

The Histopathological Study Of Various Gastro-duodenal Lesions and Their Association with Helicobacter pylori Infection.

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Abstract: Acid peptic diseases are the commonest afflictions of the gastro-intestinal tract and a major cause of patient morbidity, various etiological entities have been sought of which the most prolific being *H.pylori*; a urease producing gram negative bacilli, which colonizes the gastric submucosa and has evolved to grow and replicate in the extreme acidic milieu of the stomach.

H.pylori has been considered the most common infection of man; and has been implicated in a myriad range of upper gastro-intestinal tract afflictions which includes the innocuous gastric ulcers and gastritis to capricious malignant lesions like adenocarcinoma.

Various tests are now commercially available for the rapid detection of *H.pylori*, but most are limited in use either because of low sensitivity or exorbitant costs, inaccessibility, or want for the use of costly technologies.

Rapid urease test is a cheap, easily performed test requiring minimal resources and assets and gives fairly comparable results with histopathology, which again can be complemented by the use of special staining techniques like Geimsa and Warthin starry silver impregnation method especially on gastro-duodenal biopsy Specimens.

And prompt diagnosis has widespread ramifications in patient care and management.

Keywords- Acid peptic disease, Gastro-duodenal biopsy, *Helicobacter pylori*, Histopathological study, Rapid urease test.

I. Introduction

Acid peptic diseases are amongst the commonest causes for medical and surgical consultations worldwide. A constellation of definitive and putative etiological entities have been sought of which *Helicobacter pylori* (*H pylori*) infection is at the forefront.

H.pylori is a gram negative, urease positive, curved motile bacilli with human gastric submucosa being the preferred site of colonization. The bacilli are directly related to the development of various gastro-duodenal pathologies ranging from the innocuous but troublesome gastric ulcers to the highly capricious malignancies.

The existence of *H.pylori* was confirmed by Robin Warren, a pathologist from Perth; Australia who noted the presence of small curved bacteria in gastric biopsies obtained from the lower part of stomach in patients with acid peptic disease. Barry Marshall a young clinical fellow became interested in Warrens work and together initiated a study of gastric biopsies to isolate the elusive agent, and finally were able to isolate and grow a hitherto unknown bacillus in number of biopsies, later nomenclatured as *Helicobacter Pylori*. For their outstanding contribution they were jointly awarded the Nobel prize in Medicine in 2005. ^[1]

H. pylori; now classified as a carcinogen, is closely associated with gastritis and peptic ulcer disease a chronic inflammatory condition associated with significant morbidity, and also malignant lesions like gastric MALToma and gastric adenocarcinomas. ^[2]

Various invasive and non-invasive tests to diagnose *H.pylori* are in practice and, upper Gastro-intestinal endoscopy or gastroduodenoscopy is a routinely performed outpatient procedure, with minimal patient morbidity or mortality. Apart from direct visualization of the lesion, biopsies can be obtained and subjected to various ancillary tests and histopathological examinations.

Once this organism is identified, curative treatment regimens can be started that can ameliorate the patient agony and can have a dramatic impact on patients disease course.

II. Aim And Objectives

- a. To study the demographic profile of patients with acid peptic symptomatology.
- b. To study the spectrum of lesions diagnosed by histopathology in specimens obtained by gastroduodenoscopic biopsies.

- c. To study the association between H.pylori infection and various upper gastrointestinal lesions.
- d. To evaluate the role of rapid urease test on biopsy specimens for the detection of H. pylori.
- e. To evaluate the role of histopathological studies for the detection of H. pylori.
- g. To evaluate the use of various special staining techniques for the rapid detection of H. pylori in biopsy specimens.

III. Material And Methods

In this prospective cross-sectional study conducted at the department of Pathology;Dr.S.C.GMC, Nanded, a total of 110 patients presenting with symptom complex of acid peptic disease were evaluated by a detailed clinical history, personal history with special emphasis on food habits, smoking and tobacco abuse, and alcoholism . Drug history for NSAIDS use and family history were noted.

