General Surgery

THE STUDY OF HELICOBACTER PYLORI AND ACID PEPTIC DISORDERS IN URBAN INDIAN SCENARIO

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ABSTRACT It has been more than two decades since the discovery of the Helicobacter pylori as the culprit behind the acid peptic disorders. Medical professionals need to be updated with the current trends of the advanced and convenient modality of diagnosis and treatment of the peptic ulcer diseases. Medical treatment is the accepted treatment for the peptic ulcer disease worldwide. Various method to diagnose H. Pylori infection are grouped as an invasive method and non-invasive method. This study was attempting to find the current trend of the prevalence, associated patient factors, convenient diagnostic methods and current sensitivity and resistance of the drugs used for the treatment. This study is representing the current diagnosis and treatment of the acid peptic disorders in urban India.

KEYWORDS : Diagnosis, RUT, Histopathology, sensitivity, specificity

INTRODUCTION:

It's been more than two decades since the discovery of the Helicobacter pylori as the culprit behind the acid peptic disorders. Medical professionals need to be updated with the current trends of the advanced and convenient modality of diagnosis and treatment of the peptic ulcer diseases. Medical treatment is the accepted treatment for the peptic ulcer disease worldwide. Various method to diagnose H. Pylori infection are grouped as an invasive method and non-invasive method. The invasive method requires endoscopic biopsy of the stomach Antrum. The non-invasive method comprises urea breath test and serology. This study was attempting to find the current trend of the prevalence, associated patient factors, convenient diagnostic methods and current sensitivity and resistance of the drugs used for the treatment. This study is representing the current diagnosis and treatment of the acid peptic disorders in urban India. Present study investigated the clinical profile of acid peptic disorder, role of gastroscopy in establishing the diagnosis, the efficacy of microbiological tests for detection of H. Pylori infection.

MATERIALS AND METHODS:
STUDY DESIGN:

The present study was carried out on the patients attending surgical OPD as well as the surgical ward from January 2014 to June 2015 in Government medical college and hospital, Nanded, Maharashtra. For the level of significance and increase the power of the study, the sample size was decided to be 200. Patients were selected randomly based on the upper gastrointestinal complaints like the pain in the epigastric region, nausea, vomiting, hematemesis, Malena, etc. Detail history of patients was taken with regards to the symptoms, their duration, and severity, other complaints, drug history. Thorough clinical examination and routine hematological examination of patients was done. After the establishment of the clinical diagnosis of the acid peptic disorder, the patient was posted for endoscopy. The total number of patients who underwent endoscopy were 366, out of which 200 patients were included in the study based on inclusion and exclusion criteria.

Inclusion criteria:
- Patients who had symptoms of acid-peptic disorder
- Patients who had chronic upper abdominal pain.
- Patients who showed symptoms of dyspepsia.
- Patients who had chronic gastritis, gastric/ duodenal ulcers. on the previous gastroduodenoscopy.

Exclusion criteria:
- Patients who were on proton pump inhibitors.
- Patients who were known cases of chronic pancreatitis.

- Patients on NSAIDs for more than 1-month duration.
- Patients who had received anti-Helicobacter pylori treatment.
- Unwilling or unfit patients for gastroscopy.

After applying the inclusion and exclusion criteria, all the patients underwent upper gastrointestinal endoscopy. Patients were kept nil orally at night and were advised to attend endoscopy clinic early in the morning. Endoscopy was performed with Fujinon Gastroduodenoscope after obtaining informed consent. Esophagogastroduodenoscopy of each patient was done under the surface local anesthesia with 4% Xylocaine gel. Findings were noted, and biopsies from antrum and suspicious lesions were taken, this biopsy was put into glucose broth and was used for rapid urease test. Endoscopic findings were recorded as gastritis, duodenal ulcer, gastric ulcer, gastric carcinoma and endoscopically normal mucosa.

Statistical analysis:

After collecting data, Pearson chi-square test was used for statistical analysis. p-value <0.05 was considered to be significant.

Collection of samples:
Three mucosal biopsy specimens were collected from different sites of stomach and duodenum.

Collected specimens were kept in approximately 0.2 ml normal saline so that the specimens remain moist. Attention was given to transport the specimens to the laboratory within two hours. In case of any delay, specimens were stored in the refrigerator at 4 degree Celsius.

One specimen was taken in 10% formalin for histopathology.

