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Study the relation of gastric cancer with *helicobacter pylori* infection among dyspeptic patients

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Abstract: *Helicobacter pylor*i (*H. pylori*) is the main causative agent of gastrointestinal diseases including chronic gastritis, peptic ulcer associated disorders, gastric and duodenal carcinomas leading to morbidity and mortality in humans. This research was conducted to study the relation of gastric cancer with *H.pylori* infections through some invasive methods(Histology, Direct biopsy smear, Biopsy urease test and culture methods). One hundred and twenty five dyspeptic patients were subjected to esophageal gastroduodenoscopy in Baghdad Teaching Hospital/ Endoscopy unit from10/1/2013 to 8/1/2014, gastric biopsy samples were applied for microbiological analysis. The results showed that there was a relationship between *H. pylori* infection occurrence and endoscopically diagnosed Gastric cancer (3.2%); it was recorded highest *H. pylori* isolation (45.45%) from gastric ulcer patients followed by (25%) from gastric cancer patients. Histological examination recorded that active gastritis is found to be the most infected case (93.75%) with *H. pylori* followed by gastric adenocarcinoma (66.66%) gastric lymphoma (50%), so the study recorded a significant association between *H .pylori* infection and gastric cancer.

Key words: *Helicobacter pylori*, Gastric cancer, Dyspeptic.

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Introduction:

*Helicobacter pylor*i (*H. pylori*) infection is a cofactor in the development of three important upper gastrointestinal diseases, duodenal or gastric ulcers, gastric cancer and gastric mucosa-associated lymphoid tissue(MALT) lymphoma (1). *H.pylori* has been ranked as a class I carcinogen by the International Research Agency on Cancer (2).Also the principal cause of gastric cancer, the second leading cause of cancer mortality worldwide (3,4)). Also since 1994 this organism has been classified as carcinogenic to humans (5), and the World Health Organization (WHO) has added it to its list of known carcinogens(6). Although gastric colonization with *H.pylori* induces histologic gastritis in all infected individuals, only a minority develop any apparent clinical signs of this colonization, it is estimated that *H. pylori*-positive patients have a (10 to 20) % lifetime risk of developing ulcer disease and a (1 to 2) % risk of developing distal gastric cancer (7).

The infection of *H. pylori*

associates with a marked down-regulation of ghrelin synthesis (Ghrelin, the ligand of growth hormone secretagogue receptor 1a, takes part in several functions of the digestive system, including regulation of appetite, energy homeostasis, gastric acid secretion and motility (8), thus delineating a scenario in which such a defect contributes to sustain the *H. pylori*-driven pathogenic response, as ghrelin can increase the production of prostaglandins prostaglandin E2, a protective factor for the gastric mucosa, which has been also implicated in the pathogenesis of cancer (9). The gastric mucosa does not normally contain lymphoid tissue, but MALT nearly always appears in response to colonization with *H. pylori*, in rare cases, a monoclonal population of B cells may arise from this tissue and slowly proliferate to form MALT lymphoma, nearly all MALT lymphoma patients are *H. pylori* positive (10), and *H. pylori*-positive subjects have a significantly increased risk for the development of gastric MALT lymphoma (11).

Evidence that *H. pylori* increase the risk of gastric cancer development via the sequence of atrophy and metaplasia originates from various studies, in which it was shown that *H. pylori*-positive subjects develop these conditions more often than do uninfected controls (12**).** Stomach cancer is rare in persons who have never had *H. pylori* infection (13).

The aim of the research is to study the correlation between *H. pylori* infection occurrence and gastric cancer cases, using some invasive methods**.**

Materials and Methods:

A total of 125 patients suffering from dyspeptic symptoms representing different age groups from both genders. They underwent diagnostic upper gastrointestinal (G.I.) endoscopy at Endoscopy unit of Baghdad Teaching Hospital in Baghdad, Iraq at a period between 10/1/2013 to 8/1/2014. Several gastric biopsy samples were taken from the antrum and the body and they are subjected to various mucosa, which has been also bacteriological and histological examinations. Informed written consent is obtained in advance from each patient.

Diagnotic Tests (Invasive tests):

1-Histological Examination:

The biopsy samples which were collected in 10 % formalin as a fixative are transported to the Pathology Laboratory in Baghdad Medical City after proper labeling.

2- Biopsy Urease Test:

The test is not finally declared as negative till 24hrs. (14,15).

3-Direct Biopsy Smear:

The biopsy sample was crushed and smears are prepared and stained by routine gram stain protocols (16).

