https://doi.org/ 10.48047/AFJBS.6.5.2024. 10844-10847

African Journal of Biological Sciences

Cytological diagnosis of Helicobacter pylori- A Comparative study

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Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi: *10.48047/AFJBS.6.5.2024. 10844-10847*

Abstract:

Helicobacter pylori (H. pylori) is a gram-negative microaerophilic bacterium found in the mucous linings of the stomach, representing a prevalent and significant transmissible human bacterial pathogen. Timely diagnosis of infection is crucial for effective treatment and to mitigate potential complications. The gold standard for detection involves morphological assessment of gastric biopsies for H. pylori. This study aims to evaluate two cytological methods (Brush cytology and Touch imprint cytology) and compare their efficacy against the gold standard method. This study examines the efficacy of two methods for detecting H. pylori in gastric biopsies: endoscopy brush sampling and touch imprint cytology. Biopsies were obtained from dyspeptic patients undergoing routine upper gastrointestinal endoscopy. The specimens were analyzed using both methods and reviewed by three independent pathologists blinded to the cases. The cytological approach proves to be more efficient in promptly detecting H. pylori organisms compared to routine histopathology sections, exhibiting excellent sensitivity. Therefore, with adequate training and expertise, cytology can serve as a convenient bedside testing method for rapid and accurate H. pylori detection. This facilitates clinicians in promptly initiating targeted antimicrobial therapy.

Keywords: Helicobacter pylori, Imprint cytology, Brush cytology, gastric biopsies

Introduction:

Helicobacter pylori, a gram-negative flagellated bacilli has been implicated in causing a spectrum of gastrointestinal pathologies with dyspepsia as the main presenting symptom [1,2] The annual incidence of the *H. pylori* infection is 0.3-0.7% in the developed countries and it is 6-14% in the developing countries[3.] Helicobacter pylori lives in the mucus layer in close opposition to gastric epithelial cells where it causes damage to the cells[3]. The pathological lesions range from mild chronic gastritis to gastric malignancies like adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma[4]. Although culturing organism is the gold standard, it lacks sensitivity, and is technically difficult and costly. Identifying Helicobacter pylori in FFPE tissues has a turnaround time of several days which will delay the treatment as well as increase the patient's visit to the clinician for the same complaint**.** Imprint smears can be fixed and stained quickly, there by early management and reduced patient discomfort**.** In the present study, we evaluate the use of imprint cytology in identifying Helicobacter pylori organisms in gastric biopsies using the Diff-Quik stain and whether imprint cytology procedure damages the gastric biopsies in subsequent histopathological examination

Materials and methods:

This prospective study was done in a tertiary care centre. Patients with dyspepsia attending routine upper gastrointestinal endoscopy at our centre were enrolled in the study. About 100 patients were included in this study after obtaining written consent. Two biopsies were placed on a clean glass slide separately and the imprints were taken by gently touching the mucosal surface of the biopsy on the glass slide. Then the same biopsy tissue is placed in 10% neutral buffered formalin for routine histopathological examination. The imprint slides were air- dried and stained with Diff – Quik stain. The entire staining duration is approximately 2 minutes. The stained slides were air dried and examined under high power and oil immersion for Helicobacter pylori. The biopsy specimen was fixed in formalin and dehydrated in grades of alcohol, embedded in paraffin wax, sectioned at 3- 4 μm thick and stained with hematoxylin and eosin. Two individual pathologists examined the imprint slides and histology slides separately. The sensitivity, specificity, positive, and negative predictive values for the imprint cytology in diagnosing Helicobacter pylori were calculated in accordance with the standard methods

Results:

Among the 100 enrolled patients, 52% were males and 42% were females with a median age of 45 yrs (age range 18 - 79 yrs). Of the 100 patients, 30 were diagnosed to have Helicobacter pylori infection by histology. The imprint smears has produced good cytological details. Though many bacteria were stained with Diff Quik stain, the characteristic spiral shape of Helicobacter pylori was considered for identification (FIGURE 1a, b). 28 cases were diagnosed to have Helicobacter pylori in imprint cytology. There were two false negative cases in imprint cytology

