Original Article

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# Comparison of Efficacy of One-minute Endoscopy Room Test and Giemsa Stain in Detecting *Helicobacter Pylori* in Chronic Gastritis

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## Abstract

**Background:** *Helicobacter pylori* is the most common cause for gastritis in patients with dyspepsia where gastric mucosal biopsies are indicated to identify different forms of gastritis. The most commonly used classification of gastritis is updated Sydney system. This study was done to find the efficacy of two tests - one-minute endoscopy room test (OMERT) and Giemsa stain for the identification of *H. pylori* in biopsies with chronic gastritis.

**Objectives:** To detect the efficacy of OMERT and Giemsa stain in the identification of *H. pylori* infection in patients undergoing endoscopy with histopathological diagnosis of chronic gastritis.

**Materials and Methods:** The endoscopic biopsies from November 2013 to May 2015 were included for the study. One of the biopsied tissues was directly immersed in OMERT solution and remaining were fixed in 10% formalin and processed and stained with hematoxylin and eosin, and Giemsa stain. The sensitivity and specificity of *H. pylori* detection by OMERT and Giemsa stain in histopathologically diagnosed cases of chronic gastritis were compared. *In-situ* and invasive carcinomas, polyps, and gastroesophageal junction biopsies were excluded from the study.

**Results:** Out of 53 cases studied, chronic non-specific gastritis was the most common type accounting for 21 cases, followed by 12 cases of chronic superficial gastritis, 10 cases of *H. pylori* gastritis, 9 cases of chronic active gastritis, and 1 case of chronic atrophic gastritis. *H. pylori* was positive in 13 (24.5%) cases by OMERT and 10 (18.9%) cases were positive by Giemsa stain. Both the techniques showed similar accuracy (83%).

**Conclusion:** In the present study, both OMERT and Giemsa stain were found to be having similar accuracy (83%). The sensitivity of OMERT was higher (78%), whereas Giemsa stain showed more specificity (92%). To avoid diagnostic pitfalls, the combination of two techniques is preferable rather than a single technique.

**Key words:** Chronic gastritis, Giemsa stain, *Helicobacter pylori*, Histopathology, One-minute endoscopy room test

## INTRODUCTION

Gastritis is defined as inflammation of the gastric mucosa and is the most common non-functional cause for dyspepsia. There are various classifications of gastritis taking into account morphology, topography, epidemiology, and endoscopy. Most of the gastritis was deemed idiopathic, until the discovery of *Helicobacter pylori*.[1-3]

The most common cause of gastritis is *H. pylori*. It has been linked with benign, premalignant, and malignant lesions of digestive system including chronic gastritis, intestinal metaplasia, adenocarcinomas of the distal part of stomach, and lymphomas of mucosa-associated lymphoid tissue.[4,5]

Marshall and Warren discovered *H. Pylori,* in 1983, using Warthin-Starry silver stain.[1] Various stains including hematoxylin and eosin (H and E), Giemsa, toluidine blue, and genta have been used in detecting *H. pylori*.[6]

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The histological method was considered as the "gold standard" for demonstrating *H. pylori* in endoscopic biopsies. A heavy bacterial load is apparent on routine H and E stained sections but a low density of organisms, requires special staining techniques.[7]

*H. pylori* can be easily identified as a purple curve shaped microorganism against a blue background on Giemsa stain.[6]

One-minute endoscopy room test (OMERT) is an invasive endoscopy test, which does not need complex procedures and stains. The presence of *H. pylori* can be diagnosed within maximum of 5 min, which helps in immediate initiation of treatment and it has a high sensitivity.[8]

Since *H. pylori* is one among the treatable causes of gastritis, and it is a part of the etiological classification of chronic gastritis in updated Sydney system, it is mandatory to document whether *H. pylori* is present or absent in a given gastric biopsy.[9] Hence, the present study was conducted to compare the accuracy of Giemsa stain and OMERT test to identify the bacilli in chronic gastritis.

## MATERIALS AND METHODS

This study was undertaken in the Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry. The study was conducted from November 2013 to April 2015 for 18-month after obtaining clearance from the Ethical Committee. Clinical details including age, gender, clinical diagnosis and endoscopy findings were noted on histopathologically diagnosed cases of gastritis.

### Inclusion of Samples

The gastric biopsy specimens, taken from antrum, fundus, corpus, and incisura angularis of the stomach, received in the Department of Pathology were included.

### Collection of Specimen

Biopsies were taken with a flexible fiberoptic gastroscope OLYMPUS.

