Paper

Evaluation of histology as a Helicobacter pylori detection method and analysis of associated problems

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Abstract

Helicobacter pylori is regarded as a common cause of gastritis and peptic ulcer disease. The most commonly used H. pylori detection method in Sri Lanka is histology. However, the detection rate of H. pylori in routine histology practice is low. Therefore, we conducted the following study to evaluate the diagnostic efficacy of histology and to analyze the possible problems associated with H. pylori detection. Multiple endoscopic gastric biopsies were obtained from a sample of 205 patients detected to have endoscopic gastric erythema or ulcers. Biopsies were formalin fixed and paraffin embedded and stained with haematoxylin and eosin, toluidine blue and immunohistochemistry. Serum was collected for screening of anti H. pylori antibodies using an immunochromatography based kit method. Diagnostic efficacy of histology was evaluated against immunohistochemistry as the gold standard. Inter observer variation among four pathologists was assessed using the intraclass correlation coefficient. Haematoxylin and eosin showed a sensitivity of 100% and specificity of 99% and toluidine blue had 100% sensitivity and 98.5% specificity. Average measures of intra class correlation coefficient for H&E was 0.428 (95% CI 0.228 – 0.588) and for toluidine blue stain 0.320 (95% CI 0.085 – 0.513). The sero prevalence of anti – H. pylori antibodies was 4.9%. In conclusion, sensitivity, specificity and negative predictive values of histology in detecting H. pylori are shown to be high. Main limitations were, low positive predictive value and unsatisfactory interobserver agreement. Sampling errors and exposure to antibiotics appeared to be an unlikely cause of the low detection rate with histology.

Key words: Helicobacter pylori, diagnosis, diagnostic efficacy, histology,

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Introduction

Helicobacter pylori is a spiral shaped bacterium which resides beneath the mucous layer of the gastric mucosa often adherent to the surface epithelium.[1,2] H. pylori has been reported to have a strong aetiological relationship with chronic gastritis, peptic ulcer disease, gastric carcinoma and lymphoma[1 -3] A high prevalence of H. pylori infection is observed among the developing countries and East Asian countries.[4] The H. pylori prevalence in the South Asian region, such as India and Bangladesh, has also been reported to be relatively high.[4.5]

No single test has proven to be ideal for H. pylori detection. Commonly available test methods, which require endoscopic biopsies, include histology, rapid urease tests such as CLO test, culture and polymerase chain reaction (PCR). Serology, urea breath test and stool antigen test are minimally invasive test methods. Of these, urea breath test has been recognised as the best method.[6,7] Histology, haematoxylin and eosin stain combined with a special stain for H. pylori (Giemsa or toluidine blue), is the most commonly used detection method for H. pylori in Sri Lanka. Often, this is the only method available in the government hospitals. However, apart from one study, the general experience of pathologists in Sri Lanka is that H. pylori detection rate by histology is low.[8,9] The possible factors that can affect the H. pylori detection with histology include expertise of the pathologist and the density and distribution of H. pylori in the gastric mucosa. Exposure to antibiotics, even for other reasons can reduce the bacterial density giving rise to false negative results. Furthermore, long term use of proton pump inhibitors are known to promote migration of the organisms to the body region giving rise to false negative results with biopsies taken only from the antrum.[10] Coccoid form of H. pylori has been described as an adaptive morphological transformation of the organism in a less favourable environment.[11,12] In histology, the pathologist depends on the spiral shape morphology and their distribution pattern for identification of H. pylori. Therefore, a high prevalence of coccoid forms could lead to a false negative result on histology.

Accordingly, we conducted the following study to evaluate the diagnostic efficacy of histology in detecting H. pylori infection, taking immunohistochemistry as the gold standard. The other contributory factors that can give rise to false negative results such as sampling errors, prior exposure to antibiotics, prevalence of coccoid forms and inter -observer variability among pathologists were also evaluated.

Materials and methods

Two hundred and five consecutive patients with dyspeptic symptoms who underwent upper gastro-intestinal endoscopy (UGIE) and detected to have "endoscopic inflammation" (presence of mucosal erythema, erosions or ulcers) at the Teaching Hospital Peradeniya were recruited for the study. All patients underwent a standardised biopsy protocol which included multiple biopsies from the lesion, antrum, incisura angularis and the body. The reason for sampling the entire stomach was to assess the degree of sampling error when only the lesion is biopsied. Biopsies were collected in 10% formaldehyde. None of the patients were treated with H. pylori eradication therapy for the past two years.

All biopsies were formalin-fixed paraffin embedded and stained with Harris's Haematoxylin and eosin (H&E) stain and toluidine blue stain. Immunohistochemical staining was performed using indirect immunoperoxidase method with anti H. pylori antibodies. (DAKO B0471) on formalin-fixed paraffin embedded tissue sections.

