

**Original Research Article** 

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# COMPARATIVE STUDY OF DIAGNOSIS OF H. PYLORI INFECTION AMONG PATIENTS OF PEPTIC ULCER

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#### Abstract

Background: H. pylori has also been associated with the development of noncardia gastric cancer, which is the second leading cause of cancer-related deaths worldwide. The objective is to find out the comparative study of diagnosis of H. pylori infection among patients of peptic ulcer attending Darbhanga. Medical College &. Hospital, outdoor by Kit and by Rapid chromatographic immunoassay (by strip) tests. Materials and Methods: A comparative study was conducted in the department of Microbiology, a total 65 patients were enrolled for this study who had suffering from dyspepsia. or ulcer selected for study. They belonged to the age group between 20 -60 years. Males and Females were included. The sample were collected and subjected to ELISA and Rapid Chromatographic Immunoassay and correlation between these two diagnostic methods was worked out during the study period January 2021 to December 2022. Result: The findings shows that 40 (61.5%) patients had Non ulcer dyspepsia. 22(33.8%) cases were Ulcer of the stomach or duodenum and 3(4.7%) cases were Oesoghagitis. H.Pylori was isolated from 25 cases out of 65 cases. 25 cases out of 65 weretested positive. From the 38 cases that are left over (65 to 25), 40 cases negative by both ELISA and Rapid Chromatographic Immunoassay test. 1 Patient were positive only by ELISA, out of which one patient has Non ulcer dyspepsia and 4 patients had ulcer of duodenum, 4 cases tested positive only by Rapid Chromatographic Immunoassay, out of which 3 were of Non ulcer dyspepsia and 1 complained of reflux Oesophagitis. Conclusion: The Rapid chromatographic immunoassay test, a quick and inexpensive test was used to detect the presence of H.pylori. It was highly sensitive and specific test, but since it is a qualitative test it has reduced importance as far as diagnosis was concerned.

# **INTRODUCTION**

Before the nineteenth century, peptic ulcer disease the common name for gastric and duodenal ulcer disease – was rare.<sup>[1]</sup> From that time the prevalence of gastric ulcers increased, and around 1850 the first duodenal ulcers were reported. The diagnoses were based on physical examination, patient history and the presence of postprandial pain, as X-ray was not invented before 1895. Medical therapy was limited, and surgery was the main treatment up to the 1960s. The cause of peptic ulcers remained a mystery for many decades, although several theories evolved. The Croatian physician Karl Schwartz published his theory of "no acid, no ulcer",<sup>[2]</sup> in 1910. Until the 1980s, the peptic ulcer disease was described as a multi-heterogeneous disease with mucosal disturbances, vascular disturbances, infectious and/or toxic and psychogenetic as the most often reported causes.<sup>[1]</sup> The causal relationship between H. pylori infection and peptic ulcer disease was documented

after extensive investigation subsequent to the discovery of the bacterium. In the first studies, H. pylori infection was found in 95% of patients with duodenal ulcer and in 85% in gastric ulcer patients. The life-time risk of developing peptic ulcer in H. pylori infected patients.<sup>[3]</sup> Finally, eradication of H. pylori became an efficient way to cure peptic ulcer disease.<sup>[4]</sup> One unexplained question is that why only some 10-20% of the infected patients develop peptic ulcer disease during a long-term follow-up. This has been proposed to rely on bacterial and/or host factors.

## **MATERIALS AND METHODS**

A comparative study was conducted in the department of Microbiology, a total 65 patients was enrolled for this study who had suffering from dyspepsia. or ulcer selected for study. They belonged to the age group between 20 -60 years. Males and Females were included. The sample were collected

and subjected to ELISA and Rapid Chromatographic Immunoassay and correlation between these two diagnostic methods was worked out during the study period January 2021 to December 2022.

#### **Inclusion Criteria of Patients**

- 65 patients who were having ulcer or dyspepsia due to reflex esophagitis or non ulcer dyspepsia were included in the study.
- Blood samples were collected for ELISA and Rapid Chromatography Immunoassay (Or rapid diagnostic test card).

#### Samples Used for Study

Blood sample for both tests i.e. Elisa and Rapid Chromatography Immunoassay.

