Imprint cytology: a simple, cost effectiveness analysis for diagnosing Helicobacter pylori, in west of Iran

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Abstract

Background: This study was to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) and agreement between two methods of the stained gastric imprint cytology smears and stained gastric specimen biopsy mucosal methods for detection of H. pylori.

Methods: Air-dried imprint smears of gastric biopsies from 330 patients were stained by the Grunwald-Giemsa method in the endoscopy suite and examined for H. pylori, providing results within minutes. The grade of H pylori infection was documented. The same biopsy was processed and stained with H&E and Grunwald-Giemsa stains, and reviewed by two different pathologists blind to the imprint cytology results.

Results: Ninety-four of the 238 patients were male with a mean age of 46 (±16.4) years. Based on histology, the H. pylori prevalence was very high at 77.87% and according to cytology H.Pylori prevalence was high at 75.45% in this region our country. The sensitivity and specificity of imprint cytology in the detection of H. pylori were 96.88% and 90.12%, respectively. The PPV and NPV were 96.88% and 90.12%, respectively. The agreement between two diagnostic methods was 95.26% which confirms reliability of imprint cytology method for ion of H.pylori detection.

Conclusion: Gastric imprint smears stained with Grunwald-Giemsa method is a rapid and cost effective method in addition to histology for detecting H. pylori in patients undergoing upper gastrointestinal endoscopy and biopsy. It does not require any additional biopsy.

Keywords: Imprint cytology, H. pylori, Diagnosis, Endoscopy, Iran.

Introduction

Since Helicobacter pylori (H. pylori) infection was accepted as an important pathogen in the upper gastrointestinal tract, this microorganism has been sought with increasing frequency in patients undergoing upper gastrointestinal endoscopy (1-5).Two or more tests are often used to improve diagnostic accuracy, but such a strategy results in increasing cost (6-8). This study was designed to evaluate the valve of imprint cytology (IC) in the diagnosis of H. pylori infection. The results of IC were compared with those of histology.

Helicobacter pylori colonization of the gastric mucosa is associated with the pathogenesis of gastritis, peptic ulcer disease, and gastric malignancy. As eradication of this organism has become part of clinical practice, much research has been done in assessing the sensitivity and reliability of the available diagnostic methods for the detection of this organism (9). Although culturing organism is the gold standard, it lacks sensitivity, and is technically difficult and costly. Urease tests and histological examination of gastric specimens are frequently used methods in our country, while histology is the only method employed in our in-

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stitution with a turnaround time of several days. Cytology of gastric brushings and imprint smears have been described as reliable methods for detection of H.Pylori.

The Grunwald-Giemsa staining method is even more rapid, cheap, and can be performed in the endoscopy suited for with the identification of H.pylori within minutes.

The aim of this study was to determine the sensitivity, specificity, positive and negative predictive values of Grunwald-Giemsa stained gastric imprint smears for the detection of H. pylori in comparison with histology of the same biopsy used for imprint. It was also hoped that its usefulness, as a diagnostic method, could be varified in the west of Iran, an area with a high prevalence of H. pylori infection. The agreement of two methods or detection of H.pylori was also explored.

Methods

Three hundred thirty patients with dyspepsia (238 men and 92 women, with mean age of 46 years, and range 18-67 years) attending for upper gastrointestinal endoscopy were recruited for the study. Eligibility criteria were consisted of absence of upper gastrointestinal malignancy, no prior gastric surgery, and no consumption of antibiotics, bismuth or alcohol preparations within four weeks of endoscopy.

During the endoscopy, two antral biopsy specimens were taken from the antral mucosa with 3-4 cm proximal to the pylorus. The imprint was prepared by keeping the first biopsy sample on a glass slide and pressing it lightly. Imprints were air-dried and then stained by Grunwald-Giemsa method. The imprinted and second biopsy specimens were fixed in 10% formalin, thereafter 4 μ paraffin embedded sections were prepared and stained with hematoxylin and eosin for tissue study and a modified Giemsa stain for detecting H. pylori.