One of the bits was immersed in urea solution for OMERT. This test utilizes 10% urea solution at pH 6.8 and addition of 1% freshly prepared phenol. The presence of *H. pylori* is indicated by the change of color of the solution from yellow to pink within 5 min (Figure 1).[8]

Remaining tissue bits were fixed in 10% buffered formalin and were taken for conventional processing. After processing, tissues were embedded in paraffin wax. Sections were cut serially at a thickness of 4-5 μ. Multiple sections were taken from each block and were stained for hematoxylin and eosin (H and E) and Giemsa.

The H and E stained slides were analyzed, and the samples which were histopathologically diagnosed as chronic gastritis only were included for the study. The classification was based on Sydney system.

### Exclusion Criteria

The biopsies with *in-situ* or invasive carcinoma on histopathologic examination were excluded from the study. Furthermore, the biopsies taken from the esophagogastric junction, inadequate biopsies, and gastric polyps were excluded.

## RESULTS

A total of 53 endoscopic gastric biopsy specimens diagnosed as gastritis of any type across all ages were taken for the study over a period of 18-month. Of the total 53 cases, 21 cases were diagnosed as chronic non-specific gastritis, 12 cases were chronic superficial gastritis, 10 cases of chronic *H. pylori* gastritis, 9 cases of chronic active gastritis, and a single case diagnosed as chronic atrophic gastritis.

Among these 53 cases, *H. pylori* was positive in 13 cases by OMERT and 10 cases were positive on Giemsa stain (Figure 2).

In comparison to Giemsa stain, OMERT showed more number of positive cases (24.5%) (Table 1).

Among the two techniques used in the study, OMERT was more sensitive (70%) while Giemsa stain was more specific with 92% specificity. However, the accuracy level of both the tests was equal (83%) (Table 2).

In 70% of the *H. pylori* positive cases, the mucinous epithelium was found to be intact with significant *P* value (Table 3 and Figure 3).

Both *H. pylori* and non - *H. pylori* gastritis showed mild degree of vascularity with moderate degree of inflammation. Moderate activity was seen in *H. pylori* gastritis, whereas in non-*H. pylori* gastritis, majority showed nil activity. Intestinal metaplasia was more frequently seen

**Table 1: *H. pylori* positivity on OMERT and Giemsa stain**

| *H. pylori* | *n* (%) | |
|---|---|---|
| | OMERT | Giemsa |
| Positive | 13 (24.5) | 10 (18.9) |
| Negative | 40 (75.5) | 43 (81.1) |
| Total | 53 (100) | 53 (100) |

*H. pylori: Helicobacter pylori,* OMERT: One-minute endoscopy room test

**Table 2: Level of accuracy of OMERT in comparison with Giemsa**

| Technique | Positive | Negative | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|
| OMERT | 13 | 40 | 70 | 86.05 | 53.85 | 92.5 | 83.02 |
| Giemsa | 10 | 43 | 53 | 92 | 70 | 86 | 83 |

OMERT: One-minute endoscopy room test, PPV: Positive predictive value, NPV: Negative predictive value

| | |
|---|---|
| True positive: 7 | False positive: 3 |
| True negative: 37 | False negative: 6 |

**Table 3: Nature of epithelium in relation to *H. pylori* status**

| *H. Pylori* on Giemsa | Mucinous epithelium *n* (%) | | | Statistics |
|---|---|---|---|---|
| | Intact | Ulcer | Total | |
| Positive | 7 (70.0) | 3 (30.0) | 10 (100) | $\chi^2$=4.121 |
| Negative | 15 (34.9) | 28 (65.1) | 43 (100) | df=1 |
| | | | | *P*=0.042 |
| Total | 22 (41.5) | 31 (58.5) | 53 (100) | |

*H. pylori: Helicobacter pylori*

**Table 4: Comparison of morphological parameters with *H. pylori* status**

| *H. pylori* | *n* (%) | | Statistics |
|---|---|---|---|
| | Positive (*n*=10) | Negative (*n*=43) | |
| Inflammation | | | |
| Mild | 2 (20) | 8 (18.6) | $\chi^2$=1.792 |
| Moderate | 7 (70) | 22 (51.2) | df=2 |
| Severe | 1 (10) | 13 (24.5) | *P*=0.4082 |
| Activity | | | |
| Mild | 3 (30) | 6 (14.0) | $\chi^2$=3.502 |
| Moderate | 5 (50) | 3 (7.0) | df=2 |
| Severe | 2 (20) | 1 (2.3) | *P*=0.1736 |
| Vascularity | | | |
| Mild | 6 (60) | 22 (51.2) | $\chi^2$=0.6099 |
| Moderate | 2 (20) | 14 (26.4) | df=2 |
| Severe | 2 (20) | 7 (16.3) | *P*=0.7372 |
| Metaplasia | | | |
| Mild | 2 (20) | 17 (39.5) | $\chi^2$=0.3041 |
| Moderate | 1 (10) | 5 (11.6) | df=2 |
| Severe | 0 (0) | 1 (2.3) | *P*=0.8589 |
| Dysplasia | | | |
| Nil | 10 (100) | 40 (93.0) | $\chi^2$=0.7395 |
| Severe | 0 (0) | 3 (7.0) | df=1 |
| | | | *P*=0.3898 |

*H. pylori: Helicobacter pylori*

in non-*H. pylori* gastritis. No dysplasia was seen in *H. pylori* positive gastritis (Table 4).

