The role of *Helicobacter pylori* in esophagitis and peptic ulcer disease in Iraq

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ABSTRACT

Objective: The objectives of this study are to determine the prevalences of *Helicobacter pylori (H.pylori)* infection in symptomatic, but endoscopically normal patients as well as in patients with endoscopically and histologically proven esophagitis, gastritis, duodenitis, duodenal ulcer, and gastric ulcer.

Methods: The study extended over the period November 1999 through June 2000. Biopsy specimens were harvested from intact areas of gastric antral mucosa, duodenal bulb, gastric body and lower third of esophagus of each one of 200 patients undergoing eseophago-gastro-duodenoscopy in endoscopy unit of Tikrit General Hospital (TGH), Salahuddin Governorate, Tikrit City, Iraq. The biopsies were submitted for histopathological, cultural and biochemical investigations. Seven biopsy samples were taken from each patient. Written consent was taken from each patient. The patients were pooled from various districts of the governorate.

Results: *Helicobacter pylori* was detected in antral biopsies of the following categories of patients: in 73.9% of patients with endoscopic gastritis, in 75% of patients with gastric ulcers, in 86% of patients with endoscopic duodenitis, in 88.6% of patients with duodenal ulcers and in 57.7% of patients with endoscopic esophagitis, but absent in all patients with totally normal endoscopies.

Conclusions: Although *H.pylori* has no role in the development of esophagitis, it is a prevalent pathogen and is associated with many gastro-intestinal diseases and has an important role in the pathogenesis of peptic ulcer disease and gastritis in our district.

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Helicobacter pylori is one of the most common bacterial infectious agents worldwide.[1] It is associated with chronic gastritis, peptic ulcer disease and gastric carcinoma and may be linked to non-ulcer dyspepsia[2-4] and B.cell lymphoma.[2] *Helicobacter pylori* seems to be responsible for more than 90% of duodenal ulcers and 65% of gastric ulcers.[5,6] Although there is no direct evidence that *H.pylori* infection precedes the development of duodenal ulcer, it produces an exaggerated gastrin response, duodenitis and gastritis. *Helicobacter pylori* is thought to spread by feco-oral route and nasocomial route.[5-7] The ingestion of *H.pylori* causes acute gastritis that can undergo transformation into type B chronic gastritis. *Helicobacter pylori* associated gastritis dramatically causes ulceration in approximately 30% of all patients.[8] In western countries, 20% of the population below the age of 40-years and 50% of those above 60-years of age are infected.[5,8] It is uncommon in young children and it is more common in families with low socio-economic status. In underdeveloped countries, more than 70% of the population are infected. Acquisition occurs in 10% of the children per annum between the ages of 2-8-years, as infection occurs early in childhood.[5,8,9] *Helicobacter pylori* apparently colonizes only regions of mucosa with gastric mucosal surface cells; thus, *H.pylori*

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infection is limited to areas where gastric epithelium is present, most commonly stomach, duodenum and esophagus.[5] *Helicobacter pylori* infection alters gastric and duodenal architecture and function and it appears to be able to penetrate tight junctions between epithelial cells.[5] However, the frequency of chronic peptic ulcer disease is much less than the prevalence rates of *H.pylori* infection.[5] After eradication of *H.pylori,* re-infection is uncommon in adults and recurrent symptoms are rarely due to this.

Methods. The hospital, in which the study was conducted, is a 400-beds secondary care hospital in the main city. It serves more than 1,000,000 population in the governorate. Patients attending it belong to various socio-economic classes and occupations. Usually, endoscopy is carried out for an average of 12 patients per week; that is around 624 patients per year. Hence 200 patients would constitute around 32% of this total. Hence, it is found to be a representative figure of the whole patients attending endoscopy unit of the hospital. Over an 8-month period, every third patient endoscoped was randomly chosen to be involved in the study.