After informed written consent, gastroduodenoscopy performed via flexible, fiber-optic fuginon gastroduodenoscope and endoscopic findings recorded as gastritis, gastric ulcer, duodenal ulcer, neoplastic growth and endoscopically normal study.

Gastroduodenal biopsies from in and around suspicious lesions were obtained via specialized endoscopic biopsy forceps, and wherever possible multiple bits from multiple sites were obtained.

The biopsy samples were divided and introduced into

- a. Glucose broth (transport media) for rapid urease test.
- b. 10% buffered formalin for histopathological examination and special staining.

Rapid urease test: Urease test is based on the principle of urease enzyme by H. pylori. Samples obtained by endoscopic biopsy were incubated in an urea containing media;urease breaks down urea into ammonia(NH₃) and carbon dioxide (CO₂), ammonia being strongly basic increases the pH of the media and subsequent color change of the indicator media.^[3]

Urea broth- 10gms of urea is dissolved in 80mL of distilled water and final volume up to 100mL is made, and to it is added 0.002gm of phenol red indicator and final pH adjusted to 6.4-6.8 using N HCl.

Immediately after obtaining the biopsy specimen, the samples were incubated in urea broth for 1-1^{1/2} hours at 37C. Positive test was indicated by the development of pink color in the pale yellow broth.

Histopathological study: A part of the biopsy specimen was put in 10% buffered formalin for fixation for at least 6 hours, after processing paraffin blocks prepared and 5µ sections cut and stained by

- a. Routine eosin and haematoxylin stains.
- b. Geimsa stain.
- c. Warthin starry (silver impregnation) stain.

The results and observations were organized and interpreted in light of demographic, clinical and laboratory findings.

IV. Results And Observations

A total of 110 cases presenting with acid peptic disease were evaluated by endoscopic gastric biopsies.

4.1. Age and sex distribution- The most common age group afflicted was 31-40 years with 38(34.54%) cases, followed by 21-30 years with 24(21.87%). And the mean age of presentation was 40.6 years, The male to female ratio was 1.89:1

4.2. Clinical presentation-The presenting features were myriad and the most common presenting complaint was abdominal (epigastric) pain reported in 89(80.90%) of the respondents, followed by nausea in 62(56.36%) of cases, also noted were dyspepsia in 40(36.36%), weight loss in 20(18.18%), reflux in 18(16.36%), hematemesis and decreased appetite were noted in 12(10.90%) cases each, and finally malaena in 7(6.36%).

4.3. Personal history and addictions- Personal habits and lifestyle choices have a direct relation to the evolution and final outcome of acid peptic and upper gastro-intestinal tract pathologies. Of the 110 respondents 95(86.36%) gave a history of consumption of hot spicy meals and erratic meal timings, closely followed by excessive consumption of tea and coffee given by 88(80%) of respondents. 51(46.36%) gave history of tobacco use i.e smoking and chewing, and 35 (31.81%) gave a history of alcohol abuse.

Table 1: Depicting personal habits and addictions in the cases.

Personal history	No. of cases
Hot spicy meals/erratic meal habits	95(86.36%)
Tea and coffee	88(80%)
Tobacco use	51(46.36%)
Alcohol abuse	35(31.81%)
Excessive NSAIDS use	8(7.27%)

4.4. Rapid urease test- A total of 67(60.90%) of specimens gave a positive result on rapid urease test. The average sample turnover time was 1^{1/2} hours.

4.5. Histopathological examination-All 110 biopsy specimens were studied using Eosin haematoxylin staining technique, also special stains like Geimsa and Warthin starry silver impregnation stains were also used as deemed necessary.

On routine histopathological examination the most common diagnosis was chronic gastritis observed in 35(31.81%) of cases, followed by duodenal ulcer in 23(20.90%), gastric ulcer and gastric neoplastic lesion in 14(12.72%) of cases, Duodenitis in 10(9.09%), gastric polyp in 3(2.72%) and finally no specific pathology in 11(10%) of cases.

4.6. Special stains-Geimsa and Warthin starry (silver impregnation) stains were used for the definitive identification of bacteria.

On special stains H.pylori are observed as dark staining, slightly elongated rods lying submucosally, in small clusters and discretely.

