# **ORIGINAL ARTICLE**

# Comparison of two Diagnostic Methods for Detection of H pylori i.e., Gastric Biopsy by H&E & Giemsa and Serum Anti H pylori Antibodies

RASHEED AHMED SOOMRO\*, ABDUL KHALIQ ABRO\*\*, IKRAM DIN UJJAN\*\*\*, ZAHID IBRAHEEM\*\*\*\*, MUHAMMAD FAROOQ\*\*\*\*

### **ABSTRACT**

**Objective**: To study and compare two different techniques for diagnosis of H.pylori i.e., endoscopic biopsy, and antibody detection.

Study of design: Institution based prospective comparative study

**Place & duration of study**: The Department of Pathology & Medicine and Surgery Liaqat University of Medical and Health Sciences, Jamshoro from Oct. 2009 to Feb 2011.

**Methods**: 100 patients of all age groups and both sexes with complaint of dyspepsia, or having the clinical suspicion of H. pylori associated gastritis were included in the study with gastric biopsies and serum samples.

**Results**: Out of 100 gastric biopsy cases, H. pylori were seen in 90 (90%) cases. Amongst which 52(57.7%) were male and 38(42.3%) were female, and on serum analysis ELISA technique, H. pylori antibodies were in 68(68%) cases, amongst which 38(55.9) were male and 30(44.1%) were female. The sensitivity of this technique was seen 75.55% and specificity 60%.

**Conclusion**; H. pylori positivity is highly significant in histological examination and the detection of anti-H. pylori antibodies from serum by ELISA Method is simplest and least expensive.

Key words: H pylori, gastric biopsy, giemsa stain

## INTRODUCTION

The study of gastric bacteriology, for so long a neglected field, has gained significant importance since the isolation of spiral Like Organisms Campylobacter from antral biopsy specimens<sup>1</sup>. The human stomach was not supposed to have a resident bacterial flora, yet H. pylori colonizes the gastric mucosa of at least one in four of the adult population<sup>2</sup>. The identification of H. pylori by Warren and Marshall at the Royal Perth Hospital (Australia), there was skepticism about the proposed role of H. pylori in the pathogenesis of chronic active gastritis and peptic ulcer disease<sup>3</sup>

The organism has been implicated for causing acid peptic disease is increasingly being reported from different parts of the world<sup>4</sup>. It has a world-wide distribution with a high prevalence rate in developing countries with increasing age. It is causally related to chronic active antral gastritis and is highly associated with duodenal and gastric ulcers<sup>5</sup>. It is able to survive in the acidic environment of the stomach by means of

Correspondence to: Dr. Rasheed Ahmed Soomro, Pathology Department, UMHS, Jamshoro

urease enzyme, which converts urea into ammonia and  $CO_2$ , enabling itself to neutralize acid in its immediate environment<sup>6</sup>. Some of the infected individuals develop duodenal ulcer where as others develop gastric ulcer have prevalence of infection is different in between developing and developed nations<sup>7</sup>.

The infection by *H. pylori* is also proposed to be involved in the development of gastric cancer<sup>8</sup>. There is six fold increased risk of gastric cancer in populations with *H. pylori* infection as compared with population that have no gastric infection<sup>9</sup>. *H. pylori* have also been identified as a risk factor for gastric cancer, Nearly 100 % of patients with duodenal ulcer<sup>10.</sup> However, 70% of those with gastric ulcer, and more than 80% of patients with gastric cancer, have H. pylori infection<sup>11</sup>.