4-Biopsy Culturing:

The second minced biopsy is inoculated in each of selective (Columbia, Brain - heart infusion and Brucella) agar media plates and non-

selective Chocolate agar media plates that are used for primary isolation of *H. pylori*. The cultures are incubated at 37°C under microaerophilic conditions (5% O_2, 10% CO_2, 85 %N_2) in an anaerobic jar with a gas generating kit. Plates are examined for positive growth for intervals of (3-7) days before discarding as negative. For positive growth, the colonies must be tiny, glistering, translucent or gray and covered with entire edges (15,17,18).

Identification of *H. pylori* (19):

1-Gram Stain:

2-Biochemical Tests of *H.pylori*:

A. Rapid Urease Test (RUT) of Colonies
B. Catalase Test
C. Oxidase Test.

Results and Discussion:

Endoscopic Findings of Clinical Cases:

The results reported by physician as shown in table (1), the highest Percentage of endoscopically diagnosis was gastritis seen in 57 (45.6%) from the total 125 patients followed by peptic ulcer 25 (20%) that includes duodenal and gastric ulcers, 14(11.2%) and 11(8.8%) respectively, then gastroduodenitis was seen in 16(12.8%) and 8(6.4%) for Gastroesophageal Reflux Disease (GERD). While the lowest percentages were 4 (3.2%) of patients had gastric cancer, and for others who did not show any alteration at endoscopic examination (Non ulcer dyspepsia) was 15 (12%).

Table (1): Distribution of Endoscopy Diagnosed Dyspeptic Patients

Endoscopic diagnosis	No.	Percentage (%)
Gastritis	57	45.6
Peptic ulcer		
1.Doudenal ulcer	14	11.2
2. Gastric ulcer	11	8.8
Gastrodoudnitis	16	12.8
Gastroesophageal Reflux Disease	8	6.4
Gastric cancer	4	3.2
Non ulcer dyspepsia	15	12
Total	125	100

Similar studies in Iraq were obtained by (20,21,22) who indicated that gastritis was the highest prevalence among endoscopically diagnosed patients. While (23) indicated that duodenal ulcer patients were most prevalence among endoscopically diagnosed patients followed by gastritis cases.

Laboratory Investigations of Biopsy Samples (Invasive methods):

1- Histological Examination.

Table (2) has shown active gastritis to be the commonest histological finding in the gastric biopsies of dyspeptic patients in 43.63%, active Superficial gastritis in

27.27% of the dyspeptic patients figure (1), followed by chronic gastritis 16.36%, gastric adenocarcinoma 5.45%, figure (2), while gastric lymphoma and chronic superficial gastritis ,figure (3) were the lowest histological findings 3.63% .Also as shown in table (2), *H. pylori* was highly detected in active gastritis 45(93.75%) out of 48 cases, followed by active superficial gastritis 28 (93.33%) out of 30, chronic gastritis 16 (88.88%) out of 18 cases, gastric adenocarcinoma in 4 (66.66%) out of 6, gastric lymphoma in 2(50%) out of 4 and 1 (25%) out of 4 with *H. pylori* in chronic superficial gastritis and 1(25%) out of 4 with *H. pylori* in chronic superficial gastritis.

Table (2): Histological Diagnosis of Dyspeptic Patients in Association with *H.pylori* Infection.

Histological Aspect	Cases		Infection with *H.pylori*	
	No.	%	No.	%
Active gastritis	48	43.63	45	93.75
Active superficial gastritis	30	27.27	28	93.33
Chronic gastritis	18	16.36	16	88.88
Chronic superficial gastritis	4	3.63	1	25
Gastric lymphoma	4	3.63	2	50
Gastric adenocarcinoma	6	5.45	4	66.66
Total	110	100	96	

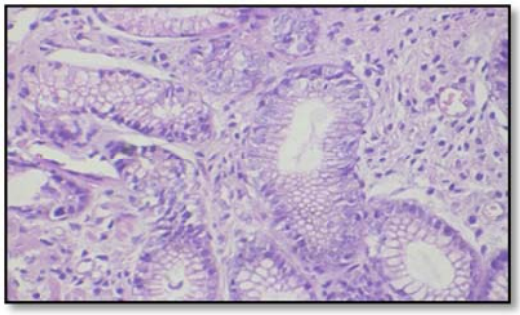

Figure (1): *Helicobacter pylori* of Active Superficial Gastritis in Section of Mucosal Layer of the Stomach Stained by H and E Stains (1000xs).

