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Original Research Article

A Study on the Seroprevalence of *Helicobacter Pylori* in Patients with Gastrointestinal Diseases Attending a Tertiary Care Center

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ABSTRACT

Background: Infection with *Helicobacter pylori* (*H. pylori*) is associated with significant morbidity and mortality. Till now, the seroprevalence of *H. pylori* infection from this part of India has not been reported.
Aim: To study the seroprevalence of *H. pylori* infection in patients with gastrointestinal disorders undergoing upper gastrointestinal endoscopy at a tertiary care government setup.
Settings and Design: It is a single hospital based, cross-sectional, prospective study.
Materials and Methods: The demographic data, blood sample to detect presence of *H. pylori* IgG by ELISA and gastric biopsy specimen for rapid urease test (RUT) were collected from eighty (80) patients.
Statistics: Data analysis was carried out with GraphPad InStat statistical program. Prevalence rates are expressed as percentages. Unpaired t test was used to compare the continuous variable. *P* value < 0.05 was taken to be statistically significant. Fischer exact test was used to analyze categorical data.
Results: Seroprevalence of *H. pylori* infection was 27.5% (22/80) among the patients screened. Thirty three (41.25%) among all the studied patients showed positive response in RUT. Maximum seroprevalence was seen in the age group of 40-49 years. Majority [97.5 %, (78/80)] of patients had some form of abnormality as detected by endoscopy that could be accounted for their symptomatology.
Limitation: The limitations of the study are discussed.
Conclusion: There is a lower prevalence of antibodies to *H. pylori* among the study population compared to other parts of India.

Key words: Enzyme-Linked Immunosorbent Assay; Endoscopy, Digestive System; *Helicobacter pylori*; Prevalence; Rapid Urease Test

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) is reported to be associated with various gastrointestinal disorders *e.g.* gastritis, peptic ulcer, gastric mucosa associated lymphoid disease and gastric cancer. [(1)] There is a geographical variation in the prevalence of *H. pylori* infection. [(2)] Even in the same geographical location, prevalence varies due to socioeconomic status, living conditions during childhood, standard of hygiene and sanitation, presence of contaminated drinking water, smoking status, use of non-steroidal anti-

inflammatory drugs, and frequent consumption of outside food *etc*. [1-3]

In 1964, Malhotra SL *et al* reported a high incidence of active and passive gastric ulcer (8.8 % in office workers and 15.1 % in manual workers) among the railway employees of this state of India. [4] We could not retrieve any recent published data on the epidemiology of *H. pylori* from this region. [*] Although any report on the incidence of stomach cancer in our state could not be retrieved by us, our state has one of the highest age-standardized cancer mortality rate per 1,00,000 population. [5] Compared to western countries, the incidence of gastric cancer in India is relatively lower. [6] However, Mizoram, one of the neighboring states, is reported to have the highest incidence of gastric cancer in India. [7] Although Khanna *et al.* reported that there was no statistical significance between the prevalence of *H pylori* in patients with gastric cancer and age matched healthy historical controls, screening for *H. pylori* has been proposed as a prescreening strategy for gastric cancer in Japan. [6,8] Moreover, recently a review article has emphasized on the public health implications of infection with *H. pylori*. [8]

Therefore, data regarding *H. pylori* infection in this part of India is very important in understanding the epidemiology of the infection and *H. pylori*-related diseases. We conducted the current study with the below mentioned objectives.

Objectives:

1. The primary objective of this study was to find out the seroprevalence of *H. pylori* infection, as detected by immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA), in patients with gastrointestinal disorders undergoing flexible upper gastrointestinal endoscopy (UGIE) at the Department of Gastroenterology of an academic tertiary care hospital.
2. The secondary objective was to determine the prevalence of positive rapid urease test (RUT) of the gastric biopsy samples of that patient group.

MATERIALS AND METHODS

Study design:

This single institution based, observational, cross sectional study was carried out among unrelated individuals. Data collection and sampling were taken from August 2012 to July 2013. Permission from Institutional Ethics Committee of our institute (No. MC/108/2012/89) was obtained to carry out this study. The study procedures were carried out in accordance with the ethical standards of institutional ethics committee and with the Helsinki Declaration of 1975, as revised in 2000. [†]

Sample size calculation:

Arora U *et al.* reported a seroprevalance of 76% in dyspeptic patients undergoing endoscopy at Amritsar, India. [9] Thus, with an assumed infinite population size and predefined precision of ± 0.10, the calculated sample size is seventy one (71). Considering an estimated 10% drop out, we took a sample size of eighty (80).

