Prevalence and factors associated with H. pylori infection in Saudi patients with dyspepsia

Mohammed Akeel[1], Erwa Elmakki[2], Atef Shehata[3,4], Ahmed Elhafey[5,6], Thanaa Aboshouk[7], Hussein Ageely[2], Mohammed Salih Mahfouz[8]

[1] Department of Anatomy, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia
[2] Department of Internal Medicine, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia
[3] Department of Microbiology and Immunology, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia
[4] Department of Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[5] Department of Pathology, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia
[6] Department of Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[7] Department of Clinical Biochemistry, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia
[8] Associate Professor, Department of Family and Community Medicine, Faculty of Medicine, Jazan University, Jazan, Kingdom of Saudi Arabia

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Abstract
Background: Helicobacter pylori (H. pylori) is a major cause of peptic ulcer disease (PUD) and chronic active gastritis that may progress to gastric cancer. Globally, it has been estimated that 50% or more of the world's population is infected by H. pylori, making it the most widespread infection across the globe.
Objectives: To determine the prevalence of H. pylori infection and to identify factors associated with H. pylori infection in Saudi patients presenting with dyspepsia.
Methods: In this prospective cross-sectional study, a total of 404 gastric biopsies were endoscopically obtained from 404 patients with dyspepsia from September 2014 to April 2016 (Jazan Province, Saudi Arabia). The specimens were analyzed using the real-time polymerase chain reaction (PCR). The data was examined using descriptive statistics as well as determining the prevalence, and employing Chi square and Fisher exact test. A p-value of ≤0.05 was considered statistically significant in examining the research hypotheses.
Results: The overall prevalence of H. pylori in Jazan Province was 46.5% (95% CI: 41.7-51.4) and the prevalence was lower among those > 55 years old. Prevalence was higher among urban (50.0%; 95% CI: 43.1-56.8) versus rural (42.1%; 95% CI: 35.1-49.3), but with no significant difference. Prevalence did not show significant difference among different Body Mass Index (BMI) categories, ranging from 40.2% to 47.7%. The prevalence of H. pylori in females was 47.1% (95% CI: 40.4-53.9) versus 45.6% (95% CI: 38.7-52.6) in males. Histopathology findings were associated with H. pylori infection with prevalence of 58.1% among patients with chronic active gastritis, compared to 24.1% and 34.8% among mild and chronic gastritis, respectively.
Conclusion: Our results indicate that there is a high prevalence of H. pylori among Saudi patients with dyspepsia. Prevalence of H. pylori was high in ages below 55 years. Chronic active gastritis was significantly associated with H. pylori infection. In depth studies are needed to determine associated factors with of H pylori infection in the region
Keywords: H. pylori, PCR, Prevalence, Jazan province

1. Introduction
H. pylori (HP) is spiral-shaped gram-negative bacterium, known to colonize, mainly the antral portion of the human gastric mucosa. H. pylori infection is correlated with the development of chronic active gastritis, peptic ulcer disease; mucosa associated lymphoid tissue lymphoma (MALT) and gastric adenocarcinoma (1, 2). Globally, it has been estimated that 50% or more of the world's population is infected by H. pylori, making it the most widespread infection across the globe (1, 2). Actual infection rates vary from one country to another however, the developing world has much higher infection rates than the developed one (3). On the other hand, up to 85% of individuals infected with H. pylori are asymptomatic and have no complications (4). H. pylori infection has been reported to be hyper- endemic in Saudi Arabia. Some studies on H. pylori in Saudi Arabia have shown a high prevalence in various age groups of patients, including individuals with non-ulcer dyspepsia (5). More recent studies on H. pylori infection among Saudi children had shown high infection prevalence among Saudi children in the cities of Jeddah and Riyadh (6). The World Health Organization (WHO) considers H. pylori as a carcinogen (7). It has been reported that infection with H. pylori accounts for 75% of non-cardia gastric malignancy worldwide (8). The H. pylori-induced gastritis can lead to atrophic gastritis. Atrophic gastritis in turn may progress to intestinal metaplasia, dysplasia and neoplasia, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (1, 9, 10). Although there are many studies on H. pylori prevalence conducted in different regions of Saudi Arabia (5, 6, 11-15), few studies have been conducted in Jazan province dealing with H. pylori infection (14). The objectives of this study were to evaluate the prevalence of H. pylori infection in patients presenting with dyspepsia and to identify factors associated with H. pylori infection in Jazan province, south west Saudi Arabia.

