Basrah Journal of Surgery

Bas J Surg September, 11, 2005

IDENTIFICATION OF *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH UPPER GASTROINTESTINAL DISEASES, AND ITS ANTIMICROBIAL SENSITIVITY

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Abstract

H.pylori is the cause of duodenal ulcer, and a lot of other gastrointestinal diseases, the aim of this study was to see the extent of this microorganism in our patients and to study its antimicrobial sensitivity. The study included 283 patients(148 males and 135 females) with upper gastrointestinal complaints including dyspepsia, heartburn, bleeding, and malabsorption to evaluate the presence of *Helicobacter pylori* (*H.pylori*) infection using rapid urease test (RUT) and culture to see there sensitivity to different antibiotics.

The study revealed that 199 patients (70.3%) have positive RUT compared to 218 patients (77.0%) showed positive culture results. The positive culture results were confirmed by positive results of biochemical tests (oxidase, catalase and urease).

Antibiotic sensitivity testing was performed in 28 cultures of *H. pylori*, and the results showed that 26 cultures (92.9%) were sensitive to amoxycillin, followed by clarithromycin, rifampicin and cephalexin with figures of 23 (82.1%), 22 (78.6%) and 21 (75.0%) respectively. Whereas, 16 cultures (57.1%) were resistant to metronidazole, 15 (53.4%) to erythromycin and 9 (32.1%) to both cephalothin and tetracycline.

It is concluded that, *H.pylori* infection is a predominant etiological factor of upper gastrointestinal diseases, Also, RUT represent simple, convenient and reliable mean for the rapid diagnosis of *H.Pylori* infection. Antibiotic sensitivity was the highest with amoxycillin and clarithromycin while the most resistant antibiotic strains were encountered with metronidazole.

Introduction

The discovery of *Helicobacter pylori* (*H.pylori*) infection as infective causative agent of peptic ulcer disease twenty years ago[1], has revolutionized the etiology, pathogenesis and treatment of that disease. Now, and beyond any doubt, *H.pylori* infection represent the principal etiological factor of peptic ulcer[2, 3] and even gastric cancer[3].

The pathogenecity of *H.pylori* infection depends on the presence of virulence factors and the host response. The microorganisms resist gastric acidity and local host defense mechanisms[4].

H.pylori is able to live in the acidic medium of the stomach due to its ability to produce the enzyme "Urease" which hydrolyzes urea into one molecule of carbon dioxide and two molecules of ammonia which converted into ammonium hydroxide, which in turn, raises the pH of the surrounding medium[5].

H.pylori is the most common urease producing microorganism, next to *Ureaplasma urelyticum*. The toxic effect of urease to the host is related to the production of ammonium hydroxide resulting from urea hydrolysis. Ammonium hydroxide is toxic to the gastric cells, where the cellular viability is reduced with increasing ammonia concentrations generated from urea catabolism. The cellular viability

restored with addition of urease inhibitor, acetahydroxamic acid, which reduces the enzyme activity, and there by, the number of ammonia molecules generated[6].

There is a widely accepted evidence that *H. pylori* is sensitive to a broad spectrum of antimicrobial agents including amoxycillin, metronidazole and clarithromycin.It is resistant to low number of antimicrobials such as sulphonamides, nalidixic acid and vancomycin[7,8].

The aim of this study was to determine the prevalence of *H.pylori* infection among patients with upper gastro-ntestinal complaints, using rapid urease test (RUT) and microbiological identification, and its antibiotic sensitivity.

Patients and Methods

This is a prospective study carried out from July, 2000 throughout December, 2002, and included 283 patients with various upper gastrointestinal complaints including dyspepsia, heartburn, bleeding, and malabsorption. They were 148(52.4%) males and 135(47.6%) females. The age range 18-70 years of age. Patients were subjected to upper gastrointestinal endoscopy at Al-Sadder Teaching Hospital, and then two biopsies from the gastric antrum were taken. The first one was used for the RUT while the second was retained to culture *H. pylori* in a microphilic conditions depending on a native gas pack which was qualified in culturing this bacterium[9].

RUT was done by dissolving 10 g of urea in 100 ml of distilled water, then 0.02g of phenol red indicator is added.20 ML of the solution is placed in plastic container, and the biopsy specimen is immersed in that container and the color change from yellow to pink is observed for an hour[10].

H. pylori are identified by several means, including Gram stain[11], biochemical tests including oxidase[12], catalase [12] and urease [7] tests.

Antibiotic sensitivity testing was performed in 28 cultures of *H. pylori*, according to the availability of antibiotic discs[13].

Results

Table I resents the diagnosis at endoscopy, where 46.3% of patients have gastritis, followed by duodenal ulcer (13.8%), gastritis and duodenal ulcer (11.3%), duodenitis (6.4%) and gastritis and duodenitis (6.4%). Normal endoscopy was confirmed in 9.2% of patients.

As shown in TableII, RUT revealed that 199 patients (70.3%) were infected with *H.pylori* compared to 218 patients (77.0%) confirmed by microbiological means.

Table III present findings biochemical tests in positive cultures (N = 218), where all showed positive tests of oxidase, catalase and urease.

