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The Role Of Imprint Cytology Stains In Helicobacter Pylori Detection : A Comparative Study In a Tertiary Care Center In India

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Abstract:

Background*: H. pylori* has been related to gastrointestinal pathologies ranging from mild chronic gastritis to gastric cancers. There are numerous invasive and non-invasive techniques used to diagnose *H. pylori* infection. Imprint cytology simple, quick, and requires minimal additional procedures for the clinician. Smears made from biopsy samples before regular histological processing significantly reducing the waiting time for results. This rapid turnaround allows clinicians to initiate treatment during the same visit, potentially improving patient outcomes by starting therapy immediately after endoscopy. In this study, the various stains used in impression cytology for *H. pylori* detection were compared to RUT and traditional histopathology biopsy evaluation.

Methodology : This prospective study was conducted in Department of Pathology.Gastric biopsy from the patients above 18 years were included in the study. Three gastric biopsies were obtained the antrum. One of the biopsy tissues was subjected to the rapid urease test, while others were first used for imprint cytology by Giemsa and toluidine blue stain, then fixed in 10% neutralized formalin and then paraffin-embedded for routine tissue processing. Finally examined by H&E and Giemsa stain.Giemsa stain on histology was used as the gold standard to compare imprint smear and RUT results.

Reults: In this study of 176 gastric biopsy specimens, 50 cases had imprint smears analyzed. All cases underwent a RUT. Giemsa histology showed H. pylori positive in 86 cases and negative in 90, while RUT showed positive in 84 and negative in 92 cases. There were 2 false positives and 4 false negatives. Among the 50 smears, Giemsa had 22 positive and 28 negative cases, while Toluidine Blue Stain showed equal results (25 positive and 25 negative). Toluidine blue had a higher sensitivity (95.8%) compared to Giemsa (97.7% specificity), with Toluidine blue's NPV (95.6%) being better and Giemsa's PPV (95.4%) higher.
Conclusion: To conclude imprint cytology is a sensitive tool for diagnosing *H. pylori* infection as it is quick, inexpensive, and simple screening method compared to other tests.
Key words: Imprint cytology, Girmsa stain, Toluidine blue stain, rapid urease test

Introduction

H. pylori, a gram-negative flagellated bacillus, has been related to gastrointestinal pathologies ranging from mild chronic gastritis to gastric cancers such as adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. [1,2] *H. pylori* is thought to be the most prevalent bacterial infection in the world, with an estimated 75% of people in developing nations having the illness even at a young age, compared to a smaller percentage of people in developed nations [3]
There are numerous invasive and non-invasive techniques used to diagnose *H. pylori* infection. Urea breath tests, stool antigen tests, urine and saliva antibody tests, and serological immunoglobulin G and immunoglobulin M detection are examples of non- invasive tests. Endoscopy-based invasive tests include polymerase chain reaction, brush and imprint cytology, rapid urease test (RUT), histopathological analysis, and other cytological tests. [4]
Since non-invasive diagnostics, such as serology, cannot differentiate between past and present infections, they are not very useful in high-prevalence areas. In contrast, invasive tests have a high sensitivity and specificity of >90%.[5]
Imprint cytology simple, quick, and requires minimal additional procedures for the clinician. Smears made from biopsy samples before regular histological processing significantly reducing the waiting time for results. This rapid turnaround allows clinicians to initiate treatment during the same visit, potentially improving patient outcomes by starting therapy immediately after endoscopy. Both imprint cytology and RUT are faster, and results are available while the patient is still in the endoscopy unit; however, RUT costs more than imprint. [4-6]
In this study, the various stains used in impression cytology for *H. pylori* detection were compared to RUT and traditional histopathology biopsy evaluation.