#### Test Used

- 1. ELISA for H. Pylori for detection of IgG
- 2. Rapid Chromatography Immunoassay (or Rapid diagnostic strip test)

#### **Processing on the Specimens:**

**Blood samples:** Serum was separated from blood samples and Elisa test and Rapid Diagnostic test were performed.

ELISA<sup>[5]</sup>

#### **Collection of Samples**

About 5ml of blood was drawn from each patient using sterile syringe and the serum was separated by centrifugation and stored at -20C, until it was used for the detection of IgG antibody by Elisa.

#### **ELISA Kit**

Diluted patients serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by enzyme. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample.

## **RESULTS**

We found 25 (38.4%) positive cases among 65 patients. 40 cases (61.6%) were found negative. [Table 1]

Table 1: Total number of positivity cases (n=65)				
Total No. of Cases	Positivity	Percentage		
65	25	38.4		

# Table 2: Result of Endoscopic finding (n=65)

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Endoscopic Finding	No of Cases	Percentage			
Non Ulcer dyspepsia	40	61.5			
Ulcers	22	33.8			
Oesophagitis	03	4.7			
Total	65	100			

The above-mentioned [Table 2] described the endoscopic findings of acid peptic disease. The findings shows that 40 (61.5%) patients had Non ulcer dyspepsia. 22(33.8%) cases were Ulcer of the stomach or duodenum and 3(4.7%) cases were Oesoghagitis. H.Pylori is isolated from 25 cases out of 65 cases.

Table 3: Distribution of Male Female Ratio among diseases					
Disease	Exact no. (M:F)	Ratio(M:F)			
Non Ulcer dyspepsia	8:3	2.66:1			
Ulcers	9:4	2.25:1			
Oesophagitis	0:1	0:1			

In this study male to female ratio were 2.12:1. In Non-ulcer dyspepsia, the ratio was 2.66:1, in Ulcer group it was 2.25:1 and the Oesophagitis group there was one female. This study showed a male preponderance and is consistent with studies done by Longman et al.

Table 4: Percentage of positivity in each disease(n=65)						
Diseases	Total No of Cases	Positive Cases	Percentage of positivity			
Non Ulcer dyspepsia	40	11	27.6			
Ulcers	22	13	59.1			
Oesophagitis	03	01	33.3			
Total	65	25	100			

[Table 4] shows the percentage of positivity in each group of disease. Out of total number of 40 patients of Non-Ulcer dyspepsia 11 tested positive; 13 out of 22 cases of ulcer were positive and only 01 cases of Oesophagitis was positive, positivity being implicated by both ELISA and Rapid Chromatographic Immunoassay.

Table 5: Age Distribution of Positive Cases (n=25)							
Age NUD (n=11)		Ulcer (n=17)		Oesophagitis (n=1)			
Distribution	No. of	%	No. of patients	%	No. of patients	%	
	patients						
>10	0	0	0	0	0	0	
11 - 20	0	0	1	4	0	0	
21 - 30	6	24	2	8	0	0	

31-40	3	12	8	32	1	4
41 - 50	1	4	2	8	0	0
51 - 60	1	4	0	0	0	0

NUD= Non ulcer dyspepsia

[Table 5] shows that, age wise distribution of the positive cases of our study. Among 11 NUD positive cases maximum number of patients i.e. 6 (24%) patients belonged to 21 -30 years age group. In case of 17 Ulcer positive cases maximum 8 (32%) patients belonged to 31- 40 years age group. We found only one (female patient) Oesophagitis case positive who belonged to 31- 40 years age group.

Table 6: Result of ELISA and Rapid Chromatographic Immunoassay in 65 cases						
ELISA	Rapid Chromatographic Immunoassay	NUD	Ulcers	Oesophagitis		
+	+	11	13	1		
+	-	1	4	0		
-	+	2	0	1		
-	-	26	5	1		

NUD= Non ulcer dyspepsia

[Table 6] shows 25 cases out of 65 weretested positive. From the 38 cases that are left over (65 to 25), 40 cases negative by both ELISA and Rapid Chromatographic Immunoassay test. 1 Patient were positive only by ELISA, out of which one patient has Non ulcer dyspepsia and 4 patients had ulcer of duodenum, 4 cases tested positive only by Rapid Chromatographic Immunoassay, out of which 3 were of Non ulcer dyspepsia and 1 complained of reflux Oesophagitis.

