

# Prevalence of *Helicobacter pylori* among dyspeptic patients in a tertiary care centre in South Kerala, India

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## ABSTRACT

**Background:** *Helicobacter pylori* (HP) is the most common bacterial infection in humans. It affects > 50% of world population and remains the major aetiological agent of ailments of upper gastrointestinal (GI) tract of humans. HP eradication prevents pre-neoplastic changes of gastric mucosa and regression of gastric mucosa-associated lymphoid type lymphoma. For these reasons and many others, it is worthwhile testing patients with dyspepsia for HP, who might benefit from eradication therapy. **Objective:** To study the prevalence of HP infection among dyspeptic patients in a tertiary care centre in South Kerala. **Materials and Methods:** Gastric biopsy specimens were collected from referred patients with dyspepsia from Southern districts of Kerala (Thiruvananthapuram, Kollam and Pathanamthitta) attending the Gastroenterology Outpatient Department, who underwent upper GI endoscopy, during a one-year period from December 2009 to November 2010. The serum samples were collected for doing HP IgG enzyme-linked immunosorbent assay test. The biopsy specimens were used for doing rapid urease test (RUT) and culture was done in patients showing urease positivity and/or high level of IgG antibody titre. **Results:** The study was conducted on 250 consecutive dyspeptic patients, with an age range of 7–73 years. Significant IgG titres (>37.5 IU/ml) were detected in 89 cases (35.6%), while the RUT was positive in 30 cases (12%). Subjects were considered positive for HP infection by combined IgG antibody and/or urease test positivity and negative when both the tests were negative. In this study, ninety patients (36%) were positive for HP infection, and 160 (64%) were negative. Culture was done in patients showing urease positivity and/or high level of IgG antibody titre and proportion of culture positivity among this group was 34%. **Conclusion:** Seroprevalence of HP infection in South Kerala was found to be low (35.6%). Low infection rates were seen in children, similar to that in developed countries, and it is likely that the prevalence of HP may fall in the coming years. Due to the lower percentage of HP infection in this area, a test and treat policy is applicable as in many developed countries.

**Key words:** Gastric biopsy, *Helicobacter pylori*, rapid urease test

## INTRODUCTION

*Helicobacter pylori* (HP) has been the subject of intense investigation since its culture from gastric biopsy in 1982.<sup>[1]</sup> It is now well accepted that the most common gastrointestinal (GI) disease, peptic ulcer disease is an infectious disease, and the causative agent, HP must be treated with antibiotics.<sup>[2]</sup>

HP causes >90% of duodenal ulcers and 80% of gastric ulcers.<sup>[3]</sup> Majority of persons infected with HP never manifest with any symptom, but it still causes chronic active, persistent or atrophic gastritis. There is

a two-fold to six-fold increase in the risk of developing gastric adenocarcinoma and mucosa-associated lymphoid type (MALT) lymphoma compared to the uninfected.<sup>[4]</sup> It is estimated that if a person has persistent HP infection for 20–30 years, it can lead to cancer of the stomach.<sup>[3]</sup>

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In India, like any other developing country, the prevalence of HP infection is extremely high. Around 70%–90% of patients with duodenal ulcer and 50%–80% of patients with non-ulcer dyspepsia or healthy asymptomatic adults are HP positive.<sup>[5]</sup> HP eradication prevents pre-neoplastic changes of gastric mucosa and regression of gastric MALT lymphoma.<sup>[6]</sup>

Dyspepsia is a broad term used to describe a variety of symptoms referable to upper GI tract such as discomfort, indigestion, fullness of stomach, upper abdominal pain and heartburn excluding dysphagia, jaundice and acute abdominal conditions such as bleeding and perforation.<sup>[7]</sup>

### Relevance of the study

Endoscopic biopsy is routinely done in dyspepsia in our institution for primary diagnosis of infection by HP when a visual analysis of upper digestive tract is necessary and/or to obtain biopsy samples for histological analysis, rapid urease test (RUT) and/or culture. These tests have been traditionally used as gold standards because they demonstrate directly or indirectly the presence of bacteria. Culture is the most specific method to diagnose HP infection of gastric biopsies. HP is a fastidious slow-growing organism that requires special culture conditions. Hence, relying on culture alone can mean missing a proportion of infected cases for technical reasons, including overgrowth of other bacteria and low bacterial load. This study used a combination of serological detection of IgG by enzyme-linked immunosorbent assay (ELISA) and RUT with the biopsy specimen. Positivity of either test was taken as HP positive.