2. Material and Methods
2.1. Setting, sampling, and selection criteria
A prospective cross-sectional study was conducted in Jazan province during the period from September 2014 to April 2016. A sample size of 440 was calculated to conduct this study building on prevalence of H. pylori in Jazan 60% (14), 95 % confidence interval, error not more than 5% and nonresponse rate of 15%. The main inclusion criterion in this study was patients 12 years and above with dyspepsia who were willing to participate in the study, while those who received proton pump inhibitors (PPI) two weeks before endoscopy were excluded from the study. Gastric biopsies were collected from all study participants, using upper gastrointestinal endoscopy during the study period at different hospitals in Jazan province.

2.2. Measures
2.2.1. Checklist
In addition to clinical data, the study collected information related to H. pylori from all participants. Information involved age, gender, and upper gastrointestinal symptoms, symptoms related to other systems, level of education, occupation, living standard, history of smoking, khat use, alcohol intake, Body Mass Index (BMI), past medical history and drug history. Trained nurses were responsible for collecting such information; some information was filled using the patients' medical records. The BMI was categorized according to World Health Organization (WHO) guidelines. These guidelines suggest that individuals with (BMI<16.0 kg/m^2) are severely underweight, (BMI=16.0-18.4 kg/m^2) as underweight, (BMI=18.5-24.9 kg/m^2) as normal weight, (BMI=25.0-30.0 kg/m^2) as overweight, and (BMI ≥30.0 kg/m^2) as obese.
2.2.2. Histopathological examination
A total of 404 gastric biopsies were examined using the routine hematoxylin and eosin (H&E) stain to study histopathological changes.
2.2.3. Detection of H. pylori using real time PCR
All gastric biopsies obtained from the study population were submitted for DNA extraction using the DNeasy blood & tissue kit (Qiagen). All extracted DNA samples were tested for H. pylori by real time PCR amplification using primer-probe based "genesig Quantification of Helicobacter pylori" kit (Primerdesign Ltd., UK). The 20 µl reaction mixture consisted of 3 µl of the extracted DNA, 10 µl of "oasig™ 2× qPCRMastermix" (Primerdesign Ltd.), 1 µl H. pylori specific primer/probe mix, 1 µl internal control primer/probe mix, 2 µl of internal control DNA, and 3 µl RNase/DNase free water supplied with the kit. The reactions were performed using the SmartCycler (Cepheid, Italy). Each PCR run contained positive control (H. pylori DNA supplied with the kit) and negative control (RNase/DNase free water instead of template DNA) reactions. The PCR cycling conditions were according to manufacturer's protocol.

2.3. Data management and statistical analysis
Data were analyzed by IBM© SPSS© Statistics version 20 (IBM© Corp., Armonk, NY, USA), using descriptive statistics, prevalence, Chi square and Fisher exact test. The Yates correction term was also used to ensure the accuracy of the Chi square test. P-values less than 0.05 were used to indicate statistical significance.

2.4. Ethical Considerations
The study was approved by the research ethics committee (REC) at Faculty of Medicine - Jazan University (Ref: FMRERC-2012). Written consent was obtained from all the participants prior to enrollment. Purpose, potential risk and benefits of the study were communicated in the Arabic language, and consent was documented for all study participants.

3. Results
The response rate was 91.8 % (404 out of 440) patients. Table 1 provides background characteristics of the study participants including age, gender, education, etc. The table showed that most participants (39.9%) were (25-39) years old, (33.9%) were housewives, and (50.5%) lived in rural areas and 20.5 % were smokers. Underweight patients accounted for 4.2 %, and obese patients were 21.8% of the group. The mean weight for males (71.7 kg) and females (67.5 kg) showed significant differences (p<0.05). The mean BMI for all study participants was 27.4 (kg/m²) with no significant difference between males and females (Table 1). Table 2 shows H. pylori among the sample of patients. The overall prevalence of H. pylori in Jazan Province was (46.5 %; 95 % CI, 41.7-51.4) and lower among those >55 years old. Prevalence was higher among urban (50.0%) (95% CI: 43.1-56.8) versus rural (42.1%) (95% CI: 35.1-49.3); but with no significant difference. Prevalence according to BMI did not show significant difference among different BMI categories ranging from 40.2% to 47.7%. H. pylori according to gender showed prevalence among males (45.6%) (95% CI: 38.7-52.6) versus females (47.1%) (95% CI: 40.4-53.9). Table 3 presents factors that may be associated with H. pylori infection among the study population. According to the table, age was significantly associated with H. pylori infection. The prevalence of H. pylori infection is declined by increase in age, the prevalence among age group (13-29) years is 53.3%, 47.0% among the age group (30-49) years and 36.4% among the population 50 years and above. Histopathology findings were also associated with H. pylori infection with a prevalence of 58.1% among patients with chronic active gastritis, compared to 24.1% and 34.8% among mild and chronic gastritis, respectively.

