THE HISTOPATHOLOGICAL STUDY OF GASTRODUODENAL BIOPSIES AND HELICOBACTER PYLORI INFECTION IN ACID PEPTIC DISEASE PATIENTS

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ABSTRACT: Acid peptic disease is the most common condition affecting the gastrointestinal tract. H. pylori, which colonizes the gastric submucosa, being the most prolific etiological agent and responsible for various gastrointestinal lesions like gastritis to gastric ulcer and sometime malignant lesion.

The present study deals with the spectrum of lesions caused by H. pylori and their diagnosis by rapid urease test & histopathological examination of gastroduodenal biopsies.

Total 100 patients with symptoms of acid peptic disease were evaluated and endoscopic biopsies from gastroduodenal site were obtained. The biopsy specimens were processed for rapid urease test & histopathological examination using H&E and special staining (Warthin-starry and Giemsa stain) techniques. Among the 100 cases, common age group of presentation was 31-40 years & male to female ratio was 1.78:1. On histopathological examination the most common lesion was chronic gastritis (33 cases) followed by duodenal ulcer (21 cases), gastric ulcer (12 cases) & gastric neoplastic lesion (14 cases). Among neoplastic lesion, adenocarcinoma was most common finding. Rapid urease test showed positive results for H. pylori in 61% cases and on histopathology in 63% of cases.

H. pylori infection has significant role in the pathogenesis of acid peptic disease, of which gastritis was most common condition. Rapid urease test is simple and cost-effective. Histopathological examination using H&E and special stains are the gold standard tools for the diagnosis of H. pylori infection.

INTRODUCTION: Upper gastrointestinal tract diseases are one of the commonest entities in surgical practice. Peptic ulcers are the diseases for which multiple etiological factors have been described and proposed; one of them is Helicobacter pylori infection. Existence of H. pylori in stomach was confirmed by Robin Warren (born 1937), a pathologist from Perth, Australia who observed small curved bacteria colonising the lower part of stomach (antrum) in about 50% of patients from which biopsies had been taken.¹ Barry Marshall (born 1951), a young clinical fellow, became interested in Warren's findings and together they initiated gastroduodenal biopsies from 100 patients. After several attempts, Marshall succeeded in cultivating a hitherto unknown bacterial species (denoted H. pylori) from several of these biopsies.¹ For their outstanding contribution, they were awarded Nobel Prize in Physiology or Medicine in October 2005.¹

H. pylori commonly causes gastritis and peptic ulcer, a chronic inflammatory condition of stomach and duodenum, presenting as recurrent abdominal pain. It is a major cause of morbidity in infected patients as it is associated with 90% of duodenal ulcers and 80% of gastric ulcers. H. pylori infection is also associated with gastric mucosa associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma.²

Various methods are available to diagnose *H. pylori* infection and are grouped as (a) Invasive methods and (b) Non-invasive methods. The invasive methods are based on collection of endoscopic gastric biopsy specimens that are subjected to urease test, staining, culture, histology and molecular diagnostic techniques. The non-invasive methods comprise of urea breath test and serology.² Whole gastroenterological community is now trying to accept and utilise this knowledge in their practice. Our study also tries to find out, whether we also get corroborative results.

MATERIAL & METHODS: The present study was carried out in the Department of Pathology, Dr. Shankarrao Chavan Government Medical College and Hospital, Nanded during the period of one and half year from Jan 2009 to June 2010. The study comprises of endoscopic mucosal biopsies obtained from 100 patients presenting with complaints of acid peptic diseases to Medicine and Surgery OPD as well as wards. Patients with complaints of acid peptic diseases who underwent endoscopy were biopsied. Only adequate biopsies were included in the study. Patient's detailed clinical history especially dietary, drug and history regarding life style like smoking, alcohol etc was obtained.

Biopsies were taken from suspicious lesions; wherever possible multiple biopsies were taken. The biopsy sample was divided into two parts. One part was put immediately into glucose broth (used as transport medium) and processed further. This sample was used for rapid urease test. Remaining portion of the biopsy sample was used for histopathological study.

