METHYLENE BLUE STAINING OF GASTRIC TISSUE FOR THE IDENTIFICATION OF *HELICOBACTER PYLORI*

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Abstract. This study compared Giemsa (GM) and methylene blue (MB) stains for the diagnosis of *Helicobacter pylori*. Gastric biopsy specimens obtained from January 2001 to December 2005 were reviewed. They were all stained with hematoxylin and eosin, GM and MB stains. The slides were examined on a blinded basis. Direct comparisons were made between the both stains. Two hundred thirty-six cases were studied with a concordance rate of 98.3% (Kappa value = 0.951, $p<0.05$), showing good agreement. MB stain can be substituted for GM stain, and is preferred because it is cost-effective, less time-consuming, less complicated to perform, accurate and widely available. Useful hints to reduce false negativity with MB stain are discussed.

INTRODUCTION

Helicobacter pylori (HP) is an organism associated with a variety of gastric diseases, such as chronic gastritis, gastric ulcer disease , gastric carcinoma and low grade B-cell gastric (MALT type) lymphoma (Covacci and Rappuoli, 2003; Blaser and Atherton, 2004). Antibiotics are used to eradicate the organism. Various methods and stains, both invasive and noninvasive means have been used to detect HP. Noninvasive means, such as the Clo test, saliva IgA and serology have been used (Kullavanijaya *et al*, 2004). Histology is an important and useful invasive test used to detect the organism. To establish the diagnosis on a surgical specimen, the pathologist should identify the organism on gastric biopsy. Although HP is readily seen on hematoxylin and eosin (HE) stained slides of biopsy specimens, for better identification of the organism, special stains have been used, such as Warthin-Starry, Giemsa (GM) and Steiner silver stain (Garvey *et al*, 1985; Kolts *et al*, 1993), Genta stain, toluidine blue stain, carbol fuchsin stain and immunohistochemical studies (Goodwin *et al*, 1997). The sensitivities, specificities and accuracies of each method have had varying results. No clear advantage of one method over the others has been demonstrated (Toulaymat *et al*, 1999; Jhala *et al*, 2002). Some studies found Giemsa preferable to HE stain (Fallone *et al*, 1997; Laine *et al*, 1997). However, GM stain is not easy to perform. MB stain is a simple stain for microbiologic studies (Fischbach, 1996). It is used to diagnose *Neisseria gonorrhoea*. MB was studied because it has a low cost, is easy to perform, and is easily available. It is therefore appropriate for small, rural laboratories. We evaluated the concordance rate between MB stain with GM stain.

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MATERIALS AND METHODS

Gastric biopsy specimens taken January 2001 to December 2005 were obtained from the surgical pathology department of BMA General Hospital. Endoscopic biopsy samples were taken from the gastric antrum and body of patients with complains of abdominal pain

and dyspepsia. Cases with only necrotic ulcer, cancerous tissue or inadequate tissue volume were excluded.

The specimens were processed routinely and stained with hematoxylin and eosin (HE), MB and GM stain. The organisms were identified by individual codes and examined by a single pathologist, blinded to patient identification and results of the other stains. Concordance rate between and individual percentages of the MB and GM stains were obtained and Kappa statistics were performed to assess agreement between the MB and GM stains.

RESULTS

Two hundred fifty-five specimens were enrolled in the study. Nineteen cases were excluded from the study. Ulcerative necrotic tissue was found in 14 cases, cancerous tissue in 3 cases and inadequate tissue volume in two cases. Therefore, only 236 cases were included in this study. HP stain bright blue with MB stain. Both stains (GM and MB) revealed HP in 51 cases and were negative for HP in 181 cases. Four discordant cases were found, two were HP-positive on GM and HP-negative on MB. The other two were HP-negative on GM and HP-positive on MB. This gave a concordance rate of 98.3% and a discordance rate of 1.7%. This reveals good concordance between GM and MB stains (Kappa value = 0.951, $p<0.05$). The results of the MB and GM stains are summarized in Table 1.

Table 1
Summary of HP-positive and -negative cases using GM and MB stains.

		GM	
		Positive	Negative
MB	Positive	51[a] (21.6)[b]	2 (0.85)
	Negative	2 (0.85)	181 (76.7)

a=The number of cases, b=Percentage

DISCUSSION

The adverse consequences of HP infection make diagnosis essential. Histology is an invasive diagnostic tools. Although HP is readily visualized on routine HE stain, it is better identified by special stains. Different stains have been proposed, such as Warthin-Starry, GM, Steiner silver (Garvey *et al*, 1985; Kolts *et al*, 1993), Genta , toluidine blue, carbol fuchsin and immunohistochemical stain (Garvey *et al*, 1985; Kolts *et al*, 1993; Goodwin *et al*, 1997). Some stains can demonstrate both the organism as well as highlighting the metaplastic morphology (El-Zimaity *et al*, 1998). Some pathologists use special stains to detect the organism on biopsy specimens. Each stain has its own advantages and disadvantages in terms of cost-effectiveness, time-consumption, technical level, accuracy and availability. Warthin-Starry stain, for example, can detect HP easily but has a higher cost, is difficult to prepare and is time-consuming. GM stain is easier to perform but has a higher cost. Immunohistochemical studies are reliable but need sophisticated preparation and are costly. In rural areas it is more practical to use stains with lower cost, are easier to perform and readily available. Since MB stain has all those features and as a simple stain for microbiologic studies, it is an appropriate stain for rural use. In this study we compared MB with GM stain for the ability to detect HP. GM has been reported to detect HP better (Fallone *et al*, 1997; Laine *et al*, 1997). Both stains found 51 HP- positive cases and 181 HP-negative cases. There were 4 discordant cases. Thus, the agreement rate are 98.3%. The Kappa value is 0.951 ($p<0.05$), showing good concordance between the two stains.

