Prevalence study to elucidate the transmission pathways of Helicobacter pylori at oral and gastroduodenal sites of a South Indian population

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ABSTRACT

Introduction: Since the discovery of *Helicobacter pylori (H. pylori)*, much progress has been made worldwide in the field of its epidemiology. In spite of these advancements, many aspects of epidemiology still remain unclear, particularly among populations with low socio-economic status. The present study was designed to elucidate the different routes of transmission of *H. pylori* in the Hyderabad (South India) population and to investigate the impact of certain factors, such as age, gender, and lifestyle.

Methods: Samples used for the study included saliva and biopsy samples of 400 symptomatic subjects from Hyderabad, India. The patients were retrospectively grouped, based on histopathology of the biopsy and 16S rRNA amplification of both saliva and biopsy as *H. pylori* positive and negative.

Results: This study showed that the prevalence of *H. pylori* in both saliva and biopsy samples increased with age. In addition, the *H. pylori* infection was found more commonly in the saliva and biopsy samples among males (64 percent and 60 percent, respectively) than females (53.3 percent and 64 percent, respectively). Similarly, 71.6 percent and 73.5 percent of those who consumed municipal water acquired *H. pylori* (which were respectively found in their saliva and biopsy samples) compared to a lesser proportion (12.6 percent and 12.6 percent, respectively) of those who consumed boiled or filtered water. The study also found that subjects who preferred home-cooked food (57.1 percent and 57.7 percent) showed a lower prevalence of *H. pylori* in saliva and biopsy samples, respectively, compared to those (80 percent and 88 percent) who frequently ate out.

Conclusion: The results of the present study suggest that besides the oral-oral route, the transmission of *H. pylori* also takes place through the consumption of food prepared under unhygienic conditions. Consumption of municipal tap water also has a high impact in the transmission of *H. pylori*.

Key Words: gastrointestinal disease, *Helicobacter pylori*, 16S rRNA, saliva, water disease transmission

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INTRODUCTION

Helicobacter pylori (H. pylori) is now thought to be one of the most important factors in the pathogenesis of upper gastroduodenal diseases[1-3]. Eradication of *H. pylori* is gaining profound significance for the treatment of many gastroduodenal diseases[4-6]. Therefore, elucidation of its transmission routes is necessary to prevent patients from being re-infected with *H. pylori* after successful eradication therapy. *H. pylori* infection is mainly acquired in early childhood, particularly in developing countries, although the precise mode of transmission is still unclear[7-10]. As suggested by a high prevalence of infection among persons living in institutions and within familial clusters[11,12], person-to-person contact is considered to be the most likely transmission route of *H. pylori*[13]. As *H. pylori* has been isolated from dental plaque, saliva, faeces, and vomitus[14-16], the transfer of this micro-organism from the stomach of one person to that of another is thought to be by oral-oral, faecal-oral and gastro-oral routes[13,17].

In developing countries, there is an increasing evidence for both oral-oral and faecal-oral modes of transmission. In the same way, food- and water-borne transmission of *H. pylori* is also under consideration[18,19]. The evidence for person-to-person transmission is supported by observations of factors, such as low socioeconomic status, lower levels of education, poor hygiene and sanitation along with household crowding, resulting in a higher

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prevalence of *H. pylori* infection. Many reports also support transmission of *H. pylori* via zoonosis, i.e. persons in close contact with domestic animals have a higher probability to acquire this infection[20,21]. Many DNA-based polymerase chain reaction (PCR) assays have been developed for detecting *H. pylori*.

Based on the difficulty of culturing from sites other than gastric mucosa[22], and the need for the non-invasive diagnostic methods, the interest has grown in the use of molecular techniques for the detection of this species. The use of gene-specific probes has been described for the detection of *H. pylori* in biopsy specimens[22,23], and progress has been made with the use of PCR, which provides a specific and highly-sensitive means of detecting microbial pathogens in clinical material. PCR assays have detected *H. pylori* DNA in fresh gastric biopsy specimens[24-28], in faeces[29,30], in saliva[27,28], and in dental plaque. These assays are mostly based on the urease gene sequences, the 16S ribosomal RNA gene and adhesin gene. The 16S rRNA gene of *H. pylori* is a highly specific target for amplification and has been used previously to help reclassify the organism[31, 32]. Weiss et al[33] demonstrated the specificity of unique *H. pylori* gene primer to identify the organism in paraffin-embedded gastric biopsy specimens.