Method of Rapid Urease Test:
Urea broth was used as the medium for the test. It consists of urea, phenol red indicator and distilled water. 10 gm of urea was dissolved in 80 ml of distilled water, and final volume made up to 100ml. To it, 0.002 gm of phenol red was added. pH was adjusted up to 6.4 - 6.8 using dil. HCI. The broth was sterilized by steaming at 100°C for 20 min. The medium was distributed in the quantity of 1.5 to 2.0 ml in aliquots. Inoculation of one biopsy piece was carried out after collection into 1.5 to 2 ml of urea broth. It was incubated in the incubator at 37°C for one and half hour. The change in color of the broth from pale yellow to deep pink was taken as a positive test.

Culture:
Homogenization of one biopsy specimen was done with mortar and pestle. The homogenized specimen was used for plate inoculation and gram staining.

The Columbia agar base was procured from Himedia laboratories. The addition of 5%-7% prepared the media defibrinated blood, and they were made selective by the addition of the Campylobacter supplement (Skirrow's) – FD-090 from Himedia Laboratories. The selective and media were inoculated with the homogenized material, and they were incubated at 37°C in an incubated in CO_2 jar with multiple wax candles lighted to create the microaerophilic atmosphere. The jar was closed tightly with the help of petroleum jelly. Incubation Jar temperature was maintained at 37degree C for 5-7 days and inspected on 3rd, 5th and 7th day. Water soaked cotton pads were used to keep microenvironment humidified. Colonies were further subjected to gram staining, catalase, oxidase and urease tests.

Direct Gram Stain: The biopsy specimen was ground in a ground-glass grinder. A loopful of ground biopsy material was compressed between two sterile glass slides, air dried, heat fixed and stained with Gram's stain.

Histopathology: The biopsy specimens which were collected in 10 % formalin as a fixative were transported to the Pathology Laboratory after proper labeling and with the requisition form for the Haematoxylin and eosin staining. After this, they were observed and reported by the pathologists.

Antimicrobial Susceptibility Testing: The isolates of H pylori subculture were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion method on Muller-Hinton agar plate supplemented with 10% blood. BHI broth with turbidity McFarland 3 was used for the inoculum of H pylori. Mueller -Hinton blood agar plates with following antibiotics were used for seeding of inoculum. The antibiotics were Metronidazole (5 µg), amoxicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), levofloxacin (5 µg), norfloxacin (5 µg), cotrimoxazole (10 µg) were placed on the plates. The plates were incubated at 37°C in CO_2 jar for 3–4 days. The results were interpreted as per Clinical Laboratory Standards Institute (CLSI) 2015 guidelines.

Treatment: In current study, Triple therapy which consists of Amoxicillin, metronidazole and one proton pump inhibitor was used for treatment. The treatment was given for 14 days. Then follow up of patients was taken for symptomatic relief and review endoscopy was done after six weeks for the assessment eradication of Helicobacter pylori.
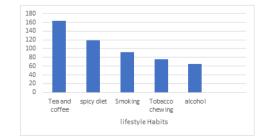
RESULTS:
The present study was conducted over the period of one and half year. A total of 200 patients presenting with the symptoms of acid peptic diseases to surgery OPD as well as the wards were included in the study. The observations are mentioned below,

It was observed that 77(38.5 %) out of 200 patients belonged to the age group between 41-50 years followed by 31-40 years and 21-30 years. Mean age ± Standard Deviation at presentation was 42.91 ± 12.05 years. Males constitute 146(73%), and 54(27%) were females, Male: Female ratio is 2.7:1.

Most common complaint with which the patients presented was the pain in the epigastric region 158 (79 %), followed by nausea 102 (51%). Many of the patients had multiple clinical symptoms which included dyspepsia (76%), vomiting (56%), haematemesis (22%) and Malena(12%).

Out of 200 patients, 164 had the habit of excess consumption of tea or coffee, 120 had the habit of spicy diet, 92 were smokers while 76 and 64 patients gave the history of tobacco chewing and alcohol respectively. (Figure 1)

Figure 1. Distribution of patients according to lifestyle habits

Endoscopic findings: In the present study, the most common finding on gastroduodenal endoscopy was gastritis 78(39%), followed by duodenal ulcer 56(28%) and gastric ulcer 36(18%). Other findings were duodenitis 14(7%), gastric polyp 4 (2%). 12(6%) patients had clinical symptoms of acid peptic diseases, but endoscopic findings were unremarkable.

Out of 200 patients presenting with complaints of acid peptic diseases RUT was positive in 122(61%) cases. RUT was positive in 78.57% (44 out of 56) cases of duodenal ulcer, 72.22% (26 out of 36) cases of gastric ulcer and 53.85% (42 out of 78) cases of gastritis. In 2 out of 12 cases (16.67%), endoscopy did not reveal any abnormality still RUT was positive on biopsy specimen. P-Value was less than 0.001 hence rapid urease is highly significant.

