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INVESTIGATION FOR THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH GASTRIC CARCINOMA IN MADRAS, INDIA

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SUMMARY: The most common type of gastric carcinoma, namely the intestinal type, has been proposed to result from a precancerous process in which chronic gastritis, atrophy, intestinal metaplasia and dysplasia develop in a sequential manner. *Helicobacter pylori* is considered as the main cause of chronic atrophic gastritis and thus may play a role in the gastric carcinogenesis process. The present study was aimed at investigating the prevalence of *H. pylori* in the gastric carcinoma cases. Urease tests and ELISA developed in our laboratory and culture were used to assess the prevalence of *H. pylori* in the study group. The positivity of *H. pylori* by various tests ranged from 56.0 to 62.6% in the gastric carcinoma group and 37.3 to 46.6% in the control subjects, the difference being statistically significant. This suggests that *H. pylori* infection could be associated with an increased risk for gastric carcinoma, but only a very small percentage of the infected persons develops gastric carcinoma. Therefore, it is suggested that along with other critical risk factors, *H. pylori* may act as a cofactor in the pathogenesis of gastric carcinoma.

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INTRODUCTION

Gastric cancer is estimated to be the world's second most common cancer, next to lung cancer (1). The role of *Helicobacter pylori* infection in the pathogenesis of gastric cancer is an important but unresolved issue. Recent epidemiological surveys have revealed the importance of *H. pylori* infection in gastroduodenal diseases which indicate the association of *H. pylori* infection with the incidence of gastric carcinoma (2,3). Authentic evidence suggests a close association of *H. pylori* in the pathogenesis of gastric carcinoma (4).

Despite ethnic diversity, cancer subjects have been documented to have a higher risk of prior infection with *H. pylori* (5). It has been suggested that mucosal alterations due to *H. pylori* infection leads to chronic gastritis, atrophy and metaplasia of gastric epithelium, which has been suggested to favor the genesis of gastric carcinoma (6,7). Nevertheless, the incidence of gastric carcinoma varies dramatically from place to place and it has been hypothesized that its incidence is influenced also by environmental factors (8,9). Hence, the present study is designed to investigate the prevalence of *H. pylori* in gastric carcinoma cases of the Indian population by means of simple, cost-effective and indigenously standardized urease tests, culture and ELISA.

MATERIALS AND METHODS

Subjects: 75 pathologically confirmed cases of gastric carcinoma and 75 healthy age, sex-matched control subjects were included in the study. Thus, a total of 150 subjects entered the study. Of them, 68 were from males and 82 were from females. The control subjects were age and sex matched to the patient group and the age range was from 40 to 65 years. The control and the patient groups included in the study belonged to the low socioeconomic status. Written consent was obtained from each patient before endoscopy at Department of Gastroenterology, Anna Nagar Peripheral Hospital, Madras, with an Olympus GIF XQ 10 endoscope (Olympus Co. Ltd., Tokyo). Upper gastrointestinal endoscopy was performed on four or five mucosal biopsy specimens in all the patients, approximately 3 by 3 mm in size, from the apparently healthy area of the antrum and also from the tumor area; 10 ml of blood was collected and sera were separated and stored at -20°C .

Diagnostic methods *Urease tests:* Urease activity was tested by the urease tests standardized in our laboratory (10). The tests are comparable with

that of the commercially available CLO test. Color change from yellow to pink within 4 hr indicates a positive test for *H. pylori* urease.

Culture: Culture was performed by streaking the biopsy specimen onto Brain Heart Infusion agar (HiMedia, Bombay, India) with 7% horse serum and Butzler antibiotic supplements (HiMedia). The inoculated plates were incubated under microaerophilic conditions in the gas pack system (Oxoid, Hampshire, UK) for 48 to 72 hr at 37 C and the characteristic *H. pylori* colonies were confirmed by oxidase, catalase, urease and hippurate hydrolysis tests. Further examinations of the organisms included microscopical morphology and motility in wet-film preparations.