The study is an analytical, descriptive and prospective one and the protocol was approved by the Departments of Microbiology and Medicine, and the local ethical committee in Tikrit University College of Medicine. According to a questionnaire format, each patient was asked in detail regarding his symptoms, previous endoscopy, x-rays, past medical history, drugs and family history. The instrument used was Olympus GIF Q20 fiberoptic gastroduodenoscope, with 2 biopsy forceps labelled 1 and 2. The endoscope was thoroughly washed and disinfected after each procedure (with hibitane followed by plain tap water). The forceps were disinfected with 70% ethyl alcohol for 10 minutes, and then washed with normal saline after each use. The light source was Olympus SLE, and a double-chambered vacuum sucker was used. The patients were fasting overnight. No anesthesia or pre-medications were used. A consent was obtained from each patient, written down in the case notes. Seven biopsies were obtained from each patient during endoscopy. Two antral biopsies within 2cm from pylorus [one was sent for histopathology, the second for microbiology (urease test and culture)]. Likewise, two duodenal biopsies were obtained from the first part of duodenum, and also 2 esophageal biopsies were obtained from the lower third of the esophagus, and were managed in the same way as antral biopsies. One biopsy was obtained from stomach body, sent for histopathology.[10] The biopsy specimens were transported to bacteriology laboratory by sterile 2 ml tube containing brain heart infusion broth. They were kept at 4^{o}c until processing. Processing was carried out usually 1-3 hours post biopsy.[11,12] For microbiological purposes, each biopsy specimen was minced with sterile disposable surgical blade in sterile petri dish under sterile conditions. One piece was handled by sterile loop and inoculated a solid medium for culture. Another part was handled by another sterile loop and inoculated in urea slant for direct urease test. The rest of the specimen was used for Gram stain. Three types of solid culture media were used[11,12] namely: 1. Brain heart infusion agar with 5-7% human blood; 2. Blood agar base number 2 with 5-7% human blood; 3. Muller Hinton Agar with 5-7% human blood. All were supplemented with vancomycin 6mg/lit, Nalidixic acid 20 mg/lit and Griseofulvin 2 mg/lit to avoid contamination.[11,12] The inoculated plates were kept in a Co2 jar with gas generating kit (Oxoid, England), liberating 8% O_2, 5-8% Co_2 and 80% H_2 (micro-aerophilic condition) and were left for 5-7 days at 37^{o}C in the incubator. They were examined for *H.pylori* colonies after this period. After inoculation of the minced specimen in urea slant, the time was recorded for any change in color to develop (red), and reported as the following: within 10 minutes, within 20 minutes, within one hour, within 2 hours, within 3 hours, overnight, and up to 24 hours. The tubes were kept in the incubator at 37^{o}c. Gram stained slides were examined for gram-negative spiral bacteria. To confirm the diagnosis, from each positive culture plate, a small number of colonies was tested by Gram's stain, urease test, oxidase test and catalase test. The histological biopsies were processed routinely and were stained with Hematoxylin[8] Eosin and Giemsa stains. The first stain was utilized to show histological changes and to show *H.pylori*.

Patients with recurrent or chronic symptoms (such as >3-months), were included. Only the patients, who were referred by their caring physicians, were involved to avoid self-reporting of acute upper gastro-intestinal symptoms that may be caused by viruses (hepatitis) infections (food poisoning) or drug poisoning. Patients were excluded from the study when antibiotic or bismuth or antisecretory compounds have been used within 2 weeks before endoscopy, a major gastro-intestinal surgery has occurred recently, evidence of major organ disease or failure (heart, brain, liver, and kidneys) is present. Also, excluded were patients who were less than 14-years-old, patients with acute gastrointestinal emergency (bleeding, persistent vomiting or diarrhea or pain), prior endoscopy was carried out within the study period and if pregnancy were diagnosed or suspected. The sensitivities and accuracy rates were calculated according to the known formulae. All the results were handled and tabulated by the computer. Criteria of endoscopic diagnoses:

Table 1 - The frequency of the presenting symptoms and signs.

Presenting symptoms	n of patients (%)	Frequency of *H.pylori* positive (%)
Epigastric pain	186 (93)	24.9
Dyspepsia	128 (64)	17.1
Heartburn	140 (70)	18.7
Waterbrash	47 (23.5)	6.3
Bleeding	13 (6.5)	1.7
B-signs Epigastric tenderness/ or pointing sign	173 (86.5)	23.1

Table 2 - The relation between number of lesions endoscopically and frequency of *Helicobacter pylori* positivity.

