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Original Article

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A study on optimal biopsy site in patients presenting with dyspepsia for *Helicobacter pylori* by using the Sydney System of grading

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Abstract

***Background & Aims*:** *Helicobacter pylori* (H. pylori) infection, a common organic cause of dyspepsia, often lacks macroscopic mucosal lesions, necessitating distinction from functional dyspepsia. This study assessed H. pylori prevalence, optimal biopsy sites, histopathological changes, and infection density in dyspeptic patients.

Materials & Methods: Gastric mucosal biopsies from 100 dyspeptic patients were formalin-fixed, paraffin-embedded, and sectioned (5µm). Hematoxylin and eosin (H&E) and Giemsa stains were used for histopathological evaluation, with Giemsa specifically applied for H. pylori grading.

Results: Patients (mean age: 44.37 years; range: 11–80) showed peak H. pylori prevalence in the fourth (28%, n=28) and fifth (29%, n=29) decades, with a male-to-female ratio of 1.26:1. Common symptoms included nausea (76%, n=76), epigastric discomfort, abdominal pain, and bloating. Chronic gastritis was identified in 89% (n=89), with 82% (n=82) testing H. pylori-positive, all exhibiting chronic active gastritis. The pyloric antrum was the predominant colonization site (86.58%, n=71/82), followed by the fundus (84.14%, n=69/82) and body (74.39%, n=61/82). Per the Sydney System, inflammation severity was mild (42.68%, n=35), moderate (48.78%, n=40), or severe (8.54%, n=7). H. pylori density was graded as mild (36.59%, n=30), moderate (54.88%, n=45), or dense (8.53%, n=7).

Conclusion: Early H. pylori detection and eradication alleviate symptoms and prevent complications. Giemsa stain proved optimal for H. pylori identification due to its cost-effectiveness and rapidity. The pyloric antrum, followed by the fundus and body, is the primary biopsy site for diagnosing H. pylori-associated gastritis. These findings emphasize targeted biopsy protocols and efficient diagnostic methods in managing dyspepsia.

***Keywords*:** Dyspepsia, Gastritis, Haematoxylin and Eosin, *H.pylori*, Sydney System

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Introduction

Dyspepsia is a set of upper gastrointestinal symptoms that primarily affects the adult population. The global prevalence of uninvestigated dyspepsia ranges from 7 to 45%, while functional dyspepsia ranges from 11 to 29.2% (1). Several populational studies conducted in Asia have identified a prevalence of undiagnosed dyspepsia ranging from 8% to 30% and of

functional dyspepsia ranging from 8% to 23% (2). Dyspepsia may have organic origins, but the majority of patients present with non-ulcer or functional dyspepsia, which accounts for approximately 60% of cases (1).

Helicobacter pylori (*H.pylori),* an infectious bacterium, colonizes the gastric antrum of nearly half of the world's population. The discoveries by Marshall and Warren, including the first robust culture of *H. pylori* in 1982, marked a new era in medicine. Their finding that *H. pylori* was associated with an increased likelihood of developing gastric cancer served to reinforce the hypothesis positing the role of *H. pylori* as a human pathogen (3). In 1982, J. Robin Warren and Barry J. Marshall made a seminal discovery when they identified *H.pylori* as the primary causative agent of gastric and duodenal ulcers, gastric cancer, and gastric B-cell lymphoma of mucosa-associated lymphoid tissue, earning them the Nobel Prize in Medicine and Physiology in 2005 (4). *H. pylori* is primarily transmitted through fecal-oral and oral-oral routes, reflecting its widespread dissemination in human populations (5,6).

There is a correlation between *H.pylori* infection and the development of acute and chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (7). In 1994, the World Health Organization (WHO) classified *H. pylori* infection as a confirmed cause of human gastric cancer (8).

H. pylori detection methods are classified as invasive or noninvasive. Noninvasive tests include serology, stool antigen testing, and carbon-labeled urea breath testing, while invasive options include the rapid urease test, endoscopic biopsy, polymerase chain reaction, and culture (9). Histopathology is highly valued for *H. pylori* detection due to its ability to provide detailed histological evaluation, complementing other accurate diagnostic methods (10). Detection stains for *H. pylori* include Giemsa, Diff-Quik, Wright Giemsa, Toluidine blue-Alcian blue, Sayeed, Giemnez, Genta, Cresyl violet, Steiner, Warthin-Starry, and immunohistochemistry (*IHC*) (11).