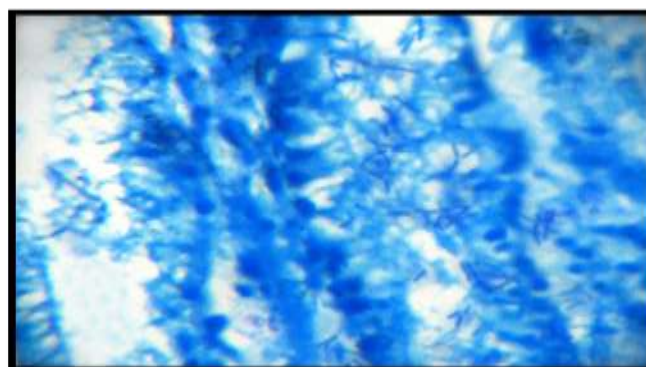


Figure1: H. pylori (blue coloured) are seen along the luminal side of gastric glands. (Giemsa stain, 10X).

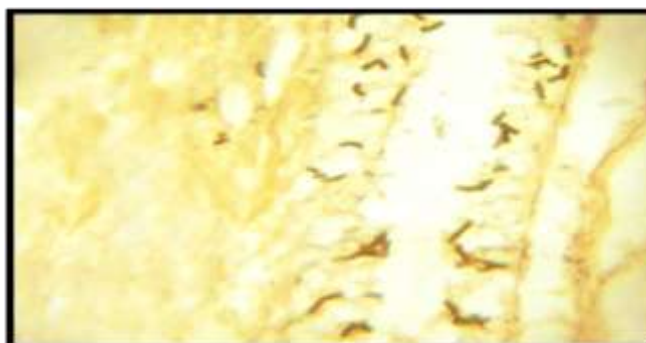


Figure 2: Spiral shaped H. Pylori are seen along the luminal side of gastric glands. (warthin- Starry silver stain, 40X)

H.pylori positivity was observed in 69 (62.72%) patient samples.

H pylori positivity on special staining technique was observed in 32(91.42%) cases of chronic gastritis, followed by 20(86.95%) of duodenal ulcer, followed by 10(71.42%) of cases of gastric ulceration, followed by 4(40%) of duodenitis., 2(18.18%) of endoscopically normal study gave a H. pylori positivity and finally 1(7.14%) of gastric adenocarcinoma gave a H. pylori positivity.

4.7. Table 2: Depicting the H.pylori positivity rates in various gastroduodenal lesions.

Histopathological diagnosis	No. of cases	Rapid urease test positivity	Histopathological examination using special stains
Chronic gastritis	35	25(71.42%)	32(91.42%)
Duodenal ulcer	23	20(86.95%)	20(86.95%)
Gastric ulcer	14	10(71.42%)	10(71.42%)
Duodenitis	10	04(40%)	04(40%)
Gastric polyp	03	00	00
Neoplastic lesion	14	01(7.14%)	01(7.14%)
No specific lesion	11	07(63.63%)	02(18.18%)
	N=110	67(60.90%)	69(62.72%)

V. Discussion

In this prospective, cross-sectional study we try to find an association between H.pylori infection and various gastroduodenal pathologies and also evaluate the efficacy of various ancillary techniques for the definitive identification of the organism.

A bewildering array of tests are now available, which includes Urease breath test, Rapid urease test, Bacterial culture, Serological tests, PCR, and Molecular techniques, but most techniques are plagued by lack of sensitivity and specificity or are limited in use by their exorbitant cost and inaccessibility.

5.1. Of the 110 patients presenting with upper gastro-intestinal complaints; abdominal (epigastric) pain was reported in 89(80.90%) of the cases, followed by nausea in 62(56.36%) of cases, also noted were dyspepsia in 40(36.36%), weight loss in 20(18.18%), reflux in 18(16.36%), hematemesis and decreased appetite were noted in 12(10.90%) cases each, and malaena in 7(6.36%), these results are similar to the findings of Tri H Lee^[4](2008), Michael G Lee^[5](2007) and Tom Richard Okello^[6](2006).