Methodology

This prospective study was conducted in Department of Pathology, Adichunchanagiri Institute of medical science, B G Nagar. Ethical clearance was obtained from Institutional ethics committee before commencing the study. Gastric biopsy from the patients above 18 years were included in the study. Patients under the age of 18, those who were mentally unstable, those who had been taking antibiotics for the previous month, and those with malignant conditions were all excluded from the study. History and endoscopic findings were noted.
Three gastric biopsies were obtained the antrum. One of the biopsy tissues was subjected to the rapid urease test, while others were first used for imprint cytology by Giemsa and toluidine blue stain, then fixed in 10% neutralized formalin and then paraffin-embedded for routine tissue processing. Finally, 3-5 micrometre thick sections were examined by H&E and Giemsa stain.
A rapid urease test was performed in the endoscopic room, and the findings were evaluated within

an hour. For RUT, Gastric biopsy samples were placed on agar gel with urea and pH indicator, such as phenol red. H. pylori's urease degrades urea in the gel, producing ammonium and bicarbonate ions that raise the media's pH. The phenol red hue changes from yellow to red, reflecting the alkalinity. [7]

Two separate imprint smears were made from the biopsy specimens by lightly rolling them over a glass slide with the help of a needle and then air dried. One imprint specimen was stained with 0.5% Toluidine blue stain and washed with water after 1 min. The second specimen was stained with Giemsa working solution for 15 min before washing with running water. [8] Then the stained slides were dried and mounted using DPX mounting medium. The slides were observed under ×400 magnification using a light microscope.

Finally, biopsy specimens processed routinely for H and E and Giemsa stain and examined. Additional finding in the form of grades of gastritis and reparative changes, intestinal metaplasia was noted on histopathological examination.

Giemsa stain on histology was used as the gold standard to compare imprint smear and RUT results.

Data analysis

Data were entered in Microsoft excel sheet. Descriptive statistics in from of mean and range were calculated. Then, sensitivity, specificity, positive predictive value and negative predictive value for imprint smears and RUT were calculated by taking Giemsa stain on histology as gold standard.

Results

In this prospective study a total of 176 gastric biopsy specimen were received in the pathology department during study period. Out of which for 50 cases imprint smears were studied. All the 176 cases had undergone rapid urease test. Age of the patient ranged between 20 and 78 years with the mean of 45 years. Female patients were predominant with a Female to male ratio 1.3: 1.

On Giemsa histology, *H. pylori* was positive in 86 cases and negative in 90 cases. (Figure 1) On RUT, *H. pylori* was positive in 84 cases and negative in 92 cases. There were 2 false positive and 4 false positive cases.

Of the 50 imprint smears studied, 22 and 28 cases, respectively, displayed Giemsa stain positivity and negative for *H. pylori*. (Figure 2) There were 3 false positive cases and single false negative case. In contrast, 25 cases each displayed positive and negative results on Toluidine Blue Stain. (Figure 2) There were 2 false positive cases and single false positive case.

Histology Giemsa was taken as gold standard and sensitivity, specificity, PPV, NPV was calculated. (Table 1) Toluidine blue had higher sensitivity of 95.8% whereas Giemsa had higher specificity 97.7%. The sensitivity of Toluidine blue was almost equal to RUT. PPV was more for Giemsa (95.4%) whereas NPV was more for Toluidine blue (95.6%).

On histologically majority of the cases showed mild chronic gastritis (40.3%), mild active gastritis (40.3%). *H. pylori* was positive most commonly in moderative active gastritis. (Table 2) In addition, 22 cases showed the reactive atypia changes and 5 cases showed the intestinal metaplasia.

Table 1: Table showing Sesitity, specificity, PPV and NPV of various diagnostic tests.

Method		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Imprint	**Giemsa**	87.5	96.2	95.4	89.3
	Toluidine blue	95.8	92.3	92	96
Rapid urease Kit		95.3	97.7	97.6	95.6

Table 2: Table showing H. pylori status in various types of gastritis.

