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Helicobacter pylori Infection: Seroprevalence and Detection of *H. Pylori* IgG by Using ELISA

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Abstract: Background: *Helicobacter pylori (H. pylori)*, one of the most common bacterial pathogens of humans, colonizes the gastric mucosa. *H. pylori* is a major factor in inflammatory and malignant diseases of the gastrointestinal tract, where it appears to persist throughout the host's life unless the patient is treated. Materials and methods: A study population was carried out through 100 patients who had gastrointestinal symptoms in Hail region. Seroprevalence of *Helicobacter pylori* was carried out by detection of *H. pylori* IgG in patient serum by using Enzyme Linked Immunosorbant Assay (ELISA). Results: Among the patients, *H. pylori* positivity percentage was found to be (57%) (57/100) in our study. It was found that, the average age of the patient was (one to 70 years old), 68% of them were male and 32% female, the majority came from Hail city. Conclusion: high *H.pylori* positivity ration (57%), that obtained from these study indicates that *H.pylori* infection is still a common problem among people in Hail region-Saudi Arabia. The titer of IgG antibody to *H.pylori* in patient serum can be used as non-invasive tests for the presence of gastric *H.pylori* infection and gastritis.

Keywords: *Helicobacter pylori*, IgG, Seroprevalence, ELISA

1. Introduction

H. pylori (previously Campylobacter pylori) causes infections in humans worldwide. *H. pylori* is a microaerophilic gram negative curved bacillus with a terminal flagellum that is found in the mucous layer lining the gastric epithelium.(8)

Various studies have indicated that the presence of *H. pylori* is strongly associated with chronic (Type B) gastritis. *H. pylori* colonization is usually chronic in nature. If the organisms are eradicated, the histological inflammation improves. When the organisms reappear inflammatory changes recur. The presence of *H. pylori* has also been associated with gastric and duodenal ulcers. (7) Humans are the known major host. The majority of the infected are asymptomatic, but a limited number develop digestive symptoms. About 30% to 60% of duodenal ulcers and 70% of gastric ulcers are associated with *H. pylori* infection that can eventually lead to gastric adenoma and lymphoma (9).

In these populations, colonization occurs at very young ages. Evidences show that infection at a young age is a risk factor for gastric cancer later in life. Transmission of *H. pylori* occurs from person to person through fecal-oral and oral-oral route, as well as through consumption of contaminated water. Risk factors for *H. pylori* infection include birth or residence in developing countries, low socioeconomic and health status, improper.(10)

H. pylori express lipopolysaccharides and flagellin that do not activate efficiently Toll-like receptors and express dedicated effectors, such as γ-glutamyl transpeptidase, vacuolating cytotoxin (vacA), arginase, that actively induce tolerogenic signals. In this perspective, H. pylori can be considered as a commensal bacteria belonging to the stomach microbiota. However, when present in the stomach, H. pylori reduce the overall diversity of the gastric microbiota and promote gastric inflammation by inducing Nod1-dependent pro-inflammatory program and by activating neutrophils through the production of a neutrophil activating protein. The maintenance of a chronic inflammation in the gastric mucosa and the direct action of virulence factors (vacA and cytotoxin-associated gene A) confer pro-carcinogenic activities to H. pylori.(5)

The presence of *H. pylori* specific IgG antibodies in human serum has been shown to be associated with past or present *H. pylori* colonization.(1,2). *H.pylori* IgG Enzyme-Linked Immuno Sorbent Assays (ELISA) is intended for the detection and qualitative determination of IgG antibodies to *Helicobacter pylori* in human serum. (4). These study was aimed to estimate the incidence of *H. pylori* in Hail region and detection of H.pylori IgG titer in patient serum by using ELISA Assay.

