

Histologic Demonstration Of Helicobacter Pylori In Gastric Biopsies: Which Is The Best Staining Method?

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Citation

F Kacar, N Çulhac?, V Yükselen, ? Meteo?lu, E Dikicio?lu, E Levi. *Histologic Demonstration Of Helicobacter Pylori In Gastric Biopsies: Which Is The Best Staining Method?*. The Internet Journal of Pathology. 2003 Volume 3 Number 1.

Abstract

Aims: We have investigated the specificity and sensitivity of different histochemical and immunohistochemical methods for the detection of Helicobacter pylori (HP) on tissue sections of gastric biopsies. In addition, interobserver agreement of different staining methods was also evaluated in order to define the most reliable, the cheapest and most easily applicable method for the detection of HP.

Methods: In this study 60 cases of HP positive and 10 HP negative cases were selected based on the results of urease test, C urease breath test and histopathologic examination of the tissue sections. Histopathologic examination was performed by Hematoxylin-Eosin (H&E), Toluidine Blue (TB), modified Giemsa (G) and HP immunohistochemistry. The sections were evaluated by two blinded pathologists. The interobserver agreement of the two pathologists was analyzed by Kappa (κ) statistics.

Results: The agreement between two observers was κ: 0.772 (96%) for HP immunostain; κ: 0.752 (92%) for modified Giemsa stain; κ: 0.487 (76%) for TB; and κ: 0.477 (80%) for H&E stain. The sensitivity and specificities for the stains were as follows: HP immunostain; 100% /100%, Giemsa stain; 97 %/90%, TB stain; 73%/90%, H&E; 97%/80%.

Conclusion: HP can be detected on tissue sections regardless of the stain performed. However, the best results are obtained by the immunohistochemical stains and the modified Giemsa stain. The costs, applicability and the reliability of the Giemsa stain make it a perfect candidate as an adjunct to diagnosis presence of HP on gastric biopsies.

INTRODUCTION

The existence of bacteria colonizing the gastric mucosa has been recognized for a long time. In the last years there have been numerous publications revealing the role of HP in the pathogenesis of gastric carcinomas, gastric M.A.L.T. lymphomas and peptic ulcer disease (1,2,3,4,5,6,7,8,9,10,11). HP infection was classified by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) in 1994 as a group-1 carcinogen in humans. This conclusion was based mainly on epidemiological findings (10,11). HP is one of the most prevalent infections in the world. The incidence of HP in healthy people in their 3rd decades is 10% while the incidence of HP rises to over 60% among people in their 6th decades (1,2,3,4,5,6,7). Gastric cancer is a multifactorial disease, where environmental, genetic factors and HP interact in a complex way (3,4,7,8,10,11). Only a minority of HP-infected individuals develops gastric cancer. Furthermore, it has been shown that this bacterium is not directly mutagenic in an Ames test. Then, it has been proposed that HP may act as a tumor promoter by changing

proliferation and apoptosis of the gastric epithelium.

Spontaneous eradication of HP has not been reported.

HP is usually localized in the apical portion of the foveolar glands and does not penetrate the gland cytoplasm. HP colonization usually triggers an inflammatory reaction in the lamina propria. This process eventually leads to loss of mucin and atrophy of the glands (6,8). Urease activity of the bacteria leads to production of ammonia that is cytotoxic to the mucosa. HP is also known to produce extracellular toxins. In summary, HP metabolic products possibly cause transformation of the mucosa while the immune response is thought to play a role in the carcinogenesis associated with HP (8,13,14,15).

Considering the role played by HP in the various processes mentioned above, it is critical to establish diagnostic tests which are both sensitive and specific enough to enable detection of HP. Currently there are several serologic, biochemical, molecular and histochemical methods available such as rapid urease test, C-urease breath test and

histochemical and immunohistochemical stains for tissue sections (16,17,18,19). Histopathological detection of HP is accepted as a reliable and reproducible method. In order to detect HP on tissue sections, several cytochemical stains have been tested, such as modified Giemsa, Warthin Starry, Gimenez, Genta and immunohistochemical stains. HP immunohistochemistry is accepted as the gold standard for the detection of HP in tissue sections in articles (3,16,20). There are few studies performed for comparison of the various staining methods that have been used for the detection of HP (16).

In this study, we compare the sensitivity and specificity as well as the interobserver agreement of different staining methods for the detection of HP in gastric biopsies.

MATERIALS AND METHODS

Biopsies were obtained from patients who had endoscopic evaluations for gastrointestinal complaints. HP status had been determined by urease test, C-urea breath test in addition to histopathologic examination (H&E, toluidine blue, modified Giemsa, HP immunohistochemistry). Seventy antral biopsies were selected. Biopsy materials were fixed in buffered formalin, embedded in paraffin and 5 m sections were obtained. The sections were stained with Hematoxylin&Eosin (H&E), toluidine blue (TB), modified Giemsa for histopathologic examination. HP immunohistochemistry was performed by utilizing the Avidin-biotin complex method (Dako, Denmark). The polyclonal HP antibody was used a dilution of 1:10. The stained sections were evaluated by two blinded pathologists independently. Five biopsies were excluded because they were too small to perform histochemical or immunohistochemical techniques.

