Helicobacter pylori in Sri Lanka

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Helicobacter pylori, a gram negative spiral organism was first demonstrated in the gastric mucosa by Marshall and Warren[1,2] in the early nineteen eighties. Since then, numerous reports have appeared in the world literature on the epidemiology, methods of isolation, pathogenicity and aspects of treatment related to the organism.

The incidence of Helicobacter varies geographically and where studies have been done, age and the socioeconomic status have been shown to affect the incidence of gastritis[3,4].

The aim of this study was to determine the incidence of Helicobacter in gastric biopsies of Sri Lankans using the methods available to us at the present time, to demonstrate the organism.

Material and methods

Gastric biopsies were obtained from the first four patients endoscoped at the Endoscopy Unit at SJGH, every Monday, Wednesday and Friday, for a period of five months from the beginning of May to the end of September 1990.

Four biopsies were obtained within five centimetres of the pylorus, from each patient. The first two specimens were used for preparing Gram stains immediately in the Endoscopy room following which they were placed in sterilized bottles containing 0.5 ml. of modified Christensens broth, to demonstrate urease activity. These were then placed in an incubator at 37.C to facilitate optimal urease activity, within half an hour of obtaining the specimen. Colour changes in the medium were observed at two, six and twenty four hours.

Biopsy forceps were washed in tap water and cleaned with 2% glutaraldehyde between patients.

Two specimens were placed in formol saline, embedded in wax and sections were prepared for staining with haematoxyilin and eosin (H & E.)

Gram stained smears and sections were reported on by a trained pathologist unaware of the endoscopy findings.

The density of organisms were graded as follows.

0. No organisms seen.
1. Few organisms seen, but not in all high power fields.
2. Organisms seen in all high power fields.
3. Numerous organisms seen in all high power fields.

The endoscopy findings were classified as follows:

A. Normal gastric mucosa
B. Superficial gastritis — erythema of the gastric mucosa without a breach.
C. Erosion — breach in the mucosa less than five mm wide and superficial.

D. Ulcer — breach in the gastric mucosa wider than 5 mm.

Other pathology such as varices, oesophagitis was recorded.

Results

A total of 175 patients were biopsied, there were 111 males and 64 females.

Table I shows the age distribution of patients, which ranged from 16 to 78 years. Helicobacter pylori was demonstrated fairly uniformly in all age groups.

Table I
Age distribution

Age distribution (in years)	*No*
15 - 25	21
26 - 35	26
36 - 45	39
46 - 55	25
56 - 65	37
66 - 75	23
over 75	4

The endoscopic diagnoses are shown in Table II. Nearly 30% had a normal endoscopy and a little over 20% had superficial gastritis. On endoscopy these two therefore accounted for over 50% of patients.

Table III shows the positive cases of Helicobacter using the three methods that were available, together with the organism density graded 1, 2 and 3 as applicable to gram staining and histology. The organism was demonstrated in over 80% of the gastric biopsies, but were in very low density in 98 of the 145 positive cases. There were 11.4% positive using the biopsy urease test and gram staining.

The histological appearence of the gastric mucosa is shown in Table IV, where the severity of gastritis appears to increase with age.

In Table V, the correlation between the endoscopic diagnosis, presence of Helicobacter demonstrated on H & E staining is shown.

Table II

Endoscopic findings	*No.*	*%*
Normal	56	32 %
Superficial gastritis	41	23.4 %
Erosive gastritis	18	10.2 %
Duodenitis	1	0.58%
Gastric Ulcer	16	9.14%
Duodenal ulcer	13	7.4 %
Oesophagitis	18	10.2 %
Hiatus hernia	4	2.3 %
Pyloric stricture	1	0.58%
Healed ulcer	3	1.76%
Varices	3	1.7 %
Carcinoma	1	0.58%

Table III

Helicobacter pylori positive cases

	+	++	+++	Negative
Histology	98	36	11	30
Gram stains	1	2	3	Neg:
	7	4	9	155
Biopsy	positive			Neg:
	20			155

Table IV

Histological appearances of gastric biopsies

Histology	*No.*	*Average age (in years)*
Normal	45	41
Mild gastritis	96	48
Moderate gastritis	17	54
Severe gastritis	15	54
Cancer	2	