Venous blood was obtained from all patients to detect the presence of serum anti H. pylori antibodies. Immunochromatography based qualitative commercially available kit device (SD BIOLINE. H. pylori test device/ multi-device) was used for this purpose. This method collectively detects all types of anti H. pylori antibodies. According to the manufacturer, the method has a high sensitivity (95.5%) and relatively low specificity (89.6%). Therefore, the test was used to screen for the presence of anti H. pylori antibodies to assess the exposure rate to the H. pylori in the study population.

The diagnostic efficacy of histology was evaluated in terms of sensitivity, specificity and positive and negative predictive values using immunohistochemistry as the gold standard. Histology results of an investigator who had undergone a special training in gastrointestinal pathology were used for this purpose.

H&E and toluidine blue stained sections were assessed for the presence or absence of H. pylori organisms by three independent pathologists and a trainee pathologist to assess the degree of inter-observer variation. The inter-observer variation of the histological results were analysed using the intra-class correlation coefficient. The confidence interval was set at 95% for all statistical methods used.

Results

There were 5 cases positive for H. pylori with H&E stain, 6 with toluidine blue stain and 3 with immunohistochemistry. Six cases were positive on histology (H&E and toluidine blue combined). All the cases detected with

immunohistochemistry were spiral shaped organisms and there were no coccoid forms.

Evaluation of haematoxylin and eosin stain

Results of H&E stain against immunohistochemistry are given in Table 1. Accordingly, the H&E stain had 100% sensitivity (95% CI, 0.3 – 1); 99% specificity (95% CI, 0.96 – 0.99); 60% positive predictive value (PPV) (95% CI, 0.15 -0.93), and 100% negative predictive value (NPV) (95% CI, 0.98 – 1).

Table 1. Comparison of H. pylori detection rate with haematoxylin and eosin stain with the gold standard test (immunohistochemistry).

	H. pylori positive (IHC)	H. pylori negative (IHC)	Total
H&E positive	3	2	5
H&E negative	0	200	200
Total	3	202	205

IHC, immunohistochemistry; H&E, haematoxylin and eosin

Evaluation of of H.pylori by toluidine blue stain

Results of toluidine blue stain against the gold standard method are given in Table 2. The toluidine blue stain had 100% sensitivity (95% CI, 0.3 – 1); 98.5% specificity (95% CI, 0.96 – 0.99); 50% positive predictive value (PPV) (95% CI 0.12 -0.88), and 100% negative predictive value (NPV) (95% CI, 0.98 – 1).

Table 2. Comparison of H. pylori detection rate with toluidine blue stain results with immunohistochemistry.

	H. pylori positive (IHC)	H. pylori negative (IHC)	Total
Toluidine blue positive	3	3	6
Toluidine blue negative	0	199	199
Total	3	202	205

IHC- immunohistochemistry

Assessment of possible causes of false negative results with histology

All H. pylori positive cases had positive results with histology and immunohistochemistry in the lesional biopsies. Inclusion of additional biopsies from the incisura and body did not increase the detection rate. Serology for anti H. pylori antibodies was positive in 10 /205 (4.9%). Table 3 provides the detection rate of H. pylori by the four observers. Intra class correlation coefficient (ICC) for H&E stain, for single measures was 0.157 (95% CI 0.069 – 0.23) and average measures was 0.428 (95% CI 0.228 – 0.588). The ICC for toluidine blue stain for single measures was 0.105 (95% CI 0.023 – 0.208) and average measures was 0.320 (95% CI 0.085 – 0.513). The inter observer correlation in detecting H. pylori infection with H & E and toluidine blue stain were not satisfactory among the participants. The interobserver agreement is considered best when it is ≥ 0.8.

Table 3. H. pylori detection rates with haematoxylin and eosin and toluidine blue by four observers

Observer	H&E	Toluidine blue
A	12	21
B	3	4
C	22	38
D	5	6

Discussion

The most commonly used H. pylori diagnostic test in Sri Lanka is histology, which includes the H & E stain combined with a special stain such as Giemsa or toluidine blue when necessary. Evaluation of the diagnostic efficacy against immunohistochemistry showed a sensitivity of 100% and a specificity of 99% and 98% for H & E and toluidine respectively. The negative predictive value of 100% with both H&E and toluidine blue is possibly due to the absence of false negative results. These high efficacy parameters indicate that the low H. pylori detection rates with histology observed in Sri Lanka is less likely to be due to false negative values and is possibly due to the low H. pylori prevalence rate as indicated by immunohistochemistry (1.5%) and the low sero prevalnce of anti H. pylori antibodies (4.9%). In fact, the main shortcoming of histology was the low positive predictive value (60% for H&E and 50% for toluidine blue) was due to the presence of false positive results. Compared to immunohistochemistry, H. pylori detection by all investigators were high (Table 3). The predictive values of tests are known to be affected by the disease prevalence; hence the low positive predictive value in histology may be attributed to the low prevalence of H. pylori in the sample.

