INCIDENCE OF *HELICOBACTER PYLORI* IN PATIENTS WITH GASTRODUODENAL DISORDERS.

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ABSTRACT

Introduction *Helicobacter pylori* infection is now recognised as a worldwide problem. It is the most common cause of chronic gastritis, and is strongly linked to peptic ulcer disease and gastric cancer.

Material And Methods Blood and antral biopsy specimens from 70 dyspeptic patients and 10 control subjects were collected. All the specimens were processed within 2-3 hours. Rapid urease test, Primary smear, Culture was done on freshly prepared brain-heart infusion agar supplemented with 7% horse blood, vancomycin and amphotericin B. Plates were incubated at 37°C under microaerophilic conditions. Histology was done using Heamatoxylin & Eosin staining and Giminez staining. Serology was also performed using a commercial kit - Novum *Helicobacter pylori* IgG and IgA ELISA manufactured in Germany.

Results The age of the study population of 70 patients ranged from 18-70 years and of 10 controls between 22-45 year. There were 54.16% males and 63.63% females. In the present study, there were 03 patients with endoscopically evident gastritis and duodenal ulcer with incidence rate of *H.pylori* i.e 66.6%. Maximum cases had gastritis(72.8%) followed by 15.7% cases of normal mucosa . Forty (57.1%)were found to have *H. pylori* in their antral biopsies by histology.

Conclusions *H.pylori* infection is acquired early in life in the Indian population The *H.pylori* infection has a high morbidity rate, but a low mortality rate and is curable with antibiotic therapy.

INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral, Gram-negative bacterium that chronically infects more than half of the world's population, and is currently recognized to play a causative role in the pathogenesis of gastritis, gastroduodenal ulcer, gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. Infection with *H. pylori* almost always results in chronic gastritis, but more severe diseases such as peptic ulcer and gastric cancer develop in only a small proportion of infected patients, suggesting that the clinical outcomes are probably determined by the interaction of bacterial virulence, host genetic susceptibility and environmental factors.[1] Despite a general decline in the incidence of gastric cancer, it remains the fourth most common cancer and second leading cause of cancer-related deaths worldwide.[2]

However, most individuals never develop clinical disease. Childhood represents the major period of acquisition of infection in the third world, but infection is rare in children in developed world. [3]

In developing countries, the *H. pylori* positive rate for young people is low, but the rate increases by about 1% for each additional year of age. Whereas in developing countries, the prevalence of *H.pylori* infection is high in young people reaches a peak between the age of 20-29 years. These differences in *H.pylori* positive rates may be due to differences in environmental sanitation levels [4].

MATERIAL AND METHODS

The present study was conducted during a period of one year in the department of Microbiology, T.N. Medical College and BYL charitable hospital, Mumbai.

Selection of cases: Patients were selected from those referred for upper gastrointestinal endoscopy and included both outdoor and indoor patients.

Patients- 70 patients with dyspeptic symptoms were selected. Patients on antibiotics and NSAIDs for the past 15 days prior to endoscopy, chronic liver diseases, severe cardio-pulmonary or renal diseases were excluded from this study.

Controls- 10 patients who were also attending gastroenterology clinic with lower gastrointestinal problems were included in this study.

Collection of specimens:

The specimens collected for the study were blood and three gastric biopsies from each patient.

Blood :- Prior to endoscopy 5-6 mL of blood was collected aseptically from the antecubital vein of each patient. Blood

was transferred to sterile glass test-tubes and allowed to clot and serum was separated. Serum was stored in sterile plastic vials and kept at -20° C until tested.

Biopsy :- Three biopsies were taken from each patient from antrum (within 2 cm of pylorum) for microbiological and histopathological examination.

- One biopsy was placed immediately in a tube of brucella broth [5]. For culture and Gram's staining and was transported to the laboratory in a flask containing ice-cubes (4°C) within two hours of collection.
- One biopsy in 10% formalin for histological examination, and
- One biopsy was inoculated in the endoscopy room, in christensen's urea agar for rapid urease test (RUT) [6]

Processing of Sample :

All the specimens were processed within 2-3 hours of collection. Following test were performed-

i. Rapid urease test [7],
ii. Primary smear stained with Gram's stain[8],
iii. Culture- ground biopsy material was inoculated on freshly prepared brain-heart infusion agar supplemented with 7% horse blood, vancomycin (6 mg/L) and amphotericin B (2 mg/L). Plates were incubated at 37°C for 7 days in McIntosh fildes jar under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) devoid of any catalyst [9]. Plates were examined on the 3rd, 5th and 7th day. Colonies of *H.pylori* from primary culture usually took 3 to 5 days to appear and were less than 2 mm in diameter, greyish, circular, low convex, translucent and weakly hemolytic.