2. Materials and Methods

2.1. Study Population

The study population was 100 patients who had Gastrointestinal symptoms at King Khalid Hospital and Maternity hospital in Hail city, Saudi Arabia, from October 2013 to February 2014. The age of patients was average from one to 70 years old, all of these patients were of the local population or were workers who have been living in the local community of Hail City for more than one year. Patients who had received antibiotics, bismuth, proton pump inhibitors (PPI) or had gastroscopy in the previous 2 months were excluded from these study. Since the st

dy population were patients, the purpose and procedures of the study were explained written informed consent was obtained from all patients before the procedure.

2.2. Demographic Data and Medical History

Demographic data, previous history of coffee drinking, water drinking, NSAIDs use, and gastrointestinal disturbances were obtained by direct interview and filling questionnaires.

2.3. Questionnaires

The questionnaires included questions on Age, gender, Family income, crowded family living, occupation and educational level, the presence of gastric symptoms, family history and hygienic behaviors. The written approvals were collected and face-to-face interviews were conducted with the participating patients, during which the questionnaire form was filled out. The data was obtained through the questionnaire after receiving the necessary permission from the institution, the data collection process began.

The questions on the questionnaire elicited the following information:

- The individual characteristics of the patients including their background (age, place and region of birth, the longest lived place and the region, place of accommodation, income level of patients and number of family members) were recorded.
- Whether the patients had experienced any gastrointestinal problems for the last one month.
- What Patients habits were including, cigarette smoking, consumption of drinks such as coke, tea, coffee and use of analgesic and anti-rheumatism drugs. Patients were also questioned about the source of their drinking water.
- Patients were asked about, whether they apply simple hygiene.
- Patients were asked whether they or their family members had any previous gastrointestinal diseases, symptoms and helicobacter positivity.
- The result of the *helicobacter* test applied to patients, was recorded.

Patients were asked whether they had experienced any gastrointestinal problems to research the association between current gastrointestinal symptoms and *H .pylori* positivity, in addition to, we questioned patients about their previous gastrointestinal symptoms.

2.4. Statistical Analysis

Statistical analysis was performed by using SPSS for Windows version 12.0 (SPSS INC., Chicago, III., USA). Data was presented as mean ± SD. Chi-square analysis μ^2 was used in findings on comparison of Helicobacter positivity according to individual characteristics. Evaluation was carried out at the 95-99% confidence interval and $P< 0.05$ was considered statistically significant.

2.5. Detection of IgG in Patient Serum by Using Elisa

Five milliliters of blood was drawn from each case, were collected from hospital of King Khalid, samples were centrifuged for separation of serum. Levels of IgG were measured by the ELISA method by using the Q-1DIAPLUS kit manufactured by United Diagnostic industry. REF EG125. Micro titration plate reader capable of absorbance measurement at 450 nm Levels above 10 U/mL were considered positive. Data were analyzed using SPSS (version 13; SPSS, Chicago, IL, USA). For univariate analysis the odds ratios, their 95% confidence intervals, and chi-square tests were used (9).

3. Results

During October 2013 to February 2014, 100 patients were enrolled in this study. Among these patients, *H. pylori* positivity percentage was found to be (57%) (57/100) in our study. It was found that, the average age of the patient was (one to 70 years old), 68% of them were male and 32% female, the majority came from Hail city, as shown in Table (1).

Table (1). *Comparison of demographic characteristics and Helicobacter positivity.*

Demographic characteristics		Helicobacter + (n=57) N0.(%) [1)]	Helicobacter – (n= 43) N0.(%)[2)]	χ^2	P*
Age	1-10	2(3.5%)	4(9.3%)	3.667	0.722
	11-20	8(14.03%)	7(16.3%)		
	21-30	25(43.9%)	20(46.5%)		
	31-40	7(12.3%)	5(11.6%)		
	41-50	7(12.3%)	5(11.6%)		
	50-60	4(7.02%)	1(2.3%)		
	61-70	4(7.02%)	1(2.3%)		
Gender	Male	39(68%)	5(11%)	32.084	0.00001
	Female	18(32%)	38(88%)		
Family situation	Unmarried	26(46%)	11(26%)	4.22	0.040
	married	31(54%)	32(74%)		
Occupation	Job	45(79%)	34(79.1%)	0.0002	0.988
	No job	12(21%)	9(20.9%)		
Educational level	Primary	12(21.1%)	29(67.4%)	21.978	0.00002
	high school	32(56.1%)	9(20.9%)		
	university	13(22.8%)	5(11.6%)		

1) Percentage in *H.pylori* (+)s.
2) Percentage in *H.pylori* (-)s.
*Percentage statistically significant difference (P>0.05).