RAPID UREASE TEST: Rapid urease test takes advantage of the fact that *H. pylori* is a urease producing organism. Samples obtained on endoscopy are placed in urea containing medium; if urease is present, the urea will be broken down to carbon dioxide and ammonia, with a resultant increase in the pH of the medium and subsequent colour change in the pH dependent indicator. The advantages of this test are being inexpensive, fast and widely available. It is limited by the possibility of false positive results; decreased urease activity, caused by recent ingestion of antibiotic agents, bismuth compounds and PPI can contribute for false positive results.³

UREA BROTH: 10 gm of Urea is dissolved in 80 ml of distilled water and final volume upto 100 ml is made. To it 0.002 gm of Phenol red indicator added. pH was adjusted to 6.4 - 6.8 using diluted HCl. Immediately after collection one piece of biopsy was introduced in 1.5 to 2.0 ml of urea broth in a test tube. The test tube was incubated at 37°C for one and half hour. Development of pink colour from yellow was taken as positive test.²

HISTOPATHOLOGICAL STUDY: The biopsy samples were kept in 10% formalin for atleast 6 hours for proper fixation. The samples were further processed for histopathology. Slides were stained with routine stain - Hematoxylin and Eosin and Special stains –i) Warthin-Starry Silver stain ii) Giemsa stain.

RESULTS: 100 patients presenting with complaints of acid peptic diseases were included in this study. Endoscopic examination was carried out and biopsies were taken from suspected lesions. The biopsy samples were tested for *H. pylori* by using rapid urease test and histopathology.

The most common age group affected in acid peptic disease was 31-40 years (33%), followed by 21-30 years (22%). Mean age at presentation was 40.6 years. Males were commonly affected than

females. Male to female ratio was 1.78:1. Most common complaints with which patients presented was epigastric pain (81%) followed by nausea (56%) and dyspepsia (36%).

Most of the patients (82%) had habit of excess consumption of tea/ coffee. Habit of smoking was observed in 46% of the patients. 32% patients had given the history of alcohol abuse.

On endoscopic examination gastritis was the most common finding (33%) followed by duodenal ulcer (21%) and growth in pylorus (14%). 12% cases found to have gastric ulcer. Rapid urease test showed positive results for *H. pylori* in 61% cases and on histopathology in 63% of cases. On histopathological study, most common finding was chronic gastritis (33%). 14% cases having antral growth were found to have gastric malignancy. Adenocarcinoma was the most common type (78.57%).

Three staining methods were used (routine H & E, Giemsa and Warthin starry silver stain) showed presence of *H. pylori* bacteria along the lumen of mucosal glands. *H. pylori* positivity was seen in 90.9% cases of gastritis, 85.71% cases of duodenal ulcer and 75% cases of gastric ulcer on histopathology.

Histopathological examination is sensitive as well as specific method to diagnose the *H. pylori*, than Rapid urease test. It is therefore considered as “gold standard test” to diagnose *H. pylori* infection.

DISCUSSION: The present study tries to find out an association between *H. pylori* infection and acid peptic diseases. Various techniques are available to diagnose this infection like rapid urease test (RUT), urease breath test (UBT), histo-pathology, serological test, bacterial culture, polymerase chain reaction. We used histopathology and rapid urease test in our study.

Most common complaint of the patients was pain in the epigastric region (81%), followed by nausea (56%). Other complaints were dyspepsia (36%), vomiting (28%), hematemesis (11%) and melena (6%). Most of the patients presented with multiple symptoms. These findings were in accordance with those of Tri H Lee⁴ (2008), Michael G Lee⁵ (2007) and Tom Richard Okello⁶ (2006).

In present study, 82% of the patients had history of excess tea and/or coffee consumption. 60% patients had history of taking spicy diet. Habit of smoking was observed in 46% of the patients. 32% patients had history of alcohol abuse. In relation to drug history, 30% patients had history of consumption of NSAIDs. According to Sharma B et al⁷ (2006) smoking habit was present in 33% of the patients presented with dyspepsia in his study. According to Michael G. Lee⁵ (2009), out of 30 patients presented with dyspepsia, 23% were regular alcoholic while 10% were cigarette smoker. Number of smokers and alcoholics were more in our study as compared to other studies.