Confirmation of *H.pylori*

Suspected colonies of *Helicobacter pylori* were identified by colony morphology, secondary smear stained with Gram's stain [8], oxidase test [10], catalase test [11] ,and urease test [6]

Histology –

The formalin fixed specimens were sectioned and stained with heamatoxylin & eosin (H&E) and Gimenez stain [12]. Light microscopic examination was carried out specifically looking for *H.pylori* and inflammatory cell infilterate in the antral mucosa. Gastritis was graded as nil, mild, moderate and severe.

Serology -

Serum samples were tested for the prescence of IgG and IgA antibodies to *Helicobacter pylori*. Commercial kits used were- Novum *Helicobacter pylori* IgG and IgA ELISA manufactured in Germany. The procedure was followed as per the instruction manual of the manufacturer.

Evaluation of parameters –

A subject was defined as *H. Pylori* positive if the bacteria were identified by Histology (Gold- standard). The results of individual diagnostic methods were compared with the histology and evaluated for its sensitivity, specificity, positive predictive value and negative predictive value. For

comparison, concordance of four, five and six parameter was also examined.

Statistical Analysis–

Data was statistically analysed by using spss/pc + (statistical package for social sciences). Chi-square test (χ^2) and chi- square with yate's correction were applied whenever possible.

RESULTS

The age of the study population of 70 patients ranged from 18-70 years with a mean age of 35.61 ± 13.57 years and of 10 controls between 22-45 years with a mean age of 31.40 ± 9.48 years. (P > 0.05, not significant)

The study population (70 patients) comprised of 48 males and 22 females and in the control group (10 subjects) 7 were males and 3 were females. There were 25 patients from 21-30 years of age group, followed by 12 patients each in the age group of 31-40 years and 41-50 years. There were only 3 patients above the age of 60 years while 10 from 11-20 years in the patient group. While in control subjects 5 were in the age group of 21-30 years, 3 in 31-40 years and 2 in 41-50 years of age group.

Based on the presence of endoscopy findings, the patient group was divided into 5 subgroups depending on the type of endoscopic lesion as normal, gastritis, duodenal ulcer, gastric ulcer and gastric carcinoma (Table I). Maximum cases had gastritis (n=51) with a percentage of 72.8% followed by 11 cases of normal mucosa with a percentage of 15.7% (Table II). Control group (10 subjects) were found to be normal on endoscopy. Among the 70 patients, 40 (57.1% with 95% confidence interval) were found to have *H. pylori* in their antral biopsies by histology (histology was taken as a gold standard in our study) (Table I).

There were 59 patients with abnormal endoscopic findings (Table II) of which 51 patients revealed gastritis alone, 3 had duodenal ulcer, 4 were of gastric ulcer while gastric carcinoma was seen in one patient only. The highest percentage of positivity for *H. pylori* was found in patients with duodenal ulcer (66.6%) followed by gastritis (56.8%) and (25%) in gastric ulcer. The case of gastric carcinoma was found to be negative for *H. pylori*. The overall positivity rate was found to be 54.23% (32/59) (Table II).

The maximum number of patients positive for *H. pylori* (13) were from 21-30 years of age group followed by 9 from 31-40 years of age group.

There were 54.16% males and 63.63% females among the total *H. pylori* patients 57.14% (P> 0.05, not significant).

Rapid urease test gave 100% positivity in gastric carcinoma followed by gastric ulcer and duodenal ulcer as 75% and 66.6% respectively. Gram's stain was 100% positive in duodenal ulcer cases, followed by 75% in gastric ulcer. Culture was positive in 33.3% in duodenal ulcer and 3.9% in gastritis cases. Serum IgG was 100% positive in duodenal ulcer and gastric carcinoma. IgA serology was 100% positive in gastric carcinoma patient followed by 81.8% in normal cases (Table III).

Table-I ***Endoscopic findings in patients with upper gastrointestinal symptoms***

S.No.	Endoscopic findings	Male	Female	Total	Positive for H.Pylori
1.	Normal	8	3	11 (15.7%)	8 (72.7%)
2.	Gastritis	33	18	51 (72.8%)	29 (56.8%)
3.	Duodenal ulcer	3	0	3 (4.28%)	2 (66.6%)
4.	Gastric ulcer	3	1	4 (5.7%)	1 (25%)
5.	Gastric carcinoma	1	0	1 (1.4%)	--
	Total	48	22	70	40 (57.14%)

Table-II ***H.pylori infection in patients with abnormal endoscopic findings***

Sr.no.	Endoscopic findings	Total	H.Pylori Positive		H.Pylori negative	
			No.	%	No.	%
1.	Gastritis	51	29	56.8	22	43.13

2.	Duodenal ulcer	3	2	66.6	1	33.33
3.	Gastric ulcer	4	1	25	3	75
4.	Gastric carcinoma	1	--	--	1	100
	Total	59	32	54.23	27	45.76