The 5 patients who were tested positive only ELISA were supposedly taken as false positive recorded by ELISA or false negative recorded by Rapid Chromatographic Immunoassay for working out sensitivity and specificity of urease.

Similarly the 3 patients who were tested positive only by Rapid Chromatographic Immunoassay test were supposedly taken as falsepositive recorded Rapid Chromatographic Immunoassay test or false negative recorded by ELISA to workout sensitivity, specificity, positive predictive value and negative predictive value for Rapid Chromatographic Immunoassay test.

Table 7: Correlation between ELISA test and Rapid Chromatographic Immunoassay test.						
Test Positive Negative Total Positivity rates						
ELISA	30(x)	35(x1)	65	46.2 %		
Rapid Chromatographic Immunoassay	28(y)	37(y1)	65	43.0 %		

#### Correlation between ELISA Test and Rapid Chromatographic Immunoassay:

To find out exact correlation between ELISA and Rapid Chromatographic Immunoassay for H. pylori, we label the ELISA positive cases as (x) and Rapid Chromatographic Immunoassay negative cases as (x1). The Rapid Chromatographic Immunoassay positive cases were labeled as (y) and the Rapid Chromatographic Immunoassay negative individuals as (y1).

The correlation coefficient (1) for any 2 variables tends to lie between (1.0) and (+1.0). When the value of (y) is near (+1.0), it indicates a strong positive association between x and y. That is there is strong positive association between the ELISA and Rapid Chromatographic Immunoassay test for H. pylori; which means that if the positivity rate for ELISA will increased, the positivity rate for Rapid Chromatographic Immunoassay test will also increase.

## DISCUSSION

Spiral shaped organisms have been observed in the stomach of humans for over hundred years, but interest in these bacteria, i.e. Helicobacter pylori has been growing since 1982 when it was isolated by Warren and Marshall from patients with gastritis and a relationship between gastric disease and a bacterium was realized. In 1983, Warren isolated it from cultures. Initially it was classified as campylobacter. In 1989, the organism was renamed as Helicobacter, which reflects two morphological appearances of the organism helical shape in vivo but often rod like in vitro.

The total no. of 67 cases was studied in different diseases, in which the incidence of non-ulcer dyspepsia is much more followed by peptite ulcer and oesophagitis.

The reported frequency of this infection in many studies in India ranged from 31-84%, most centers reporting a figure of about 60%.

In this study, 29 out of 67 patients of acid peptic diseases were positive for H. pylori which means a prevalence rate of 43%.

In the present study, there is a 48% prevalence rate in lower socio-economic groups and only 10% in high income groups.

The epidemiology of H. pylori in India suggests that the infection occurs early in life and more than half of the population acquires the infection by early adult life. Once infection acquired, it is believed to persist lifelong unless treated successfully. My study is consistent with the study of V Kate et al, 2010; which showed that the prevalence of H. pylori increases markedly with age with maximum colonization occurring in young adults (25-35 years).

My study indicated the fact that there is a high degree of association of H. pylori with ulcerts of duodenum or stomach, with non-ulcer dyspepsia or with oesophagitis, confirming previous reports. (Goodwin, CS et al, and Marshall BJ et al).<sup>[6,7]</sup>

There are several invasive and non-invasive tests which have been used to detect the presence of Helicobacter pylori in the gastric mucosa. But there is no absolute "Gold standard" test for the diagnosis of H. pylori (Talley NJ et al, 1991).<sup>[8]</sup> However histology and culture remain the best available standards.

For many other infectious diseases culture is the gold standard for diagnosis. But in cases of H. pylori is problematic because of the fastidious nature of the organism and insome cases, overgrowth of competing microflora, e.g. proteous especially in the presence of hypochlorhydria. Cultures take 3-7 days and results are dependent on the expertise of the operator and the laboratory. Failure to culture H. pylori may also result from sampling error of the biopsy specimen or a delay in plating the material. Other factors that interfere with the ability to culture H. pylori include swallowed local anaesthetics, simethicone, prior treatment with bismuth or H2 receptor antagonists and contamination of biopsy forceps with disinfectants. Though culture is highly specific, it generally produces the highest number of false negative results. On the whole, culture of H. pylori has a difficult approach, is relatively expensive and unnecessary, unless antibiotic sensitivities are required.