Geography of the place of study:

The state in which this study was conducted is situated at the Northeast part of India. As per Government of India census in 2011, the total population of this state is 3,11,69,272 with 85.92% residing in rural area.[‡] Using Tendulkar methodology, it is estimated that 34.40% (in the period 2004-2005) of the population are below poverty line.[‡] The institution where the study was carried out is the only government tertiary care center for a sizable population living in the western part of this state.

Study participants:

The study population consisted of clinically diagnosed cases of acid peptic

disorders, dyspepsia or gastroesophageal disorders were scheduled for flexible UGIE. All patients provided informed consent for the procedure as well as the study and underwent the endoscopy as per the protocol of the Department of Gastroenterology. Demographic information, blood sample, gastric biopsy samples were collected and endoscopic findings were recorded. Patients who were scheduled for therapeutic UGIE procedure, previous surgery in the upper gastrointestinal tract and patients admitted in intensive care unit were excluded from this study.

Sample collection and testing:

All the eighty (80) patients who met the inclusion criteria underwent flexible UGIE carried out by senior faculty members of the Department of Gastroenterology. Mucosal biopsy tissues were taken using a biopsy forceps and at least two tissue (one from the antrum of stomach) samples were collected and placed in a small vial containing rapid urease test broth (Rapid Urease Test Broth, M1828, HIMEDIA®, Mumbai, India).[§] It was then incubated at 37^0C and observed for colour change. [(10)]

For serological testing, 3ml blood was collected in clot vials from all the patients using sterile precautions. The blood samples drawn were then centrifuged and serum was separated, properly labelled and were stored at -70^0C. *H. pylori* seropositivity was evaluated with a commercially available ELISA kit (*Helicobacter pylori* IgG ELISA, Calbiotech Inc, Spring valley, CA, USA) according to the manufacturer's instructions. [‖]

Statistical analysis:

Data were imported into GraphPad InStat (GraphPad software Inc, vesion 3.1, CA, USA) statistical program for analysis. Prevalence rates are expressed as percentages. Unpaired t test was used to compare the age related data. *P* value < 0.05 was taken to be statistically significant. Fischer exact test was used to analyze the categorical data.

RESULTS AND OBSERVATIONS

Sero Prevalence of *H. pylori* Infection among the study patients:

Eighty (80) patients, between the age of twelve (12) and seventy three (73) years participated in the study, of which fifty four (54) were males and twenty six (26) were females. The mean age (± standard deviation) of male and female patients were 36.11 (±12.889) and 39.385 (± 13.983) years respectively without any statistical difference (*P* value 0.3) between these two groups. The overall seroprevalence of *H. pylori* among patients was 27.5% (22 cases) with no difference between men and women (*P* = 1.0). The prevalence among different age groups in both males and females are presented in Table No: 1. Maximum seroprevalence [8.75%, (7/80)] was noted in the age group of 40-49 years, irrespective of gender.

Table No- 1: Prevalence of *H. Pylori* in different age groups by serology and Rapid urease test (RUT)

Age group in years	Total number of cases	Sero Prevalence (no of cases)		Seroprevalence in both the sexes among total no. of cases (no of cases)	Prevalence by RUT (no of cases)		Prevalence by RUT in both the sexes among total no. of cases (no of cases)
		Male¶	Female**		Male¶	Female**	
10-19	7	3.70 (2)	3.85 (1)	3.75 (3)	3.70(2)	3.85 (1)	3.75 (3)
20-29	14	3.70 (2)	3.85 (1)	3.75 (3)	11.11(6)	3.85 (1)	8.75 (7)
30-39	24	7.40 (4)	7. 70 (2)	7.50 (6)	12.96(7)	7.69 (2)	11.25 (9)
40-49	22	9.26 (5)	7. 70 (2)	8.75 (7)	14.81(8)	7.69 (2)	12.5 (10)
50-59	9	3. 70(2)	3.85 (1)	3.75 (3)	3.70 (2)	3.85 (1)	3.75 (3)
60-69	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
70-79	3	0 (0)	0 (0)	0 (0)	1.85 (1)	0 (0)	1.25 (1)

¶ Prevalence in that age group among total number of males

** Prevalence in that age group among total number of females

Endoscopic findings:

Among the eighty patients, maximum number [41.2%, (33/80)] of patients were diagnosed with duodenal ulcer on endoscopy. The percentages of patients with different endoscopic diagnoses is presented in Figure No: 1. The distribution of endoscopic findings in patients with different IgG ELISA and RUT status is presented in Table No: 2.