Chronic gastritis is associated with intestinal-type metaplasia of the gastric mucosa, a precancerous lesion with adverse outcomes. Metaplasia is prevalent in both *H. pylori*-infected patients and heavy smokers (12). Chronic atrophic gastritis is linked to a fivefold increased risk of gastric cancer (13).

This study's objectives were evaluating the prevalence of *H. pylori* infection in dyspeptic patients, assessing the optimal biopsy site for *H. pylori* detection, addressing ongoing debates about site-specific diagnostic yield, studying the histopathological changes in the stomach due to *H. pylori* infection, as well as grading and correlating the density of *H. pylori* infection according to the Sydney grading system, providing local data to refine its clinical application.

Materials & Methods

This investigation was conducted prospectively in the Department of Pathology on 100 endoscopic stomach biopsy specimens from dyspeptic patients, obtained from the Medical Gastroenterology Department of Narayana Medical College, Nellore. The study spanned from September 2018 to September 2020. All endoscopic gastric mucosal biopsies from dyspeptic patients with normal-appearing gastric mucosa and a positive rapid urease test were considered as the inclusion criteria and forwarded to the Department of Pathology.

The exclusion criteria involved gastric biopsies from patients diagnosed with tumor or tumor-like lesions within the stomach, gastric mucosal biopsies with a negative rapid urease test, biopsies with inadequate sampling or from patients currently undergoing or recently completed *H.pylori* eradication therapy, biopsies from patients with recent use of proton pump inhibitors (PPIs) or antibiotics within 4 weeks before endoscopy, as these may interfere with *H. pylori* detection.

Processing of Sample

One hundred endoscopic stomach mucosal samples were labeled, preserved in 10% aqueous formalin solution, and processed for tissue analysis. The processed tissue bits were embedded in paraffin, and 5-

micron-thin sections were prepared using a LEICA microtome. The sections were stained with Haematoxylin and Eosin (H&E) and examined under a microscope to assess histological alterations in the gastric mucosa. Additionally, *Giemsa* staining was performed on all sections to identify *H.pylori*. Grading was conducted by experienced pathologists, evaluating specific parameters including inflammation, activity (neutrophilic infiltration), atrophy, intestinal metaplasia, and *H. pylori* density. For both H&E and Giemsa stains, the Sydney System was applied, with each parameter graded on a scale of 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe). Inflammation, activity, atrophy, and metaplasia were primarily assessed on H&E-stained sections, while *H. pylori* density was specifically graded on Giemsa-stained sections for enhanced visualization of the bacteria.

Results

The patients' ages ranged from 16 to 80 years, with an average of 44.37 ± 5.27 years. The majority of patients (n = 29, 29%) were between the ages of 41 and 50. The age group between 16 and 20 years had the fewest cases (2%).

In this study, the sex ratio (M:F) was 1.38:1, with 58 (58%) male and 42 (42%) female patients.

Chief Complaints

The most frequent principal complaint in the current investigation was nausea, which accounted for 76% of cases individually (n = 76), followed by epigastric discomfort (n = 37, 37%). The least prevalent primary complaint in this study was abdominal distention (n = 10.10%).

Evidence of Gastritis

The majority of the cases had gastritis on histology (n = 89, 89%). Out of 89 individuals, 77 (86.52%) showed mild inflammation, whereas 12 (13.48%) had significant inflammation. Eleven cases (11%) showed no indication of gastritis.

Nature of the Inflammation

The majority of cases had chronic inflammatory cell infiltrates in histopathological sections (n = 72, 80.90%), followed by mixed inflammation in 17 individuals (19.10%).

