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Original article

Relationship between Helicobacter Pylori Morphological Forms in Gastric Biopsy and Helicobacter Pylori Stool Antigen

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Abstract

Background: Helicobacter pylori (H. pylori) is a gram-negative, spiral-shaped microorganism and is responsible for colonization in the gastric microniche of more than 50% of the world population. This work aimed to compare H. pylori stool antigen titer in different H. pylori morphological forms in gastric biopsy. **Patients and Methods:** This study was carried out on 120 patients with dyspepsia. All patients were subjected to full history taking, upper GI endoscopy, and gastric biopsies according to Sydney protocol for histopathology and stool antigen test by using the H. pylori Antigen (ELISA) quantitative test kit. **Results:** Of the 120 patients there were 37 males and 83 females with a mean age of (29.57 ± 9.17), H. pylori morphological form was divided into two forms: Coccoid form (n = 27) and Bacillary form (n = 91). H. pylori gastritis was more common in females (69.17%) than males (30.83%), and common above the age of 30 years (56.7%), and more patients were nonsmokers (89.2%). H Pylori stool antigen was significantly higher in the case of the bacillary form of H. pylori than in the coccoid form. **Conclusion:** H. pylori stool antigen is higher in bacillary forms of H. pylori than in coccoid forms.

Keywords: Helicobacter Pylori, Coccoid, Bacilli, Stool Antigen.

Introduction:

Helicobacter pylori is a spiral, microaerophilic, noninvasive, gram-negative bacterium that colonizes the human gastrointestinal tract, primarily the stomach [1]. H. pylori is one of the most common causes of human infection, especially in developing countries, where the incidence can be up to 90% of the population [2]. H. pylori infection often persists throughout life. This organism has been identified as an etiological agent of chronic active gastritis, peptic ulcer disease [3], gastric adenocarcinoma [4], and mucosa-associated lymphoid tissue

(MALT) lymphoma [5]. The type and severity of diseases depend on many factors, among them: the status of the host's immune system, the pathogenicity of H. pylori strains, and the presence of environmental factors (diet, stress, hygiene level, or the presence of co-infections)[6]. H. pylori infection can be diagnosed by noninvasive tests such as H. pylori antigen in stool specimen, UBT (Urea Breath Test), serology, and invasive tests such as PCR (polymerase chain reaction), culture, and histology which require upper GI endoscopy and biopsy specimens[7]. Histopathological examination of biopsy material can provide important information about morphological features indicating the severity of gastritis and evidence for dysplasia. However, the accuracy of histology may be variable due to the density of H. pylori and sampling error and subjective to the experience of the pathologist [8]. H. pylori exists mainly in two different morphological forms in histopathology, spiral, and coccoid. Coccoid forms of H. pylori have been described as 'viable but non-culturable (VBNC). These cells may be viable and can revert to culturable forms in mice, but are no longer culturable on conventional media [9]. Spiral H. pylori has been reported to be associated with chronic active gastritis, and coccoid H. pylori is more frequently associated with chronic inactive gastritis in symptomatic adult patients [10]. Coccoid forms are less virulent than spiral ones [11]. Additionally, they are also poorly responsive to antibiotic therapy[12]. H. pylori coccoid forms may be hidden within the biofilm (complex composed of bacteria and extracellular matrix of polyanionic polysaccharides). Some studies demonstrated the beneficial role of N-acetylcysteine for its mucolytic and bacteriostatic properties. So, N-acetylcysteine could be used as a pre-treatment followed by antibiotic therapy [11]. H. pylori antigen also appears in the stool. Stool tests have the advantage of being noninvasive and the specimen is easily obtainable. H. pylori stool antigen (HpSAg) which is based on the detection of Ag by ELISA technique has been proven to be clinically useful with sensitivities and specificities of more than 90% and is advantageous to confirm eradication [13]. It can be used as a routine diagnostic tool for H. pylori infection because it seems to overcome the limitations of conventional invasive techniques (14). HpSAg may be useful particularly in the selection of the cases requiring endoscopic examination, in monitoring the response to treatment, and in epidemiological studies (13).

The relationship between the morphological form of H. pylori and the detection of H. pylori stool antigen has not been studied before. This work aimed to compare H. pylori stool antigen titer in different H. pylori morphological forms in gastric biopsy.