Out of the 200 isolates, Gram stain was positive in 92(46%) of the cases. Gram stain was positive in 40(71.43%) out of 56 cases of Duodenal ulcer while it was positive in 16(44.44%) in Gastric ulcer and 26(33.33%) in Gastritis. In duodenitis, it was positive in 6(42.86%) out of the 14 cases while in 12 cases of the endoscopically normal findings it was positive in 4(16.67%). P-Value is 0.000 is less than 0.001 hence Gram Staining is highly significant.

Out of the total 200 samples Culture, positive samples were 24(12%) The highest isolates of Helicobacter pylori were found in duodenal ulcer cases with 14(25%) out of 56. Other isolates were found in Gastric ulcer 4(11.11%) and Gastritis 6 (7.69%). P-Value is 0.013 is less than 0.05 hence Culture is significant.

On histopathological examination, out of total 200 patients presenting with symptoms of acid peptic diseases, 128 were positive for H. pylori. Highest positivity for H. pylori was found in duodenal ulcer (78.57%), followed by gastritis (66.67%)and gastric ulcer (55.55%). Out of 4 cases of malignancy, 2 (50%) case of was positive for H. pylori. Surprisingly 4(33.33%) patients with normal endoscopic findings were positive for H. pylori.

The positive cases showed the presence of curved bacilli along the lumen of the mucosal glands with Routine H & E stain used. P-Value is 0.0129 is less than 0.05 hence Histopathology is significant. The above results are compiled in Table 1.

Table 1. Comparison of h. pylori positivity between staining, culture, rut and histopathology according to endoscopic findings.

Endoscopic findings	No. of cases (n)	No. of H. pylori positive cases by Gram staining (% out of each n)	No. of H. pylori positive cases by Culture (% out of each n)	RUT positive cases (% out of each n)	No. of H.pylori Positive cases by histopathology (% out of each n)
Gastritis	78	26(33 %)	6(8 %)	42(54 %)	52(67 %)
Duodenal Ulcer	56	40(71 %)	14(25 %)	44(79 %)	44(79 %)
Gastric ulcer	36	16(44 %)	4(11 %)	26(72 %)	20(56 %)
Duodenitis	14	6(43 %)	0(0 %)	6(43 %)	6(43 %)
Endoscopically normal mucosa	12	4(33 %)	0(0%)	2(17 %)	4(33 %)
Growth in Pylorus	04	0(0 %)	0(0 %)	2(50%)	2 (50 %)
Total	200	92(46%)	24(12%)	122 (61%)	128 (64 %)
P value		<0.0001	0.0013	<0.0001	0.0129

$p < 0.05$ = Significant $p < 0.001$ = Highly Significant $p > 0.05$ = Non-Significant (Insignificant

The resistance pattern observed for Metronidazole, Clarithromycin, Amoxicillin Tetracycline, Ciprofloxacin and Furazolidone were 62.5%, 20.83%, 12.5%, 54.16%, 50% and 12.5% respectively (table 2).

Table 2. Percentage of Resistance:

Antibiotic	No. of Isolates	Percentage %
Metronidazole	15	62.50%
Clarithromycin	5	20.83%
Amoxicillin	3	12.50%
Tetracycline	13	54.16%
Ciprofloxacin	12	50%
Furazolidone	3	12.50%
Metronidazole + Clarithromycin	4	16.67%
Metronidazole + Tetracycline	12	50%
Metronidazole + Clarithromycin + Tetracycline	2	8.33%

DISCUSSION:

Age distribution of patients with acid peptic diseases: The age range was 15-80 years. People between age 41-50 years (35%) were most commonly affected followed by 31-40 years (28%). Mean age of presentation was 40.6 years. The mean age at presentation of the patients who had symptoms of acid peptic diseases in current study was following other reference studies. [1-5]

Sex wise distribution: In current study, it was observed that, acid peptic diseases were more common in males as compared to females. Male to female ratio was 2.7: 1. Male sex preponderance found in current study was following other reviews. [2, 3, 6-9]

Many of the patients presented with multiple symptoms. Michael G Lee et al. (2007) in their research found that the most common symptoms were the epigastric pain, nausea, vomiting, and dyspepsia. [10] Tri H Le et al. (2008) in their study found that the most common symptoms were the pain in the epigastric region, nausea, vomiting, and dyspepsia [11]. Epigastric pain was the most common symptom of the study done by Tom Okello et al. [12]. S M Ayana et al. (2014) in their study found that Epigastric pain was reported by 179 (86.1%) of patients with dyspepsia. Other presenting symptoms were heartburn (58.2%), nausea (46.6%). [7] The findings in the present study were following these studies.