Two pathologists evaluated the IC and histology were not aware of the clinical diagnosis of the patients. Histologic evaluation was carried out according to the Syd-

ney system (9). Increase in lymphocytes and plasma cells in the lamina propria characterized the gastritis as chronic and activity in the context of chronic gastritis referred to the density of neutrophil leukocytes in the lamina propria, gastric pits, and surface epithelium. The density of H. pylori was graded. The presence of any H. pylori was considered as evidence for infection.

Statistical analysis

Statistical evaluation was made using SPSS v. 18.0. Data were shown as frequency (percentage) or mean±SD. Agreement between outcomes of different tests was defined by determining Kappa coefficient. The agreement considered high when the kappa coefficient was >0.5. This agreement was considered intermediate when the kappa coefficient was between 0.3 and 0.5. The p values less than 0.05 were regarded as significant. Sensitivity, specificity, PPV, NPV and accuracy were calculated by the following formulas:

$$\text{Sensitivity} = \frac{\text{True positive}}{(\text{True positive} + \text{False negative})}$$

$$\text{Specificity} = \frac{\text{True negative}}{(\text{True negative} + \text{False positive})}$$

$$\text{PPV} = \frac{\text{True positive}}{\text{Positive cases}}$$

$$\text{NPV} = \frac{\text{True negative}}{\text{Negative cases}}$$

$$\text{Accuracy} = \frac{(\text{True positive} + \text{True negative})}{\text{Total cases}}$$

Results

Of the 330 patients studied, 249(75.45%) showed presence of H. pylori by imprint method (Fig. 1). The H. pylori infection was found by histological examination of biopsy specimens of 257(77.87%) of these 330 patients. 73(22.12%) patients negative for H. pylori by imprint method, were also negative by histopathology. There were 8 false negative cases on imprint cytology. There is no positive case on imprint smear,

that was initially identified as negative on histology.

The density of H.pylori was graded on histology as: 29.96%(77/257) grade I, 37.87%(125/257) grdae II, and 16.66% (55/257) grade III. The agreement of two methods on grading of H.pylori density were: 92.20% grade I, 98.40% grade II, 100% grade III which indicated an increase in agreement power between imprint smears and histology methods with increasing of the density of H.pylori infection. Results of the IC and histology for the diagnosis of H. pylori infection are depicted in Table 1.

The time to carry out the two tests and their cost were also calculated. Sensitivity of histology (100%) was significantly higher than that of the IC (96.88%; p<0.001), Specificity of histology (100%) was significantly higher than that of the IC (90.12%; p<0.003), PPV of histology (100%) was significantly higher than that of the IC (96.88%; p<0.001), and the NPV of histology (100%) was significantly higher than that of the IC (90.12%; p<0.003). Therefore, both the imprinted specimens and directly processed specimens were used for tissue study. The results are shown in Table 2. The agreement between two diagnostic methods considered high when the kappa coefficient was >0.5.

Discussion

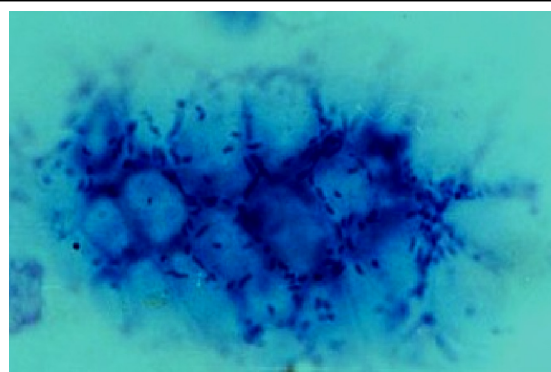Four tests were used to detect H. pylori (10-12):

Fig.1. Imprint cytology showing presence of H.pylori (Giemsa stain, x400)

Blood antibody test checks to see whether your body has made antibodies to H. pylori bacteria.

Urea breath test checks H. pylori bacteria in your stomach. This test can show if you have an *H. pylori* infection.

Stool antigen test checks substances that trigger the immune system to fight an H. pylori infection are present in your feces (stool).

Stomach biopsy. A small sample is taken from the lining of your stomach and small intestine during an endoscopy.