Patients and Methods:

This cross-sectional study was carried out on 120 patients aged 18 or above who were presented to the outpatient clinic of Hepatology and Gastroenterology of Shebin Elkom Teaching Hospitals with dyspeptic symptoms. The study was done from November 2022 to May 20 after approval from the Ethical Committee Shebin Elkom Teaching Hospitals, Benha, Egypt. Informed written consent was obtained from the patients.

Approval code of REC: Ms.30.8.2022

Exclusion criteria included patients who received antibiotics during the last month, Colloidal bismuth compounds during the last month, proton pump inhibitors (PPI), or H2 blockers during the last 2 weeks, refused to give consent.

All patients were subjected to complete history-taking, complete general examination, and routine laboratory investigations.

Specific Investigations: Stool antigen test by using H. pylori Antigen (ELISA) quantitative test kit (Sure Bio-Tech (USA) Co., Ltd): The exclusion criteria for the stool samples were diarrhea, inadequate amount, and delayed delivery of the samples after collection.

A solid fecal sample was collected as follows: Unscrew the lid of the sample extraction tube, randomly insert the sample collection rod into at least 3 different parts of the fecal sample and collect about 30 mg of sample (equivalent to 1/4 pea), and then transfer to the sample extraction tube. Collection of liquid fecal sample: Take 2 drops (about 50µl) of the fecal sample vertically from a special dropper, transfer to the sample extraction tube, tighten the lid on the sample extraction tube, and shake vigorously to mix the sample with physiological saline then samples were stored at -20 °C and recovered to room temperature before detection.

All reagents were allowed to reach 20°C-30°C for 15 minutes before the wash buffer was diluted at the rate of 1:40 dilution with distilled water before use. 50µl H.P reference material and 50µl specimen were added into the corresponding wells. 50µl conjugate was added into each well then shaken gently to mix and incubated at 20°C-30°C for 60 minutes with the sealing plate membrane sealing the plate. At the end of the incubation, remove and discard the plate cover was removed and discarded then wash buffer was added to each well and Repeated 5 times. Substrate A (50µL) and Substrate B (50µL) were added, gently shaken, and mixed, and the color was developed at room temperature for 10 minutes. 50µl Stop Solution was added to each well to stop the reaction.

The microplate reader was Set and read the absorbance of each well using dual wavelength 450nm/630nm. Using a four-parameter fitting method, a standard curve and calculation of the H.P antigen content of the sample were established.

Pelvi- abdominal ultrasound: to exclude other causes of dyspepsia such as gall bladder stones.

upper GI endoscopy: and gastric biopsies according to updated Sydney protocol[15].: 3cm from the pylorus, greater curvature, 3cm from the pylorus, lesser curvature, Incisura angularis, body, lesser curvature, body, greater curvature.

Histopathological examination: gastric biopsies for detection of H. pylori morphological forms (spiral or coccoid form).

Sample Size Calculation:

From a previous study, it is estimated that 40% of H. pylori forms were bacilli form. Assuming that the bacilli form has 65% H. pylori stool antigen positive, and the cocci

form has 40% H. pylori stool antigen positive, a minimum sample size of 60 patients in each group will be needed to detect a significant difference and a total sample size of 120 patients is assigned.

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The significance of the obtained results was judged at the 5% level.

1 - Chi-square test:

For categorical variables, to compare different groups.

2 - Fisher's Exact:

Correction for Chi-square when more than 20% of the cells have an expected count of less than 5.

3- Student t-test:

For normally distributed quantitative variables, to compare between two studied groups.

4 - Mann Whitney test:

For abnormally distributed quantitative variables, to compare between two studied groups.

- A p-value of >0.05 was considered statistically non-significant.
- A p-value of <0.05 was considered statistically significant.
- A p-value of <0.001 was considered statistically highly significant.

Results:

This table shows that: **Table 1**

- The study was done on 120 patients with a mean age of 29.57±9.17 with 37 males and 83 females.
- The upper git symptoms and clinical signs among the studied patients including epigastric pain (100%), hematemesis (13.3%), nausea& vomiting (35%), bloating (15.8%), heartburn (32.5%), weight loss (4.2%), melena (12.5%), pallor (9.2%), epigastric tenderness (91.7%).
- Past history among the studied patients including previous H. pylori treatment (14.2%), previous endoscopy (6.7%), NSAID abuse (6.7), hypertension (3.3%), and diabetes mellitus (1.7%).