3.1. Comparison of Hygiene Applications and Helicobacter Positivity

Among the participant patients there were 44 eating meals with their hands without using of fork, 17 wash their hands before eating, 25 wash their hands after toilet, 27 share forks and spoon with other during eating,13 eat unwashed vegetables and fruits and 28 shared their beds with others, as shown in Table (2).

Table (2). *Comparison of hygiene applications and Helicobacter positivity.*

Some Hygiene application[1)]	Helicobacter + (n=57) no. (%)[2)]	Helicobacter – (n= 43) no. (%)[3)]	χ^2	P*
I like eating meals with my hands without using fork and spoon.	44(77.1%)	20(46.5%)	10.014	0.002
I wash my hands before the meals.	17(29%)	40(93.0%)	39.941	0.00001
I wash my hands after toilet.	25(43%)	41(95.3%)	28.957	0.00001
I share materials such as fork, spoon and knife with someone else during the meals.	27(47.3%)	10(23.2%)	6.114	0.013
I eat vegetables and fruit without washing them	13(22%)	0(0%)	11.272	0.001
I share my bed with someone else.	28(49.1%)	36(83.7)	12.734	0.0004

1) Ones who answered yes in hygiene applications were considered.
2) Percentage in *H.pylori* (+)s.
3) Percentage in *H.pylori* (-)s.
*Percentage statistically significant difference (P>0.05).

3.2. Comparison of Knowledgement about Helicobacter Infection and Helicobacter Positivity

The obtained data revealed that there are 50 participants know about *H.pylori* infection, while 45 patients know symptoms of *H.pylori* infection and 50 patients know about method of infection, as shown in Table (3).

Table (3). *Comparison of Knowledgement about Helicobacter Infection and Helicobacter Positivity*

Knowledgement about Helicobacter infection[1)]		Helicobacter + (n=57) no. (%)[2)]	Helicobacter – (n= 43) no. (%)[3)]	χ^2	P*
I know about *Helicobacter* Infection		50(87.7%)	12(27.9%)	37.218	0.00001
I Know about the symptoms of *Helicobacter* infection		45(78.9%)	10(23.2%)	30.715	0.00001
My knowledgment about Helicobacter infection from	Journal	16(28.1%)	6(13.9%)	2.865	0.239
	Physician	25(43.9%)	22(51.2%)		
	T.V.	16(28.1%)	15(34.9)		
I Know about method of Helicobacter infection		50(87.7%)	22(51.1%)	16.247	0.0001

[1)]Ones who answered yes in knowledgment about *Helicobacter* infection were considered.
[2)]Percentage in *H.pylori* (+)s.
[3)]Percentage in *H.pylori* (-)s.
*Percentage statistically significant difference (P>0.05).

3.3. Comparison of Knowledgement about Symptoms of Helicobacter Infection and Helicobacter Positivity

Collected data detected 35 patients suffer from stomachache immediately after meals, while 45 of them suffer from sour stomach and 39 patients defecate bloody stool, as shown in Table(4).

Table (4). *Comparison of Knowledgement about Symptoms of Helicobacter Infection and Helicobacter Positivity.*

Gastrointestinal symptoms developed[1)]	Helicobacter + (n=57) no. (%)[2)]	Helicobacter – (n= 43) no. (%)[3)]	χ^2	P*
Stomachache immediately after meals	35(61.4%)	12(27.9%)	11.04	0.001
Sour stomach, burning	45(78.9%)	10(23.2%)	30.713	0.00001
Bloody and black stool	39(68.4%)	2(4.7%)	41.204	0.00001

[1)]Ones who answered yes in Knowledgement about symptoms of *Helicobacter* infection were considered.
[2)]Percentage in *H.pylori* (+)s.
[3)]Percentage in *H.pylori* (-)s.
*Percentage statistically significant difference (P>0.05).