Habits: Excess tea and coffee consumption was the most common habit, and 82 % of the population was addicted to same. This habit was followed by spicy diet (60%) and smoking (46%). According to Sharma et al. 33% smokers suffer from dyspeptic symptoms [13], while this proportion was comparatively less in research did by Michael G. Lee et al. [10]. The number of smokers and alcohol consumers were more in current study as compared to other studies.

In the study conducted by Mustapha S.K et al. (2007), common findings on endoscopy were gastritis including erosions (60%) and duodenitis (27.9%). Gastric ulcer was most common and found in 6.4%. The next pathology was the duodenal ulcer(4.8%) and gastric cancer (1.7%) [3]. Hashemi Mehmood Reza et al. (2006) observed that endoscopic signs of gastritis were evident in 56.8%, duodenal and gastric ulcers in 0.3% patients. 16.1% patients had normal endoscopic results. [2] A study conducted by Emad Mohamed Abdallah et al. (2015) observed that common endoscopic findings were Gastritis in 52% and Duodenal ulcer in 22% followed by the Gastric ulcer in 14%, Duodenitis in 10% and Growth in pylorus in 2%. [9] Trend of pathologies found in the current study was similar to international studies.

In the present study, RUT was positive in 112 out of 200 patients. The overall positivity of RUT in current study correlated well with reports by M Sharma et al. (2012) and Maimomma et al. (1994). It was more than the study done by Sadidulla et al. and was lower than that reported by MDU Islam et al. (2010) [14-19] Patchy distribution could be the reason behind missing biopsies. In the present study, the sensitivity of RUT was 95.08%, specificity 92.30%, positive predictive value 95.08% and negative predictive value 92.30%.

Benjamin C.Y et al. (1997-98) found that rapid urease test had limited sensitivity (70-90%). [20] Vandana Berry et al. (2005) in their study found that RUT had high sensitivity (90-95%) and cent percent specificity [6]. Specificity and positive predictive value of 100% was seen in the study done by Said et al. while the diagnostic accuracy was 99% [21].

The present study correlates with the gram stain findings of the various studies mentioned. In the present study, the sensitivity of Gram Stain was 71.86%, specificity 100%, positive predictive value 95.08% and negative predictive value 66.67%. The sensitivity of Gram's stain in current study was 71.86% which is low compared to Montogomery and Co-worker (92%), Maimooma et al. (80.8%) the specificity of Gram's stain in current study was 100%. [15, 22]

In current study, [table1] culture was positive in 12%, whereas Malik et al. and Oyedeji et al. have reported in22.5% and 19.3% respectively. Prevalence of protozoal infestations is high in India and metronidazole is frequently used for it, that could have caused low isolation of organisms in the study of Malik et al. In studies done by Kaori et al. and Vagareli et al. it is 8 % and 2.5 %. [22-27] Gupta et al. cited that, Hypochlorhydria could have lead to overgrowth of other organisms and suppressed the organism under study [28]. Failure of culture may also result from sampling error of the specimen or delay in plating the material; other factors include swallowed local anesthetics, simethicone, prior treatment with bismuth, antibiotics or H2 receptor antagonist and contamination of biopsy forceps with disinfectants. Though culture is highly specific, it produces the highest number of false-negative results. [7]

In the present study, the sensitivity of Culture was 19.67%, specificity 100%, positive predictive value 100% and negative predictive value 44.31%. In a survey by Destura et al., [29] H. pylori culture showed a sensitivity of 45%, the positive predictive value was 97%, and the negative predictive value was 55% which matches with current study.

In the present study, histopathological examination showed H. pylori in 64% cases. The overall positivity of histopathology in current study correlated well with reports from other studies. [3,4,9,15]

In the present study, 64% cases were positive for H. pylori on histopathology, while 61 % were positive on RUT, 46% were positive on Gram stain and 12% in Culture. Barry J. Marshall advocates "If the H. pylori diagnosis is going to make an important contribution to management, then the most sensitive test should be used. Currently, this is histology." [24] This conforms with our observation that histology detected most of the infected patients who underwent endoscopy in current study. CDC recommends that histopathology [H and E] should be taken as gold standard. [26]

CONCLUSION:

In Indian scenario, gastritis was the most common endoscopic diagnosis followed by duodenal ulcers which were confirmed on histopathological examination. Histopathology detected all the H. Pylori cases; thus, it was superior to rapid urease test. It is considered as 'gold standard test ' to diagnose H. Pylori infection. A rapid urease test is easy to perform and interpret, requires less time and cost-effective, we recommend it for the diagnosis of H. Pylori associated dyspeptic symptoms.

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