## MATERIALS AND METHODS

A cross-sectional study was conducted among dyspeptic patients attending the Gastroenterology Outpatient Department during one-year period from December 2009 to November 2010.

### Inclusion criteria

- Incident cases of dyspepsia aged between 5 and 75 years who underwent upper GI endoscopy before receiving antimicrobial therapy to eradicate HP infection
- Those already on proton pump inhibitors (PPIs), H<sub>2</sub> receptor antagonists and antacids underwent endoscopy, after two weeks of discontinuation of these drugs.

### Exclusion criteria

Patients with history of use of H<sub>2</sub> receptor antagonists, PPIs bismuth and antimicrobial agents, within two weeks before endoscopic examination, pregnant women, those with chronic renal and liver diseases and malignancies were excluded.

### Collection of gastric biopsy specimen

During endoscopy, biopsies were taken from two sites of the antrum within 2 cm from the pylorus and were used for RUT and culture.

Half an hour after endoscopy and breakfast, about 3 ml blood was drawn aseptically in a sterile bottle and allowed to clot at room temperature. The serum was separated, labelled and stored at –20°C for ELISA test.

### Laboratory procedures

#### Urease test

The bit of tissue for urease test was put immediately after endoscopy in a labelled transparent 2 ml plastic vial containing 0.5 ml freshly prepared 10% w/v urease broth in deionised water at pH 6.8 with two drops phenol red as indicator and incubated at 36°C. A positive result was recorded if the colour of the broth changed from yellow to pink within 2 h.

#### Culture

Biopsy specimens for culture were taken from patients showing urease positivity and/or high level of IgG antibody titre. Brucella broth with 20% glycerine was used as the transport medium. Samples were stored at –70° until culture. Specimens were sent to National Institute of Cholera and Enteric Diseases, Kolkata, and isolation and identification of HP were done there.

#### *Helicobacter pylori* IgG enzyme-linked immunosorbent assay

The test was done according to the manufacturer's instruction provided with the ELISA kit. Test was interpreted as positive when IgG antibody titre was >37.5 IU/ml, the receiver operating characteristic cut-off level obtained during kit evaluation by the manufacturer.

Data were entered in Microsoft Excel 2007 and analysed by SPSS version 16.

## RESULTS

The study was conducted among 250 consecutive dyspeptic patients ageing from 7 to 73 years. Of these patients, 159 (63.6%) patients were males and 91 (36.4%) were females. Subjects were considered positive for HP infection by combined IgG antibody and/or urease test positivity and negative when both the tests were negative. In this study, ninety patients (36%) were positive for HP infection, and 160 (64%) were negative [Table 1]. Significant IgG titres (>37.5 IU/ml) were detected in 89 cases (35.6%), while the RUT was positive in 30 cases (12%).

A majority of the patients who were positive for HP were from rural area, i.e., 75/90 (83.3%), and of

these, 82/90 (91.1%) belonged to low socio-economic status [Table 2]. Most of the HP positive cases, i.e., 26/90 (28.9%), were in the 30–39 years age group [Table 3].

The prevalence of HP infection was significantly higher in semiskilled and unskilled workers (48.8%;  $P = 0.025$ ) and least among professionals [Table 4].

