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"ULCERATIVE LESIONS OF THE STOMACH AND ITS ASSOCIATION WITH HELICOBACTER PYLORI – HISTOPATHOLOGICAL AND BIOCHEMICAL ASSESSMENT OF ENDOSCOPIC BIOPSIES"

Pathology

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ABSTRACT

Background and Objectives: Diseases of the gastrointestinal tract are the major causes of morbidity and mortality. Endoscopic biopsy is an integrated investigation of GI lesions. The present study was undertaken to find the association of H. pylori with ulcerative lesions of the stomach, H. pylori detection by rapid urease test and reconfirmation by histopathology, and to analyze its relationship with the duration of symptoms. **Methods:** It is a prospective study, from June 2012 – Jan 2015 carried out in the Department of Pathology in collaboration with the Department of Surgery at SDM College of Medical Sciences and Hospital, Dharwad. All endoscopic gastric mucosal biopsy specimens, with a diagnosis of gastric ulcer were included. **Results:** A total of 103 were studied. Non-neoplastic lesions (55.3%) outnumbered the neoplastic lesions (44.6%). Chronic non-specific gastritis (29.8%) was the most common non-neoplastic lesion and well-differentiated adenocarcinoma (39.6%) was the most common neoplastic lesion. H. Pylori bacilli was detected histologically in 17 specimens. 16 were reconfirmed by rapid urease test and 1 was false negative. In H. pylori-positive biopsy specimens, the most common finding was chronic gastritis (47.1%). H. pylori was found in 1 case of diffuse infiltrating adenocarcinoma. 7 of 17 H. Pylori-positive cases (41.1%) had symptoms duration of less than 6 months **Conclusion:** Non- neoplastic lesions are more common than neoplastic lesions. Rapid Urease Test is a simple economical and quick test in identifying H. Pylori. Association between H Pylori and malignancy is low in this part of the country and most H Pylori confirmed patients had less than 6 months' duration of symptoms.

KEYWORDS

GI lesions, endoscopy, H pylori, Ulcerative stomach lesions, Rapid urease test

INTRODUCTION

The discovery of Helicobacter pylori in 1982 by Marshall and Warren was the starting point of a revolution in the concepts and management of Acid peptic diseases.[1] Helicobacter pylori, in a very short period, has become one of the most important etiological agents causing gastrointestinal infections like duodenal ulcer diseases, chronic antral gastritis, gastric ulcer diseases, and, possibly non-ulcer dyspepsia.[2] Helicobacter was named as a "definite biological carcinogen" by the World Health Organization in 1994.[3] This bacterium is Gram-negative, motile, and microaerophilic and can withstand the acidity of the stomach for survival. H. pylori infection is the major cause of chronic gastritis and is important in the pathogenesis of duodenal and gastric ulcers, atrophic gastritis, MALT lymphoma, and distal gastric adenocarcinoma. The previous operation theatre lists which contained Gastro Jejunostomy and Vagotomy are replaced by endoscopic biopsy in the current scenario. Endoscopic biopsies with the invention of Helicobacter pylori (H. pylori) infection have almost replaced the old theory of hurry, worry, and curry for peptic ulcer disease. Treatment for Acid Peptic disease by H. pylori kit has almost eradicated it. H. Pylori organism which is a preventable cause for cancer.[4] The prevalence of H. Pylori varies in different geographic areas, being higher in developing countries and lower in developed. A higher incidence is seen in low socioeconomic status, poor hygienic conditions and crowded living conditions in childhood in developing countries. The prevalence of H. pylori infection in the Indian population ranges from 31 to 84% or more in rural areas and the prevalence among children and adults is 87% and 88% respectively. More than 20 million Indians are estimated to suffer from peptic ulcer disease, which is the most commonly recognized manifestation of H. pylori infection in India.[5]

Several techniques have been developed to diagnose H. pylori infection. Endoscopic diagnostic tests include histopathological examination of gastric mucosa, culture of gastric mucosal biopsy cells, and biopsy urease test. The present study was undertaken to document the association of H. Pylori in benign and malignant ulcerative lesions of the stomach.