Lesions n category	Frequency of each category n (%)	*H.pylori* positive n (%)
Single lesion	85 (44.7)	53 (62.3)
Double lesions	78 (41)	36 (46.1)
Multiple lesions	27 (12.2)	14 (51.8)
Normal	10 (5)	0 (0)
Total	**200 (100)**	

Gastritis. Endoscopic examination describes the visible changes of gastric mucosal lining such as edema, erythema, friability, exudates, flat erosions, nodularity, raised erosions, rugal hyperplasia, rugal atrophy, visibility of vascular pattern, and intramural bleeding.[13-17] Topographically, gastritis is divided into gastritis of antrum (B), pangastritis (antrum predominates or corpus predominates) or gastritis of corpus (A).[12,15]

Esophagitis. Edema, erythema, exudates and erosions were utilized to diagnose esophagitis.[18] The extent of the findings were utilized to determine the grade of inflammation.

Duodenitis. Same gross features as in gastritis were utilized to diagnose duodenitis.[19]

Ulcer. Whether esophageal, gastric or duodenal is obviously the mucosal discontinuity with which the base is deep and the margin is surrounded by edema and erythema.

Results. Two hundred patients were involved in the study, 88 males, with a mean age of 40.2-years, and 112 females with a mean age of 36.2-years. The age range was 14-100 years with a mean of 38.2-years. The frequencies of isolation of *H.pylori* in various age groups were 2.2% in 60-69-years, 60.6% in 50-59-years, 52.5% in 40-49-years, 42% in 14-39-years. The frequency at large was 51.5%. It was found that 93% had epigastric pain, 64% had dyspepsia, 70% had heartburn, 23.5% had waterbrash, as presenting symptoms, while 86.5% had epigastric tenderness or positive pointing signs **(Table 1)**. Ninety-five percent of the patients had abnormal endoscopic findings (such as 190 patients) **(Table 2)**. Also 73.1% of patients with endoscopic gastritis had positive antral biopsies for *H.pylori,* but 45.2% of patients with endoscopically normal stomachs had positive antral biopsies for *H.pylori* **(Table 3)**. It was found that 44.7% of patients had single lesions (endoscopically or histologically), 41% had double lesions and 14.2% had multiple lesions. Of those with single lesion, 62.3% were positive for *H.pylori*, 46.1% of those with double lesions were positive for *H.pylori,* and 51.8% of those with multiple lesions were positive for *H.pylori* **(Table 2)**. Only ten patients (5%) had normal looking esophagi, stomachs, and duodena endoscopically. All these were negative for *H.pylori* (100%) and had no histological evidence of inflammation. Out of 57 patients with endoscopic duodenitis, 86% had positive antral biopsies for *H.pylori*, by histopathology [47.3% by culture], and out of 35 patients with duodenal ulcers, 88.6% had positive antral biopsies for *H.pylori* by histopathology [85.7% by culture], but out of 108 patients with endoscopically normal duodena, 21.3% had positive antral biopsies for *H.pylori* by histopathology [17.6% by culture]. Also, out of 52 patients with endoscopic esophagitis, 57.7% had antral biopsies positive for *H.pylori*, proved by histopathology [only 28.8%, were positive by culture], but out of 148 patients with normal esophagi, 49.3% had positive antral biopsies for *H.pylori*, proved by histopathology [41.2% proved by culture]. Esophageal biopsies were positive for *H.pylori* [by culture] in 42.3% of patients with endoscopic esophagitis [and only 1.9% were positive by histopathology]. They were negative for *H.pylori* in endoscopically normal oesophagi. Endoscopy showed various types of gastritis. Antral biopsies were positive for *H.pylori* in 65.2% of those with mild gastritis, 100% of those with chronic severe gastritis (histologically diagnosed), but 11.4% of duodenal ulcer patients were positive for *H.pylori* in duodenal biopsies, 15.8% of those with duodenitis had *H.pylori* in duodenal biopsies. All duodenal biopsies from endoscopically normal duodena were negative for *H.pylori*. Gastritis was diagnosed

Table 3 - Endoscopical findings of the stomach mucosa versus *Helicobacter pylori* positivity of antral biopsies.

Endoscopical findings	Patients n (%)	Culture of *H.pylori* n (%)	Histological examination for *H.pylori* positive n (%)	Frequency of *H.pylori* isolation (%)
Gastritis	46 (23)	23 (50)	34 (73.9)	73.9
Gastric ulcer	4 (2)	4 (100)	3 (75)	100
Atrophic gastritis	2 (1)	0 (0)	0 (0)	0
Tumor	2 (1)	0 (0)	0 (0)	0
Normal	146 (73)	49 (33.6)	66 (45.2)	45.2
Total	**200 (100)**	**76 (38)**	**103 (51.5)**	