**Table 1: Overview of two tests employed in the detection of *Helicobacter pylori* infection among the study population**

Test parameter	Positive (%)	Negative	Total
IgG ELISA test (cut-off level >37.5 IU/ml)	89 (35.6)	161	250
Rapid urease test	30 (12)	220	250

ELISA: Enzyme-linked immunosorbent assay

**Table 2: Basic demographic characteristics of the study population in terms of *Helicobacter pylori* status**

Characteristic	Sub-division	HP positive (%)	HP negative	Total
Gender	Male	61 (67.7)	98	159
	Female	29 (32.2)	62	91

HP: *Helicobacter pylori*

**Table 3: Age distribution of the study population**

Age group	HP positive (%)	HP negative	Total
<9	0	2	2
10-19	1 (1.1)	7	8
20-29	15 (16.7)	27	42
30-39	26 (28.9)	37	63
40-49	21 (23.3)	39	60
50-59	16 (17.8)	34	50
60-69	9 (10.0)	11	20
>70	2 (2.2)	3	5
Total	90	160	250

HP: *Helicobacter pylori*

**Table 4: Basic demographic characteristics of the study population in terms of *Helicobacter pylori* status**

Occupation	HP positive (%)	HP negative	Total
Professional	3 (3.3)	14	17
Service provider*	22 (22.4)	20	42
Semiskilled and unskilled*	44 (48.8)	73	117
Homemaker, student and unemployed	21 (23.3)	53	74
Total	90	160	250

\*Service providers (clerk, teachers, minor professionals and administrators); semiskilled and unskilled workers (uneducated farmers, and manual labourers, fisherman, salesperson). HP: *Helicobacter pylori*

Culture was done in patients showing urease positivity and/or high level of IgG antibody titre and proportion of culture positivity among this group was 17/50 (34%) [Table 5].

## DISCUSSION

HP infection is a transmissible disease and a major public health problem, especially in a developing country like India, for which treatment should be provided when diagnosed.<sup>[8]</sup>

Two hundred and fifty dyspeptic patients residing in southern part of Kerala were tested for HP status by serology and RUT. The prevalence of HP infection among dyspeptic patients in our study is 36%.

In hospital-based studies from different parts of India, the prevalence of HP ranges from 45% to 92%, but none of them has shown such low prevalence as found in this study.<sup>[9]</sup>

Prevalence rates are quite high (70%–90%) in Africa, Central/South America and Asia and low (30%–50%) in Western Europe, United States and Canada.<sup>[10]</sup> The low prevalence rate in this study, comparable to that in developed countries, may be due to several reasons. One is probably due to better hygiene practices in households of Kerala. Several of these studies have also demonstrated an inverse relationship between HP prevalence and educational level of the population studied.<sup>[11]</sup> Another probable reason could be the higher usage of PPIs and antisecretory drugs; both over the counter and by physician prescription.<sup>[12]</sup> This lower prevalence of HP disease in Kerala is in agreement with standards of higher childhood living conditions, household hygiene, improvements in social conditions and easy access to healthcare facilities.<sup>[13]</sup>

Among ninety cases who were positive for HP infection, 61 (67.7%) were men and 29 (32.2%) were women. As expected, higher proportion of men with dyspepsia attend the gastroenterology clinics than women. In some studies, a significant association of HP with male gender was seen. A meta-analysis of population-based prevalence survey of 18 adult and 10 paediatric populations worldwide reported in 2006, each with sample size more than 500, has shown that, in adults, male gender was found to be associated with HP infection. They suggested that differential antibiotic exposure and different protective immunity between genders explain the variations.<sup>[14]</sup>

In this study, none of the children aged zero to nine years was HP positive, and low prevalence was seen in the age

**Table 5: Relation of culture positivity with serology and urease tests**

Culture	Serology positive	Serology negative	Serology positive (high titre >60 IU/ml)	Total
	Urease positive	Urease positive	Urease negative	
Culture positive	10	1	6	17
Culture negative	19	0	14	33
Total	29	1	20	50

group of 10–19 years. Increase in prevalence with age was significant, and age group with highest frequency belonged to 30–39 years. Population-based studies from developing countries show a sharp increase in prevalence until 20 years and then remain more or less constant in later years.<sup>[15]</sup>