Objectives

- To study the association of H. pylori with ulcerative lesions of the stomach which are due to benign and malignant causes.
- Detection of H. pylori by performing rapid urease test and reconfirmation by Hematoxylin and Eosin and Giemsa stain.
- To analyze the relationship between the duration of symptoms and the presence of H. pylori infection.

MATERIAL AND METHODS

It is a prospective study, from June 2012 – Jan 2015 which was carried out in our Department of Pathology in collaboration with the Department of Surgery at SDM College of Medical Sciences and Hospital, Dharwad. A detailed clinical history and complete clinical examination were done as per the proforma. Informed consent was obtained from all the patients who were undergoing endoscopic biopsies. All endoscopic gastric mucosal biopsy samples obtained from patients with ulcerative lesions in the stomach, during the study period, were included.

A total of 103 patients who had ulcerative lesions on endoscopy were included in this study. Patients who have undergone gastric surgery in the past, patients with a recent history of H. Pylori eradication therapy, Patients not fit to undergo endoscopy and biopsy, patients with a history suggestive of sub-acute intestinal obstruction, and patients with associated duodenal or pancreatic tumors were excluded from this study. The patients were subjected to the following procedures-

Collection Of Biopsy Samples:

Before the procedure, the patient was told to fast for at least 6 hours. The patient was asked to lie on a trolley on his/her left side. A local anesthetic liquid such as xylocaine was given half an hour before the procedure. After that, a mouth guard was placed between the patient's teeth to protect the endoscope from damaging and biting.

Followed by the introduction of an endoscope under visual control by the surgeon. A gastric cavity was seen for signs of gastritis. Into the operating channel of the endoscope, standard biopsy forceps were introduced and pushed from the outside to the level of the gastric cavity.

The forceps were then opened and pushed against the gastric wall.

Once it was firm against the mucosa, the forceps were closed and then pulled back into the endoscope taking a small piece of mucosa. The forceps were then removed from the endoscope and again opened. The small piece of mucosa (1-4mm in diameter) was then removed from the forceps. Antral biopsies were taken from the prepyloric region 10-20 mm in front of the pylorus, preferably from different sites (as H. Pylori can be patchy in distribution) From the body, samples were taken in the same manner from the greater curvature and lesser curvature[6].

Several biopsies taken for this study were a total of five samples per patient: One for rapid urease from antrum along the greater curvature.

Two for histopathology from the anterior and posterior wall of the antrum, 2 cm from the pylori duodenal junction. The other two histopathology, from the anterior and posterior walls of the mid-body.

Processing Of Biopsy Samples:

Rapid Urease Test:

The biopsy specimen taken from the antrum was placed immediately in the Eppendorf tube containing 0.5 ml urease test reagent (yellow colored) and sealed with the cap. Then the tubes were kept in an incubator at 37^0c to improve the sensitivity.

The color change was observed at 0,3,6,12 and 24-hour intervals. The development of red color (from yellow) was considered as a positive test indicating the presence of H. Pylori

Histopathology:

Biopsies were transported in Bouins solution. Then tissue was fixed immediately in 10% formalin to prevent tissue shrinkage. After fixation of the biopsy specimen, it was wrapped in a piece of filter paper and processed in a perforated capsule.

Routine processing was done. It was then unwrapped and embedded in paraffin wax with the mucosal surface facing the cutting edge of the knife. 4 mm thick sections were cut perpendicular to this surface. Serial four to five sections were taken on each slide. A minimum of two slides were prepared and multiple sections were studied.

Staining:

Sections of the antrum and body on the first slide were stained with routine Harris Hematoxylin and Eosin stain. The second slide was stained using the Giemsa technique for demonstration of H. pylori

Histopathological evaluation of stained sections was studied for H. pylori-associated gastritis. Without knowing the Rapid Urease Test result, two observers graded independently. No inter-observer variation occurred in the severity of gastritis.

(1) The histological basis of grading of chronic gastritis was followed as per the criteria –

Grade 1- Presence of few mononuclear inflammatory cells (mainly lymphoplasmacytic) in the lamina propria.

Grade 2 – Presence of a fair number of mononuclear cells with or without polymorphonuclear infiltration but without *Cryptitis.