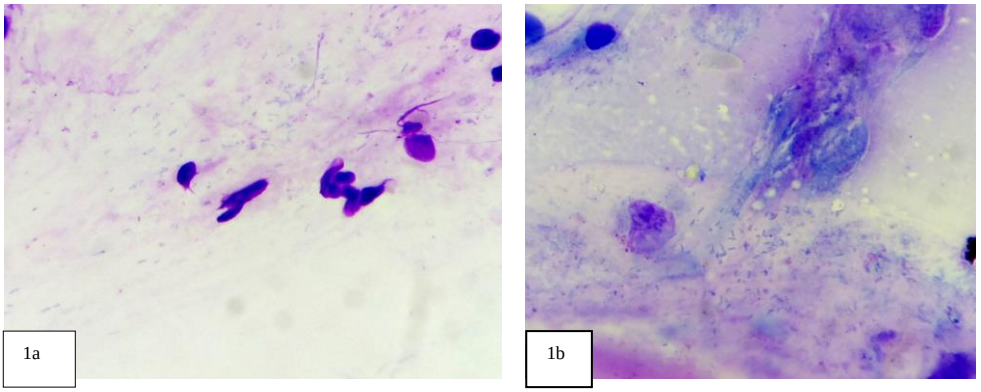

Figure 1a,b H pylori organisms seen in imprint cytology using Diffquik stain

We also observed that the histological details of all the gastric biopsies from which the imprint was taken were not compromised. In our study, the specificity, sensitivity, PPV, NPV were found out to be 100%, 93.33%, 100%, 97.22% respectively (Table 1)

IMPRINT CYTOLOGY FOR H PYLORI	Gastric biopsy histology positive	Gastric biopsy histology negative	TOTAL
POSITIVE	**28**	**0**	**28**
NEGATIVE	**2**	**70**	**72**
TOTAL	**30**	**35**	**100**

Table 1- Comparison of imprint cytology and their respective histology in identifying Helicobacter pylori

Discussion:

The available methods for the detection of H. pylori organisms are many and broadly categorized as invasive and non-invasive methods. Histology is often accepted as the 'gold standard' in diagnosing H. pylori infection as culture has marked limitations. As H. pylori is found in the gastric mucus on the surface epithelium and gastric foveolae, cytology of imprint smears and brushings have proven to be reliable methods for its detection[5]

In our study, the incidence of H pylori among the biopsied patients was 30%. The specificity, sensitivity, PPV, NPV were 100%, 93.33%, 100%, 97.22% respectively which was comparable with previous studies [5-9]

Two imprint smears showed false negatives, probably due to very low bacterial load which was also noted in previous studies [6, 7]

In our study the inflammatory changes on imprint smears did not correlate well with the respective histology. We believe that histological examination of the biopsy is essential to provide accurate information on inflammation, metaplasia, atrophy, etc.

The quality of the gastric biopsies from which the imprints were made was not adversely affected, and proper histological examination could be done, validating previous studies [5]

Performing imprint smears of gastric biopsy specimens before routine histological processing added no extra procedure or inconvenience to the endoscopist or to the patient. Comparing imprint smears and matching histology was ideal in eliminating sample bias. Staining the imprint slides with Diff-Quik stain is a simple and rapid procedure for identifying helicobacter pylori with a turnaround time is about 15-20 mins when compared to routine histopathological examination which takes about 3-5 days, thereby providing an advantage for the clinician and the patient, by immediate initiation of therapy for the patient on the same day of visit

Conclusion

From this study, we conclude that gastric imprint smears stained with Diff Quik stain is a simple, rapid and inexpensive test and can rapidly and accurately diagnose *H pylori* infection, thereby early initiation of treatment and reduced patient discomfort, in resource poor settings. Maximum sensitivity can be achieved when combined with histological examination.

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