Helicobacter pylori in Dyspeptic Children and Adults: Endoscopic, Bacteriologic and Histologic Correlations

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Using different bacteriological (urease test, Gram staining and culture) and histological (Steiner staining and modified Giemsa staining) techniques, we searched for the presence of Helicobacter pylori *in the gastric antrum of 200 dyspeptic Brazilian patients (106 females and 94 males aged 19 days to 81 years). The presence of bacteria was then correlated with the endoscopic and histological findings. H. pylori was present in 59.5% of the population studied. In Brazil, colonization occurs early, involving 37% of the dyspeptic population by 20 years of age. The presence of* H. pylori *in the gastric antrum was strongly associated with duodenal ulcer (P<0.001) and a normal endoscopic examination did not exclude the possibility of colonization of the gastric antrum by* H. pylori. *The most sensitive test was the preformed urease test (89%). We conclude that more than one diagnostic method should preferably be used for the detection of* H. pylori *and that the presence of* H. pylori *is closely correlated with active chronic gastritis (P<0.001).*

Key words: *Helicobacter pylori* - gastric ulcer - duodenal ulcer - adults - children

Helicobacter pylori has been associated with gastritis, peptic ulcer, non-ulcerous dyspepsia and, more recently, has been considered as a potential risk factor for the development of gastric cancer (The Eurogast Study Group 1993). Despite these observations, however, the clinical significance of *H. pylori* continues to be obscure.

Many studies have been published in the international literature (Marshall & Warren 1984, Price et al. 1985, Von Wulffen et al. 1986, Lamouliatte et al. 1987, Itoh et al. 1987, Marcheggiano et al. 1987, Gnarpe et al. 1988), whereas information about this topic is scarce in Brazil (Coelho et al. 1987, Ferrari et al. 1989). Questions such as ethnic variations, incidence and associated digestive pathologies, especially in the younger age ranges, continue to be unexplained. On this basis, the objective of the present study was to determine the incidence of *H. pylori* in the gastric antrum of dyspeptic children and adults using different bacte-riological and histological techniques, and to correlate the presence of bacteria with endoscopic and histological findings.

MATERIALS AND METHODS

Patients - The study was conducted on 200 consecutive patients with complaints related to the upper digestive tract who were referred to three endoscopy services located in the municipality of Rio de Janeiro for diagnosis. The sample consisted of 106 females and 94 males ranging in age from 19 days to 81 years. All the patients were inquired if they were taking antibiotics or bismuth compounds during ten days before the procedure.

Endoscopy and collection and transport of gastric biopsies - All patients or the persons responsible for them gave informed consent to participate in the study. All patients were examined after a fast of more than 6 hr. Children were examined with an Olympus GIF XP10 fibroscope under general inhalation anesthesia (halothane-nitrogen protoxide and oxygen). The adults were examined with an Olympus XQ fibroscope after anesthesia of the oropharynx by local spraying with 10% lidocaine hydrochloride and sedation with a combination of the ansiolytic

drug diazepam (0-10 mg) and of the hypnoanalgesic drug meperidine (50 mg) administered intravenously.

After judicious evaluation of the esophagogastroduodenal tract, four biopsy fragments of gastric antral mucosa were taken 1 and 2 cm from the pylorus of children and adults, respectively. The fragments were immersed in the following solutions: urea buffered with an indicator (0.05 ml) for the presumptive test for the presence of *H. pylori* by the method of Hazell et al. (1987); 20% glucose (0.5 ml), the transport medium for bacteriological analysis; 10% formalin, a histological preserver (two fragments).

Bacteriology - The preformed urease test (PFUT) was started in the endoscopy room and monitored for 24 hr in order to observe urease activity, revealed by the change in color of the pH indicator. The transport medium containing the biopsy fragment was maintained under refrigeration at 4°C in an isothermal container and sent to the laboratory for processing within less than 3 hr. The fragment was inoculated by rolling onto the surface of the selective medium (Goodwin et al. 1985) and incubated at 37°C in a jar with a humid microaerophilic atmosphere (Gas Pak BBL 70304 envelope, an H_2 and CO_2 generator, with no catalyser). After inoculation, a smear was obtained from the same fragment for Gram bacterioscopy.