Grade 3 – Presence of heavy mononuclear cells along with polymorphonuclear infiltration in the lamina propria with *Cryptitis.

*Cryptitis – If inflammatory cells are found infiltrating the glandular lining epithelia.

Grade 4 – Presence of lymphoid follicles in the lamina propria with or without (follicular) atrophic/metaplastic changes.

RESULTS:

Table 1: Age-wise distribution of gastric lesions

Age	Number of cases		Total
	Male	Female	
0-10	0	0	0
11-20	0	0	0
21-30	3	4	7
31-40	8	5	13
41-50	16	7	23
51-60	12	7	19
Above 60	37	4	41
Total	**76(73%)**	**27(26%)**	**103(100%)**

Out of 103 patients, 41 were more than 60 years of age group

Table 2: Age and sex incidence in H. Pylori positive cases:

Age in year	No. of Cases		Total
	Male	Female	
0-10	0	0	0
11-20	0	0	0
21-30	1	1	2
31-40	1	2	3
41-50	2	0	2
51-60	4	1	5
60 & above	2	3	5
Total	**10**	**7**	**17**

In total 17 H. Pylori-positive cases, 10 were male and 7 were female.

Table 3: Distribution of lesions in males and females

Lesions	Males	Females	Total
Nonneoplastic	40	17	57
Neoplastic	39	07	46
Total	**79**	**24**	**103**

Out of 103 cases, 46 cases were Neoplastic and 57 cases were Nonneoplastic.

Table 4: Distribution of non-neoplastic lesions

LESIONS	NUMBER	%
Peptic ulcer	13	22.8%
Chronic nonspecific Gastritis	17	29.8%
H. Pylori induced acute Gastritis	06	10.5%
H. Pylori induced chronic Gastritis	8	14.03%
H. Pylori induced atrophic Gastritis	1	1.7%
Hyperplastic polyp	7	12.29%
Atrophic gastritis	3	5.27%
Others	2	3.51%
Total	**57**	**100%**

Out of 57 non-neoplastic lesions, 17 (29.8%) were chronic non-specific gastritis,

Table 5: Distribution of neoplastic lesions

Lesions	Number	Percentage
Adenomatous polyp	2	3.7%
Infiltrating adenocarcinoma	7	13%
Intramucosal Adenocarcinoma	1	1.8%
Mucinous adenocarcinoma	2	3.77%
Non hodgkins lymphoma	1	1.8%
Poorly differentiated Adenocarcinoma	3	5.6%
Moderately differentiated Adenocarcinoma	6	11.3%
Well differentiated Adenocarcinoma	21	39.6%
Signet ring cell carcinoma	10	18.8%
Total	53	100%

In the 17 H. pylori positive cases, 10% cases were males and 6.7% of cases were females with a M: F ratio = 3:1 Commonest age incidence was between 51-60 and above 60 years.

Severity of chronic Gastritis – Severity was assessed by grading of chronic gastritis as mentioned by Choudhary et.al[2]. According to them, chronic gastritis was graded as Grade I, II, III, IV.

Table 6: Severity Of Chronic Gastritis

Grades	No. of Cases	Percentage
I	04	16%
II	12	48%
III	06	24%
IV	03	12%
Total	25	100%

Out of 25 cases of chronic gastritis, maximum were Grade-II (48%) followed by Grade-III (24%) gastritis.

Table 7: Duration Of Symptoms With Detection Of H. Pylori

Duration of symptoms	Number	Percentage
6months	7	41.18%
6 months – 1 year	4	23.52%
1 year and above	6	35.29%

Total	17	100

Out of 17, H. Pylori positive cases, 35.2% have 1 year and above duration of symptoms.

Table 8: Comparative Results Of Helicobacter Pylori By Rapid Urease Test (rut) And Histopathology

No. of cases	H. Pylori	
	RUT	Histopathology
16	+VE	+VE
01	-VE	+VE

Out of 17 H. Pylori positive cases, 16 were positive by both RUT and histopathology. But 1was RUT negative but positive for H. Pylori by histopathology.