Gastric lesions	Endoscopical diagnosis n (%)	Histopathological diagnosis n (%)
Gastritis	46 (23)	127 (63.5)
Gastric ulcer	4 (2)	0 (0)
Atropic gastritis	2 (1)	3 (1.5)
Intestinal metaplasia	0 (0)	2 (1)
Tumor	2 (1)	1 (0.5)
Normal	146 (73)	67 (33.5)
Total	**200 (100)**	**200 (100)**

Table 4 - Endoscopical versus histological diagnosis of gastric findings.

endoscopically in 24% of patients, but it was diagnosed histologically in 65% of them. Likewise, a normal stomach was diagnosed in 73% of patients (excluding cancer and ulcer) endoscopically, but histology showed gastritis in 33.5% of these (**Table 4),** but figures for endoscopic and histologic diagnoses of duodenitis [57%, 60%,] duodenal ulcers [17.5%, 17%] and normal duodena [54%, 53%], were close to each other. Endoscopic and histologic diagnoses of esophagitis were 26% [for the former] and 33.5% [for the later]. For normal esophagi, the figures were 74%, and 66.5% in the same sequence. *Helicobacter pylori* also was found (by any mean and in any site) in 64.9% of those with positive family history for chronic dyspepsia and in 46.1% of those with no such family history. Likewise, *H.pylori* was found in 61.5% of smoking patients and in 45.1% of non-smoking ones. *Helicobacter pylori* was also found in 47.6% of patients living in rural area, and in 53.3% of those living in urban areas. *Helicobacter pylori* was also found in 81.4% of those with blood group O and this percentage varied between 34.4-36.8% for other blood groups. It was found in 70.6% of alcoholic patients and in 49.7% of non-alcoholic patients and was found in 63.8% of illiterate patients, 64% of those who read and write only, and 40% of secondary school and university graduated patients. Finally, no osophageal or duodenal malignancies were encountered, nor esophageal ulcers.

Discussion. There is an obvious increase in the frequency of *H.pylori* infection with increasing age.[5,8,20-22] Previous studies showed that 20% of persons below 40-years and 50% of those above 60-years are infected.[8,20] In our study, >72% of those above 60-years are infected. Some studies stated that the frequency of infection is higher in developing countries.[8,22] However, our study showed that 51.5% of the examined patients at large are infected, but these are pooled into the hospital from various districts and they are symptomatic people.