Although attempts were made earlier to focus on the epidemiological aspects of this pathogen, most of the studies were done on adults and only few attempts were made to explain the epidemiological mechanism among infants and children[36]. As the prevalence rate of *H. pylori* is increasing with age, there is a need to unearth the exact sources, which augment the transmission of *H. pylori*. The aim of the present study was to elucidate every possible route of transmission of *H. pylori* to find out the most likely mode of transmission in this population.

METHODS

A total of 400 symptomatic subjects (250 males and 150 females, mean age 14 years, range 0-29 years) referred for upper gastrointestinal endoscopy at the Deccan College of Medical Sciences and Research Centre, Hyderabad, India, were enrolled in this study. Informed consent from all 400 dyspeptic patients, and approval from the Department of Biotechnology, Government of India, were obtained.

All subjects were interviewed by means of a questionnaire for general demographical details and socioeconomic circumstances, history of peptic ulcer, duodenal ulcer, or dyspepsia. Questions about their food intake at home or outside, drinking habits like consumption of municipal tap water, boiled or filtered water, were asked. Patients were also inquired about the consumption of alcohol. Similarly, data were also collected for the smoking habits of the subjects under study.

The selection criteria for the 400 dyspeptic patients excluded subjects who were treated with antibiotics and proton pump inhibitors (PPI) at the time of endoscopy or within the previous two weeks. Returning patients with the same problem were treated as the patients with antibiotics or PPI, and were excluded from the study.

Samples to assess *H. pylori* infection included saliva of the subjects along with three gastric biopsies (two from the antrum and one from the corpus). Saliva samples (1 ml) were collected from subjects prior to endoscopy and were placed in a sterile container containing digestion buffer (100mM NaCl, 10mM Tris-HCl [pH 8.0] 0.5% SDS). The three gastric biopsies were collected, one in urea solution for rapid urease test (RUT), one in 10% buffered formalin for histological analysis and one in phosphate buffer saline for DNA isolation for PCR assay.

Genomic DNA was isolated from all samples as per the standard protocol[34] by cetyltrimethyl ammonium bromide (CTAB) method. Two 20-base oligonucleotide primers designated 16S rRNA-F (5'-TAAGAGATCAGCCTATATGTCC-3') and 16S rRNA-R (5'-TCCCACGCTTTAAGCGCAAT-3')[35] were selected. The amplified product of these two primers with DNAs prepared from the clinical isolates and from the type strain of H. pylori (ATCC 26695) was a 534 bp fragment.

PCR amplification was performed, through initial denaturation at 95°C for five minutes, 40 cycles with one cycle consisting of 30 s at 94°C, 30 s at 52°C, one minute at 72°C. The final cycle included a ten-minute extension step to ensure full extension of the PCR products. Amplification was performed in a thermalcycler (M J Research Inc, Watertown, USA). DNA of the ATCC type strain was used as a positive control in each set of PCR assays while negative control consisted of all the reagents of the master mix except the template DNA. The PCR-amplified products were analysed by agarose gel electrophoresis; samples were scored as positive when a band of 534bp could be detected on agarose gel.

Statistical analysis of the obtained data was done by odds-ratio (OR), and 95% confidence interval (CI). Risk factors, which are significant in the univariate analysis, were used in the multiple regression models. These models helped to assess the relative importance of *H. pylori* risk factors. The data were analysed using Statistical Package for Social Sciences (SPSS) version 12.0 (Chicago, IL. USA) (Table I).

Table I. Multivariate analysis of different variables and its association with *H. pylori*.

Variables	Odds-ratio	95.0% C.I. for odds-ratio	
Age (in years)			
0-4	Referent		
5-9	2.474	0.555	11.031
10-14	2.688	0.759	9.517
15-19	7.072	1.883	26.567
20-29	17.272	5.119	58.282
Sex			
Male	.010	.002	.050
Female	Referent		
Eating habits			
At home	Referent		
Outside	5.315	1.251	22.586
Drinking habits			
Tap (municipal water)	259.766	54.933	1228.374
Boiled or filtered water	Referent		
Smoking habits			
Smoker	.622	.285	1.357
Non-smoker	Referent		
Alcohol consumption			
Yes	Referent		
No	8.885	2.360	33.450

TABLE II. Prevalence of *H. pylori* in the oral cavity and stomach of dyspeptic subjects, grouped according to age.