Table 1. Topography of histopathologically diagnosed cases of gastritis

Topography of gastritis	Number of patients (n = 89)	Percentage (%)
Fundus	79	88.76
Body	77	86.52
Greater curvature	71	79.77
Lesser curvature	69	77.53
Antrum	81	91.01

Histopathological study revealed that the topography in the majority of gastritis cases was in the incisura (n = 81, 91.01%). The fundus and body were the next most common, accounting for 88.76% (n = 79) and 86.52% (n = 77), respectively (Table 1).

Table 2. Presence and grading of neutrophilic activity in patients with gastritis

Neutrophilic activity	Number of patients (n = 89)	Percentage
Absent	72	80.90
Present	17	19.10
Mild	15	88.24
Moderate	2	11.76
Severe	0	0

Neutrophilic activity was seen in 19% of the patients (n = 17). In 15 of these 17 cases (88.24%), mild activity was seen, whereas only two individuals (11.76%) showed moderate activity (Table 2). Lymphoid follicles were seen in three (3.37%) of the 89 cases with gastritis. Among the 89 cases with gastritis, mild mucosal atrophy was observed in one case (1.12%) and three cases (3.37%) showed intestinal metaplasia. All three cases (3.37%) had mild intestinal metaplasia.

Table 3. Presence and grading of *H.pylori* in patients with gastritis

H.pylori	Number of patients (n = 89)	Percentage
Absent	7	7.86
Present	82	92.13
Mild (1+)	50	60.97
Moderate (2+)	25	30.49
Severe (3+)	7	8.54

Of the 89 cases of gastritis, 82 (92.13%) included *H.pylori*. *H.pylori* density was determined to be 1+ in 50 cases (60.97%), 2+ in 25 cases (30.49%), and 3+ in 7 cases (8.54%) (Table 3).

Table 4. Incidence of *H.pylori* positive cases

H.pylori	Number of patients (n = 100)	Percentage (%)
Positive	82	82.0
Negative	18	18.0
Total	100	100

Standard histologic investigation and detection of *H. pylori* was performed on a total of 100 endoscopically guided biopsies from patients with dyspepsia using the specific stain Giemsa. Of these, 82 patients (82.0%) tested positive for *H. pylori* (Table 4).

H.pylori infection of the stomach was more common in males 48 (48.0%) than females 34 (34.0%), with a male to female ratio of 1.41:1. The age range of all 82 cases was 16 to 80 years, with a mean age of 44.3 years; the peak incidence was noted in 31-40 years, followed by 41-50 years. Gastritis tends to increase as one ages.

Table 5. Topography of histopathologically diagnosed cases of *H.pylori* gastritis

Topography of *H. pylori* gastritis	Number of patients (n = 82)	Percentage (%)
Fundus	69	84.15
Body	61	74.39
Greater curvature	58	70.73
Lesser curvature	56	68.29
Antrum	72	87.80

Among 82 cases *with H.pylori* gastritis examined histopathologically, the majority had topography in the pyloric antrum (n = 72, 87.80%). The fundus and body were the next most common, accounting for 84.15% (n = 69) and 74.39% (n = 61), respectively (Table 5). We noticed that the occurrence of *H.pylori* was more usually connected with the kind of inflammation ($P = < 0.001$), pyloric antral location ($P = 0.002$), and fundus ($P = 0.007$).