The increasing use of two or more diagnostic methods for detecting H. pylori infection is adding significant cost to the care of patients. Histology is a reliable method for diagnosing H. pylori infection and can also yield information about mucosal structure, but is relatively expensive (10-14). A second inexpensive test is Brush cytology which is rapid with high sensitivity and specificity (15-17). However, brushing may some-

Table1. Comparison of gastric imprint smear to matching histology in H. pylori detection based on the graded density of organism.

Imprint Cytology	Histology			
	Grade I	Grade II	Grade III	Total
Positive	71	123	55	249
Negative	6	2	0	8
Total	77	125	55	257

Table 2. Comparison of gastric imprint smear to matching histology in H. pylori detection.

Imprint Cytology	*Histology*		
	Positive	Negative	Total
Positive	249	0	249
Negative	8	73	81
Total	257	73	330

times be improperly carried out, resulting in samples unsuitable for cytological examination (7,16,17). Rey et al (18) reported that brushing and imprint cytology yield the same sensitivity for identifying H. pylori and that the latter is easier to perform and overcomes most problems of the former. Touch cytology (biopsy sample is firmly pressed and rolled on a glass) and rapid urease test are also inexpensive, rapid methods, with high sensitivity and specificity (7, 19,20).

Touch imprints were common practice, while the stain used included Grunwald-Giemsa, We decided that performing imprint smears of gastric biopsy specimens before routine histological processing added no extra procedure or inconvenience to the endoscopist or patient. Comparing imprint smears and matching histology was ideal in liminating sample bias.

Imprint smears provided good cellularity and one smear per patient was adequate. Epithelial and inflammatory cells, as well as H. pylori, were easily visualized with the Grunwald-Giemsa stain. Other bacteria often present in de-acidified stomachs may be confused with H. pylori. However, the characteristic morphology of H. pylori can be readily made at 400x magnification. Noetheless, both touch cytology and rapid urease test require additional biopsies.

The Grunwald-Giemsa staining procedure is simple and rapid, requiring no additional staff besides the pathologist who stained and interpreted the imprint smears. The turnaround time was an average of 10 minutes, compared with three to five days for a histological report. This provides a tremendous advantage, since therapy can be commenced before the patient leaves the endoscopy suite on the same day.

Misra et al (21) report that IC has a sensitivity and specificity equal to that of histology (100%), which was taken as the gold standard. Ninety three patients were included in Nazligil et al study and Nazligil reports that IC has sensitivity and specificity of 93.7%, 92.3%, respectively (22). In our study, the sensitivity and specificity in 330 patients of IC were lower than histology

(96.88%, 90.12%). Sentürk et al (23) reported that the sensitivies of histology, brushing cytology and IC were 88.24%, 85.88% 85, 88%, respectively in their study of five methods. In another study, Misra and colleagues (23) reported that H. pylori positivity of imprinted and directly used specimens were the same, and that preparing imprint smears did not damage the biopsy specimens for subsequent histologic examination. We also found that preparing imprint smears did not damage the biopsy specimens for subsequent histologic examination. But the agreement between the specimens in tissue diagnosis was relatively underestimated (95.26%) because of patchy distributions of microorganism and inflammation. The patchy distribution can be overcome by multiple biopsies taken from each patient (20.24,25). In present study, the H. pylori infection prevalence rate of 77.87% among an endoscoped population considered unusually high. The predominant Iranian population in this part of the country had higher the prevalence rates. However, with the higher index of suspicion of H. pylori infection among Iranian people, imprint cytology may prove to be of great value since infected patients can begin therapy immediately.

Conclusion
Air-dried , Giemsa stained gastric imprint smears method provide a simple, fast , and reliable method for the detection of H. pylori, with the enormous advantage of immediate commencement of eradication therapy. This technique can achieve maximum sensitivity with useful information when combined with histological examination, especially in our ethnic groups that have a higher prevalence of the infection. However, we offer gastric imprint smear preparation before histologic study for each patient to reach the best and prompt therapy and leads to faster recovery of patient who suffers from clinical gastric problems because of H. pylori infection.

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