The jar was kept under observation for seven days and was opened on the 3rd and 7th days. Growth was characterized on the basis of colony morphology, Gram stain, and the production of urease, catalase, and oxidase according to Czinn et al. (1989).

Histology - Staining with haematoxylin and eosin (H & E) was used for the diagnosis and classification of gastritis (Warren 1983) and the methods of Steiner and Steiner (1949) and modified Giemsa (Gray et al. 1986) were used for visualization of spiral forms.

Statistical analysis - Data were analysed by the chi-square test for the determination of association between variables using contingency tables and Yates correction when necessary.

RESULTS

Endoscopy of the esophagogastroduodenal tract revealed as main diagnosis 62 normal examinations, 59 cases of chronic gastritis, 48 cases of peptic ulcers, 11 cases of duodenitis, 10 cases of esophagitis, 5 cases of gastric erosion, and 5 miscellaneous examinations.

The presence of *H. pylori* was determined by bacteriological techniques (PFUT, Gram staining and culture) and/or histological techniques (Steiner staining and modified Giemsa staining) in the gastric antrum of 119 patients (59.5%), with an incidence of 67.8% in the population older than 20 years and an incidence of 37.0% in the under 20 group and a similar distribution among males an females (Table I). *H. pylori* was detected in all age ranges studied, except for that of less than 30 days (Table II).

When we calculated the correlation between endoscopic data and presence of *H. pylori* in the gastric antrum, the microorganism was found to be present in high percentages in most of the upper digestive pathologies diagnosed (Table III). *H. pylori* was significantly associated with duodenal ulcer (P<0.001; X^2 = 15.79) and with gastric ulcer (P<0.01; X12 = 6.66) but not with gastritis (P>0.05; X^2 = 0.02).

Comparison of the bacteriological and histological methods used to demonstrate the presence of *H. pylori* (Table IV) showed a clear superiority of the preformed urease test (89%). The rate obtained by histological staining was similar to that obtained by culture.

Histology of gastric biopsies stained with H & E was highly correlated with the presence of *H. pylori* in patients with active chronic gastritis (91.4%) and less markedly correlated with chronic gastritis (47.1%). Both correlations were statistically significant at the P<0.001 level (X^2 = 83.72 for active chronic gastritis and X^2 = 12.68 for chronic gastritis), as shown in Table V.

TABLE I

Distribution of patients with *Helicobacter pylori* in the gastric antrum by age and sex

Population	Sex				Total	
	Male		Female			
	No.	%	No.	%	No.	%
Adult	45(66)	68.1	54(80)	67.5	99(146)	67.8
Less than 20 years	10(28)	35.7	10(26)	38.4	20(54)	37.0
Total	55(94)	58.5	64(106)	60.3	119(200)	59.5

TABLE II

Distribution of patients with *Helicobacter pylori* in the gastric antrum by age range

Age range	Present No.	Present %	Absent No.	Absent %	Total
< 30 days	-	-	1	100.0	1
> 30 days - 1 year	1	25.0	3	75.0	4
> 1-6 years	5	23.8	16	76.1	21
> 6-12 years	10	45.4	12	54.5	22
> 12-19 years	4	66.6	2	33.3	6
20-29 years	4	40.0	6	60.0	10
30-39 years	25	62.5	15	37.5	40
40-49 years	20	74.0	7	25.9	27
50-59 years	22	73.3	8	26.6	30
60-69 years	20	76.9	6	23.0	26
70-79 years	7	70.0	3	30.0	10
80-89 years	1	33.3	2	66.6	3
Total	119	59.5	81	40.5	200

