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## **A study on association of H. pylori in patients with dyspepsia of any cause in a tertiary private hospital**

Dr. Vudumula Vijaya Lakshmi, Assistant Professor, Dept of General Surgery, Government Medical College Ananthapuramu, A.P., India

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### **ABSTRACT**

The study of gastric bacteriology has gained significant impetus since the isolation of H.pylori from gastric mucosal biopsies in 1983 by Barry Marshall and Robin Warren in Perth (Australia). They are able to demonstrate a strong association between the presence of H.pylori and the finding of inflammation on gastric biopsy. To investigate the association of H.pylori in patients with dyspepsia of any cause i.e., in the form of gastric ulcer, duodenal ulcer, gastritis, carcinoma stomach and non-ulcer dyspepsia with the help of following parameters- Direct biopsy urease test, Gram stained biopsy smear examination, Culture, Histopathological examination. This study was under taken at the Department of surgery, Narayana medical college, Nellore from January 2007 to July 2008. A total of 150 patients with duodenal ulcers, gastric ulcers, antral gastritis, gastric carcinoma and dyspepsia of any kind were studied. Evidence for H.pylori infection as decided by the laboratory tests gradually increased from 21 years to 50 years and showed decline thereafter. Incidence is more in males than females.

**Key Words:** H.pylori, dyspepsia, gastric bacteriology, Direct biopsy urease test

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### **INTRODUCTION**

Helicobacter pylori (H. pylori) has a role in the multifactorial etiology of peptic ulcer disease. A link between H. pylori infection and duodenal ulcer disease is now established. Other contributing factors and their interaction with the organism may initiate the ulcerative process. [1] The role of H. pylori as a gastric pathogen is dependent on virulence factors and pathogenic mechanisms. Virulence factors are those that allow H. pylori to survive in the hostile environment of the gastric lumen which includes its spiral shape, motility, adaptive enzymes, proteins, and ability to adhere to gastric mucosal cells and mucus. [2]

The study of gastric bacteriology has gained significant impetus since the isolation of H.pylori from gastric mucosal biopsies in 1983 by Barry Marshall and Robin Warren in Perth (Australia). They are able to demonstrate a strong association between the presence of H.pylori and the finding of inflammation on gastric biopsy [3]. Multiple studies show that H.pylori is associated with the development of gastritis, Gastric and duodenal ulcer, MALT lymphoma and gastric adenocarcinoma. Effective eradication therapy of

H.pylori infection had resulted in over 90 % of cure rate of gastric and duodenal ulcers.

In recent years comparative studies were made in various combinations to estimate the specificity and sensitivity of tests. Among them combination of four tests are occupying prime place.

- Urease test
- Direct Gram stained biopsy smear
- Culture and
- Histological examinations.

Since the discovery of H. pylori, several diagnostic methods have been developed for the aim of accurate detection of this organism. These tests include noninvasive method—serology, urea breath test, or stool antigen test—and invasive methods, such as, culture, histological examination, and rapid urease test, which require upper gastrointestinal endoscopy to obtain gastric biopsy samples [4,5]. Among these, histological examination is one of the most useful diagnostic tests for H. pylori infection [6].

**Objective:** To investigate the association of H.pylori in patients with dyspepsia of any cause i.e., in the form of gastric ulcer, duodenal ulcer,

gastritis, carcinoma stomach and non-ulcer dyspepsia with the help of following parameters- Direct biopsy urease test, Gram stained biopsy smear examination, Culture, Histopathological examination.

## MATERIALS AND METHODS

This study was under taken at the Department of surgery, Narayana medical college, Nellore from January 2007 to July 2008. A total of 150 patients with duodenal ulcers, gastric ulcers, antral gastritis, gastric carcinoma and dyspepsia of any kind were studied.

### Criteria for selection of patients

1. Consent of the patients taken prior to admitting for study.
2. Age ranging from 20 to 70 years and above.
3. Patients without previous gastric surgery.
4. Patients free of other concurrent infection or symptomatic illness.