ELISA: Sera collected from the subjects were screened for *H. pylori*-specific antibodies (IgG) by enzyme-linked immunosorbent assay (ELISA) standardized in our laboratory as described previously (11). In brief, ultracentrifuged whole cell sonicate was used as the antigen (12). Polystyrene microtiter plates were coated with the antigen (2 µg/well) and incubated overnight at 4 C. Nonspecific binding sites were blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS, pH 7.4) for 90 min at 37 C. PBS containing 0.1% BSA was used for washing throughout. Patient serum was diluted in PBS supplemented with 2% Tween-20 and 1% BSA.

The test serum (100 µl) was added to the antigen-coated well and the plates were incubated at 37 C for 90 min. The plates were washed twice, 100 µl of 1:2,000 diluted rabbit antihuman IgG - peroxidase conjugate (Bangalore Genei Pvt Ltd., Bangalore, India) was added, and incubated at 37 C for 90 min. The substrate (100 µl) containing 0.04% of O-phenylenediamine dihydrochloride (Abbott Laboratories, Chicago, IL) and 0.04 ml of 30% H₂O₂ in PBS (pH 5.0) were added to the plates, which was incubated at 37 C in dark for 15 min. Sulfuric acid (2.5 M) (0.05 ml) was added to stop the reaction. The plates were read in ELISA reader (Bio-Tek Instruments Inc., Winooski, VT) at 492 nm. Two positive and two negative serum samples served as controls for calibration and quality control.

Serum samples from the subjects were counter checked by a commercially available test kit (Hycor-Pyloragen, Garden Grove, CA).

Histopathology: Formalin fixed biopsies were embedded in paraffin, histological sections cut with 4 µm thickness and stained with hematoxylin and eosin. All the specimens were screened for the presence of *H. pylori*. The investigator was blinded as to the patient identity or results of other tests for *H. pylori*.

Statistical analysis: Chi-square test was used to analyze the significance of difference between proportions of the two groups.

RESULTS

Positivity of the tests for *H. pylori* in the patients with gastric carcinoma and in the healthy controls is shown in Table I. The patients were subgrouped based on the endoscopic finding as shown in Table I.

The positivity of *H. pylori* by various tests in the patient and control groups ranged from 56 to 62.6% and 37.3 to 46.6%, respectively; the difference was found to be statistically significant. In control subjects, biopsy was taken from the antral region, since *H. pylori* is predisposed to the antral region (13). *H. pylori* was not demonstrable in the biopsies from the site of carcinoma by histopathology or by other methods.

Table I. *H. pylori* positivity in the patients with gastric carcinoma and in the healthy controls

Subject	Biopsy	Number of specimens	Number of specimens positive for (%)			
			b)Liquid Urease	c)Biopsy Urease	Culture	ELISA
a)Gastric carcinoma	Antrum	40	27(67.5)	27(67.5)	25(62.5)	27(67.5)
	Body	25	14(56.0)	16(64.0)	16(64.0)	16(64.0)
	Antrum & Body	10	1(10.0)	4(40.0)	2(20.0)	4(40.0)
Healthy Controls	Antrum	75	29(38.6)	33(44.0)	28(37.3)	35(46.6)

a)Positivities of *H. pylori* in total biopsy specimens for liquid urease, biopsy urease, culture and ELISA was 56.0, 62.6, 60.0 and 62.6%, respectively.

b)Liquid urease test medium consisted of 10% urea and phenol red indicator in 0.5 ml of 0.005% monobasic phosphate buffer.

c)Biopsy urease test medium consisted of 10% urea and phenol red indicator in a semisolid agar base.