(H. pylori header spans Present and Absent columns)

TABLE III

Correlation between endoscopic data and presence of *Helicobacter pylori* in the gastric antrum

Endoscopic data	Population Adult		Population Less than 20 years		Total	
Normal	17/34	(45.9)[a]	7/25	(28.0)	24/62	(38.7)
Chronic gastritis	30/46	(65.2)	4/13	(30.7)	34/59	(57.6)
Active duodenal ulcer	14/14	(100.0)	1/1	(100.0)	15/15	(100.0)
Healed duodenal ulcer	7/7	(100.0)	1/2	(50.0)	8/9	(88.8)
Active gastric ulcer	10/11	(90.9)	0/1	-	10/12	(83.3)
Healed gastric ulcer	8/9	(88.8)	-	-	8/9	(88.8)
Active gastric/duodenal ulcers	2/2	(100.0)	-	-	2/2	(100.0)
Healed gastric/duodenal ulcers	1/1	(100.0)	-	-	1/1	(100.0)
Duodenitis	4/7	(57.1)	3/4	(75.0)	7/11	(63.6)
Gastric erosions	3/4	(75.0)	1/1	(100.0)	4/5	(80.0)
Esophagitis (grades II/III)	1/4	(25.0)	2/6	(33.3)	3/10	(70.0)
Gastric neoplasia	1/2	(50.0)	-	-	1/2	(50.0)
Gastric polyps	0/1	-	-	-	0/1	-
Gastric lymphnode hyperplasia	-	-	1/1	(100.0)	1/1	(100.0)
Hiatus hernia	1/1	(100.0)	-	-	1/1	(100.0)
Total	99/146	(67.8)	20/54	(37.0)	119/200	(59.5)

[a] : patients with *H. pylori*/total no. of patients (percentage).

DISCUSSION

When the sample studied was divided into populations older than 20 and younger than 20, the rates of *H. pylori* colonization were 67.8 (99/146) and 37.0% (20/50), respectively. The percentage obtained for the adult population, although slightly lower than that detected by other Brazilian investigators (Coelho et al. 1987, Ferrari et al. 1989), was much higher than that obtained in de-veloped countries (Marshall & Warren 1984, Price et al. 1985, Von Wulffen et al. 1986, Lamouliatte et al. 1987, The Eurogast Study Group 1993). This difference was more marked in the under 20 group (37.0%) for which Hill et al. (1986) and Drumm et al. (1987) detected rates of 15.7% (6/39) and 10.4% (7/67), respectively.

It should be pointed out that in Brazil there is early colonization of the gastric antrum, that probably occurs more frequently among preschoolers

TABLE IV

Performance of the different methods used for the detection of *Helicobacter pylori* in gastric biopsies according to origin

Method	Population				Total	
	Adult = 99[a]		Less than 20 years = 20			
	No.	%	No.	%	No.	%
Performed urease test	91	91.9	15	75	106	89.0
Gram staining	76	76.7	12	60	88	73.9
Steiner/modified Giemsa staining[b]	62	62.6	13	65	75	63.0
Culture	60	60.6	14	70	74	62.1

[a] : total number of patients with *H. pylori*.
[b] : Steiner staining was used for the initial 119 patients and detected *H. pylori* in 68% (51/75), whereas modified Giemsa staining was used for the remaining 81 patients and detected *H. pylori* in 54.5% of them (24/44).

TABLE V

Correlation between gastric biopsies stained with H & E and presence of *Helicobacter pylori* according to origin

Histology data	Population		Total
	> 20 years	< 20 years	
Normal	4/27 (14.8)[a]	4/26 (15.3)	8/53 (15.0)
Chronic gastritis	19/40 (47.5)	6/13 (46.1)	25/53 (47.1)
Active chronic gastritis	76/79 (96.2)	10/15 (66.6)	86/94 (91.4)
Total	99/146 (67.8)	20/54 (37.0)	119/200 (59.5)

[a] : patients with *H. pylori*/total no. of patients (percentage).