Table 1. Socio-demographic description of the studied patients

Characteristics		n	%
Age groups (year)	13-24	58	14.4
	25-39	161	39.9
	40-54	142	35.1
	≥55	35	8.7
	Not stated	8	2.0
Gender	Male	194	48.0
	Female	207	51.2
	Not Stated	3	0.7
Place of Residence	Urban	185	45.8
	Rural	204	50.5
	Not Stated	15	3.7
Smoking	Yes	83	20.5
	No	313	77.5
	Not Stated	8	2.0
Khat chewing	Yes	87	21.5
	No	308	76.2
	Not Stated	9	2.2
BMI Categories	Underweight	17	4.2
	Normal weight	90	22.3
	Overweight	88	21.8
	Obese	88	21.8
	Not stated	121	30.0
Total		404	100

Table 2. Prevalence of H. pylori among sample of patients

Characteristics		Prevalence	95% CI	p-value
Age groups (year)	13-24	50.0	37.5-62.5	0.014
	25-39	49.7	42.0-57.3	
	40-54	50.0	40.8-60.1	
	≥55	29.5	20.5-40.4	
Gender	Male	45.6	38.7-52.6	0.765
	Female	47.1	40.4-53.9	
Mode of living	Urban	50.0	43.1-56.8	0.787
	Rural	42.1	35.1-49.3	
BMI Category	Underweight	47.1	26.0-96.2	0.762
	Normal weight	42.2	32.5-52.6	
	Overweight	47.7	37.6-58.0	
	Obese	40.2	30.5-50.7	
Educational level	Illiterate	35.5	25.6-46.7	0.050
	Primary	59.1	47.0-70.1	
	Intermediate	43.9	34.8-53.4	
	Secondary	52.7	39.4-65.3	
	University and above	44.3	33.8-55.3	
Occupational status	Housewife	48.5	40.2-56.8	0.117
	Employee- Private sector	40.9	29.8-52.9	
	Employee- Public sector	53.3	43.1-63.1	
	Laborer	50.0	26.5-73.4	
	Others	37.8	27.6-49.2	
Smoking	Active Smokers	42.2	32.1-52.9	0.408
	Non-Smokers	47.3	41.8-52.8	
Khat chewing	Khat chewers	46.0	35.9-56.4	0.422
	Non Khat chewers	46.4	40.8-52.0	
Overall prevalence		46.5	41.7-51.4	

Table 3. Factors associated with H. pylori infection

Characteristics		Positive for HP	Negative for HP	p-value
Gender	Male	88 (45.6)	105 (54.4)	0.765
	Female	97 (47.1)	109 (52.9)	
Age groups (year)	13-29	64 (53.3)	56 (46.7)	0.034
	30-49	77 (47.0)	87 (53.0)	
	50+	40 (36.4)	70 (63.6)	
Living standard	High	14 (51.9)	13 (48.1)	0.751
	Medium	134 (47.2)	150 (52.8)	
	Law	31 (43.7)	40 (56.3)	
Endoscopic findings	Normal	26 (49.1)	27 (50.9)	0.791
	Gastritis	114 (44.0)	145 (56.0)	
	Gastric ulcer	14 (45.2)	17 (54.8)	
	Duodenal ulcer	8 (47.1)	9 (52.9)	
Histopathology findings	Mild Chronic gastritis	7 (24.1)	22 (75.9)	0.000
	Moderate chronic gastritis	46 (34.8)	86 (65.2)	
	Severe Gastritis	1 (100.0)	0 (0.0)	
	Chronic Active Gastritis	122 (58.1)	88 (41.9)	

4. Discussion
The overall prevalence of H. pylori among the studied patients was 46.5%, based on real-time PCR. This is in agreement with what was reported in past literature. Ayoola et al. reported a prevalence of 54.9% among Saudi patients with dyspepsia in the Jazan region of Saudi Arabia in 2004 (14). Another two large studies on Saudi patients have reported prevalence rates of 28% and 70%, with an average of approximately 50% (15, 16). Alazmi et

al. reported that the prevalence of H. pylori infection in Kuwaiti patients with dyspepsia was 49.7% (16). Furthermore, several studies from the Middle East have demonstrated that prevalence ranges between 44 %, and 49% (17, 18). In many studies worldwide (United States, Brazil and China), the prevalence of H. pylori among subjects with dyspepsia was 28.9%, 57%, and 84% respectively (19-21). These variations in prevalence rates of H. pylori in different studies across the world might be attributed to different contributing factors including socioeconomic status, living standards, ethnicity and geographical location (22). In addition to the variability in the methods of H. pylori detection, size of the study and exclusion of prior antibiotic use, all these can play roles in these variations. In the present study, the intention was to exclude antibiotics use, but their usage cannot be guaranteed. Despite the lower prevalence of H. pylori infection in developed countries, there are higher rates of gastric carcinoma, contrary to that of developing counties (23). The highest rate of H. pylori infection in this study (53.3%) was seen in the age group between 13-29 years, 47% among the age group between 30-49 years and 36.4% in participants equal to or more than 50 years of age. Many studies have shown the decreased rate of H. pylori infection with an increase in age (22, 24). In contrast, other studies have demonstrated positive correlation between H. pylori prevalence rates and increase in age (25, 26).