This table shows that**: Table 2**

- H. pylori stool Ag was positive in 95 patients.
- Hemoglobin level ranges from 6.9 to 15.1 g/dL.
- Platelet count ranges from 170 to 410 /mm3.
- White blood cell counts range from 3.8 to 13 cells /mm3.

Table 3 shows that:

- Ultrasound-positive data among the studied patients including bright hepatomegaly (11.7%).
- Upper GI endoscopic findings among the studied patients including gastric mottling (100%), gastric ulcerations (16.7%), gastric erosions (32.5%), hyperemia (78.3%), reflux esophagitis (13.3%), and hiatus hernia (7.5%).

Table 4 shows that:

- H. pylori stool antigen is significantly higher in the case of the bacillary form of H. pylori than in the coccoid form with a P-value (0.002).
- Melena is significantly higher in the case of the bacillary form of H. pylori than in the coccoid form with a P-value (0.02).

Table 5 shows that gastric ulceration is significantly higher in the case of the bacillary form of H. pylori than in the coccoid form with a P-value (0.041).

Table 2: Distribution of the studied cases according to demographic, clinical data, and past history (n = 120):

		Patients (n = 120)
Age (years)		29.57 ± 9.17
Age (years)	**<30**	68(65.7%)
	≥30	52(43.3%)
Sex	**Male**	37 (30.83%)
	Female	83 (69.17%)
Smoking	**Negative**	107 (89.2%)
	Positive	13 (10.8 %)
Complain		
Epigastric pain		120 (100%)
Hematemesis		16 (13.3%)
Nausea& vomiting		42 (35%)
Bloating		19 (15.8%)
Heartburn		39 (32.5%)
Weight loss		5 (4.2%)
Melena		15 (12.5%)
Clinical signs	**Pallor**	11 (9.2%)
	Epigastric tenderness	110 (91.7%)
Past history	**H. pylori treatment**	17(14.2%)
	Endoscopy	8(6.7%)
	NSAID	8(6.7%)
	HTN	4(3.3%)
	DM	2(1.7%)

Data are presented as frequency (%).

Table 2: Distribution of the studied cases according to investigations, (n = 120):

	No.	%
H. Pylori stool Ag	25	20.8
Negative		
Positive	95	79.2
Mean ± SD.	49.25 ± 43.80	
Median (IQR)	36.60(0.0 – 181.46)	
▪ HB **g/dL**		
Min. – Max.	6.90 – 15.10	
Mean ± SD.	11.54 ± 1.52	
Median (IQR)	11.55 (10.70 – 12.50)	
Plat **/mm^3**		
Min. – Max.	170.0 – 410.0	
Mean ± SD.	271.68 ± 51.30	
Median (IQR)	270.0 (230.0 –307.5)	
WBCS **cells/mm^3.**		
Min. – Max.	3.80 – 13.0	
Mean ± SD.	7.05 ± 2.13	
Median (IQR)	7.15 (5.30 – 8.50)	

Table 3: Distribution of the studied cases according to ultrasound, upper GI endoscopy:

		No.	%
Ultrasound	**Gallbladder pathology**	0	0.0
	Liver (Bright)	14	11.7
	Pancreas pathology	0	0.0
Upper GI endoscopy	**Gastric mottling**	120	100.0
	Ulceration	20	16.7
	Erosions	39	32.5
	Hyperemia	94	78.3
	Reflux esophagitis	16	13.3
	Hiatus hernia	9	7.5

Data are presented as frequency (%).

Table 4: Relation between H. pylori morphological forms and H. pylori stool antigen titre and between H. pylori morphological form and clinical data (n = 120):