5.2. Of the 110 respondents queried about their personal habits and addictions, 95(86.36%) gave a history of consumption of hot spicy meals and erratic meal timings, closely followed was excessive consumption of tea and coffee as given by 88(80%) of respondents.

51(46.36%) respondents gave history of tobacco abuse i.e smoking and tobacco-chewing., as elaborated by Sharma B et al^[7] (2006) who found smoking habit was present in 33% of the patients presenting with dyspepsia, while Michael G. Lee^[5] (2009) found an incidence of 10% among patients presenting with dyspepsia.

In our study 35 (31.81%) gave a history of alcohol abuse, Michael G Lee^[5] (2009) found an incidence of alcohol abuse in 23% of the patients.

5.3. In the present study positivity on rapid urease test was noted in 67(60.90%) of the biopsies, and this correlated well with the test results of Sharma B et al^[7] (59%) and Mastaghni A A et al^[8](59%), but it was much lower than that reported by Sengupta et al^[9] i.e. 96%.

And the reason for this variability could be the patchy distribution of the bacilli in the submucosa and its subsequent absence on biopsy.

5.4. H.pylori positivity was observed in 69 (62.72%) patient samples.

H pylori positivity on special staining technique was observed in 32(91.42%) cases of chronic gastritis, followed by 20(86.95%) of duodenal ulcer, 10(71.42%) of cases of gastric ulceration, 4(40%) of duodenitis, 2(18.18%) of endoscopically normal study gave a H. pylori positivity and finally 1(7.14%) of gastric adenocarcinoma gave a H. pylori positivity.

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] i.e. (57%).

Demonstration of H.pylori was possible by all the three staining methods, but were particularly appreciable in Warthin starry staining and Geimsa stain, but none gave a clear advantage over the other, as similarly observed by Jhala N.et.al^[12] (2002), and Toulaymat M.et.al^[13] (2009).

Thus Histopathology can be considered as gold standard technique for the demonstration of H.pylori in the biopsy specimen.

Table 3: Comparison of Disease wise Frequency Of H. Pylori Positivity In Our Study With Other Studies

Disease	Our Study (2011-12) %	Shah Sattar Khan et al ^[14] (2008) %	Mustapha S. K et al ^[15] (2007) %	Hashemi M. R et al ^[10] (2007) %	Wyatt JI .Semin Diagn pathol ^[16] (1991)%
Gastritis	91.42	84	89.1	70.1	90
Duodenal ulcer	86.95	100	100	86.2	95
Gastric ulcer	71.42	-	61.9	71.9	70
Duodenitis	40	100	83.3	-	-
Adenocarcinoma	7.14	0	-	-	50
No pathology	18.18	0	-	33.5	-

5.5. In the present study, sensitivity of RUT was 90.5%, specificity 89.2%, positive predictive value 93.4% and negative predictive value 84.6%, these finding are in accordance with the works of Benjamin C.Y et al^[17] (1997-98) and Vandana Berry et al^[2] (2005).

VI. Conclusion

Gastritis was the most common cause of acid peptic disease in present study followed by duodenal ulcer. Acid peptic diseases commonly affect young to middle aged economically productive age group with a clear male preponderance.

H. pylori was detected in 91.42% cases of gastritis, 86.95% cases of duodenal ulcer and 71.42% cases of gastric ulcer.

H. pylori positivity was 62.72% on histopathology and 60.90% on RUT., histopathology is superior to RUT and the gold standard method to detect H. pylori in gastroduodenal biopsy specimen.

Various histopathological staining methods like Giemsa and Warthin-Starry Silver stain are helpful as ancillary-auxiliary tests for the identification of H. pylori.

Rapid urease test is a simple, inexpensive, and easy to perform procedure with comparable sensitivity and specificity as histopathology.

Acid peptic disease is a common malady causing much morbidity and loss of useful man-hours, and the culprit being H.pylori and erratic modern lifestyle being a compounding factor, early and rapid detection of this causative organism can help us initiate anti-bacterial therapy for the eradication of this bacilli and thus has major implications in patient recovery.

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