(23.8%) and schoolchildren (45.4%) and tends to increase with age, eventually reaching rates higher than 70%. The maintenance of a chronic status for consecutive years may represent a risk factor for the development of gastric cancer, as suggested by the Eurogast Study Group (1993).

When the endoscopic data of the present patients were correlated with the presence of *H. pylori* in the gastric antrum, the bacterium was found to be present in high percentages in most of the digestive pathologies diagnosed, particularly duodenal ulcers (100%) and gastric ulcers (83.3%), in agreement with data reported in the literature. In the under 20 population, the incidence of *H. pylori* was higher in duodenal pathologies (ulcer and duodenitis).

Endoscopic diagnosis of gastritis was not well correlated with the presence of *H. pylori* (65.2% in the over 20 population, 30.7% in the under 20 population, and 57.6% in the total sample), but the gastric mucosa is subjected to aggression of a different nature, such as alcohol and medications. In this respect, Brazilian rates (Coelho et al. 1987, Ferrari et al. 1989) are also higher than interna-

tional rates (Marshall & Warren 1984, Itoh et al. 1987).

An interesting result was the presence of *H. pylori* in 38.7% of the patients with normal endoscopy, a rate lower than those reported by other Brazilian authors (Coelho et al. 1987, Ferrari et al. 1989) and by Marshall and Warren (1984) and Marcheggiano et al. (1987), but higher than most of those reported by international investigators, possibly representing an early phase of evolving digestive pathology with still undetectable major macroscopic alterations.

Among the diagnostic methods used for the detection of *H. pylori*, the performed urease test had a higher positivity rate (89.0%), followed by Gram staining (73.9%), histological staining (63.0%) and culture (62.1%). The preformed urease test is quite safe since the inclusion of sodium azide prevents the multiplication of contaminants, only permitting the action of preformed urease (in a constitutive manner) on the substrate.

Gram staining is also a good method, but the use of the same biopsy fragment after inoculation for the preparation of the smear causes impover-

ishment of the material and a consequent reduction in the number of microorganisms, impairing the results of the test or leading to negative results.

Comparison of the histological staining techniques used showed that silver staining was more effective (68.0%, 51/75) than modified Giemsa (54.5%, 24/44), in agreement with data reported by Gustavsson et al. (1987).

The fact that the positivity rate of culture was lower than that obtained with the other methods may have been due to the excessive presence of contaminants, to an escape from the micro-aerophilic atmosphere by poor jar sealing, or in some cases to inhibition of the antimicrobial agents present in the selective medium.

It should be pointed out that the distribution of *H. pylori* in the gastric antrum may be heterogeneous or even focal (Lamouliatte et al. 1987, Morris & Nicholson 1987), explaining the discrepant results obtained with different methods. Thus, it is recommended that the microorganisms be searched for in more than one biopsy and using several techniques, or at least two different methods. It is also important to certify that the patients are not using antimicrobials or bismuth compounds. We suggest the PFUT and either the Gram staining or the histological staining methods for routine.

All techniques employed, except culture, tended to be less effective for biopsies from the under 20 population. We propose that the smaller, more friable biopsy of infants and young individuals, with a thick mucus layer may explain this fact.

In the overall sample computation, histological analysis of the gastric biopsies (H & E) showed a close correlation of the microorganism with active chronic gastritis (91.4%) and a less marked correlation with chronic gastritis (47.1%). To justify the differences observed between the populations studied here or even between our data and those reported by others with respect to the presence of the bacteria in active chronic gastritis or in chronic gastritis, it is important to remember the experiments carried out on volunteers (Marshall et al. 1985, Morris & Nicholson 1987) who, after going through the acute phase with polymorphonuclear infiltrates in the gastric mucosa (active gastritis), developed chronic gastritis. Thus, the search for the bacteria may have occurred during distinct phases of colonization by the microorganism.

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