Table-III *Correlation of Endoscopy findings and results of various diagnostic tests employed for detection of H.pylori*

Sr. no.	Endoscopic findings	RUT	Gram's stain	Culture	Histology	IgG Serology	IgA Serology
1.	Normal (n=11)	5 (45.4%)	5 (45.4%)	-	8 (72.7%)	8 (72.7%)	9 (81.8%)
2.	Gastritis (n=51)	25 (49%)	20 (39.2%)	2 (3.9%)	29 (56.8%)	26 (50.9%)	34 (66.6%)
3.	Duodenal ulcer (n=3)	2 (66.6%)	3 (100%)	1 (33.3%)	2 (66.6%)	3 (100%)	2 (66.6%)
4.	Gastric ulcer (n=4)	3 (75%)	3 (75%)	-	1 (25%)	3 (75%)	2 (50%)
5.	Gastric carcinoma (n=1)	1 (100%)	-	-	-	1 (100%)	1 (100%)
	TOTAL	36	31	3	40	41	48

DISCUSSION

Since the initial report of Warren and Marshall in 1983 [13], the association of *H. pylori* with gastritis and/ or gastroduodenal ulceration has received much interest. This finding not only changed our knowledge of the microflora of the stomach, which was thought to be sterile by many other [14], but also because of the association of this bacterium with gastritis and peptic- ulceration, opened a new field for investigation.

Association of *H. pylori* has been reported in various gastroduodenal disorders from all over the world including India [13, 15, 16,17,18,19,20,21,22,23,24].

The incidence rate (57.14%), in our study, correlated well with those reported by Joshi et al [25], Maimooma et al [26], Musgrove et al [27], and Gill et al [28]. Where as it was relatively lower than that reported by Kang et al [29] (98%), Gospodarek et al [30] (90%) and Sandikci et al [31] (86%). However our study showed higher incidence rate than reported by Kunz et al [32] (47.9%) and Cheong et al [33] (34%).

In our study the incidence of *H.pylori* infection in patients who showed abnormal endoscopic findings in 59 patients was 54.23% (Table 2). Similar findings are reported by Joshi et al [25] (54.4%), Trowell et al [34] (56%) and by Morris et al [35] who reported a incidence rate of 46%.

Marshall and Warren [13] described a unique association between the presence of *H.pylori* and gastritis. The bacteria are almost always seen in the presence of type B chronic gastritis, particularly, chronic active gastritis and are rarely present in a histologically normal antral mucosa. These findings have been confirmed by reports of many workers throughout the world [36],[37],[38],[39],[40],[41],[42].

In our study, 51 patients revealed gastritis (Table II). *H.pylori* positivity reported by various workers range from 47-92%, indicating its importance as a causal agent in gastritis [25],[42],[39],[43],[44], [45],[46],[47],[48],[49]. In the present study, *H.pylori* positivity in gastritis patients was 56.8%. Similar incidence rate has been reported by McNulty et al [39], Marshall et al [13] and Hazell et al [43]. The highest percentage positivity of *H.pylori* was found to be 92% in the study done by Goodwin et al [49] and lowest of 47.8% by Joshi et al [25]. The difference in the incidence rate of *H.pylori* could probably be due to the presence of very few organisms in the biopsy tissue or due to the patchy distribution of the organism in the stomach [25].

The further evidence for the causative role of *H.pylori* in gastritis is found in therapeutic trials. In patients with gastritis and *H.pylori* when the bacterium is eradicated, the gastritis resolves in vast majority of cases [37],[50],[51],[52],[53]. Recolonisation with the bacterium leads to recurrence of gastritis. Using restriction endonuclease analysis of the bacterial DNA, before treatment and after relapse, it has been shown that the same strain or subtype is involved [54], a strong correlation between the number of *H.pylori* and the severity of chronic gastritis has been reported [55].

Above evidence suggests that *H.pylori* is strongly associated with the type B chronic non-autoimmune gastritis affecting primarily the antral mucosa.

H.pylori has been isolated from the gastric antrum as well as, from the areas of gastric metaplasia in the duodenum from patients of duodenal ulcer [38], [39], [40]. Also most of the patients with duodenal ulcer show presence of associated chronic antral gastritis[38].

Marshall and associates [4], have suggested that gastric metaplasia may be a primary phenomenon in patients at risk for duodenal ulcer. *H.pylori* are seen only in areas of the gastric metaplasia suggesting the aetiological role of *H.pylori* in duodenal ulcer. *H.pylori* were never present in histologically normal duodenum [56].

In the present study, there were three patients with endoscopically evident gastritis and duodenal ulcer with incidence rate of *H.pylori* 66.6% (Table-II).