**Sample collection:** Patients suffering from dyspepsia of any etiology were endoscoped for biopsy. Patients fasted for up to 12hours before endoscopy. Prior to specimen collection the endoscope with biopsy forceps was rinsed thoroughly with water and soaked in 2% gluteraldehyde (cidex) for 20 minutes. The endoscope was thoroughly rinsed with sterile normal saline just before collection of specimen. At the end of each day endoscope was washed and soaked in 2% gluteraldehyde for 30 minutes.

The endoscopists ascertained the diagnosis just before performing the biopsy. From each patient four biopsy specimen were taken from pylori antral mucosa within 5cms of pylorus, each piece of tissue approximately measuring 1mm in diameter under standard aseptic precaution. The four specimens were used for bacteriological study and were picked up from the endoscopic biopsy forceps with the help of sterile disposable needles. The first specimen was taken in a screw capped bottle of 5 ml capacity containing 3ml of sterile 20% glucose broth for culture. The second specimen was inoculated on to the urease test bottle, with third specimen an imprint smear was made on a sterile slide by placing it on and pressing the specimen with another sterile slide. The fourth specimen was subjected for Histopathological examination by H - E staining.

**Rapid urease test:** The biopsy specimen was tested for urease production by placing it in the Christensen's urea broth in small bottle. The urea is incorporated in the broth. The percentage of urea is 20%, phenol red is the indicator used, it also contained a buffer. After the biopsy specimen was inoculated in the urea broth medium it was examined for colour change from yellow (negative) to orange or bright pink (positive). In other words the test was read as positive when urea broth turns to red or a pink colour.

**Gram's staining:** The specimen was transformed from 20% glucose broth to a clean, new microscope slide of No.1 thickness and compressed with another same sterile slide and smears were prepared. Smears were air dried and fixed in methanol for about one hour. Gram's staining was done by using diluted carbol fuschin as counter stain. This gram stained smears were examined by light microscope under oil immersion at a magnification of 1000x up to half an hour in zigzag manner for gram negative 'S' or 'U' shaped bacilli arranged in group. Most of the times these are associated with huge number of lymphocyte infiltration.

**Culture:** The biopsy specimen was inoculated on to selected set of freshly poured media. The media used are Skirrow's medium and a non-selective medium, 6% sheep blood agar. Microscopies of colony were gram negative, straight un branched curved or unique oxbow or "Sea Gull" shapes and coccal forms were visualized. Their ends were rounded.

**Histopathological examination:** In all the subjects studied 1-2 biopsy specimens were fixed in 2 ml of 10% v/v buffered formalin, dehydrated in ethanol and embedded in paraffin. Section were cut a 5 micrometer with fine microtome and stained with Hematoxylin - Eosin. In cases where bacteria were few and difficult to recognize special stain (Warthin-starry) were performed to confirm the presence of H.pylori. The prepared slides were evaluated for the presence of H.pylori under oil immersion using light microscope.

## RESULTS

Findings of the present Laboratory study of 150 cases were here under presented and analyzed.

**TABLE 1:** Endoscopic diagnosis of the cases (n=150)

Sno	Endoscopic Diagnosis	No. studied	% of Incidence
1	Antral gastritis	60	40
2	Non – ulcer dyspepsia	45	30
3	Duodenal Ulcers	22	14.6
4	Gastric Ulcers	15	10
5	Carcinoma stomach	08	5.3

Antral gastritis was predominant (40%) followed by non-ulcer dyspepsia (30%) among the study group. Carcinoma stomach (5.3%) was comparatively less frequent.