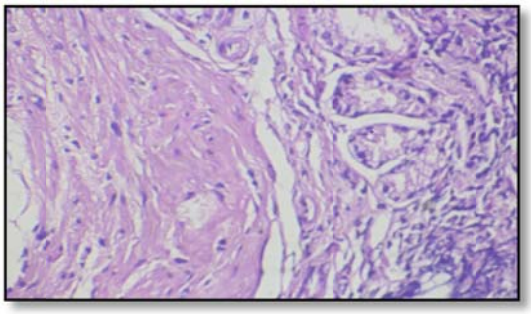

Figure (2): *Helicobacter pylori* of Gastric Adinocarcinoma in Section of Mucosal Layer of the Stomach Stained by H and E Stains (1000xs).

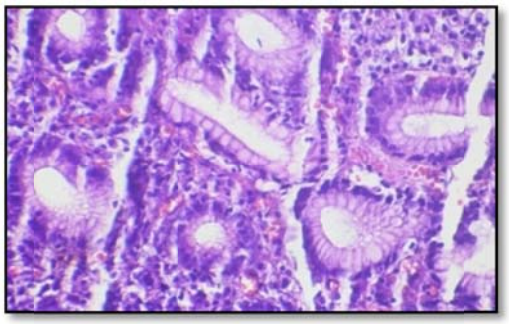

Figure (3): *Helicobacter pylori* of Chronic Superficial Gastritis in Section of Mucosal Layer of the Stomach Stained by H and E Stains (1000xs).

Histological evaluation has been the "gold standard" method for diagnosing *H. pylori* infection (24,25), although guidelines indicated that no single test can be considered as the gold standard (26). It allows for the definitive diagnosis of infection, as well as the degree of inflammation or metaplasia and the presence/absence of MALT lymphoma or other gastric cancers in high risk patients (27).

Close results are obtained by (28) indicated that the Active gastritis was the commonest histological finding in the gastric biopsies of dyspeptic patients. These results closely resemble those obtained by(29) who indicated that *H. pylori* was present in high prevalence among cases with chronic gastritis and (28) indicated that *H. pylori* was present in high prevalence among cases with active superficial gastritis. Also (28,29) indicated that *H. pylori* were present in half of gastric lymphoma cases, also they recorded *H. pylori* were present in 58% and 60% of adenocarcinoma patients and in 86.4% and 95% of chronic gastritis cases, respectively.

While (30) found that *H. pylori* was present in 75% of gastric lymphoma cases. This may be due to the difficulty to recognize these microorganisms in malignant glands due to the changes in composition and secretion of the glands (31).

2- Direct Biopsy Smear:

In this test out of 125 dyspeptic patients, 72 (57.6%) gave positive result and 53 (42.4%)gave negative for the presence of *H. pylori* using Gram stain as shown in figure (4).

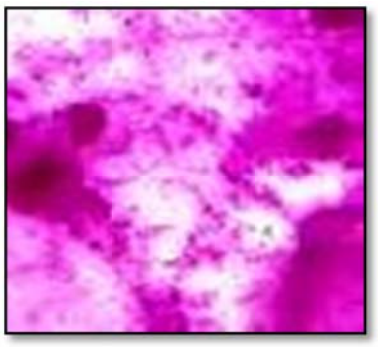

Figure (4): Direct Biopsy

Smear Stained by Gram Stain for *H.pylori*:

Gram stain is a simple relative inexpensive and faster method than the histological method (32). Stained smears of biopsy material provide an opportunity for rapid evaluation of *H. pylori* status, Gram stain make *H. pylori* visible and allowing morphology of the bacterium to be determined.

3- Biopsy Urease Test (BUT):

As shown in table (3) and figure(5), Out of 125 dyspeptic patients 58 (46.4 %) are positive by using biopsy urease test at different periods. From these, 6 (10.3%), 11 (18.96), 12 (20.68), 13 (22.41) and 16 (27.58) of samples gave positive results within 30min., after one hr., 1.5 hr., 2 hrs. and after 24hrs., respectively. These results observed that there is highly significant association between *H. pylori* infection and biopsy urease test (x^2 = 6.702, P = 0.0149) at different periods.

Table (3): Biopsy Urease Test results at Different Periods

Gastric Biopsy	Time	Result (n=125)		χ^2- value	P-value
		Positive	%		
Biopsy Urease Test (BUT)	Within 30min.	6	10.34	6.702 **	0.0149
	After 1 hr.	11	18.96		
	After 1.5 hr.	12	20.68		
	After 2 hrs.	13	22.41		
	After 24 hr.	16	27.58		
Total	---	58	100	---	