Our study was in concurrence with other researchers like Nair et al [24] (70.3%) and Kunz et al [32] (70%). In the present study, 25% positivity of *H.pylori* infection was found in four patients of gastric ulcer while patient of gastric carcinoma was negative for *H.pylori* (Table-III). The histology of antrum in this patient showed the tumor tissue but no evidence of *H.pylori*. *H.pylori* is proposed to be etiological agent for gastric adenocarcinoma by some [57], but others disagree[58]. Studies suggest that India, Madagascar and Ivorycoast have high incidence of *H.pylori* but a low incidence of gastric cancer than the developed nations [59],[60]. However, as the number of patients studied in present study were small, definite conclusions cannot be drawn and this requires a more critical evaluation in future studies.

All the control subjects were found to be endoscopically normal and were negative by rapid urease test, Gram's stain, histology and culture.

Helicobacter pylori is reported more commonly in younger age groups in developing countries. In our study, in the age group of 11-20 year, the *H.pylori* incidence was 70%. Similar high incidence rate has been reported by Gill et al [61] in young Indian subjects, while Kunz et al [32] had found no significant difference in detection of *H.pylori* between young and older patients. The incidence rate was highest in the age group of 31-40 years (75%). The frequency of *H.pylori* infection above 60 years of age was

33.3% in the present study. This is similar to findings reported by Nanivadekar et al [62] who also have shown a fall in the frequency of *H.pylori* infection from 87% to 60% above the age of 60 years.

The high incidence of *H.pylori* infection early in adult life in the present study can possibly be explained by: exposure to *H.pylori* early in life because of factors like bad hygiene and lack of proper sanitation and increased susceptibility because of a genetic predisposition.

In the present study, almost equal percentage of cases in both sexes i.e. male 54.16% and females 63.6% (P > 0.05, not significant) were associated with *H.pylori*, suggesting no evidence of sex predilection. Similar findings were reported by Marshall et al [32] and Joshi et al [25].

The antral biopsy specimen collected from each patient were subjected to rapid urease test (RUT), Gram's stain, culture and histology. Blood was collected from each patient for the determination of antibodies IgG and IgA to *Helicobacter pylori* in serum by using commercial kit-Novum *H. pylori* IgG and IgA ELISA, Germany.

In the present study, RUT was positive in 1 out of 1 patient of gastric carcinoma (100%) (Table III), followed by 75% in gastric ulcer and 66.6% in duodenal ulcer patients. Our study correlated well with the findings of Joshi et al [25]. Gram's stain was positive in all the three cases of duodenal ulcer (100%) followed by 75% in gastric ulcer cases (Table III), which matched perfectly with the findings of Hantzschel et al [63]. Culture was positive in 33.3% of duodenal ulcer cases (Table III) which was less than that reported by Sito et al [64] (50%). IgG antibodies were detected in 100% cases of duodenal ulcer and of gastric carcinoma (Table III). Our study is in concurrence with Sobala et al [65] and Adamsson et al [66]. IgA serology was positive in 100% of gastric carcinoma patients (Table III).

The best test for the determination of *H. pylori* status of a patient is that which has the greatest validity (combination of sensitivity and specificity) and the largest *J* value [67]. In our study, validity and *J* value was highest with Gram's stain (71.25% and 0.43 respectively), followed by serum IgG serology (69.15% and 0.39 respectively).

Distribution of *H.pylori* may be a patchy phenomenon and histopathology/ culture assess only a small area of the stomach. Serology in essence, assays the entire stomach. Therefore, serological test may be better in already established (past) infections where the organism may not be detected bacteriologically and histologically.

Thus, IgG serology (ELISA) could serve as an easy, sensitive, cheap and non-invasive method for screening patients before endoscopy. It is useful in management, by virtue of early detection of patients with an increased risk of chronic peptic ulceration and malignancy of stomach. However, the levels of *H.pylori* specific antibodies must be evaluated in the normal population to establish ELISA as a definitive diagnostic tool.

CONCLUSIONS

H.pylori is a causal agent in gastritis and gastroduodenal ulcer diseases. *H.pylori* infection is acquired early in life in the Indian population. There is no evidence of any particular sex predilection in *H.pylori* infection. The concordance of results of various diagnostic tests is the best available option for determining a patient's true *H.pylori* status.

The incidence of *H.pylori* infection in the study group was 57.14%. *H.pylori* incidence was 54.23% in patients with abnormal endoscopic findings. Of these 56.8% of gastritis patients, 66.6% of duodenal ulcer and 25% of gastric ulcer cases were positive for *H.pylori*. *H.pylori* positivity was 70% in 11-20 years, 75% in 31-40 years and 33.3% in 61-70 years of age group. The rate of *H.pylori* incidence was similar in male and female (p > 0.05, not significant).

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