Table 9: Rapid Urease Test Results – H. Pylori Positivity By Rapid Urease Test –

Test	H. Pylori		Total
	Present	Absent	
RUT + Ve	16	01	17
RUT – Ve	01	85	86

The specificity and sensitivity of the Rapid Urease Test is as follows- Sensitivity – 94.12%, Specificity – 98.8%

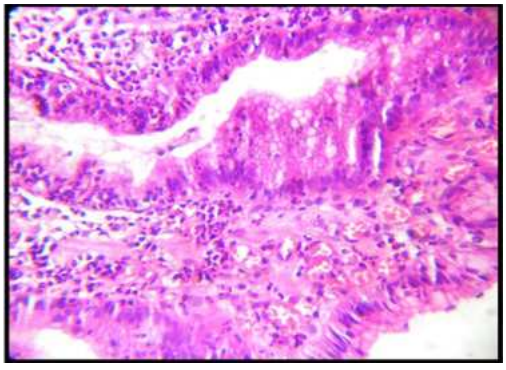

Figure 8: H&E,10x- Grade 2 Chronic gastritis, Presence of lymphocytes and neutrophils in lamina propria and intraepithelium

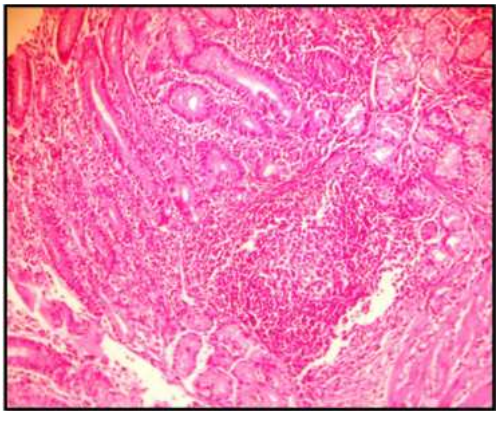

Figure 9: H&E,10x- Grade 4 Chronic gastritis, Presence of lymphoid follicles in lamina propria.

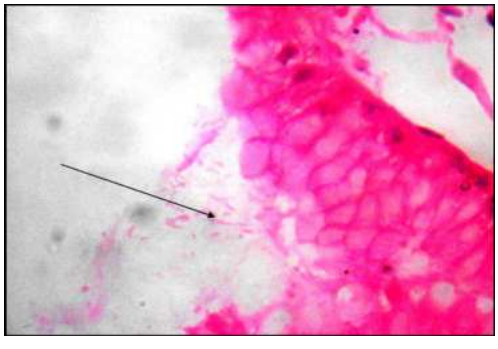

Figure 10a: H & E,40x -H. Pylori gastritis (Curvilinear bacilli)

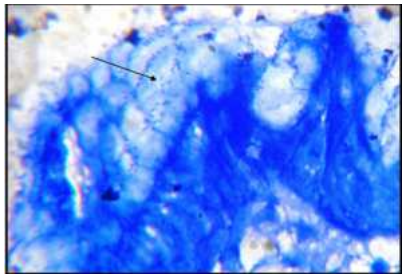

Figure 10b: Giemsa – H. Pylori

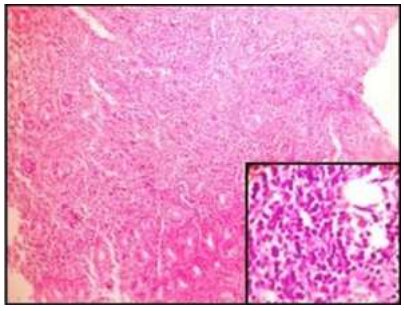

Figure 11: 10x, H&E- Non-Hodgkins Lymphoma.Inset,40x, H&E- lymphoid cells, round to oval hyperchromatic nucleus with scant cytoplasm.

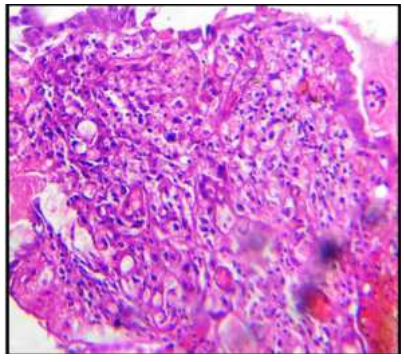

Figure 12a: H&E,10x, Diffuse infiltrating adenocarcinoma

Figure 12b: H&E,40x, H. Pylori in diffuse infiltrating adenocarcinoma.