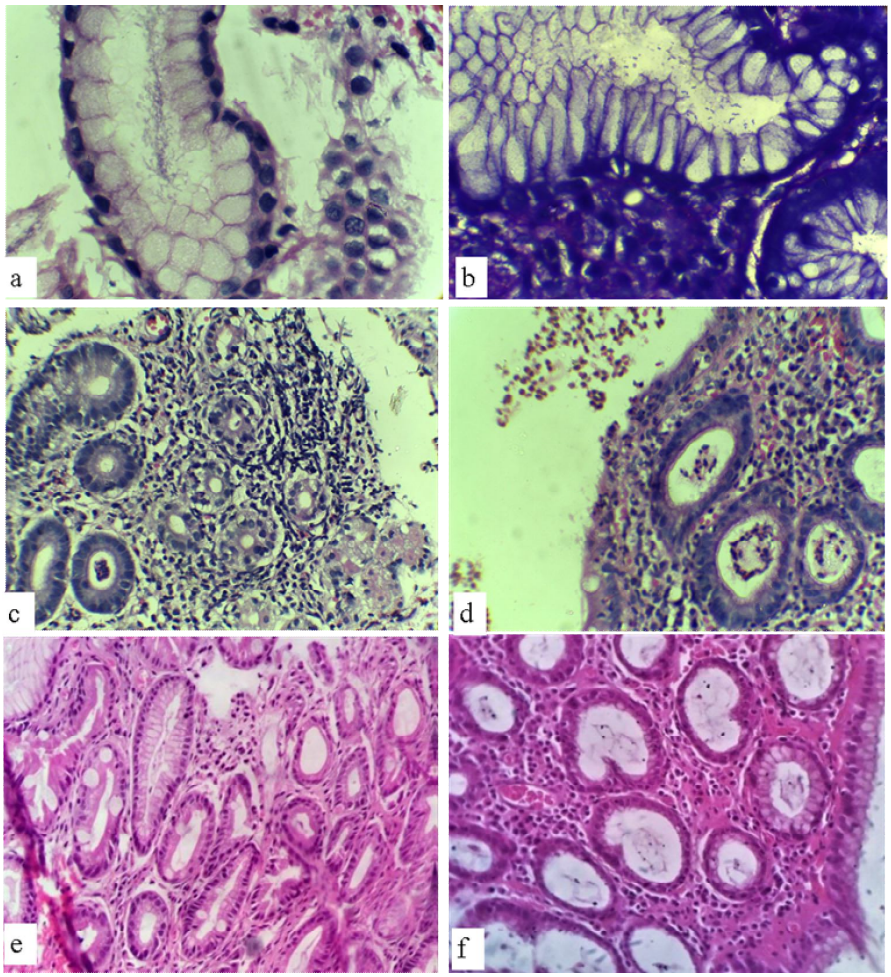

Fig. 1. a. H. Pylori 3 + (Grade III) (H & E stain - Oil immersion), **b.** H. pylori 2+ (Grade II) (Geimsa stain - Oil immersion), **c.** Lymphoid aggregates (H & E; High power), **d.** Crypt abscess (H & E; High power), **e.** Mild intestinal metaplasia (H & E; High power), **f.** Mild glandular atrophy (H & E)

Table 6. Association of various histopathological parameters according to the incidence of *H. pylori*

Histopathological parameters	***H.pylori***				***P-value***
	Absent (n = 18)		**Present (n = 82)**		
	Number	**%**	**Number**	**%**	
Nature of inflammation					< 0.0001
Absent	11	61.11	0	0	
Chronic	5	27.78	72	87.8	
Mixed (acute + chronic)	2	11.11	15	18.29	
Topography of gastritis					< 0.0001

Histopathological parameters	*H.pylori*				*P-value*
	Absent (n = 18)		Present (n = 82)		
	Number	%	Number	%	
Absent	11	61.11	0	0	
Present					
Fundus	-	-	69	84.15	0.007
Body	-	-	61	74.39	0.186
Greater curvature	-	-	58	70.73	0.899
Lesser curvature	-	-	56	68.29	0.744
Antrum	-	-	72	87.80	0.002
Neutrophilic activity					0.463
Absent	16	88.89	67	81.71	
Present	2	11.11	15	18.29	
Mucosal atrophy					0.638
Absent	18	100	81	98.78	
Present	0	0	1	1.22	
Intestinal metaplasia					0.41
Absent	18	100	79	96.34	
Present	0	0	3	3.66	
Lymphoid aggregates					0.41
Absent	18	100	79	96.34	
Present	0	0	3	3.66	

Discussion

Microscopic analysis of gastric biopsy material reveals, in addition to *H.pylori* status, the grade, extent, and topography of gastritis, as well as atrophy-related abnormalities in the stomach mucosa. Endoscopic biopsy remains a key approach for diagnosing various gastrointestinal lesions. The current investigation identified 82 positive cases of *H.pylori* out of 100 stomach biopsies (82%), a prevalence comparable to that reported by Dube et al. (14).

Patients' ages ranged from 16 to 80 years (mean age: 44.37 ± 5.27 years), with the highest occurrence in the fourth decade (26.83%), consistent with findings by Adisa et al. (15).