Many invasive and non-invasive methods have been studied with varying specificities, sensitivities and accuracies. Histology is a

widely used invasive method which can visualize both organisms and gastric morphology. The sensitivities and specificities of histology are satisfactory, ranging from 83-93.5% and 90.4-100%, respectively (Taj *et al*, 2003; Kullavanijaya *et al*, 2004). Laine *et al* (1997) concluded GM stain appears to be the preferred stain for HP diagnosis on the basis of its good sensitivity, excellent specificity, and lack of technical difficulty in preparation compared with HE and Genta stains, along with acceptable sensitivity and specificity of 90% and 98%, respectively. Wabinga (2002) reported similar sensitivity and specificity of GM stain with very good agreement with immunohistochemical studies, however Babic *et al* (2005) found better detection using immunohistochemical studies which may be a result of the low sample size. The varied sensitivities and specificities in the different studies may be due to using different gold standards (Laine *et al*, 1997; Wabinga, 2002; Taj *et al*, 2003; Kullavanijaya *et al*, 2004). El-Zimaity *et al* (1998) proposed a triple stain carbol fuchsin/Alcian blue/hematoxylin-eosin stain to visualize HP and gastric morphology simultaneously and found superior results compared to HE stain. Fallone *et al* (1997) demonstrated HE stain is an inexpensive method for HP detection but with test performance characteristics inferior to Giemsa, Genta, and silver stains. Laine *et al* (1997) suggested GM is the preferred stain. Thus GM stain is a reliable stain for the identification of HP. However, it is difficult to prepare and time-consuming using several chemical substrates. MB stain has similar sensitivity to GM and is easier to prepare, is readily available and has a low cost making it ideal for small or rural laboratories.

Factors which can influence identification of the organism are its density and the experience of the pathologist. Anim *et al* (2000) concluded that HE is adequate for initial assessment but low density of the organism, special stains should be used. Kolts *et al* (1993) stated that experienced pathologists had a better sensitivity in identification of the organism compared to rotating pathologists when using the HE stain. HP colonizes gastric mucosa in a variety of ways: free in the mucus, surface adhesion and intercellularly (Chan *et al*, 1992). If bacteria are well visualized in these areas in combination with MB stain even at low densities of HP, false negatives will be decreased.

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REFERENCES

Anim JT, Al-Sobkie N, Prasad A, John B, Sharma PN, Al-Hamar I. Assessment of different methods for staining *Helicobacter pylori* in endoscopic gastric biopsies. *Acta Histochem* 2000; 102: 129-37.

Babic T, Basic H, Miljkovic B,Kocic B, Tasic G. Detection of *Helicobacter pylori* in gastric biopsy and resection specimens. *Vojnosanit Pregl* 2005; 62: 39-43.

Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: biology and disease. *J Clin Invest* 2004; 113: 321.

Chan WY, Hui PK, Leung KM, Thomas TM. Modes of Helicobacter colonization and gastroepithelial damage. *Histopathology* 1992; 21: 521-8.

Covacci A, Rappuoli R. *Helicobacter pylori* : after the genomes, back to biology. *J Exp Med* 2003; 197: 807.

El-Zimaity HM, Ota H, Scott S, Killen DE, Graham DY. A new triple stain for *Helicobacter pylori* suitable for the autostainer: carbol fuchsin/ Alcian blue/hematoxylin-eosin. *Arch Pathol Lab Med* 1998; 122: 732-6.

Fallone CA, Loo VG, Lough J, Barkun AN. Hema-

toxylin and eosin staining of gastric tissue for the detection of *Helicobacter pylori*. *Helicobacter* 1997; 2: 32-5.

Fischbach FT. Microbiologic studies. In: A manual of laboratory and diagnostic tests. Chap 7. 5th ed. Philadelphia, New York: Lippicott, 1996: 473.

Garvey W, Fathi A, Bigelow F. Modified Steiner for the demonstration of spirochetes. *J Histotechnol* 1985; 8: 15-7.

Goodwin CS, Mendall MM, Northfield TC. *Helicobacter pylori* infection. *Lancet* 1997; 349: 265-9.

Jhala N, Lechago S, Lechago J, Younes M. Is immunostaining for *Helicobacter pylori* superior to the special stain thiazine in detecting small numbers of *H. pylori* in gastric biopsies? *Appl Immunohistochem Mol Morphol* 2002; 10: 82-4.

Kolts BE, Joseph B, Achem SR, Bianchi T, Monteiro C. *Helicobacter pylori* detection. A quality and cost analysis. *Am J Gastroenterol* 1993; 88: 650-5.

Kullavanijaya P, Thong-Ngam D, Hanvivatvong O, Nunthapisud P, Tangkijvanich P, Suwanagool P. Analysis of eight different methods for the detection of *Helicobacter pylori* infection in patients with dyspepsia. *J Gastroenterol Hepatol* 2004; 19: 1392-6.

Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of *Helicobacter pylori*. *Gastrointest Endosc* 1997; 45: 463-7.

Taj Y, Essa F, Kazmi SU, Abdullah E. Sensitivity and specificity of various diagnostic tests in the detection of *Helicobacter pylori*. *J Coll Physicians Surg Pak* 2003; 13: 90-3.

Toulaymat M, Marconi S, Garb J, Otis C, Nash S. Endoscopic biopsy pathology of *Helicobacter pylori* gastritis. Comparison of bacterial detection by immunohistochemistry and Genta stain. *Arch Pathol Lab Med* 1999; 123: 778-81.

Wabinga HR. Comparison of immunohistochemical and modified Giemsa stains for demonstration of *Helicobacter pylori* infection in an African population. *Afr Health Sci* 2002; 2: 52-5.