.6% by serology. Similarly, Marie [9] reported a prevalence of 67% by serology *H. pylori* IgG among outpatients suffering from GIT symptoms. In addition, Akbar and Eltahawy [16] detected HP in 70% by histology and 78% by ELISA.

A negative HP serology test whereas biopsy sampling is simultaneously positive, may be due to the presence of a recent infection; or the presence of IgA against helicobacter instead of IgG in 2% of total cases [71]. A positive HP serology test whereas biopsy sampling is simultaneously negative, may be due to incorrect sampling from gastric mucosal tissue

during endoscopy procedure which can decrease the rate of HP identification and isolation [72].

In our setting, sensitivity of IgG was only 61.4% compared to the histopathology. Monteiro et al. [73] found no statistically significant difference between the invasive tests and the non-invasive tests in the diagnosis of HP infection. Laheij and his associates [74] also reported similar sensitivity and specificity ELISA results compared to the biopsy-based tests in detecting HP infection in dyspeptic patients. This discrepancy may be due to antigens used in the ELISA kits. The sensitivity and specificity of an assay in a particular population may not necessarily be applied to another [72].

In western countries, ELISA kits have been shown to have high sensitivities and specificities (80-95%) [75], whereas in developing countries, commercial ELISA kits have low specificities [76-78]. Individuals vary considerably in their antibody responses to HP antigens, and no single antigen is recognized by sera from all subjects. The accuracy of serological tests therefore depends on the antigens used in the tests making it essential that HP ELISA is locally validated [16].

The vast molecular heterogeneity among HP strains isolated from different geographic regions worldwide necessitates the use of native antigens for such serological assays to avoid false results [79,80].

A significant molecular heterogeneity is existing among Iranian and western HP strains in some bacterial conserved and nonconserved genes was reported [81,82]. Similarly, in Saudi Arabia, the molecular heterogeneity existing between Saudi and non-Saudi and may be between Saudi themselves. This emphasizes the need for use of native (local) antigens in developing ELISA assay for detecting seroreactivity toward this bacteria. Perez-Perez et al. [76] and Bodhidatta et al. [83] reported a higher sensitivity and specificity when using the antigen prepared from local HP isolates than the commercial ELISA re-agent kits. Talebkhan et al. [72] suggested that the cut off values of the imported kits should be refined for user country due to differences in the vast geographic heterogeneity among HP antigens, in order to obtain the highest sensitivity and specificity. Furthermore, they concluded that home made ELISA kit can be substituted for imported ELISA kits due to its valid serological criteria.

4. CONCLUSION

In the present study, HP-infected patients with upper GI symptoms had more abnormal endoscopic and pathological findings than those without HP infection, which might be related to gastric mucosal inflammation.

The lower sensitivity and specificity of *H. pylori* IgG ELISA compared to histopathology after endoscopy, emphasizes the need to locally consider another non-invasive test as urea breath test, immunoblot or antigen stool detection. The non invasive nature of HP IgG ELISA, practicality, low cost and easy applications, are critical characteristics of it making it more accepted than other approaches among clinicians and laboratory staff. This pushes us to recommend shifting to home made ELISA kit to substitute the imported ELISA kits in order to obtain the highest sensitivity and specificity.

CONSENT

Written informed consent was obtained from the patients before entering the study.

ETHICAL APPROVAL

Ethical committee of the king Fahd hospital and the scientific committee of Taibah University approved the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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