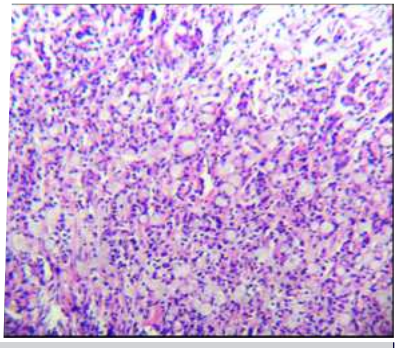

Figure 13a: H&E,10x, Signet ring cell carcinoma

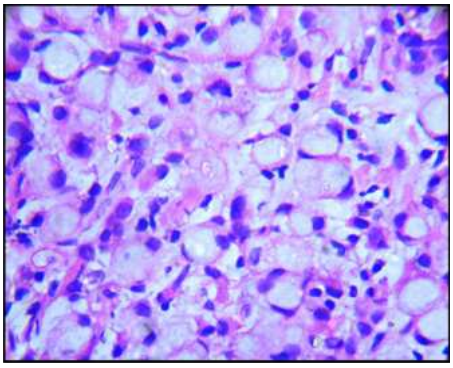

Figure 13b: H&E,40x, Signet ring cell carcinoma

DISCUSSION

The advent of fiberoptic endoscopy with biopsy facilities has helped us to obtain the biopsy specimens under direct vision from the gastric mucosa. At present endoscopy and biopsy of the stomach has created new opportunities for the study of various stomach lesions and in particular H. pylori associated lesions. Warren and Marshall described the strong association between H. Pylori and chronic gastritis in 1983.[7] The present study was designed to know the association of H. pylori with ulcerative lesions of stomach which are due to either benign or malignant causes, reconfirmation of presence of H. pylori by doing rapid urease test and to analyze the relationship between the duration of symptoms and the presence of H. pylori infection.

Age And Sex Incidence:

In our present study, a total of 103 cases with ulcerative lesions in the stomach were collected from June 2012 to January 2015. Out of the total 103 cases, males were 79(76.69%) and females were 24(23.31%). Male to female ratio was 3:1. Mean age in males was 57.3 years and in females was 45.7 years. 37 out of 79 males (46.83%) were more than 60 years old and 14 out of 24 females (58.33%) were in the age group of 40-60 years. This finding is similar to the study done by Lawa OO et al which showed the age group incidence for gastric lesions was between 41-59 years.[8]

Histological study

Out of the total 103 endoscopic biopsies, 46 were neoplastic (44.66%) and 57 were non-neoplastic (55.33%). A similar study done by Krishnappa R et al showed 19 out of 60 cases of endoscopic biopsies of the stomach were neoplastic (31.67%) and 41 out of 60 were non-neoplastic (68.33%)[9]. A study done by Venugopal LS et al showed 11 out of 47 biopsies from the stomach were neoplastic (23.4%) and 36 out of 47 cases were non-neoplastic (76.59%).[10] The incidence of neoplastic lesions was higher in males as compared to females. Out of the 46 neoplastic lesions, 39 were in males (84.79%) and 7 were in females (15.21%) Among the neoplastic lesions well-differentiated adenocarcinoma was the commonest (39.6%), followed by signet ring cell carcinoma (18.8%). The results concur with the study done by Venugopal LS et al.[10] non-neoplastic lesions were seen in 57 cases. 40 were males (70.1%) and 17 were females (29.9%). Among the non-neoplastic lesions, the most common entity was chronic nonspecific gastritis (29.8%) followed by peptic ulcer (22.8%). In the study done by Lawal OO et al most common non-neoplastic lesion in the stomach was chronic gastritis.[8]