3.4. Comparison of Serological Test for Detection of Helicobacter and Helicobacter Positivity

Data obtained from these study revealed that about 50 patients conduct tests for detection of *H.pylori*, while 47 conduct ELISA test for detection of *H.pylori* IgG in the serum as shown in Table(5).

Table (5). *Comparison of serological test for detection of Helicobacter and Helicobacter positivity.*

Serological test for detection of *Helicobacter*[1)]	Helicobacter + (n=57) no. (%)[2)]	Helicobacter – (n= 43) no. (%)[3)]	χ^2	P*
I conduct test for detection of *Helicobacter*	50(87.7%)	34(79.1%)	1.364	0.243
I conduct ELISA kits for detection of IgG in serum	47(82.5%)	18(41.9%)	17.755	0.00003

[1)]Ones who answered yes in serological fest for detection of *Helicobacter* infection were considered.
[2)]Percentage in *H.pylori* (+)s.
[3)]Percentage in *H.pylori* (-)s.
*Percentage statistically significant difference (P>0.05).

3.5. Detection of Helicobacter Pylori Igg in Patient Serum by Using Elisa

Thirty samples were examined for *H.pylori* IgG, 29 samples were positive with titer ranged from 11.11 to >100), while only one sample was negative, as shown in Table (6) and Figure (1).

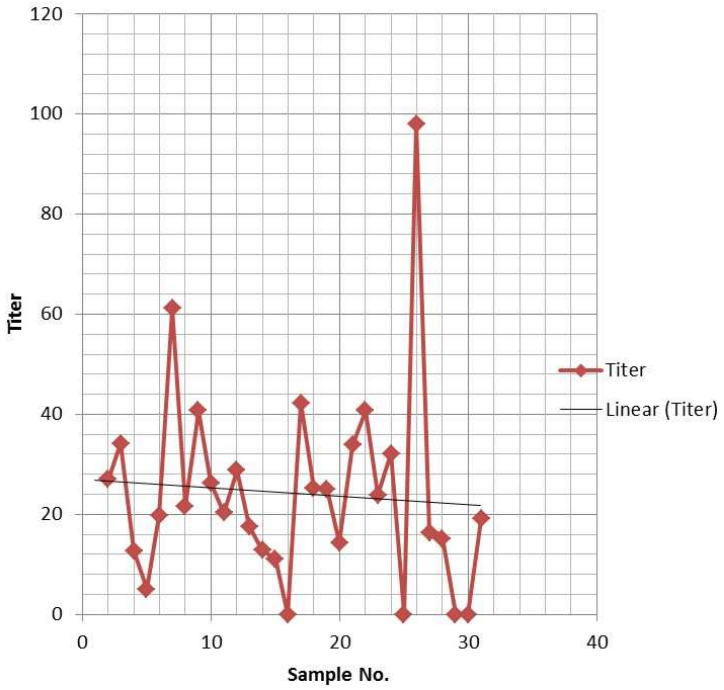

Figure 1. *Titer of Helicobacter pylori IgG in patient serum.*

Table (6). *Detection of H.pylori IgG in patients serum by using ELISA Kits.*

No.	Age	Gender	Result	Titer
1	22	F	+	27
2	26	F	+	34.2
3	13	M	+	12.7
4	28	M	-	5.1
5	47	F	+	19.8
6	26	F	+	61.2
7	21	F	+	21.6
8	22	F	+	40.77
9	21	F	+	26.25
10	21	F	+	20.3
11	48	F	+	28.84
12	27	F	+	17.6
13	2	F	+	12.8
14	26	F	+	11.11
15	30	M	+	>100
16	30	M	+	42.2
17	22	F	+	25.2
18	60	M	+	25
19	22	M	+	14.4
20	14	M	+	33.9
21	20	M	+	40.7
22	20	M	+	23.9
23	21	F	+	32.1
24	57	F	+	>100
25	52	M	+	98
26	6	M	+	16.24
27	32	F	+	15.1
28	37	M	+	>100
29	16	M	+	>100
30	19	M	+	19.1