## DISCUSSION

In our study, 53 patients with dyspepsia were evaluated on gastric endoscopic biopsies for histopathological gastric mucosal changes and *H. pylori* positivity over a period of 18-month.

**Table 5: Comparison of *H. pylori* positivity with previous studies**

| Study | Country | Positivity for *H. pylori* (%) | Method used for identification |
|---|---|---|---|
| Rokkas *et al*.[10] | England | 45 | Histopathology |
| Pettross *et al*.[11] | USA | 43 | Culture, Silver stain |
| Abdulla *et al*.[12] | Indonesia | 68 | Culture, Silver stain, Rapid urease test |
| Present study | India | 18.9 | Giemsa stain |
| | | 24.5 | OMERT |

*H. pylori: Helicobacter pylori*, OMERT: One-minute endoscopy room test

**Table 6: Comparison of *H. pylori* detection by OMERT and Giemsa stain with previous study**

| Technique | Jeelani Romshoo *et al*.[8] (%) | Present study (%) |
|---|---|---|
| OMERT | | |
| Sensitivity | 80 | 70 |
| Specificity | 92.86 | 86.05 |
| Giemsa | | |
| Sensitivity | 74 | 53 |
| Specificity | 78.57 | 92 |

*H. pylori: Helicobacter pylori*, OMERT: One-minute endoscopy room test

The biopsies taken from the esophagogastric junction, gastric polyps, carcinoma, and inadequate biopsies were excluded from the study.

In 1984, Marshall and Warren, in their study showed that among 20 cases of chronic gastritis, 12 were positive for *H. pylori* (60%).[2] Our study showed a lesser positivity which can be attributed to the following reasons.

The environmental factors in the population under study may be a factor. Furthermore, in many of the previous studies, culture also was used which was highly sensitive and capable of demonstrating the organism even when the load was less (Tables 5 and 6). Among the 53 cases in our study, 26 cases showed intestinal metaplasia and 31 cases had mucosal ulceration. Both these changes are known to alter the pH of gastric mucosa, and render it unfavorable for the growth of *H. pylori* which requires a slightly alkaline pH.[13]

Misra *et al*., in their study showed that the numbers of *H. pylori* positive cases were increased with the increasing grades of gastritis, and the association was found to be statistically significant.[14] In our study, the number of cases of *H. pylori* was seen more with moderate severity of