H. Pylori and its association with ulcerative lesions of the stomach:

In our study out of a total of 103 endoscopic biopsies 17 were H. pylori positive (16.5%). H. Pylori was detected in 10 male patients (58.83%) and seven female patients (41.17%).In a similar study done by Dandin A et al 48 out of 100 cases(48%) were positive for H. Pylori .[11] Maximum cases with H. Pylori-associated gastritis were seen in the age group above 50 years. 10 out of 17 H pylori confirmed cases were above 50 years (58.9%). Study done by Wyat JI et al shows H. Pylori associated gastritis was present in elderly patients and the prevalence of H. Pylori in adult populations increases with age, in parallel with that of chronic gastritis.[12] Of the 17 H. pylori histologically confirmed cases 6 were acute gastritis (35.29%), 8 were chronic gastritis (47.1%), 1 was atrophic gastritis (5.88%), one was hyperplastic polyp (5.88%), And 1 case was diffuse infiltrating adenocarcinoma (5.88%). These findings were similar to the study done by Dandin A et al which showed the commonest lesion with H. Pylori was chronic gastritis.[11] In Our study only one patient with H pylori infection had gastric adenocarcinoma. This is explained by the Asian enigma as reported by the study done by Singh K et al. The incidence of Gastric Carcinoma with H. Pylori is lowest in developing countries despite the high frequency of H. Pylori infection. As in countries like Japan and China where the occurrence of H. Pylori is lowest, the gastric cancer risk is the highest in the world. This is in contrast to some of the simplified models of gastric carcinogenesis resulting from H. Pylori infection which state that if the infection is acquired at an early age, particularly in the presence of malnutrition, it may lead to reduced gastric acid secretion, leading to pangastritis and ultimately gastric cancer. In contrast, infection acquired in a person with good nutritional status, in middle age, and with normal gastric acid secretion would lead to hyperchlorhydria and duodenal ulcer disease. Studies from Japan and China showed that virulence factors of H. pylori are strongly associated with gastric carcinoma. Based on the available evidence, one cannot conclude that in Asian countries, despite the high frequency of H. pylori infection, the low frequency of gastric cancer is related to infection with non-pathogenic strains. Differences in carcinogenic risk in people living in different geographical areas might be related to variations in genetic makeup among different races. Specific allelic variation of different genes (polymorphism) present in a proportion of the general population may determine variation in carcinogenic potential in different populations in response to environmental carcinogenic exposure, including that to H. Pylori infection. Genetic susceptibility of a person may be important in several carcinogenic processes that include: (1) mucosal protection against H. pylori infection and injury by other carcinogens; (2) mucosal inflammatory response to infection with H. pylori; (3) degree of apoptotic cell death [68]; (4) carcinogen activation and detoxification by various enzyme systems of the hosts;(5) variability in the repair of mutated DNA; and (6) ability of the cell to proliferate in a controlled manner to repair the damage.

Rapid Urease Test Confirmation:

Out of 17 confirmed H. Pylori cases 16 were reconfirmed by rapid urease test and 1 was negative for rapid urease test. As per our study sensitivity and specificity of the rapid urease test for the detection of H. Pylori is 94.12% and 98.8% respectively. These findings are similar to the earlier study done by Dandin A et al which showed Specificity – 94.23 % and sensitivity – 89.58%.[11]

Duration of the symptoms with H. pylori:

The natural history of H. pylori-associated gastric ulcer has been well studied. Without specific antibiotic treatment to eradicate the organism 85% will have endoscopically visible recurrence in a year and half of these will be symptomatic. With successful antibiotic therapy, the recurrence rate is 5-20% at 1 year. Most of these recurrences are due to NSAID use or reinfection with H. Pylori. In our study 7 out of the 17cases were confirmed for H. pylori and had a symptom duration of less than 6 months (41.17%). 4 cases had a symptom duration of 6 months to 1 year (23.52%) and 6 cases had a duration of symptoms of more than 1 year (35.29%).

CONCLUSION

Ulcerative lesions of the stomach are more common in males than females and are seen in elderly patients. Non-neoplastic lesions are more common than the neoplastic lesions. H. pylori bacilli was found in 16.5% of ulcerative lesions of the stomach in our present study. Rapid Urease Test (RUT) is a simple economical and quick test for identifying H. pylori for routine screening of patients. H. pylori-associated gastritis was seen predominantly in elderly patients. H. pylori-associated with malignancy was seen in one case

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