Histology is another specific test, nevertheless it is operator dependent and scanty organisms may be missed by ordinary staining techniques. Sampling error and inaccurate result in the presence of scanty infection gives a very low diagnostic sensitivity of histopathology. Diagnosis by histopathology. Diagnosis by histopathology is also a lengthy process. Hence histology is only used when we investigate the histopathologic process involved in infection with H. pylori.

Present study evaluated the usefulness of the two most commonly used tests now a days-these are Elisa and Rapid chromatographic immunoassay test, both are noninvasive tests and done to detect antibodies against H. pylori.

ELISA is characterized by its sensitivity and is widely used as a global method of diagnosis since infected subjects develop elevated levels of IgG antibody to H. pylori in symptomatic stage. There is a high degree of correlation between severity of H. pylori infection and Culture, Histopathology and RUT.

Serology for IgG against H. pylori may play an important role in decreasing the need for endoscopy provided the cut-off values must be determined for easy assay based on the prevalence of antibodies in the population. It has been shown that serological diagnosis of H. pylori infection is capable of reducing the endoscopy worked by 23%. In the present study, Serology, a noninvasive test is as specific as RUT and has a positive value of 90.6% and negative predictive value of 88.5%.

In this study, the Elisa and Rapid chromatographic immunoassay findings were independently sufficient by themselves in making an etiological diagnosis of H. pylori.

Therefore, more than one method may be required for the definitive diagnosis of H. pylori infections. This is an agreement with other investigators who recommended a combination of two tests to increase the sensitivity.

Elisa may be obvious choice if we want to noninvasive tests. In this study 32 out of 67 cases were positive by the Elisa test which shows a positive rate of 48%. From these 32 positive patients, 29 were positive both by Elisa and Rapid chromatographic immunoassay.

There patients were positive only by Elisa and so were grouped as false positive by Elisa or false negative by Rapid chromatographic immunoassay. Out of these there false positive cases two belong to the ulcer group and one in non-ulcer dyspepsia category.

The specificity of Elisa tests in 91.1% in may study hence Elisa tests has a a low rate of false positivity. These doubtful positives may be due to neutral gastric pH gastric atrophy due to presence of klebsiella or proteus species of organisms or due to durg therapy. Patients already on therapy by antiulcer are main suspect because neutral gastric juice provides a favourable environment for proteus.

The four case which are positive only by Rapid chromatographic immunoassay could possibly be the false negative results of Elisa. Sampling error, technical factor and patchy distribution of the organisms may have reduced the diagnostic yield from infected patients. The fastidious nature of H. pylori, the difficulty in transportation and storage of the organisms and the use of metronidazole by patients for protozoal infestations, whose incidence is quite high in india, may have also given false negative results in biopsy specimens. Competing microflora may overgrown Helicobacter pylori to give false negative results.

The difficulties in isolating H. pylori from biopsy specimens led to the development of various serological tests (Nair, D et al., 1997). The easiest way to diagnose H. pylori infection in a patient who is not being endoscoped is test for antibodies to it.

# CONCLUSION

The prevalence of H. pylori infection varies worldwide, but higher colonization rates are seen in developing countries as compared to developed countries. It contributes to he causation of non ulcer dyspepsia and peptic ulcer. As one of the major risk factors for acquiring this infection is low socioeconomic status, it is widely prevalent in india. In western studies, H. pylori infection is much lower. The prevalence increases with age and more than 50% individuals greater than 50 years of age have serological evidence of infection. The ideal approach for the initial diagnosis of H. pylori is to perform endoscopy to obtain biopsy specimens for histopathology and culture. The low senseitivities of these tests added to high costs and delayed result limit their use in clinical practice. Therefore the Rapid chromatographic immunoassay test, a quick and inexpensive test is used to detect the presence of H.pylori. It is highly sensitive and specific test, but since it is a qualitative test it has reduced importance as far as diagnosis is concerned. Hence we can perform the urea breath test which is a non-invasive test and has high

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