Table V

Correlation between endoscopic diagnosis, presence of gastritis and incidence of helicobacter pylori

	Histology			
Endoscopy finding	*Normal*	*Mild*	*Moderate*	*Severe*
Normal	25	22	3	2
	+18	+21	+3	+2
Superficial gastritis	6	30	3	2
	+ 5	+29	+3	+2
Erosive gastritis	3	16	nil	nil
	+ 2	+11		
Duodenitis	nil	nil	1	nil
			+1	
Gastric ulcer	nil	12	2	2
		+ 9	+2	+1
Duodenal ulcer	2	4	4	4
	+ 1	+ 3	+4	+4
Oesophagitis	6	9	nil	3
	+ 4	+ 9		+3

(*Contd.*)

Endoscopy finding	*Normal*	*Mild*	*Moderate*	*Severe*
Hiatus hernia	3	1	nil	nil
	+ 2	+ 1	nil	nil
Pyloric stricture	nil	nil	nil	1
Healed ulcer	nil	nil	2	1
			+2	+1
Varices	nil	2	1	nil
		+ 1		
Carcinoma 2				

Discussion

Methods available to demonstrate Helicobacter are:

1. Culture: Blood enriched culture media with selected antibiotics are used to culture the organism in micro-aerophilic conditions. This was not available at the time this study was undertaken.
2. Histology: Warthin-Starry silver staining is considered the standard for demonstrating Helicobacter but is expensive and not used routinely. H&E and Giemsa staining techniques are also accurate as will be shown later.
3. Biopsy Urease Test[5]: It is a simple, cheap and quick method of demonstrating the organism and is based on the high content of performed enzyme urease in biopsies positive for H. Pylori. When biopsies are placed in a medium rich in urea, the enzyme splits urea producing ammonia, increasing the PH which can be readily detected by a PH indicator in the medium. We used the broth described by Christensen. The CLO test is a commercially available kit using the same principle.
4. Demonstrating antibodies in serum using Elisa technique.
5. Breath Urease Tests[6]: Urea with radioactive labelled Carbon is ingested which is split by urease produced by the organism. The Carbon dioxide produced with labelled carbon is exhaled in the breath and is measured using a scintillating counter.
6. Gram Staining:

B. Joseph, T. Bianchi[7] and others compared the specificity and sensitivity of the different methods available to demonstrate H. pylori. They concluded that the CLO Test, based on biopsy urease activity, Giemsa and H&E all had similar sensitivities while the urease test was more specific. The 3 methods used by us therefore have been validated.

Epidemiological studies done world wide suggest the H. pylori is tranmitted by the faeco-oral route. In developed countries, the incidence increases with increasing age, which is similar to the presence of gastritis, which too increases with age. In developing countries however, the organism appears to be acquired at an earlier age, probably due to poor socioeconomic conditions. In the study too Helicobacter was demonstrated fairly uniformly in all age groups.

In this study, there was a remarkable difference in the incidence of H. pylori as demonstrated by H&E staining when compared with the biopsy urease test. False negative biopsy urease tests are seen if the organisms are scanty and if they have been exposed to antibiotics just before endoscopy. We do not have data on the drug history of our patients, but in over 67% of positive cases, organisms were scanty. This could well account for the low rate of biopsy urease tests. Are these strains with low urease activity? This is a theoretical possibility to which we have no answer.

Pathogenic mechanisms by which Helicobacter causes mucosal injury are poorly understood, but some possible methods are the production of enzymes such as urease and catalases, digestive enzymes lipases, proteases and phospholipases, production of cytotoxins and antigenic stimulation of an immune response in the mucosa which may lead to cell mediated injury. One or more of these pathogenic mechanisms could lead to gastritis resulting breakdown of mucosal resistance. Acid and other injurious agents such as NSAID's could then lead to ulceration.

We have a low incidence of ulcer disease in Sri Lanka. Though this study showed over 80% positive for Helicobacter on H&E staining, organisms were scanty and the urease test was positive in only 11%. It would be tempting to conclude that this latter finding could be tied up with the low incidence of ulcer disease in our country. More studies are needed before we can determine what role Helicobacter plays in the pathogenesis of peptic ulcer in Sri Lanka.

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