Age in years (n)	Oral cavity (n=240)	Stomach (n=246)
0-4 (30)	10 (33.3%)	6 (20%)
5-9 (30)	12 (40%)	10 (33.3%)
10-14 (70)	40 (57.1%)	30 (42.8%)
15-19 (90)	50 (55.5)	60 (66.6%)
20-29 (180)	128 (71.1%)	140 (77.7%)
Total (400)	**240 (60%)**	**246(61.5%)**

RESULTS

H. pylori was detected in biopsy samples of 246 (61.5%) subjects from 400 patients, all of whom had endoscopically-proven gastritis. Of the 400 saliva samples collected, it was observed that 240 (60%) of infected subjects gave a positive amplification with the 16S rRNA primers. When we assessed the prevalence of *H. pylori* infection in the various age groups (Table II), we found that prevalence of *H. pylori* in both the saliva and biopsy samples increased with age (from 10% and 6%, respectively, in the 0-4 year age group; to 71.1% and 77.7%, respectively, in the 20-29 year age group).

Table III. Prevalence of *H. pylori* in relation to gender, eating, and drinking habits.

Variables	Saliva	Stomach biopsy
Gender		
Male (n=250)	160 (64%)	150(60%)
Female (n=150)	80 (53.3%)	96 (64%)
Eating habits		
At home (n=350)	200 (57.1%)	202 (57.7%)
Outside (n=50)	40 (80%)	44 (88%)
Drinking habits		
Municipal (tap) water (n=321)	230(71.65%)	236(73.5%)
Boiled or filtered water (n=79)	10(12.6%)	10 (12.6%)

TABLE IV. Prevalence of *H. pylori* in relation to smoking habits and alcohol consumption.

Parameters	Oral cavity (%)	Stomach (%)
Smoking habits		
Smoker (n=100)	63 (63%)	63 (63.0%)
Non-smoker (n=300)	177 (59%)	183 (61%)
Alcohol consumption		
Yes (n=84)	39 (46%)	43 (51.2%)
No (n=316)	201 (63.6%)	203 (64.2%)

When gender was considered (Table III), we found that the presence of *H. pylori* in the saliva and biopsy samples were relatively similar, 64% and 60%, respectively, in males; and 53.3% and 64%, respectively, in females. When eating and drinking habits were considered, we found *H. pylori* to be present in the saliva and biopsy samples of 57.1% and 57.7%, respectively, of the subjects who preferred home-cooked food in contrast to 80% and 88%, respectively, of individuals who preferred outside food (Table III). When drinking habits were evaluated, we found a higher prevalence of *H. pylori* in the saliva (71.6%) and biopsy samples (73.5%) of individuals who used municipal tap water, compared to those (both 12.6%) who frequently used boiled or filtered water (Table III).

We also analysed the prevalence of *H. pylori* in saliva and stomach specimens of smokers and alcohol consumers. Out of the 400 subjects enrolled,

100 were found to be smokers and 300 were non-smokers. *H. pylori* could be detected in the saliva and biopsy samples of 63% and 63%, respectively, of smokers and 59% and 61%, respectively, of non-smokers. In terms of alcohol consumption, *H. pylori* was found in the saliva and biopsy samples of 46% and 51.2%, respectively, of those who regularly consumed alcohol, compared to 63.6% and 64.2%, respectively, of those who abstained (Table IV).

DISCUSSION

The epidemiological aspects of *H. pylori* infection remain an interesting arena of research, particularly in relation to the colonisation of *H. pylori* and different consequences of its manifestations[37,38]. In the present study, *H. pylori* was detected in 240 (60%) and 246 (61.5%) of saliva and biopsy samples of 400 symptomatic subjects by 16S rRNA PCR assay. The result of the present study demonstrated that the PCR assay was sensitive and specific for the detection of *H. pylori* in clinical specimens, as we were able to detect *H. pylori* in highly-contaminated specimens, like saliva, in almost all the infected subjects.

In the present study, most of the subjects belong to the families of low socioeconomic status, confirmed either by the occupation of parents or the subjects themselves. A previous study proved that a low level of education, or a low level of socioeconomic status, or both, could have a marked impact in the prevalence of *H. pylori* infection[39]. The study also evaluated the effect of every possible parameter, such as age, gender, eating habits, drinking habits, smoking and consumption of alcohol. As evident from Table II, the prevalence of *H. pylori* infection increased with age. However, the precise implications remain unclear.