Type of gastritis		H. pylori

	Total number of cases	Positive	Negative
Mild active gastritis	71(40.3%)	48(67.6%)	23(32.3%)
Mild chronic gastritis	85 (48.2%)	23(27%)	62(72%)
Moderate active gastritis	14(7.9%)	12(85.7%)	02(14.3%)
Moderate chronic gastritis	02(0.6%)	01(50%)	01(50%)
Severely active gastritis	04(1.2%)	02(50%)	02(50%)

Figure 1–*H. pylori* in gastric biopsy histology (Giemsa Stain, 400X)

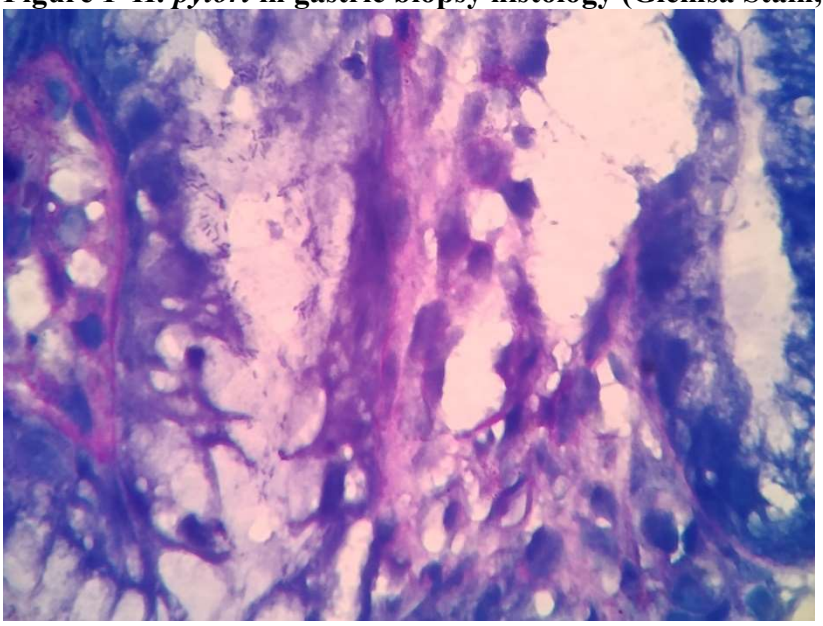
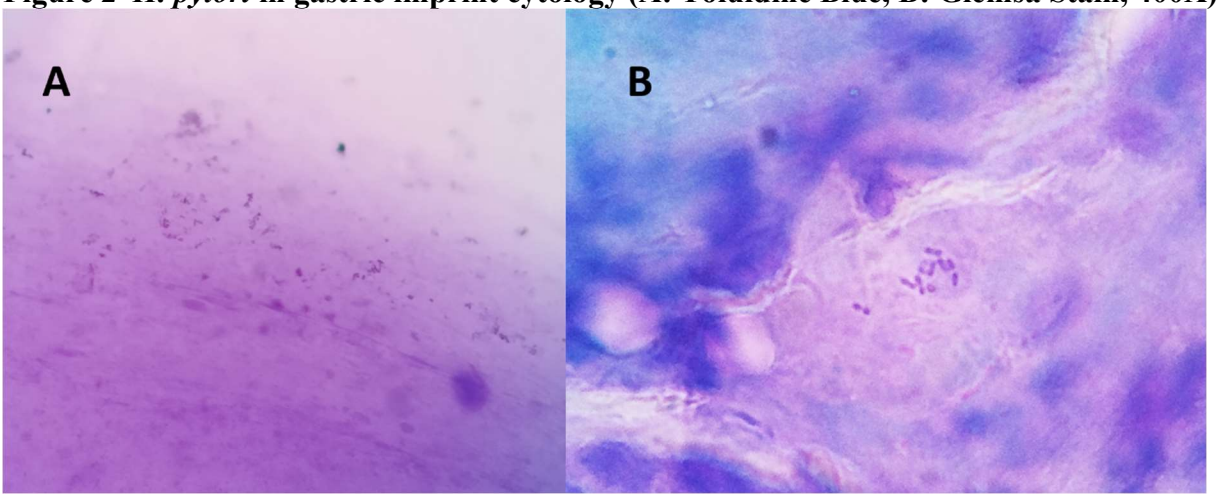