As presented Table IV amoxycillin showed the highest sensitivity rates, where 26 cultures (92.9%) were sensitive to it, followed by clarithromycin, rifampicin and cephalexin with figures of 23 (82.1%), 22 (78.6%) and 21 (75.0%) respectively. On the other hand, 16 cultures (57.1%) were resistant to metronidazole, 15 (53.4%) to erythromycin and 9 (32.1%) to both cephalothin and tetracycline.

Discussion

The diagnosis of *H.pylori* infection as a cause of peptic ulcer disease, focused the attention of researchers toward ulcer therapy and the use of antimicrobials for the eradication of infection, as it promotes the healing of ulcer and reduces the rate of it's recurrence[4].

The study revealed that 77.0% of patients have positive culture results. This finding is in agreement with other studies[14,15]. The positive results of

cultures of *H.pylori* are supported by results of biochemical tests which showed positive tests in all cultures. This is similar to the observation of others[7,9,12]. The result of cultures in this study is satisfactory, as there is no ideal culture medium for cultivation of *H.pylori*, but it differs from one medium to another[12].

Urease production by *H.pylori* represent an essential step for its colonization in the gastric mucosa, as it provide alkaline environment surrounding the microorganism suitable for its survival through urea breakdown[4]. Also, the gastric juice in *H.py*lori infected patients contains high ammonia concentrations more than urea concentrations compared to non-infected subjects[16]. The RUT result was positive in 70.3% of patient, which is comparable to other results[17-20].The urease present in the stomach of patients with gastrointestinal diseases is of infective origin, because its activity is abolished with antibiotic treatment[21]. However, negative result of RUT, in the presence of *H.pylori* infection, may be encountered in some cases due to low bacterial count in the biopsy specimen leading to false negative result[22]. The benefit of RUT arises from its convenience and feasibility, as it can be done in the endoscopy room, and the result can be obtained within one hour compared to 3-4 days of the culture results.

The study revealed very high sensitivity rate of *H. pylori* to amoxycillin, which is in agreement with several studies published elsewhere in the world[23,24], indicating a low resistance rate of *H. pylori* to this antibiotic. The sensitivity rate of *H. pylori* to clarithromycin was also high, and it is comparable to that of Realdi et al[25], while it is different from other reports[12,26]. In western countries the resistance rate of *H.pylori* to clarithromycin reached to 60% due to the prolonged use of this somewhat expensive drug[26], while it is a little bit new drug in our country. On the other hand, the widely used drug in the combination treatment for the eradication of *H.pylori* infection, metronidazole, showed the highest resistance rate among the tested antibiotics(57.1%), a result comparable to that of Bindyna[27] and Xiao et al[28], while it is distinctly higher than that of Magraud[29]. This result can be explained by the irrational use of the drug as well as it's abuse in gastrointestinal complaints. This, in turn, necessitates further studies to clarify the effectiveness of metronidazole in the eradication strategy of *H. pylori* infection.

In conclusion, H.pylori is the principal infective agent causing upper gastrointestinal disease. The RUT represent simple, rapid as well as satisfactory diagnostic tool of *H.pylori* infection. The highest antibiotic sensitivity rates were encountered with amoxycillin and clarithromycin, while the highest antibiotic resistance rates were encountered with metronidazole.

Table I. Endoscopical diagnosis of the studied Patients

Diagnosis	No.	%
Gastritis	131	46.3
Duodenal ulcer	39	13.8
Gastritis and duodenal ulcer	32	11.3
Duodenitis	18	6.4
Gastritis and duodenitis	18	6.4
Duodenitis and duodenal ulcer	7	2.5
Gastric ulcer and duodenal ulcer	5	1.8
Gastric ulcer	4	1.4
Gastritis and gastric ulcer	3	1.1
Normal	26	9.2
Total	283	100.0

Table II:RUT and Microbiological diagnosis

Diagnostic tool Result	RUT		Microbiological Diagnosis	
	No.	%	No.	%
Positive	199	70.3	218	77.0
Negative	84	29.7	65	23.0
Total	283	100.0	283	100.0

Table IIIBiochemical tests in cases with positive cultures of H.pylri (n=218).

Enzyme Result	Oxidase		Catalase		Urease	
	No.	%	No.	%	No.	%
Positive	218	100.0	218	100.0	218	100.0
Negative	0	0.0	0	0.0	0	0.0
Total	218	100.0	218	100.0	218	100.0

TableIV: antibiotic sensitivity (n=28)

Result	Amx No.(%)	Cla No.(%)	Met No.(%)	Rif No.(%)	Cep No.(%)	Ery No.(%)	Cef No.(%)	Tet No.(%)
Sensitive	26 (92.9)	23 (82.1)	12 (42.9)	22 (78.6)	21 (75.0)	13 (46.6)	19 (67.9)	19 (67.9)
Resisrant	2 (7.1)	5 (17.9)	16 (57.1)	6 (21.4)	7 (25.0)	15 (53.4)	9 (32.1)	9 (32.1)
Total	28 (100)	28 (100)	28 (100)	28 (100)	28 (100)	28 (100)	28 (100)	28 (100)

Amx: Amoxycillin, Cla: Clarithromycin, Met: Metronidazole,Rif: Rifampicin, Cep: Cephalexin, Ery: Erythromycin, Cef: Cephalothin,Tet: Tetracycline

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