**TABLE 2: Results of Different laboratory tests employed**

Sno	Laboratory test	No. of positive for H.pylori	No. of negative for H. pylori
1	Rapid urease test	118 (78.6%)	32 (21.3%)
2	Gram stained biopsy smears	105 (70%)	45 (30%)
3	Culture	80 (53.3%)	70 (46.6%)
4	Histopathological examination	61(40.6%)	89 (59.3%)

All the above tests done in this study clearly demonstrate the presence of Helicobacter pylori in approximately more than 60-70 % of all dyspeptic cases. Rapid urease test can be used as a safe, economical and reliable indicated test for H.pylori.

**TABLE – 3: Results of laboratory tests among various gastro duodenal diseases:**

S.No	Endoscopic Disgnosis	Number studied	Urease test		Gramstain positive		Culture positive		Histopathology Positive	
			No	%	No	%	No	%	No	%
1	Antral gastritis	60	59	98.3	56	93.3	45	75	35	58.3
2	Duodenal ulcer	22	21	95.4	21	95.4	12	54.5	10	45.4
3	Gastric ulcer	15	15	100	15	100	11	73.3	08	53.3
4	Carcinoma Stomach	08	07	87.5	05	62.5	02	25	02	25
5	Non ulcer dyspepsia	45	16	35.5	08	17.7	06	22.2	06	13.3

It can be seen that evidence for H.pylori infection by various laboratory tests was observed in more number of antral gastritis cases followed by ulcer diseases. Lowest positivity was observed in cases of non ulcer dyspepsia.

**TABLE – 4(A): Results of various tests among males and females of different age group**

Sno	Age	Rapid Urease test			
		Males		Females	
		+	-	+	-
1	21-30	16	3	6	3
2	31-40	21	4	9	4
3	41-50	23	7	6	2
4	51-60	18	4	4	2
5	61-70	8	2	3	-
6	>70	3	1	1	-

**TABLE – 4(B): Results of various tests among males and females of different age groups**

Sno	Age	Gram stained smears			
		Males		Females	
		+	-	+	-
1	21-30	14	5	6	3
2	31-40	21	4	8	5
3	41-50	22	8	5	3
4	51-60	14	8	3	3
5	61-70	7	3	2	1
6	>70	2	2	1	-

**TABLE – 4(C): Results of various tests among males and females of different age group**

Sno	Age	Culture			
		Males		Females	
		+	-	+	-
1	21-30	10	9	5	4
2	31-40	15	10	8	5
3	41-50	16	14	4	4
4	51-60	11	11	2	4
5	61-70	5	5	2	1
6	>70	2	2	-	1

**TABLE – 4(D): Results of various tests among males and females of different age group**

Sno	Age	Histopathology examination			
		Males		Females	
		+	-	+	-
1	21-30	8	11	4	5
2	31-40	13	12	3	10
3	41-50	10	20	2	6
4	51-60	10	12	1	5
5	61-70	5	5	2	1
6	>70	2	2	1	0

From the above table it can be inferred that evidence for H.pylori infection as decided by the laboratory tests gradually increased from 21 years to 50 years and showed decline thereafter. Incidence is more in males than females.

## DISCUSSION

In the present laboratory study of 150 symptomatic individuals undergoing endoscopy, an attempt has been made to study more than one biopsy specimens from them by more than one endoscopy based tests with a view to know the prevalence of H.pylori infection among them.

The presence of H.pylori can be detected by several different methods which can be classified as invasive and noninvasive tests. For the diagnosis of H.pylori associated gastro duodenal diseases,

histological staining and culture of biopsy specimen's has been considered "the gold standard".

H.pylori exhibits a patchy distribution pattern in the gastric mucosa and this makes endoscopy dependent tests relatively insensitive especially in the presence of scanty infection. The predictive value of diagnostic test for H.pylori can be improved by taking more than one biopsy sample or by using more than one endoscopy dependent test at the same time. Various studies have demonstrated the clinical usefulness of serological

assays for antibodies against H.pylori infected patients. Majority of these studies are from western countries.