In terms of gender, our findings indicated that, there were no statistically significant differences in the prevalence of H. pylori (45.6% in males and 47.1% in females). Some authors reported a high prevalence of H. pylori among females (25). While other authors reported a high rate of H. pylori infection among males, a large number of studies showed no gender differences (24, 27, 28). A recent meta-analysis study conducted by Zamani, et al in 2018 that involved 183 studies from 73 countries in six continents revealed that although males were predominant of H. pylori infection across all continents, none of the differences reached statistical significance. The issue of gender disparity in H. pylori infection is an intriguing topic and further research is needed to understand the mechanisms by which sex may influence the acquisition and/or persistence of infection (29). In contrast to other reports, our results showed no significant differences in the prevalence rates of H. pylori among different BMI categories (42.2% in normal weight, 47.7% in overweight, and 40.2% in obese participants). Lender et al. reported an inverse correlation between H. pylori prevalence and the rate of overweight/obesity (30). However, other authors demonstrated positive correlation between H. pylori prevalence rates and overweight/obesity (31). Therefore, the evidence of the role of H. pylori infection in human obesity is inconclusive and controversial (32).

In the present study, we found no difference in the rate of spread of H. pylori in smokers versus non-smokers, (42.2% and 47.3% respectively). This is in agreement with Khalifa et al. who found no statistically significant difference in H. pylori positivity between smokers and non-smokers (33). It should be mentioned that some arguments about the negative association between smoking and H. pylori infection suggest that the elevated acid and pepsin secretion caused by smoking protects the gastric mucosa from H. pylori infection (34). According to Hassan et al., khat chewing is positively associated with gastritis (35). Many epidemiological studies have suggested that the habit of khat chewing is deeply rooted among the Jazan population, and the current prevalence rate of khat chewing is high at (28.7%) (36). In the current study, the prevalence rate among khat chewers was 46%, whereas it was 46.4% in non-khat chewers. Almakdad et al. reported a similar result. He found no relation between khat chewing and high prevalence of H. pylori in Yemeni patients (37). In the current study, the most common endoscopic findings among the H. pylori positive subjects were gastritis (44%), duodenal ulcer (DU) (52.9%) and gastric ulcer (GU) (45.2%), whereas, in H. pylori negative subjects, the rates of gastritis, DU and GU were 56.6 %, 54.8 % and 52.9 % respectively. So we did not find association between these endoscopic findings and H. pylori. This is in disagreement with Mohammed et al. and Ayana et al. who found significant correlation between endoscopic findings and H. pylori (38, 39). However, some authors reported no or poor correlation between endoscopic findings and histological diagnosis (40, 41). These differences may be attributed to other contributing factors including socioeconomic status, living standards, ethnicity and geographical location. In the present study, the most common histopathological finding was chronic active gastritis (CAG), which was found in 58.1% of H. pylori positive subjects, compared to 41.9% of H. pylori- negative ones. In the current study, CAG was significantly associated with H. pylori, and is a good suggestion for the causative role of H. pylori in chronic active gastritis. Many clinical studies reported that H. pylori is significantly associated with CAG, atrophic gastritis, intestinal metaplasia and gastric cancer (42, 43).

5. Study limitation
The present study has some limitations; first, the study sample may not be representative of the population of Jazan, since the sampling technique was a purposive sampling without randomization. Second, a cross-sectional study design is not suitable for assessing risk factors for H. pylori. Third, no controls were included as the study relied on

the invasive method of H. pylori detection. Despite these limitations, the study provided updated information on the status of H. pylori in Jazan province.

6. Conclusions

In conclusion, the prevalence of H. pylori among patients with dyspepsia in the Jazan region is high, especially in age groups below 55 years. No correlation was found between endoscopic findings and H. pylori positivity. Chronic active gastritis was significantly associated with H. pylori. The results of this study provide important implications for public health strategies for the prevention of H. pylori infection in the Jazan region. In depth studies are needed to determine the factors associated with H. pylori infection in the region.

Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
MA, EE, AS, ASE, HA and TA conceptualized and designed the research project, and carried out the practical work. MSM performed the statistical analysis and finalized the manuscript. All authors provided significant input in the manuscript, and read and approved the final version of it.

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