The main shortcoming of histology is unsatisfactory interobserver agreement, which was more prominent in the interpretation of the toluidine blue stain (Table 3). Misidentification of thick straight bacilli, which are often contaminants of coliforms from ingested meat and debris, stain positive with toluidine blue and Geimsa stains causing a false positive result on histology. A correct diagnosis of H. pylori by histology depends on appreciation of the correct morphology and the distribution of the organism (Slender, spiral bacilli within the mucus layer on the surface epithelial cells).

However, one limitation of the study was the participation of only four observers to assess the interobserver agreement. Disagreement with even one participant can significantly affect the result when the numbers of participants are low.

Immunohistochemistry did not demonstrate any coccoid forms of H. pylori., which excluded the possibility of a high prevalence of coccoid forms giving rise to the low detection rate with histology. Furthermore, immunohistochemistry is an expensive testing method and considering the reasonable diagnostic efficacy of H&E and toluidine blue, inclusion of immunohistochemistry may not be cost effective in routine practice.

Inclusion of additional biopsies from the stomach did not increase the H. pylori detection rate indicating that sampling errors are unlikely to be responsible for the low detection rate.

The low sero-prevalence (4.9%) of anti H. pylori antibodies indicated that the exposure rate of this study population to the bacterium was low. The test method we used is a sensitive method which collectively detects all types of anti H. pylori antibodies including IgG which indicate current as well as past infection. Therefore, the low detection rate by histology cannot be attributed to a low H. pylori density due to prior exposure to antibiotics.

Conclusions

The sensitivity, specificity and negative predictive value of haematoxylin and eosin and toluidine blue stain in detecting H. pylori were shown to be high. Main limitations were, the low positive predictive value and unsatisfactory interobserver agreement. Sampling errors and exposure to antibiotics appeared to be an unlikely cause for the low detection rate by histology.

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References

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1(8390): 1311–15.
2. Warren J. R., Marshall B. J. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet.* 1983; 1(8336):1273–1275.
3. Anon. NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. *Journal of the American Medical Association* 1994; 272:65–69.
4. Alexander C. Ford, Anthony T. R. Axon. Epidemiology of Helicobacter pylori infection and Public Health Implications. *Helicobacter* 2010; 15 (Suppl. 1): 1–6.
5. Janzon A, Bhuiyan T, Lundgren A, Qadri F, Svennerholm A-M, Sjoling A. Presence of high numbers of transcriptionally active Helicobacter pylori in vomitus from Bangladeshi patients suffering from acute gastroenteritis. *Helicobacter* 2009;14: 237–47.
6. Chey W.D., Wong B.C.Y and the Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology Guideline on the Management of Helicobacter pylori Infection. *American Journal of Gastroenterology* 2007; 102:1808–1825.
7. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of Helicobacter pylori infection –the Maastricht IV/Florence Consensus Report. *Gut* 2012;61: 646–64.
8. Waidyarathne E, Mudduwa L, Lekamwasam S, Lekamwasam V, Gunawardhana H. Prevalence of Helicobacter pylori infection determined by histology in patients with different upper gastrointestinal diseases. *Ceylon Medical Journal* 2010; 55: 68 -69.
9. Wijetunge S, Halahakoon VC, Galketiya KB, Kumarasiri PVR, Tennakoon TRDSK, Herath HMDN. Detection of Helicobacter pylori by histology in biopsy specimens of patients with gastric ulcers in a selected group pf patients from teaching Hospital Peradeniya (abstract). In proceedings of Peradeniya University Research Sessions 2011
10. Moayyedi P, Wason C, Peacock R, Walan A, Bardhan K, Axon AT, et.al. Changing patterns of Helicobacter pylori gastritis in long-standing acid suppression. *Helicobacter* 2000; 5:206.
11. Azevedo NF, Almeida C, Cerqueira L, Dias S, Keevil CW, Viei MJ. Coccoid Form of Helicobacter pylori as a Morphological Manifestation of Cell Adaptation to the Environment. *Applied and Environmental Microbiology*. 2007; 73: 3423 – 3427.
12. Chan WY, Hui PK, Leung KM, Chow J, Kwok F, Ng CS. Coccoid forms of Helicobacter pylori in the human stomach. American. *Journal of Clinical Pathology* 1994; 102:503–507.
13. De Silva M Prevalence of Helicobacter pylori infection in patients with functional dyspepsia. *Ceylon Medical Journal* 1999; 44:118-119.
14. Fernando N, Holton J, De Silva VDM, Fernando D. Prevalence of Helicobacter pylori in Sri Lanka as Determined by PCR. J*ournal of Clinical Microbiology* 2002; 40: 2675–2676.
15. Fernando N, Weerasekera D, Fernando S, Liyanage N, Holton J. Helicobacter pylori serology in two MOH areas of the Western Province of Sri Lanka. *The Ceylon Journal of Medical Science* 2003; 46: 35-43.
16. Fernando N, Perera N, Vaira D, Holton J. H. pylori in school children from the Western province of Sri Lanka. *Helicobacte*r 2001; 6; 169-174.