### **METHODOLOGY**

The study was conducted at Pathology Department in collaboration with Department of Medicine, and Surgery, Liaquat University of Medical & Health Sciences, Jamshoro. The Period of study was 15-10-2009 to 28-02-2011. Hundred cases of gastric biopsies, serum samples and stool specimens of patients, were included in this Study for performing *H.* 

<sup>\*</sup>Pathology Department, LUMHS, \*\*Ghulam Muhammad Mahar Medical College, Sukkur, \*\*\*Pathology Department, LUMHS, \*\*\*\*APMO, Pathology Emergency Lab. SIMS/SHL, Lahore.

pylori diagnosis. Patients of all age groups and both sexes with complain of dyspepsia, or having the clinical suspicion of *H. pylori* associated gastritis were included in study. Gastric biopsies showing complete denudation or completely ulcerated surface epithelium, or autolytic changes. Patients having H/O NSAIDs, or eradication therapy were excluded from study. A printed Performa specially designed for recording the relevant clinical and other details of each case. Clinical findings and relevant data of patients were collected from endoscopist and directly from the patients.

# **RESULTS**

This study was conducted to compare following methods for detection of *H. pylori* regarding their specificity and sensitivity. (1).Gastric endoscopic biopsy: histological examination of tissues for detection of organisms on H & E and Giemsa stain. (2). ELISA method: serological detection of antibodies from blood samples. Out of 100 gastric biopsy cases, *H. pylori* bacilli were seen in 90(90%) cases on histological examination on H & E and Giemsa stained tissue sections. Amongst which 52(57.7 %) were male and 38(42.3 %) were female. Ages of the all patients were included in study ranged from 14to 85 years with a mean age of 47.9.

Blood samples were obtained from 100 patients included in the study for separation of serum for analysis on ELISA technique. Out of 100 cases, *H. pylori* antibodies were found in 68(68 %) cases. Amongst which 38(55.9%) were male and 30(44.1%) were female. The sensitivity of this technique was 75.55% and specificity 60%. P Value= 0.007."

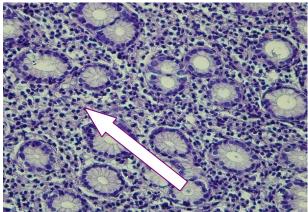


Fig 1: Microphotograph showing Helicobacter pylori, (arrow) on H & E Stain.

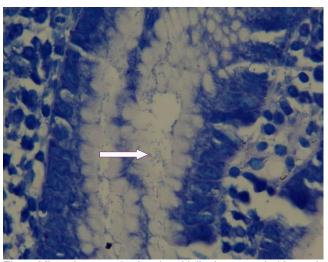


Fig 2: Microphotograph showing *Helicobacter pylori* (arrow) on Giemsa stain

Table 1: Results of gastric biopsies on H&E & Giemsa stain related to age & sex.

Gastric biopsies	Male				Female				
Age Group	14-32	33-64	65-85	Total	14-32	33-64	65-85	Total	Grand Total
Positive +++	02	03	00	05	01	02	00	03	08
Positive ++	01	02	00	03	01	04	00	05	08
Positive +	20	21	03	52	08	22	00	38	74
Negative	00	04	00	04	02	04	00	06	10
Total	23	30	03	56	12	32	00	44	100

<sup>+=</sup>Low ++=Medium +++=High

Table 2: Serum analysis for *H. pylori* antibodies related to age & sex. (n=100)

H. pylori antibodies	Male				Female				Grand Total
Age group	14-32	33-64	65-85	Total	14-32	33-64	65-85	Total	
Positive	17	20	01	38	07	23	00	30	68
Negative	06	10	02	18	05	09	00	14	32
	23	30	03	56	12	32	00	44	100

# **ORIGINAL ARTICLE**

Table3: Results of 100 gastric biopsies with serum anti *H. pylori* antibodies

Gastric biopsies	Total	+ve	%age	-ve	%age
H. pylori Positive	90	66	73.3	24	26.7
H. pylori Negative	10	02	20	80	80

Table 4: Sensitivity & specificity of serum *H. pylori* antibodies

Gastric biopsies						
Serum anti HP antibodies	Positive	Negative				
Positive	(a) 66	(b) 02				
Negative	(c) 24	(d) 08				

