J Bras Patol Med Lab, v. 51, n. 2, p. 108-112, April 2015

10.5935/1676-2444.20150019

Histological identification of *H. pylori* stained by hematoxylin-eosin and Giemsa: review for quality control

Identificação histológica do H. pylori *corado por hematoxilina-eosina e Giemsa: revisão para controle de qualidade*

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ABSTRACT

Introduction: Several special staining methods are available for *H. pylori* (Hp) identification in histological sections of chronic gastritis (CG), including the routine hematoxylin-eosin (HE) method. Some reports suggest that ancillary stains are not always needed to establish the diagnosis of Hp infection. In addition, the benefit of using them, when biopsies show minimal inflammation, is not clear. **Objective:** We performed a retrospective study to compare the usefulness of HE with Giemsa method for the histopathological diagnosis of Hp in tissue sections. **Methods:** Histological sections from 390 consecutive patients were reviewed. The patients were registered in the histopathology laboratory of Instituto Alfa de Gastroenterologia, Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil. They were divided in 4 groups according to the gastric inflammatory changes as follows: Group I, gastric mucosa with normal morphology or minimal inflammatory changes ($n = 146$); Group II, chronic gastritis (CG) with mild inflammatory activity ($n = 101$); Group III, CG with patent inflammatory activity ($n = 123$); Group IV, patients with atrophic body gastritis ($n = 20$). All histological sections were carefully evaluated by 2 examiners at the oil immersion objective (1000×). **Results:** The identification of Hp was positive by Giemsa and HE, respectively at: Group III, 111 (90.2%) and 93 (75.6%) patients ($p < 0.01$); Group II, 43 (42.6%) and 29 (28.7%) patients ($p < 0.05$). Hp was negative in Groups I and IV. **Conclusion:** The results show that Giemsa stain is superior to HE for histological identification of Hp in CG. Although Hp could be identified by HE stain in the majority of CG cases, a significant number of infected patients may be neglected, regardless the intensity of the inflammatory response.

Key words: chronic gastritis; *Helicobacter pylori*; Giemsa; hematoxylin and eosin; gastric mucosa; stomach.

INTRODUCTION

Infection with *H. pylori* (Hp) is the most common cause of chronic gastritis (CG) and it is associated with the pathogenesis of gastric cancer. Therefore, there is great interest in the detection and eradication of this bacterium. In the laboratories of Pathology, there are several special histological techniques, in addition to the routine hematoxylin-eosin (HE) stains that have been used to detect the presence of Hp in tissue sections. This microorganism can also be identified in histological sections stained by HE but the contrast with the background tissues is usually of lower quality than that observed in special stains. Some laboratories can optimize the HE staining for this purpose, and then make this the method of choice used for the majority of routine cases[1-3]. In special cases of low concentrations of bacteria in gastric mucosa, the ancillary staining would be recommended, such as in gastritis with mild inflammatory activity and in previously treated patients[4, 5]. Furthermore, to obtain more reliable results, others recommend the use of immunohistochemical methods which seems to be more specific and present smaller interobserver variation index[6, 7].

Therefore, it seems to be no consensus among the various reports about the best practice in pathology diagnostics

First submission on 20/01/15; last submission on 27/02/15; accepted for publication on 28/02/15; published on 20/04/15

laboratories for the correct assessment of Hp in histological sections. Thus, the matter is whether the use of ancillary techniques for histological diagnosis of Hp would always be necessary to obtain more reliable results[1, 3]. Furthermore, the application of these special techniques is ultimately based on the interest of each laboratory, which also depends on its technological capabilities, and the presence of an expert gastrointestinal pathologist who could recognize the microorganism more easily in routinely stained sections. Giemsa stain became one of the most worldwide method spread among the special techniques used for this purpose because it is sensitive, cheap, easy to perform, and reproducible[8]. In the present study, we aimed to carefully review endoscopic biopsies of gastric mucosa stained by HE and Giemsa methods, which are routinely performed in the laboratory of gastrointestinal pathology linked to the section of Gastrointestinal Endoscopy of a Brazilian public hospital.

METHODS

Gastric biopsies from 390 consecutive unselected dyspeptic patients performed from December 2013 to March 2014 at the Endoscopy Section of the Instituto Alfa de Gastroenterologia (IAG), Hospital das Clínicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, were analyzed. Only the patients who underwent biopsies of the gastric antrum and body with diagnosis of chronic gastritis or normal gastric mucosa were included in this study. Patients who underwent gastrointestinal surgery or diagnosed with gastrointestinal tumors were excluded.