In the present laboratory study of 150 symptomatic individuals undergoing endoscopy, an attempt has been made to study more than one biopsy specimens from them by more than one endoscopy based tests with a view to know the prevalence of H.pylori infection among them.

Extensive work made all over the world by several workers indicate that gastro duodenal diseases are more common among male members of the population. Common in the age groups between 21 to 50 years and less among children and older people. According to ozmen M.M et al., gastro duodenal diseases are uncommon in the age group below 20 years [7]. These are gradually increases to adolescent age and constant with higher incident in adults. So, overall views shows prevalence with age. (H.pylori an update for surgeons, 1995 edition). Male to female ratio for duodenal ulcer varies from 5:1 to 2:1, whilst that for gastric ulcer is 2:1 or less.6

Corroborating with this, in the present study also the male to female ratio of the patients is 3:1 showing male preponderance. Incidence has been more in age groups between 21-50 years among both sexes and later showed a gradual decline.

**Rapid urease test:** In the present study rapid urease test was positive in 78.6% of cases. Rapid urease test positivity was 98.3% among antral gastritis cases, 100% among gastric ulcers, 95.4% among duodenal ulcer cases, 87.5% among gastric carcinoma cases, and 35.5% in non-ulcer dyspepsia.

Rapid urease test with the biopsy material as reported by many workers is sensitive and specific to detect H.pylori infection. Findings of the present study, where in 76.6% overall positivity was observed are corroborative.

The accuracy of rapid urease testing is high, such that a correlation between histology and rapid urease testing provides a simple measure to gauge the accuracy of the pathology department. In a study with 143 patients, we found the sensitivity and specificity of different rapid urease tests to be in the range of 95 and 100%, respectively [8]. There is little difference among the available rapid urease tests, so that cost and availability are the prime determinants of which is used. The speed of the reaction is enhanced when large biopsies or multiple biopsies are placed in a single test well

and when a warmer is used to speed the reaction [9-12].

**Gram's staining:** Gram stain of a single antral biopsy has 65-85% sensitivity (Parsonnet et al, 1988, Montgomery et al, 1988).If tissue from both antrum and fundus is examined the sensitivity appears to increase to 92 to 100%. In our study presence of small, curved, Gram negative rods some with typical spiral appearance was observed in 105 cases out of 150 studies, thus H.pylori infection could be tentatively detected in 70% of cases. Result of our study expressed in table No.4 clearly shows that 93.3% of antral gastritis cases, 100% of gastric ulcer cases, 95.4% duodenal ulcer cases, 62.5% gastric carcinoma cases, and 17.7% of non ulcer dyspepsia cases were smear positive.

**Culture:** The culture positivity rate reported by several workers show variations. In the present study H.pylori was isolated from 80 cases. The culture positivity rate being 53.3%. Culture positivity was 75% among antral gastritis cases, 73.3% among gastric ulcers and 54.5% among duodenal ulcer cases, 25% among gastric carcinoma cases, 22.2% in non-ulcer dyspepsia cases.

**Histopathological examination:** The first tissue stain was the hematoxylin and eosin stain used early in the century (Bizzozero 1883). But the organism is barely discernible with this procedure. Freedberg and Barron (1940) introduced the use of silver staining which allows the organism to be seen in histological sections much more readily. The Warthin starry procedure was used in the landmark work of Marshall and Warren 1984 and in most studies since them. In the present study H.pylori was observed in 61 cases, Histopathology positivity was 58.3% among antral gastritis cases, 53.3% among gastric ulcer cases, 45.4% among duodenal ulcer cases, 25% among gastric carcinoma cases and 13.3% among non-ulcer dyspepsia cases.

Based on the findings of the present study it may be appropriate to conclude that for detection of H.pylori infection among symptomatic individuals, it is necessary to use "more than one test". The frequently referred gold standard tests Histological examination and Culture may not be relied at present. The usage of Rapid urease test and Gram stain smear examination stands better chance in establishing diagnosis of H.pylori infection.

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