4. Discussion

The obtained data from these study revealed that the seroprevalence of Helicobacter infection was 57% among participants in Hail region. The Seroprevalence of Helicobacter infection ranges between 12.3 and 86.5% indifferent countries (Korea,66.9% (10); Japan,63% (11) and India 34%(12)).

Socioeconomic conditions, hygiene levels and family life habits inluence the distribution of *H.pylori* in different populations. In Hail region, Saudi Arabia, *H.pylori* infection occur frequently. In a previous study, the distribution of *H.pylori* positivity, was frequently according to age groups was found to be, 96% in the 7-12 age group; 83% in the 13-18 age group; 75% in the 19-24 age group,96% in the 25-29 age group; 91% in the 30-34 age group; 83% in the 35-39 age group and 94% in the 40-65 age group (13). The average age of the patients who took part in our study is nearly similar with the literature, while the *H.pylori* positivity ratio in our study is lower than the literature.

(14) reported that the ratio of *H.pylori* (+) cases in their studies was 78.5%, and did not find a significant difference between age groups and gender distributions. However emphasized that this ration has been decreasing over the years. *H.pylori* was detected at quite high rate among our participants (57%).

The fact that the income level of the family is related to the income level and educational level of the participants. High H.pylori positivity is a result of this situation, so in these study, no significant difference was found between H.pylori positivity and this data. A study performed in china which support our results, indicate that there is no relation between educational level, income level and H.pylori positivity.

(13) Many studies were performed worldwide have shown that H.pylori infection and social class actors are interrelated. In our study there is no relation was detected between educational level, social states and H.pylori positivity (15).

In a cross sectional study performed in Japan, it was reported that, consuming salty foods was related to H.pylori infection risk. In a lot of studies, were detected that, the frequent consumption of vegetables, fruits, Vitamin C and probiotics were protective against H.pylori infection (16,17,15).

When H.pylori positivity was compared between people who had gastrointestinal problems in the last one month and who did not, it was found that the difference was not significant in the literature, in a study which analyzed H.pylori frequency and its relation with dyspeptic complains, there is no significant difference was detected in *H.pylori* positivity distributions and dyspeptic complains such as burning, bloating and sour taste in mouth were found(13,18,19), our study detected the same results of the literatures.

The result obtained from these study, suggest that in patients with gastritis, elevated *H. pylori* antibodies indicate an ongoing *H. pylori* infection. the titer of IgG antibody to

H.pylori in serum can be used as non-invasive tests for the presence of gastric *H.pylori* infection and gastritis.(20) It is, however, not known whether the titers of *H.pylori* antibodies in serum are indicative of the severity of gastritis. If so, the severity of gastritis could be assessed and followed without endoscopy. (21) detect a higher mean antibody titer in active chronic than in inactive chronic gastritis. In contrast, (22) and (23) did not find higher titer of *H.pylori* antibodies in severe gastritis, although in the last study a tendency to a higher concentration in more severe gastritis was observed. Testoni and coworkers found that 67% of patients with chronic gastritis and antral atrophy had elevated *H. pylori* IgG antibody levels in serum, suggestive of a current infection in spite of negative histology results (24).

5. Conclusion

In conclusion, the high *H.pylori* positivity ration (57%) that we obtained indicates that *H.pylori* infection is still a common problem among people in Hail region-Saudi Arabia. It was found that, the average age of the patient was (one to 70 years old), 68% of them were male and 32% female. Titer of *H.pylori* IgG ranged from 11.11 to >100 in patients serum.

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