Status of rapid urease test among the study patients:

Thirty three (41.25%) patients showed positive result in RUT. Nineteen (86.36%) of the seropositive patients were also RUT positive. Thus fourteen (14) patients were RUT positive but seronegative. There were three seropositive but RUT negative cases.

Post hoc sample size calculation:

As the seroprevalence observed during this study was much less compared to the prevalence reported by Arora U *et al*, a post hoc power analysis with prevalence rate of 27.5%, absolute precision of ± 0.10 for an infinite population, the estimated sample size is seventy seven (77). [(9)]

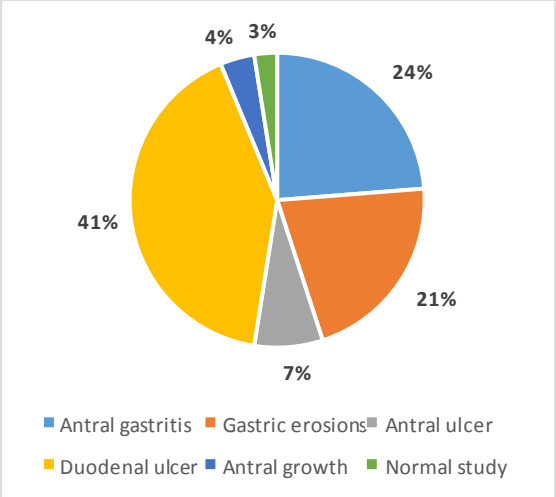

Figure No- 1: Distribution of endoscopic findings in endoscopy among the study population

Table No- 2: Distribution of endoscopic findings in patients with different IgG ELISA and RUT status

UGIE findings	Total no. of cases (%)	IgG ELISA status(% among individual group)		RUT status (% among individual group)	
		Positive	Negative	Positive	Negative
Antral gastritis	19 (23.75)	4 (18.18)	15 (25.86)	6 (18.18)	13 (27.66)
Gastric erosions	17 (21.25)	6 (27.27)	11 (18.96)	6 (18.18)	11 (23.40)
Antral ulcer	6 (7.5)	2 (9.09)	4 (6.89)	4 (12.12)	2 (4.25)
Duodenal ulcer	33 (41.2)	9 (40.90)	24 (41.38)	17 (51.51)	16 (34.04)
Antral growth	3 (3.75)	0 (0.00)	3 (5.17)	0 (0.00)	3 (6.38)
Normal study	2 (2.5)	1 (4.54)	1 (1.72)	0 (0.00)	2 (4.25)
Total	80	22	58	33	47

IgG ELISA-immunoglobulin G enzyme-linked immunosorbent assay RUT- rapid urease test

Table No- 3: Comparison of prevalence of *H. pylori* infection by different methods

Authors	Place of study	Year of publication (in print)	Prevalence (%)		
			Serology	RUT	Histology
Present study	(to be included after peer reviewing)	-	27.5	41.25	-
Kang G *et al* [12]	Vellore, Tamil Nadu	1999	60.24††	69.88	69.88
Arora U *et al* [9]	Amritsar, Punjab	2003	76	72	-
Adlekha S *et al* [13]	Ernakulam, Kerala	2013	-	57.7	59.4
Dorji D *et al* [2]	Bhutan	2014	86	-	-
Shrestha R *et al* [14]	Nepal	2014	-	-	68
Jemilohun AC *et al* [15]	Nigeria	2010	-	60.5	52.35
Ayana SM *et al* [16]	Tanzania	2014	-	65	-

†† included IgG and IgA, second generation assay RUT- Rapid Urease Test

DISCUSSION

In this study, the seroprevalence of *H. pylori* infection was 27.5% (22/80), whereas, the prevalence is 41.25% (33/80) with calculation based on positive reaction to RUT. The difference of the prevalence

obtained by these two methods can be explained by the fact that the response to RUT depends on the bacterial load, as well as intake of proton pump inhibitors, site of biopsy and the presence of intestinal metaplasia; *H pylori* is a diverse bacterial pathogen with diverse antibody response. [11]