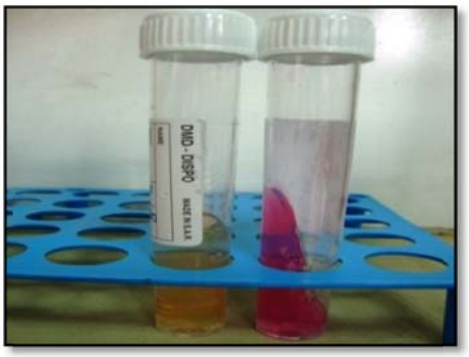

Figure (5): Biopsy Urease Test for *H.pylori*

Similar study obtained by Al-Yas, (28) indicated that 23, 38 and 40 of biopsy urease test became positive within 30 min., after 1 and 24 hrs. Respectively. The results appear a highly significant association was found between *H. pylori* infection and biopsy urease test (x^2=59.9,P= 0.000001). Hazell *et al.*,(33) reported that the time for a positive result is usually proportional to the number of *Helicobacters.*

Helicobacter count is high and more organisms seen in the biopsy, which is more likely a positive biopsy urease test within 30min. Therefore, urease test is used to identify the relative numbers of

H. pylori present at any particular site in the stomach. Laine *et al*.,(34) stated that doubling the amount of tissue (size or number) in the urease hastens the positive result by approximately (1.5 to 2) hrs. Furthermore, warming of the urease sample is recommended when final result of the test is desired within (1 to 2) hr. within such period this difference appears to be significant.

4-Isolation of *H. pylori* From Biopsy:

Results shown in table (4), revealed the highest isolation rate is 8 (32%) from 25 peptic ulcer cases, include 5 (45.45%) from 11 gastric ulcer cases gastritis cases and the lowest rate was 1(12.5%) isolated from 8 GERD cases, while there ulcer dyspepsia cases non ulcer dyspepsia cases.

Table (4): Isolation of *H. pylori* from Endoscopically Diagnosed Dyspeptic Patients.

Diagnosis	No.	*H.pylori* Isolated (No.)	*H.pylori* (%)
Peptic ulcer 1-Gastric ulcer 2-Doudenal ulcer	 11 14	5 3	45.45 21.42
Gastric cancer	4	1	25
Gastrodoudenitis	16	4	25
Gastritis	57	8	14
GERD	8	1	12.5
Non ulcer dyspepsia	15	-	-
Total	125	22	

Similar results to the present study are obtained by (28) who indicated that the highest prevalence of *H.pylori* was 66.6% and 41% in gastric ulcer and duodenal ulcer patients respectively and 28.5% for gastric cancer patients.

Different results recorded by (35) who noted that using endoscope evaluation 55% of patient with gastritis were colonized by *H. pylori*. While (36) found that *H. pylori* associated with 77% of gastritis cases.

Graham *et al.*, (37) mentioned that *H. pylori* can be cultured from about half of the patients undergoing upper gastrointestinal endoscope and *H. pylori* infection of the stomach has been associated with gastric ulcer, duodenal ulcer and gastritis. Although various methods have been developed for detecting *H.pylori* infection, bacterial culture remains extremely, *H. pylori* is fastidious organism so various factors, including bacterial density, transport conditions, culture medium and microaerophilic condition, directly influence the yield of culture (38). The presence of false negative and false positive result may be explained by the patchy distribution that *H.pylori* present in gastric mucosa, especially in the body and fundus of the stomach, so the microorganism can be present in one biopsy and absent in another from the same patient (39,40). So the false negative values by this test are caused by the patchy distribution of the bacteria. Piccolomini *et al.,* (17) stated that practical contamination is difficult to be avoided and therefore a suitable selective media with antibiotics are usually essential to detect *H. pylori*.

Identification of *H. pylori* Isolates:

1- Macroscopic Examination (Shapes of colonies):

Culture examination of *H. pylori* was done between (3-7) days of plating on culture agar media under microaerophilic conditions at 37°C, revealed that colonies appeared tiny. This means, when a positive result is obtained rapidly it is likely that the glistering, translucent or gray and convex with entire edges.

2-Microscopic Examination:

A-Gram Stain:

When a part of a suspected colony grown on the agar medium is smeared on a microscopically slide and stained by gram staining technique, cells appeared as slightly curved or straight rods to curved bacilli with a gram - negative reaction according to the discretion of (41,42).

2-Biochemical Tests:

A-Oxidase and Catalase:

Presumptive isolates of *H. pylori* are also catalase positive through air bubbles production and oxidase positive through color changing from violet to dark violet on the filter paper of presumptive isolates (43)**.**

B-Rapid Urease Test (RUT):

Isolates of presumptive *H. pylori* are also urease positive through color change from yellow to pink through few minutes in an acid medium (urea agar base + urea supplement) (44).

Conclusion:

We concluded that Gastric cancer cases include Gastric lymphoma and Gastric adenocarcinoma was seen in high prevalence among *H. pylori* endoscopically diagnosed dyspeptic patients, the detection obtained some invasive methods (Histology examination, culture and Direct biopsy smear).

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