HP infection was considered positive when two or three tests (urease, C-urease test, and histopathologic observation) were positive. Sixty cases were labeled as positive while 10 cases were considered to be negative. Once the cases were selected, they were mixed and coded without additional information to avoid a bias during the evaluation process. Two blinded pathologists independently evaluated the cases for the presence of HP and sensitivity and specificity for the different staining methods. Each stain was evaluated separately without the knowledge of the staining results for the other set of stains. The agreement between the pathologists regarding the interpretation of HP staining was calculated for each stain using Kappa statistics. Kappa statistics analysis was performed according to Landis and

Koch (21). SPSS statistics package software was used.

RESULTS

Among the 70 cases included in the study, 60 cases tested positive for rapid urease test, C-urea breath test and immunohistochemistry. 10 cases were considered negative since one of the three tests was negative for HP. The mean age was 48, with 41 males and 29 females. Among the 60 HP positive cases, 58 were positive with modified Giemsa, 58 with H&E, and 46 with TB. Interobserver agreement was good for immunohistochemical staining and modified Giemsa, while for H&E and TB agreement was fair. The sensitivity and specificity for each stain were as follows: Immunohistochemistry, 100%/ 100%; modified Giemsa, 97%/ 90%; TB, 73%/ 90%, and H&E, 97%/ 80% (Table I). For one case immunohistochemistry was reported as negative by one pathologist while the other reported it as positive. This case was then reviewed by both pathologists simultaneously and classified as positive.

Figure 1

Table 1: Comparison of the statistical values, cost and staining times for the methods

	Interobserver Agreement	κ	Interpretation	Cost	Staining time	Sensitivity	Specificity
Immuno.	96%	0.772	Good	Expensive	3.5 h	100%	100%
M. Giemsa	92%	0.752	Good	Cheap	15 m	97%	90%
TB	76%	0.487	Moderate	Cheap	4 m	73%	90%
H&E	80%	0.477	Moderate	Cheap	20 m	97%	80%

Figure 2

Figure 1: Demonstration of Helicobacter pylori by immunostain. HP immunostain x1000 (High power view, immersion)

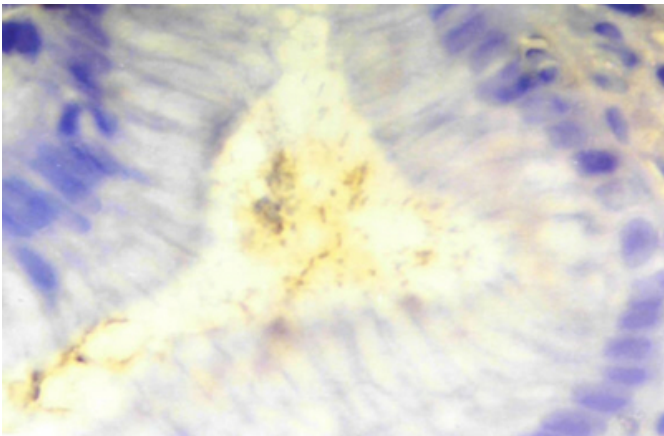


Figure 3

Figure 2: Demonstration of Helicobacter pylori by modified Giemsa stain. M. Giemsa x1000 (High power view, immersion)

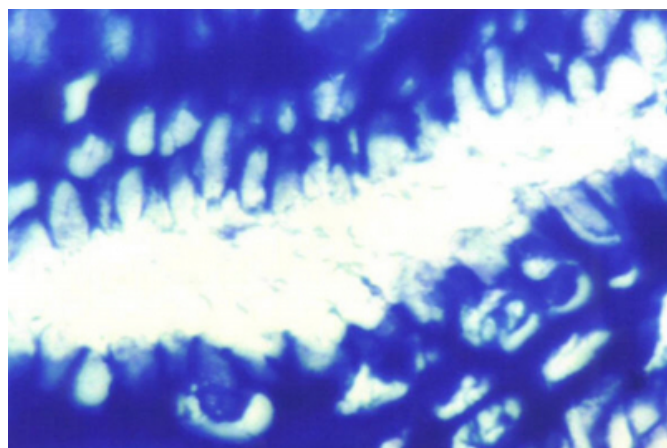


Figure 4

Figure 3: Demonstration of Helicobacter pylori by Hematoxylin & Eosin stain. H&E x1000 (High power view, immersion)