So this figure may represent the actual frequency of infection in the community. The percentage in the population at large may be higher. The highest rate of infection was noted in the age range of 20-69-years. Vomiting was not a prominent symptom as it is in other studies, but there was an obvious interlacing of symptoms, patients presented with. The most common symptom was epigastric pain, bleeding was least common, while epigastric tenderness was very common. The frequency of isolation of *H.pylori* was highest among those with epigastric pain as a symptom, and among those with pointing sign and epigastric tenderness as presenting signs. Close figures were also found in other studies.[5,8,11,23] Having 95% of patients with abnormal endosocpy indicates the high sensitivity of these symptoms and signs for the detection of *H.pylori* induced diseases, though they are non-specific features. Our study showed higher frequency of dyspeptic complaints among female patients. This fact was not found in other studies.[21,22] The high frequency of isolation of *H.pylori* (by culture, urease test, or histology) from antral mucosae of patients with endoscopic gastritis was faced by a lower (though still high) frequency of isolation of *H.pylori* from endoscopically normal stomachs. It is shown also that cases of atrophic gastritis[2] and gastric tumor[2] were negative for *H.pylori* by culture and histology. But the total number[4] is too small to draw any conclusions from. These high frequencies stress the following facts: 1. These normal stomachs (endoscopically) yet infected, may be associated with pathologies elsewhere (duodena) and 2. The *H.pylori* infection may lie dormant for variable periods of time only to cause 1. Gastritis when the stomach is exposed to an additional insult; 2. This dormant infection may be associated with a normal duodenum, which is actually passing in one of the known phases of peptic ulcer disease. Normal esophagus, stomach and duodenum do not exclude *H.pylori* infection. Actually, it has been found that normal esophagi may be associated with a higher frequency of antral *H.pylori* infection than abnormal esophagi (esophagitis), as will be discussed later. Whatever the endoscopic finding is, antral biopsy should always be obtained during endoscopy, beside other necessary biopsies. Likewise, as stated earlier, antral biopsies for *H.pylori* were positive in 65.2% of patients with mild gastritis (histopathologically) and 100% of chronic severe gastritis (histopathologically). This was also shown in previous studies,[20] but the frequency is higher in our study, possibly as the infection is more common in developing countries.[8] Some studies found that the density of *H.pylori* colonization is related to the grade of glandular atrophy.[11,24] Here it may be possible to add that the more the chronicity and or activity of gastritis, the more likelihood to get *H.pylori* positivity or higher *H.pylori* density. Our figures show very high frequency of antral *H.pylori* infection in duodenal ulcer patients and in patients with endoscopical duodenitis. These figures are comparable to figures of other studies.[25-27] Meanwhile endoscopically normal duodena also were associated with at least 21.3% rate of antral *H.pylori* infection (whether with antral gastritis or with a normal stomach). But, when duodenal biopsies were examined for *H.pylori* colonization, it is clear from our figures that only 11.4% of ulcerated duodena and 14% of inflammed duodena [duodenitis] are actually infected by *H.pylori*. This strengthens the facts that 1. The duodenal mucosa may not be suitable for the growth of *H.pylori*. This may be strengthened by the fact that *H.pylori* was best detected in the duodenal wall by culture rather than histology, which indicates the small number of *H.pylori* bacteria living in the duodenal wall. 2. Duodenal ulcers and duodenitis as such do not develop as a result of the infection of the duodenal mucosa itself. Biopsies from normal duodena (108) did not show evidence of *H.pylori* infection. 3. Hence, an inflamed or ulcerative duodenum developing due to other causes may create a milieu required for colonization of *H.pylori* (in the duodenum itself). The patients with esophagitis were found to have high level of antral colonization with *H.pylori*, however, endoscopically normal esophagi also had high level of antral colonization with *H.pylori*. The difference is not statistically significant. These figures appear higher than they are in other studies. It is probable that esophagitis actually does not parallel *H.pylori* infection. However in our study it is shown that esophageal biopsies tested for *H.pylori* infection were 40.4% positive (by culture only) in patients with esophagitis, but only 1.9% of these esophageal biopsies were positive by histology for *H.pylori*. This probably indicates that when esophagi are directly infected, the infection may be apparent in only one third of patients. Culture may be superior to histopathology in detecting *H.pylori* in esophageal biopsies. *Helicobacater pylori* bacteria may be present in only minute numbers in esophageal mucosa, so that they may not be detected histologically or by urease test. Interestingly all endoscopically normal esophagi were free from *H.pylori* infection. To summarize, fewer than half of esophagitis patients, have antral *H.pylori* infection, and esophageal *H.pylori* colonization, while > 40% of normal esophagi are associated (in symptomatic patients) with antral *H.pylori* colonization, but all normal esophagi are probably free from direct *H.pylori* infection. Other studies showed similar tendencies.[19,26,27] Actually, the problem of *H.pylori* infection in esophagitis and gastroesophageal reflux disease (GORD) was extensively studied in the guidelines of European Society for Primary Care Gastro-enterology

(ESPCG) in 1999.[28] Regarding the number of lesions seen in each patient endoscopically, it is clear that there is no linear relationship (directly or inversely proportional) between the number of lesions and frequency of *H.pylori* infection; so much so *H.pylori* infection could cause any number of lesions, at any time in the same patient. There may be variability over time and place. However, it appears that the tendency of *H.pylori* is to produce one lesion. Also, it is clear that antrum constitutes the most frequent site of colonization in the stomach (detected by histology mainly) followed by the esophagus (detected mainly by microbial culture), followed by duodenum, (detected by microbial culture as well). Hence, histology may be the most sensitive for detection of *H.pylori* infection from the antrum, but culture may be superior in detecting the infection from esophagus and duodenum. The reason for that could be the minute number of *H.pylori* bacteria residing in the esophagi and duodena. Stomach body was not cultured. Factors that may help spread infection between family members, which may better be called as we suggest [familial clustering] may be: 1. Low socio-economic status with high crowding index. This was evident in our figures (see results) and supported by other studies,[29,30] 2. Smoking may be a risk factor that helps stabilization of infection with *H.pylori*. Once it is acquired, smoking may lower local immunity of gastric endothelium, or may increase the boring power of *H.pylori*; 3. Likewise, chronic alcoholism may increase the tendency to bacterial infection in upper gastro-intestinal tract.[31] The same explanation for 2 may apply here as well. A separate study is required to confirm this hypothesis.

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