The most prevalent primary complaints in our study were nausea (76%) and epigastric discomfort (37%). Kumar et al. reported similar findings, with epigastric discomfort as the most common presenting symptom.

Chronic Inflammation with Grades of Gastritis

An increase in chronic inflammatory cells in the gastric mucosa is the primary histologic hallmark of chronic gastritis.

In all 89 (89%) mucosal biopsies examined, chronic inflammatory infiltration was observed to varying degrees. Tanko et al. (16) and Sarfraz et al. (17) reported moderate mononuclear infiltration as the most common grade, whereas the current study found mild chronic inflammation to be predominant.

H.pylori and Neutrophilic Activity

Neutrophilic activity, defined as the presence of neutrophils within the gastric mucosa indicating active inflammation, was observed in 17 (19.10%) biopsies. Of these, 15 (88.23%) were graded as mild according to the Sydney System (0 = none, 1 = mild, 2 = moderate, 3 = marked), with two (11.77%) graded as moderate. This contrasts with studies by Udoh et al. (18), Bhosale et al. (11), and Palaniappan (19), which reported a higher incidence of neutrophilic activity.

H.pylori and Intestinal Metaplasia

Intestinal metaplasia, characterized by small intestinal-like glands containing goblet cells, is a risk factor for intestinal-type gastric cancer. It was observed in 3.3% of cases in the current study.

H.pylori and Glandular Atrophy

Histopathologically, inflammation extending into deeper mucosal layers can lead to glandular destruction and atrophy. Oksanen et al. (20) and Kuipers et al. (21) reported atrophic gastritis in 31 (40.2%) and 16 (28%) of *H.pylori*-infected individuals, respectively. In contrast, the current study found a rate of one (1.12%), aligning with Rugge et al. (22), who reported a low incidence of four (5.6%).

Incidence of *H.pylori*

Our investigation found that 82% of dyspeptic patients tested positive for *H. pylori*. The highest proportion of *H. pylori*-positive patients was in the fourth to fifth decades (26.83%), with the lowest in the second decade (2.44%).

H.pylori Density Grading and Correlation

Using the Sydney System, *H. pylori* density was graded in 82 positive cases as mild in 30 (36.59%), moderate in 45 (54.88%), and marked in 7 (8.53%). Inflammation grades were mild in 35 (42.68%), moderate in 40 (48.78%), and marked in 7 (8.54%). No strong correlation was observed between *H. pylori* density and inflammation or activity grades, suggesting that bacterial load may not directly dictate the severity of inflammatory response in this cohort, possibly due to host factors or regional differences in pathogenicity.

Histopathology in Gastritis

Comparison with other studies, such as Mysorekar et al. (23) and Ahluwalia et al. (24), confirms that mild chronic inflammation predominates in our study. Rates of intestinal metaplasia and glandular atrophy were 3.37% and 1.12%, respectively.

Conclusion

H.pylori infection is associated with chronic gastritis, gastric ulcers, and gastric cancer. Early detection and eradication of *H. pylori* can relieve symptoms and reduce associated complications. Study findings indicate that the pyloric antrum is the most prevalent biopsy site for chronic gastritis and *H. pylori* colonization, followed by the fundus and body. The Sydney System grading revealed a predominance of mild to moderate *H. pylori* density and inflammation, with no significant correlation between bacterial density and inflammation severity.

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Ethical statement

The patient's initials and personal information were kept confidential. Authors followed the guidelines outlined by the Helsinki Declaration for the conduct of Research. The study protocol was approved by the institutional ethics committee, Narayana Medical College, Nellore, A.P.

Data availability

The data of this study are available upon request from the corresponding author.

Conflict of interest

The authors declare no conflict of interest.

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None declared.

Author contributions

Gopidesi Divya Tejaswi was responsible for writing the paper. K Durga contributed to the conception and design of the analysis. Gopidesi Divya Tejaswi, Recharla Madhuri, Gopidesi Divya Tejaswi, and

Jonnadula Pratima were involved in data collection. K Durga, Nandam Mohan Rao, and Vaheda Begam handled the analysis and provided supervision.

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