It is obvious that ageing does not cause infection, but there must be some external factors to which the subject is either constantly or increasingly exposed with age. The study also focused on the gender that was at a greater stake to acquire *H. pylori*. According to the results obtained, we found that males and females were at an equally high risk to acquire this infection. In contrast, most of the previous studies showed that men were at higher risk than women because men have a higher gastric acid secretory capacity as compared with women of similar age and weight[40]. Cigarette smoking may confound the effect of gender in acquiring *H. pylori*.

Evaluation of the dietary factors and drinking habits in our study revealed that subjects who consumed home-cooked food and avoided outside food were less prone to harbour *H. pylori* than those subjects who consume outside food frequently. These findings are quite similar to the other studies undertaken before[41], which reported an increased prevalence of *H. pylori* infection in those subjects who consumed food, including contaminated raw vegetables[42], sold by street vendors.

There are reports suggesting that seropositivity of *H. pylori* increased with the consumption of raw vegetables in Chile[43]. In the same way, children in rural Colombia who ate raw vegetables contaminated with irrigation water were at an increased risk of infection[44]. In our study, a comparatively high proportion of subjects, i.e. 80% and 88% of subjects, as respectively assessed by the saliva and biopsy DNA, were infected. The results were statistically significant for these subjects who most often consumed outside food.

Similarly, subjects drinking municipal tap water were more likely to acquire this infection compared to those who utilised boiled or filtered water. It was found that among the total of 400 subjects, 321 subjects consumed municipal tap water, of which 230 (71.6%) subjects were found positive for *H. pylori* from saliva samples. In contrast, of 79 subjects who used either boiled or filtered water frequently, only 10 (12.6%) were positive. Comparable results were obtained from the subjects' biopsies, which showed the presence of *H. pylori* in 73.5% of the subjects who frequently used municipal water, and 12.6% who frequently used filtered water or boiled water.

An earlier study by Klein et al[45] showed that the water-borne transmission of *H. pylori* might be an even more important source of infection in developing countries, especially if the water supply is vulnerable to bacterial contamination. Similar results were obtained by Hulten et al[46], who reported that *H. pylori*-specific DNA were detected in water from the taps and water tanks, but not in well water. The findings of the present study suggest that the people who take municipal water for drinking purposes are more vulnerable to *H. pylori* infection and showed a higher prevalence of *H. pylori* infection than those who take boiled or filtered water. Thus, these results suggest that those subjects who are consuming tap water without boiling or filtering it are at a high risk of acquiring this infection, and that tap water is also one of the chief sources of transmission of this pathogen. In a news report of the United Nations, India ranks 120th in water quality out of 122 countries[47].

Smoking has been recognised as one of the most prominent risk factors of increased incidence, delayed healing, and increased relapse of peptic ulcers[48]. The data on the effects of smoking on the gastric mucosa and *H. pylori* infection are controversial. In our study, there was not much difference in the prevalence of *H. pylori* in smokers and non-smokers. However,

previous studies reported that smokers were more likely to have *H. pylori* infection than non-smokers, but this association was only observed in those smoking more than 35 cigarettes/day[49]. Smaller cross-sectional surveys have reported an association between smoking and *H. pylori* prevalence,[50,51] but these may not have been adequately controlled for confounding factors. The conflicting results could be explained by the complexities of absorption and action of nicotine, and doses of nicotine from smoking. In particular, a study done in Italy reported an increased prevalence of *H. pylori* and peptic ulcer disease among smokers[52].

Alcohol consumption was also taken as a parameter to assess the prevalence of *H. pylori* in our study. The data obtained from our study correlated with those of other studies undertaken before[53], as we found that subjects who consumed alcohol quite frequently were less prone to acquire *H. pylori* compared to those who abstained. Thus, it could be assumed that the consumption of alcohol seems to play a protective role against *H. pylori* infection.

In conclusion, the results of the present study suggests that besides the oral-oral route, the other main important pathway of transmission of *H. pylori* in this population is through the consumption of municipal tap water. By boiling or filtering tap water, we can minimise the chances of transmission of *H. pylori*. Similarly, subjects who frequently took outside food showed a statistically higher prevalence of *H. pylori* compared to those who prefer home-cooked food. This confirms that the food prepared outside is less hygienic, and the frequent consumption of outside food definitely increases the prevalence of *H. pylori*. In this study population, except for alcohol consumption, other factors like smoking and gender do not show any great impact in the transmission of this pathogen. By being aware of the contributing factors of *H. pylori* occurrence, we can minimise the chances of recrudescence of infection after successful eradication of the pathogen by antibiotic therapy.

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