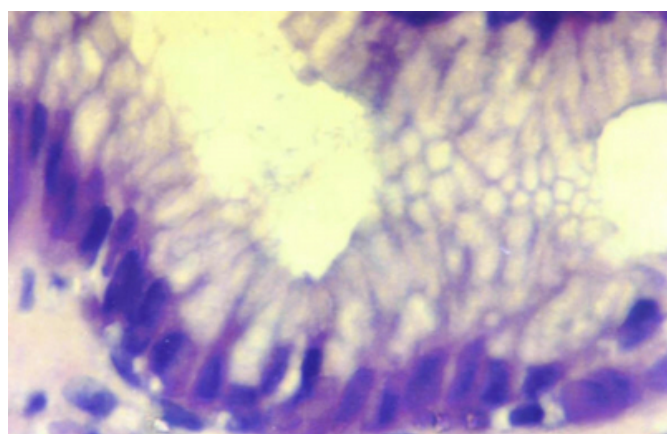
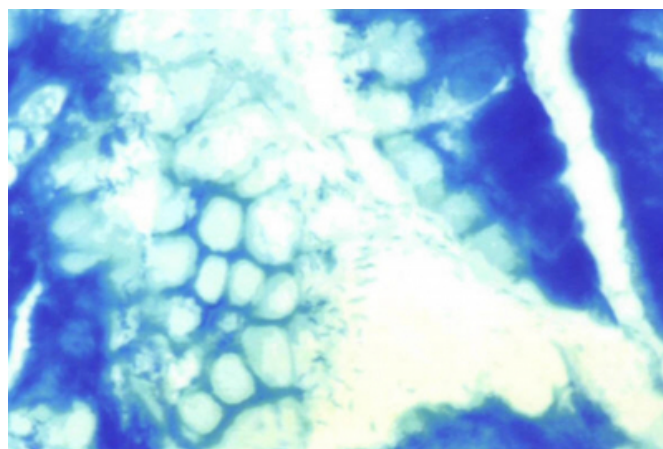


Figure 5

Figure 4: Demonstration of Helicobacter pylori by Toluidine blue stain. TB x1000 (High power view, immersion)



DISCUSSION

Following the studies revealing the important role played by HP in the pathogenesis of gastric carcinomas and lymphomas, there was an increased interest in the correct identification of HP in the tissue sections of gastric biopsies. There are several tests available for the diagnosis of HP colonization such as rapid urease test, breath test, cultures, serological tests and histopathological methods (^{13,16,17,18,19}). Culturing HP is a tedious and time consuming process therefore it has been abandoned in most labs (^{15,16}). Urease test is a rapid but relatively insensitive method. This test relies on the colorimetric detection of pH changes caused by CO₂ and ammonium ions produced by the bacteria. False negative results are common especially when the bacterial load is low. Urease breath test is an expensive test that has a relatively low sensitivity due to similar reasons. Serological identification of anti-HP antibodies is a non-invasive method. However, the antibody titers preserve their levels even after the eradication of the bacteria by antibacterial therapy. PCR methods have also been used for the detection and identification of HP bacteria. This method is expensive and requires technical support (^{16,17,18,19}).

In this study, the histochemical stains that are readily available in routine histology laboratories were analyzed and compared for their sensitivity, specificity and interobserver agreement. H&E stain is routinely performed for the evaluation of gastric biopsies, which makes it cost effective to use. However, sensitivity of the H&E stain is low probably due to the lack of contrast between the bacteria and the surrounding tissues. The specificity of the H&E is also low due to its non-specific staining of the non-HP bacteria resident in the stomach (¹⁶). Another drawback is the low

Kappa value for interobserver agreement seen in our study. We believe that H&E in combination with a special stain for the bacteria may be a cost effective way of demonstrating bacteria.

The TB stain was considered to be not so reliable stain for the detection of HP organisms due to its low sensitivity and specificity.

HP immunohistochemistry is an expensive and time-consuming technique with procedure length ranging from 1 hour to 24 hours. Obviously, since HP organisms can be easily identified in the immunoslides, therefore sensitivity and specificity are high.

Modified Giemsa is a cheap, easily applicable stain that can be performed in 15 minutes. The results are reliable and the sensitivity and specificity values are acceptable. The lack of contrast is a disadvantage of the Giemsa technique but careful observation should allow identifying the organisms (16,17).

TB stain is cheap and easily applicable with an average hands on time of 4 minutes, however its sensitivity and specificity in the current study were not as good as the other stains. In addition, interobserver agreement was relatively low (≈ 0.47).

In summary, HP immunohistochemistry had the highest sensitivity and specificity with high interobserver agreement. However, due to its cost and the hands-on time required we think that Giemsa stain is the best stain for the detection of HP due to its low cost, short hands on time required for staining and very high sensitivity and specificity combined with a high interobserver agreement.

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