The prevalence of HP infection was significantly higher in semiskilled and unskilled workers (48.8%;  $P = 0.025$ ) and was least in professionals. Further studies are needed to analyse the type of occupation and mode of transmission. This association may be due to the difference in socio-economic status between these two groups. Studies have shown that HP was common in gastro-endoscopists and endoscopy nurses.<sup>[16]</sup> Different studies among sewage workers and shepherds have also shown an increased prevalence of HP infection.<sup>[17]</sup>

At present, two different methods are needed to determine whether a patient is infected with HP.<sup>[9]</sup> In the present study, 250 dyspeptic patients residing in the southern part of Kerala were tested for HP status by serology and RUT. HP positivity by serology and RUT were 35.6% and 12%, respectively.

The difficulties associated with invasive tests and low rate of isolation of the organism make the serological tests quite important. The best antigen preparation for such a test must include CagA-coded antigen, vacuolating cytotoxin and urease complex of pathogenic HP strains. The ELISA test used in this study is based on the inactivated 128 kDa external protein (CagA).<sup>[18]</sup> The higher positivity seen with IgG is explained by the fact that the other test was based on biopsy from antrum, and there is a possibility that distribution of HP in the stomach is patchy; hence, sampling error can occur. Our finding can be explained by the fact that serology assays the systemic response and practically no sampling error can occur with serological tests.<sup>[19]</sup> Therefore, serological tests may be better in already established (chronic) infections where the organism may not be detected bacteriologically.

If the patient is infected, serology would most probably be positive unless in some rare instances, where the host could

not mount a humoral immune response to HP. The ability to produce a significant level of antibody to the organism by the host may vary. There may also be persistence of IgG antibody for few months even after eradication of infection or natural recovery from infection.<sup>[19]</sup>

Urease test was positive in 12% of the dyspeptics and was less sensitive than serology. The fallacies of this study that may explain the low urease positivity are medications that reduce the density and/or urease activity of HP, such as bismuth containing compounds, antibiotics or PPIs can decrease the sensitivity of RUT by 25%.<sup>[20]</sup> Although it was advised to stop PPI for two weeks, some patients might have taken it to reduce their dyspeptic symptoms. A high bacterial load is necessary for the urease test to be positive.

Proportion of culture positivity among patients showing urease positivity and/or high level of IgG antibody in this study was 17 (34%). The culture positivity reported from India ranges from 23.9% to 65.8%.<sup>[21,22]</sup> Low isolation rate of this organism is possibly due to patchy distribution in the gastric mucosa, mucosal atrophy, intestinal metaplasia (in stomach), administration of antibiotics for some other infection or protozoan infestation and use of PPIs. Other technical reasons include sub optimal quality of the media, difficulty in achieving the required gaseous environment, fastidious nature of the organism and overgrowth of other bacteria. Another reason may be low bacterial load and loss of viability during transportation.<sup>[23,24]</sup> Culture is not recommended for routine evaluation because of restricted availability of good laboratory facilities and potential errors, leading to false positive results. Hence, culture is done only in reference laboratories.

## CONCLUSION

HP infection is a transmissible disease and a major public health problem, especially in a developing country like India, for which treatment should be provided when diagnosed. This will not only ameliorate the symptoms but also prevent complications. In this study, seroprevalence of HP infection in South Kerala was found to be low (35.6%). Low infection rates were seen in children, similar to that in developed countries, and it is likely that the prevalence of HP may fall in the coming years. This lower prevalence of HP disease in Kerala is in agreement with higher standards of childhood living conditions, household hygiene, improvements in social conditions and easy access to healthcare facilities. There is also substantial decrease in the number of peptic ulcer perforation in emergency units of this institution and all over Kerala. Due to the lower percentage of HP infection in this area, a test and treat policy is applicable as in many developed countries

such as the UK.<sup>[25]</sup> In our population, serological detection of infection by IgG ELISA is a reliable method to detect HP infection. Being simple and cost-effective, it may be used as a screening test. Culture and susceptibility can be reserved for treatment failures

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### Conflicts of interest

There are no conflicts of interest.

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