Sensitivity=a/ (a+c) ×100=73.33% Specificity=d/(d+b) ×100=80%

### DISCUSSION

Incidence of H.Pylori organism on endoscopic biopsies was 90% in this study, these results are in favor of studies of Tzeng, et al  $(2005)^{12}$  Qureshi et al  $(1996)^{13}$  and Rotimi et al  $(2000)^{15}$  who also found 93.81%, 90 % & 87 % cases positive for H. Pylori. Although this study coordinates with above studies, but is more significant and specific. In this study, out of 90 positive cases for *H. pylori*, 52(57.7%) were male and 38(43.3%) were female. But in studies conducted by Tzeng, *et al*  $(2005)^{12}$ , 58(52.2%) were male and 53(47.7%) were female. In study of Qureshi *et al*  $(1996)^{13}$  out of 72(90%) positive cases for *H. pylori*, 48(66.6%) were male and 24(33.3%) female.

In this study , on ELI SA technique the detection of H. Pylori antibodies was 73.3%, these results are also in favour of study conducted by Satti et al (2004) $^{15}$  for detection of H. Pylori on serum analysis, Luthra et al (1997) $^{16}$  also showed 87.7% & 63.3%.

# CONCLUSION

Serum analysis for detection of circulating *H. pylori* antibodies on ELISA technique is a simple non-invasive, less expensive, easy and effective method. The tests can be performed even on stored sera.

### REFERENCES

- Dooley CP.(1988) Histological Gastritis in Duodenal ulcer: Relationship to campylobacter pylori and effect of ulcer therapy. Am. J. Gastroenterology, 83: 278.
- Skirrow M B. (1992) Campylobacter In: Medical Microbiology. Eds. Greenwood D, Slack RCB,

- Forest J. 14<sup>th</sup> Ed., New York Churchill Livingstone. Pp. 358.
- 3. Forbes K J, Fang 2, Pennington T H. Allelic variation of the Helicobacter pylori flagellin genes flaA and flaB: its consequences for strain typing schemes and population structures. Epidemiol Infect.1997; 114, 257.
- Kazi JI. (1990) A placebo controlled trial of Bismuth salicylate in H. pylori associated gastritis. JPMA. 40:154-155.
- Greenberg ER and Bank S. (1990) The prevalence of H. pylori in non-ulcer dyspepsia. Arch. Inter, Med. 150:2053-2055.
- Dye KR. (1990) Campylobacter pylori colonizing heterotopic gastric tissue in the rectum. Am. J. Clin. Pathol. 93(1): 144-147
- 7. Das J C and Nibedita paul. (2006) Epidemiology and pathophysiology of Helicobacter pylori infection in children. Indian journal of Pediatrics 74:287-290.
- 8. Forman D, Sitas F, Yarnell J W G, Burr M L, Elwood P C, Pedley S. H.pylori infection rates in relation to age and social class in a population of Welshman Gut,1991; 32 25 28.
- 9. Forman D, Webb P, Parsonnet J. Helicobacter pylori and gastric cancer. Lancet 1993; 243-343.
- Goodwin CS et al. (1993). Microbiology of Helicobacter pylori. Gastroenterol. Clin. North Am 22(6): 142-146
- 11. Grabiec J, Own DA. (1985) Carcinoma of the stomach in young persons. Cancer, 56(2): 388-396
- Tzeng JE. (2005) Comparison of four diagnostic method for Helicobacter pylori. Tzu Chi Med. J. 17(5): 339-343
- 13. Qureshi AF. (1996) Incidence of Helicobacter pylori in gastrodudenitis. Biomedica 12:19-21.
- 14. Rotimi O. (2000) histological identification of Helicobacter pylori comparison of staining methods. F Clin Pathol 53:756-759.
- 15. 15. Satti SA.(2004) Comparison between serological testing and biopsy examination of Helicobacter pylori. Pak Armed Forces Med. J. 54(2): 195-198.
- Luthra GK et al. (1997) Comparison of Biopsy and serological methods of diagnosis of Helicobacter pylori infection and the potential role of antibiotics. The American Journal of Gastroenterology 93(8):1291.