In the present study, the most common finding on gastroduodenal endoscopy was gastritis (33%), followed by duodenal ulcer (21%) and pyloric growth (14%). Other findings were gastric ulcer (12%), duodenitis (8%), gastric polyp (2%). 10 patients had clinical symptoms of acid peptic disease but endoscopic findings were unremarkable. The endoscopic findings were variable in various studies. Gastritis and duodenal ulcers were common in all the studies as observed in our study.

In present study, RUT was positive in 61 out of 100 patients. The overall positivity of RUT in our study correlated well with reports by Sharma B et al⁷ (59%) and Mastaghni A et al⁸ (59%) while it was lower than that reported by Sengupta et al⁹ (96%). The reason behind it might be the patchy distribution of *H. pylori* bacilli in gastroduodenal mucosa which might have been missed on biopsy.

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In present study, histopathological examination showed *H. pylori* in 63% cases. The overall positivity of histopathology in our study correlated well with reports by Hashemi M. R et al ¹⁰ (67.1%) while it was higher than reported by Yakoob Javed et al ¹¹ (57%). We observed *H. pylori* bacilli in all the three staining methods (routine H & E, Giemsa and Warthin Starry silver stain). Out of these three staining methods, no clear advantage of one over the other has been demonstrated. This is in accordance with study of Jhala N et al ¹² (2002) and Toulaymat M. et al ¹³ (2009).

Our results correlated with those of the study performed by Goh. K. L. et al ¹⁴ (1994). It could be considered as “gold standard” for the detection of *H. pylori*. Thus, some of the positive and negative observations of other test (RUT) not coinciding with histopathology were considered as negative. Why such false observations with RUT were noticed is not known. Further, taking sensitivity and specificity of histology (being “gold standard”) as 100%, the same was much in case of RUT.

TABLE: Comparison of Disease wise Frequency of *H. Pylori* Positivity In Our Study With Other Studies.

Disease	Our Study (2009-10)	Shah Sattar Khan et al ¹⁵ (2008)	Mustapha S. K et al ¹⁶ (2007)	Hashemi M. R et al ¹⁰ (2007)	Wyatt J.L. Semin Diagn pathol ¹⁷ (1991)
Gastritis	90.9%	84%	89.1%	70.1%	90%
Duodenal ulcer	85.71%	100%	100%	86.2%	95%
Gastric ulcer	75%	-	61.9%	71.9%	70%
Duodenitis	37.5%	100%	83.3%	-	-
Adenocarcinoma	7.14%	0	-	-	50%
No pathology	20%	0	-	33.5%	-

In present study, *H. pylori* bacilli were present in 90.9% cases of gastritis, 85.7% cases of duodenal ulcer and 75% cases of gastric ulcer. These findings were in accordance with observations made by Wyatt J.L. Semin Diagn pathol ¹⁷ (1991) and Mustapha S. K et al ¹⁶(2007). However, only one (7.14%) case of gastric adenocarcinoma was positive for *H. pylori*. This finding was less than what was reported in other studies.

In present study, sensitivity of RUT was 90.5%, specificity 89.2%, positive predictive value 93.4% and negative predictive value 84.6%. Our finding is in accordance with Benjamin C.Y et al¹⁸ (1997-98) and Vandana Berry et al ² (2005).

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FIG. 1 [A] (H&E, 10X)

FIG. 1 [B] (H&E, 40X)

FIG.1 [A & B]. Chronic gastritis: - Gastric glands lined by columnar cells. Lamina propria shows lymphocyte aggregate.

FIG. 2 [A] (H&E, 40X)

FIG. 2 [B] (GIEMSA, 40X)

FIG. 2 Spiral shaped *H. pylori* (pink coloured [A] and blue coloured [B]) are seen along the luminal side of gastric glands.(H&E,40X).

FIG. 3 [A] (10X)

FIG. 3 [B] (40X)

FIG. 3 [A & B]: Spiral shaped *H. pylori* are highlighted in Warthin- Starry silver stain.

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