DISCUSSION

The results from the present study show high prevalence of *H. pylori* infection (62.6%) in patients with gastric carcinoma than the normals. A much more higher prevalence rate of *H. pylori* in the gastric carcinoma cases of the Indian population has been shown by Graham et al (14). A variation in *H. pylori* detection between adjacent sections, related to low bacterial load, has already been reported (15). It has also been observed that *H. pylori* organisms are found in very small numbers in the gastric carcinoma cases, which may explain the variation in the detection rates of *H. pylori* in the gastric carcinoma cases. The detection of *H. pylori* in gastric biopsy specimens may also be influenced by sampling error. To minimize this, much more biopsy samples have been assessed by the urease tests. Reports suggest the use of more than one diagnostic method and examination of multiple biopsies from different areas of the gastric mucosa to detect *H. pylori* infection in patients with gastric carcinoma (16). The urease tests incorporated in the study have been found to be sensitive and cost-effective in the diagnosis of *H. pylori* (10).

A high prevalence of *H. pylori* infection has been reported in patients with precancerous lesions (17). Recent studies have shown a higher positivity of *H. pylori* in patients with carcinomas located in the antrum and body (5,18). In the present study, a correlation does exist between *H. pylori* infection and the occurrence of gastric carcinoma. *H. pylori* was isolated in 62.5 to 67.5% of cases with carcinoma of the antrum and 56.0 to 64.0% in case of carcinoma of the gastric body. In general, the prevalence of *H. pylori* was significantly higher in patients with gastric carcinoma than in controls. This suggests a role of *H. pylori* infection and subsequent chronic gastritis which may lead to gastric carcinoma over a period of time (19,20). Even though a much higher prevalence rate of *H. pylori* is observed in patients with peptic ulcer disease, it seems to be negatively associated with gastric carcinogenesis. This could be due to some cofactor, related to the ulcer disease, that might protect against the development of gastric carcinoma.

Serological tests for *H. pylori* have been developed by many investigators and have proven to be an excellent tool for the diagnosis of *H. pylori*. A good concordance has been demonstrated between the histopathological findings and IgG in serum samples for the detection of *H. pylori* infection rather than IgA or IgM (21). Positive serology can indicate previous or latent infections with *H. pylori* (22). The antigen used in the ELISA system had a specificity of 95%, but a moderate sensitivity of 84%. The sensitivity of this system has been suggested to be

increased by combining purified complex antigen of *H. pylori* with the highly antigenic 120 kDa antigen of *H. pylori* (12), but the major problem in this procedure is that the 120 kDa protein is not demonstrable in all the strains of *H. pylori*. The prevalence of *H. pylori* IgG antibodies was significantly higher in the patients with gastric carcinoma (62.6%) than in controls (46.6%). The quality of the ELISA system developed was comparable to the Hycor Pyloragen test kit.

H. pylori was not demonstrable in the biopsies from the site of gastric carcinoma by all the tests. This supports the suggestion that as the degree of atrophy increases, infection with *H. pylori* declines (22).

Epidemiological studies have shown a good correlation between *H. pylori* infection and gastric carcinoma (23). The incidence of gastric carcinoma also varies widely in different populations, and even in genetically similar subpopulations of the same geographic areas. This suggests the role of some important environmental factors in the genesis of gastric carcinoma (24). The present study indicates the possible role of *H. pylori* infection in preceeding the development of gastric carcinoma.

The results of our study indicate a higher prevalence rate of *H. pylori* in the patients with gastric carcinoma compared with the normals suggesting the possible link between *H. pylori* and gastric carcinogenesis. Only a minority of the people with *H. pylori* gastritis develops gastric carcinoma thereby indicating that *H. pylori* is not the only risk factor for the development of gastric carcinoma. Therefore, *H. pylori* could be considered as a cofactor in the gastric carcinogenesis process from the high prevalence rate in the gastric cancer subjects and also the fact that *H. pylori* is the main cause of chronic atrophic gastritis which is an inflammatory precursor to gastric carcinoma. Other critical risk factors that induce the genesis of gastric cancer after colonization with *H. pylori* need to be identified.

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