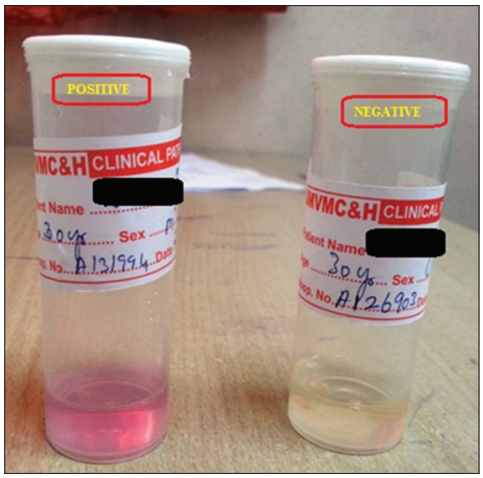


**Figure 1: One-minute endoscopy room test: Positive (pink); negative (light yellow)**

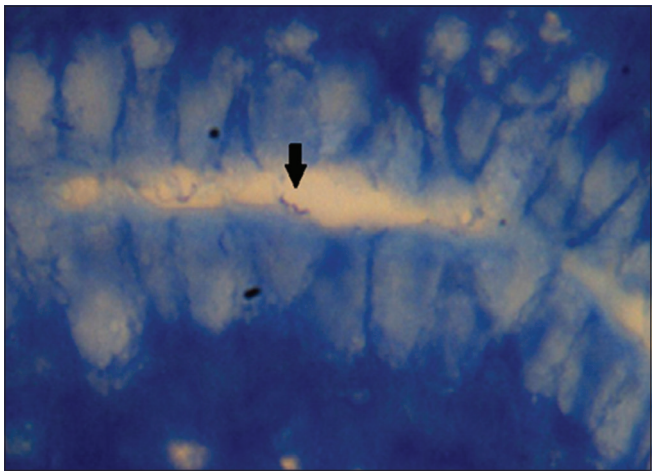

**Figure 2: Helicobacter pylori, curved metachromatic bacilli (arrow) - Giemsa, ×100**

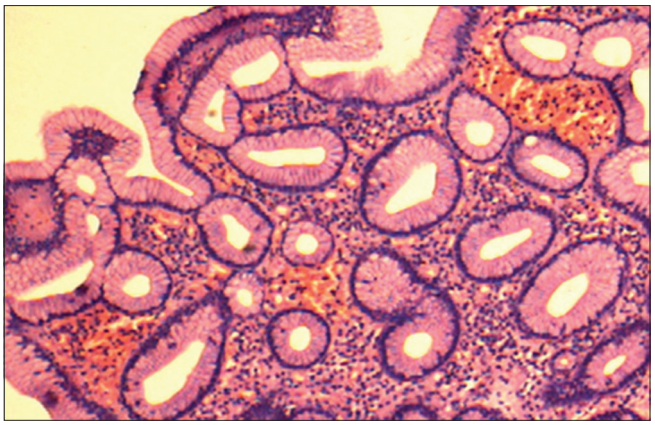

**Figure 3: Mucosa with intact surface epithelium, regular glands, mild inflammation and hemorrhage in the lamina propria, hematoxylin, and eosin, ×10**

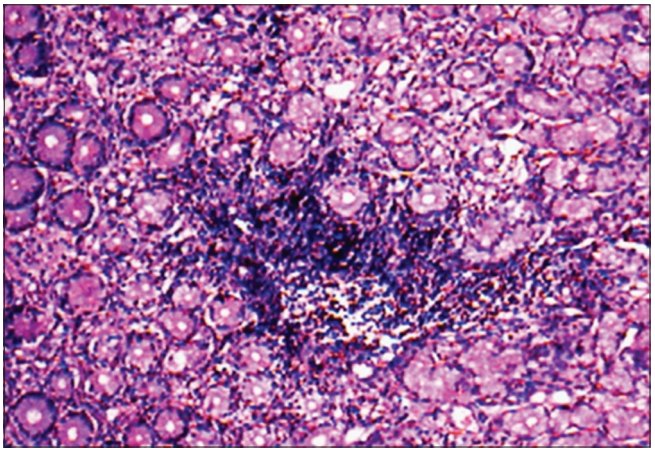

**Figure 4: Dense inflammation in the lamina propria, hematoxylin and eosin, ×10**

inflammation and was found to be statistically insignificant (Figure 4).

Although both the tests had good sensitivity and specificity with same accuracy level (83%), we encountered a few minor pitfalls.

### Pitfalls in OMERT

OMERT showed false positivity in three cases. This may be due to immersing the endoscopic biopsy forceps into the container containing the solution thereby rendering the solution alkaline, which causes color change to pink, the criteria for positivity. Hence, the biopsy bit to be immersed should be taken by a sterile needle from the scopy forceps and then placed in the solution.

### Pitfalls in Giemsa Stain

Giemsa stained sections, in few cases showed stain particles or other non-metachromatic bacteria which caused difficulty in identifying the *H. pylori*. However, *H. pylori* was identified only based on the curved architecture and metachromatic staining characteristic which made this method very specific.

In the present study, *H. pylori* was found in 18.9% and was associated with moderate inflammation and activity. Both Giemsa stain and OMERT were having similar accuracy levels (83%); however, OMERT was more sensitive, and Giemsa stain was more specific.

## CONCLUSION

The present study had 53 cases of histopathologically diagnosed gastritis for 18-month, and histopathological parameters were analyzed along with *H. pylori* status in all these patients by two methods (OMERT and Giemsa stain).

Among the 53 cases, *H. pylori* was positive in 13 (24.5%) cases by OMERT and 10 (18.9%) cases were positive by

Giemsa stain. OMERT test showed higher sensitivity (70%), whereas Giemsa stain showed higher specificity (92%). However, the accuracy levels of both techniques were similar (83%).The presence of *H. pylori* is directly proportionate to the degree of inflammation and activity in chronic gastritis.

Non-invasive tests for detection of *H. pylori* may be preferred choice for clinicians, but histopathological demonstration of the organism has the advantage of accuracy. Furthermore, it gives us a chance to study the associated histopathological changes which may be of prognostic value.

In view of avoiding, the pitfalls of each technique and to avail the advantage of morphological correlation, the combination of both techniques will be beneficial.

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