Figure 2–*H. pylori* in gastric imprint cytology (A: Toluidine Blue, B: Giemsa Stain, 400X)



Discussion

H. pylori infection is diagnosed using a variety of approaches, each with its own set of advantages and limitations. The most popular and widely utilized procedure is histological evaluation of antral samples with H and E stain. Histopathological examination is highly sensitive and specific for identifying *H. pylori* infection. It also gives useful information on mucosal architecture and atypia. It is often recognized as the most accurate diagnostic test, but its main disadvantage is the high turnaround time and cost. [9]

Imprint cytology is much simpler to perform, and imprint smears of biopsy materials prior to regular histological processing add no additional procedure or burden to the endoscopist or patient. It took an average of 10 minutes to process, whereas histology reports took three to five days. This gives a huge benefit because therapy can begin before the patient leaves the endoscopic suite on the same day. Both imprint cytology and RUT are faster, and findings are accessible while the patient is still in the endoscopy unit; however, the cost of RUT is higher than imprint. [8-9]

In the present study, we evaluated the sensitivity, specificity and predictive values of different methods. Our study found that Toluidine blue staining, used in imprint cytology, demonstrated a sensitivity of 95.8%, which was nearly equivalent to the sensitivity of the RUT. These findings align with the study by Tajalli et al., [10] who also reported high sensitivity with Toluidine blue. However, Adlekha et al. [8] observed a lower sensitivity in their study, which suggests that sensitivity may vary depending on the method or sample quality. Despite some variation in the literature, Toluidine blue remains a reliable and efficient stain for detecting *H. pylori*.

Specificity was another key parameter in our study, with Giemsa staining showing superior specificity compared to Toluidine blue. Our results were consistent with the findings of Tajalli et al., [10] and Piyumali et al., [8] who also observed higher specificity with Giemsa stain. This superior specificity may be due to Giemsa's ability to distinctly differentiate bacterial cells from epithelial cells, making it particularly useful for confirming the presence of *H. pylori* in biopsy samples.

In terms of predictive value, both Toluidine blue and Giemsa stains performed well. Toluidine blue showed a high predictive value, similar to that reported by Tajalli et al., [10] and Piyumali et al., [8] indicating that it is a reliable stain for detecting *H. pylori*. However, the positive predictive value was higher for Giemsa imprint cytology, which could be attributed to better bacterial discrimination in Giemsa staining, where bacteria take on a distinct magenta colour, while epithelial cells remain blue.

One of the significant advantages of imprint cytology is its speed, enabling treatment to begin immediately. This contrasts with the slower histological methods, where the results are often delayed. The Rapid Urease Test (RUT) is also quicker, providing results while the patient is still in the endoscopy suite, but it tends to be more expensive than imprint cytology. Despite the faster processing time, the sensitivity and specificity of imprint cytology were comparable to those of RUT, making it a viable alternative in settings where rapid and affordable testing is needed. [11-13]

However, there are some limitations to imprint cytology. One potential issue is the risk of false negatives, particularly when the bacterial load is low or when the infection is multifocal. Sparse *H. pylori* organisms can be difficult to identify against the background smear, which may lead to misinterpretation. [8,14] Additionally, while imprint cytology maximizes the use of biopsy samples for histological examination, it is crucial to handle biopsy material carefully to preserve the integrity of the tissue for further processing. [15]

Conclusion

In summary, imprint cytology is a sensitive tool for diagnosing *H. pylori* infection and a quick, inexpensive, and simple screening method. The diagnostic value of the biopsy specimen can be increased by using it for histological examinations after it has been used to produce impression smears.

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