Comparison of prevalence of *H. pylori* infection in symptomatic patients hailing from different developing nations as detected by serology, RUT and histology is presented in Table No: 4. The prevalence of *H. pylori* varies among different population. [2] To best of our knowledge, this is the first study to report on sero prevalence of *H. pylori* from this region of India.[*] The most striking finding of our study was the lower rate of seroprevalence in our study group in comparison to reports from other parts of India, some of the less developed regions of South East Asia and Africa. [2,9,12-16] It is difficult to comment on the possible reason(s) for this difference, as we do not have any previous data from our state. It is suggested to validate the cut off value for an ELISA test on local reference sera. [11] We used the cutoff value as recommended by the manufacturer due to non-availability of laboratory back up to detect the local cutoff value. [‖] The possible role of various factors *e.g*. geographical variation, influence of concomitant infection with antibody response of the host, insufficient seroconversion at the time of performance of serological test also needs to be investigated to account for this difference. [11]

In this study, the highest number of positive cases were in the age group of 40-49 years (refer to Table No: 1), both by the serological test and RUT. Moreover, we also observed an age dependent increase in the incidence of *H. pylori*, peaking in 40 -49 years and declining in the subsequent decades (refer to Table No :1) which was consistent with the observations made in another study, but in Bhutan, the peak incidence was reported in 50-59yrs age group. [2,14] The increased prevalence of infection with age can be explained by the fact that it represents a continuing rate of bacterial acquirement throughout one's lifetime and the frequency of *H. pylori* infection for any age group in any locality reflects that particular cohort's rate of bacterial acquisition during childhood years. [17] In contrast, the highest prevalence was found in <20 years age group in a study conducted among dyspeptic patients in Nepal. [14] Their report cannot be compared with ours as histology was used to diagnose the cases and the total number of cases in that age group was less in comparison to the other age groups. [14]

Foot note:

[*] Systematic review of the electronic databases (PubMed, IndMED and Google Scholar) was carried out on 15-10-2014**.** The electronic search strategy was done with the below mentioned words
Helicobacter pylori AND Assam

[†] WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Available from http://www.wma.net/en/30publications/10policies/b3/
Last accessed on 28-09-2014

[‡] Economic survey. Available from http://ecostatassam.nic.in/ads_economic%20survey.pdf
Last accessed on 28-09-2014

[§] Rapid Urease Test Broth (M1828) Available from http://himedialabs.com/TD/M1828.pdf
Last accessed on 27-09-2014

[‖] *H. Pylori* IgG ELISA Available from http://www.calbiotech.com/infectious-diseases/h-pylori-igg-elisa-detail
Last accessed on 27-09-2014

One important observation made during this study was that 97.5% (78/80) of patients had some form of abnormality as detected by endoscopy that could be accounted for the symptomatology. Similar observations (94.2%) have been reported from Kerala, but observations from Africa quote a lesser percentage. [13,15,16] Although the commonest endoscopic abnormality observed was duodenal ulcer in the present study, gastritis is the commonest endoscopic abnormality in the previously quoted studies. [13,15,16] There is conflicting results regarding whether any difference exists in the frequency of *H. pylori* infection among patients with abnormal endoscopic findings and those with normal findings. [13,15] Our study is unable to add any more evidence, as the number of patients without abnormality on endoscopic findings was very low [2.5%, (2/80)].

The following limitations may have affected the quality of the evidence being presented-

The study was carried out in a single center and the studied population is a symptomatic population presenting to a tertiary care center. Moreover, which cases among those presenting with gastrointestinal symptoms will undergo flexible UGIE was on the sole discretion of the treating gastroenterologist. Selection and referral biases are limiting factors of such studies.

A combination of two methods is often applied in studies related to epidemiology of *H. pylori.* [1] Although histology of gastric biopsy and polymerase chain reaction (PCR) based diagnostic testing for *H. pylori* has been reported to have excellent sensitivity and specificity, we choose to evaluate the sero prevalence and response to RUT only. [11]

Although precision of ± 0.05 is commonly used in epidemiological surveys, we choose a precision of ± 0.10 for our sample size calculation. It was done considering our financial constraints.

In conclusion, this study provides evidence of lower prevalence of antibodies to *H. pylori*, as measured with the IgG ELISA kit, among the study population. In majority of the patients who were selected by the gastroenterologist for flexible UGIE, etiology of their symptomology could be identified.

The data provided in this study should benefit the future researchers intending to study the seroprevalence of *H. pylori* in this part of the world. Further data regarding *H. pylori* infection detected with more specific and sensitive assays is warranted.

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