		H. pylori morphological form				Test	p
		Coccoid form (n = 27)		**Bacilli form (n = 91)**			
		No.	**%**	**No.**	**%**		
H. pylori stool antigen (normal range from 0 to < 10 ng/ml)						χ^2=10.074*	0.002*
Negative		11	40.7	12	13.2		
Positive		16	59.3	79	86.8		
Min. – Max.		1.68 – 132.0		0.0 – 181.46		U=651.50	0.001*
Mean ± SD.		24.65± 27.35		57.59 ±44.93			
Median		18.0		51.0			
Complain	**Epigastric pain**	27	100.0	91	100.0	–	–
	Hematemesis	1	3.7	15	16.5	2.901	FEp=0.11 5
	Nausea& vomiting	13	48.1	28	30.8	2.774	0.096
	Bloating	2	7.4	16	17.6	1.668	FEp=0.24 0
	Heartburn	8	29.6	30	33.0	0.106	0.744
	Weight loss	0	0.0	5	5.5	1.549	FEp=0.58 8
	Melena	0	0.0	15	16.5	5.099	FEp=0.02 1*
Clinical signs	**Pallor**	2	7.4	9	9.9	0.152	FEp=1.00 0
	Epigastric tenderness	24	88.9	84	92.3	0.314	FEp=0.69 4

Data are presented as mean ± SD or frequency (%).

Table 5: Relation between H. pylori morphological forms and Upper GI endoscopy, (n = 120):

		H. pylori morphological form				x^2	p
Upper GI endoscopy		**Coccoid form (n = 27)**		**Bacilli form (n = 91)**			
		no	**%**	**No**	**%**		
Gastric mottling	27	100.0	91	100.0	–		–
Ulceration	1	3.7	19	20.9	4.364*		FEp=0.041*
Erosions	10	37.0	29	31.9	0.251		0.616
Hyperemia	24	88.9	68	74.7	2.431		0.119
Reflux esophagitis	3	11.1	13	14.3	0.179		FEp=1.000
Hiatus hernia	2	7.4	7	7.7	0.002		FEp=1.000

Data are presented as frequency (%).

Discussion:

More than 50% of the world's population has Helicobacter pylori (H. pylori), a gram-negative, spiral-shaped microbe, in their stomachs [16]. Our results show that H. pylori gastritis was more common in females (69.17%) than males (30.83%), common above the age of 30 years (56.7%), and more patients were nonsmoker (89.2%). These results are in agreement with [17] who stated that H. pylori gastritis was predominant in females. Also, [18] had the same findings as found in his study , he reported that H. pylori gastritis is more predominant in middle age group from 30-40 years. Our results displayed that upper GIT symptoms and clinical signs among the studied patients including Epigastric pain (100%), Hematemesis (13.3%), Nausea& vomiting (35%), Bloating (15.8%), Heartburn (32.5%), Weight loss (4.2%), melena (12.5%), pallor (9.2%), epigastric tenderness (91.7%). These results are in accordance with [19] who found patients with H. pylori infection may present with different complaints such as epigastric pain, bleeding from GIT, melena, pallor due to anemia, weight loss due to ulcers increase pain with food so patients avoid food causing weight loss .Our work showed ultrasound-positive data among the studied patients including bright hepatomegaly (11.7%). The results of the study done in 2020 [20] were similar to our results as they observed the association between H. pylori infection and fatty liver which appears bight hepatomegaly by ultrasound. Contrarily, other studies [21] had different results as they found no association between H. pylori and fatty liver. Our study observes that upper GI endoscopic findings among the studied patients including gastric mottling (100%), gastric ulcerations (16.7%), gastric erosions (32.5%), hyperemia (78.3%), reflux esophagitis (13.3%), and hiatus hernia (7.5%). [22] had the same as our results, the findings of upper endoscopy of H. pylori gastritis range from normal mucosa to mottling, erosions, hyperemic, and association with symptoms of GERD with hiatus hernia with different grades. Our results show that the histopathological findings among the studied patients include H. pylori morphological forms as coccoid form (22.5%) or bacilli form (75.8%). These findings match with the results of [23] who observed that the spiral form is the most common form involved in colonization of the human stomach. Our results exhibit that H. pylori stool antigen is significantly higher in the case of the bacillary form of H. pylori than in the coccoid form. These findings match with the results of [24] who observed that H. pylori Ag in stool is significantly higher in bacillary forms than in coccoid forms which are attributed to those bacillary forms being more fragile.

Limitations: The sample size was relatively small. The study was in a single center. Lack of correlation of our results after H. pylori treatment for possible recurrence. Lack of study of the conversion of H. pylori from one form to another with therapy.

Conclusions:

The H. pylori stool antigen titer is higher in Bacilli forms of H. pylori than in coccoid forms.

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