Histological sections from the antrum and body regions had been previously stained with Harris' hematoxylin-eosin and Giemsa staining methods. Giemsa stain working solution was prepared as follows: 40 ml Giemsa stock solution with 60 ml of distilled water. Giemsa stock solution was prepared as follows: Giemsa powder 4 g, glycerol 250 ml and methanol 250 ml (Giemsa powder was purchased from Sigma-Aldrich, St. Louis, MO, USA). Histological sections were re-examined by a pathologist with expertise in gastrointestinal pathology (A.J.A. Barbosa) to standardize the classification of gastritis. For the diagnosis of Hp infection, the HE and Giemsa stained sections were examined under oil immersion objective (1,000×) by the other two team researchers. The cases in doubt were evaluated by a third examiner specialized in gastrointestinal pathology. The diagnosis of infection was confirmed only in cases of typical morphology of Hp in biopsy samples: it is observed as comma- or S-shaped bacilli (2-4 µm long and 0.5-1 µm thick). In addition, the bacteria were only observed when adhered on cell-surface or free in the mucus layer, with its typical morphology, and forming at least small colonies. When they were found only in isolated forms of Hp-like bacterium the case was considered negative by histology. Likewise, the cases not elucidated were considered negative.

After reviewing all cases, the patients were separated into 4 groups: Group I – patients presenting normal gastric mucosa or gastric mucosa with minimal inflammatory changes; Group II – chronic gastritis with mild inflammatory activity; Group III – chronic gastritis with moderate or severe (patent) inflammatory activity; Group IV – patients presenting atrophic body gastritis. The degree of inflammatory activity was based on the general pattern polymorphonuclear neutrophils (PMNs) infiltrate the lamina propria. Moderate or severe activity indicates presence of numerous PMNs diffuse along the section, frequently invading the epithelium of the glands, and sometimes forming tiny abscesses. Mild activity indicates presence of few PMNs in lamina propria, frequently with regional distribution or just forming inflammatory focus of activity.

Statistical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for comparative analysis. The p value less than or equal to 0.05 was considered significant (x^2 test).

RESULTS

The total number, mean age and gender of studied patients in each group are showed in **Figure**. The histological analysis of each group showed the following results: 146 (37.4%) patients with normal gastric mucosa or with minimal inflammatory changes (Group I); 101 (25.9%) patients presenting chronic gastritis with mild inflammatory activity (Group II); 123 (31.5%) patients presenting chronic gastritis with moderate or severe inflammatory activity (Group III); 20 patients presenting the final diagnosis of atrophic body gastritis (ABG) in whom the result confirmed negative for Hp (Group IV). Patients of the Group IV were considered a special group of Hp negative subjects and were excluded from the main comparative analysis between Giemsa and HE staining. Regardless the presences of different degrees of gastritis activity, the bacteria detected by Giemsa stain were always responsible for the largest number of patients with positive diagnoses of infection: in patients of Group II, respectively, 43 (42.6%) versus 29 (28.7%), and Group III, respectively, 111 (90.2%) versus 93 (76.0%). None of

the patients with normal gastric mucosa or with ABG showed positive diagnosis for Hp infection (**Table 1**).

The sensitivity, specificity, positive predictive value and negative predictive value of HE stained sections in relation to Giemsa staining are showed in **Table 2**.

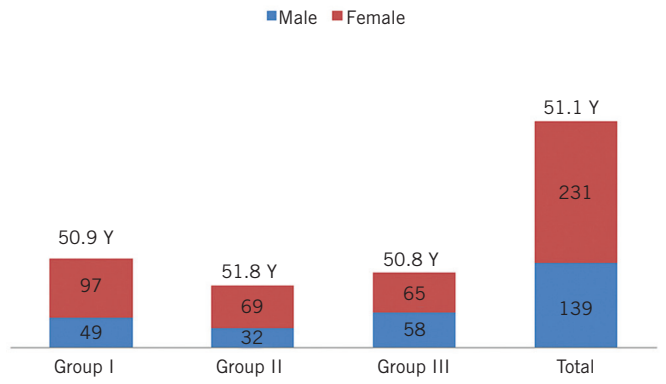

FIGURE – *Total number, mean age (y) and gender (female/male) of patients with normal gastric mucosa (Group I), chronic gastritis with mild inflammatory activity (Group II), and chronic gastritis with moderate/severe inflammatory activity (Group III). The 20 patients with atrophic body gastritis are not represented in the Figure*

TABLE 1 – Distribution of the 390 patients according the histology of gastric mucosa (Groups I-IV), gender, and Hp positive and negative staining results by Giemsa and HE methods

Patients group	*n* (%)	Gender		Giemsa stain		HE stain	
		M	F	Hp+ (%)	Hp- (%)	Hp+ (%)	Hp- (%)
I	146 (37.4)	49	97	0 (0)	146 (100)	0 (0)	146 (100)
II	101 (25.9)	32	69	43* (42.6)	58 (57.4)	29* (28.7)	72 (71.3)
III	123 (31.5)	58	65	111** (90.2)	12 (9.8)	93** (75.6)	30 (24.4)
IV	20 (5.2)	9	11	0 (0)	20 (100)	0 (0)	20 (100)
Total	390 (100)	148	242	154 (39.5)	236 (60.5)	122 (31.3)	268 (68.7)

Patient groups: Group I – normal gastric mucosa; Group II – chronic gastritis with mild inflammatory activity; Group III – chronic gastritis with moderate/severe inflammatory activity; Group IV – atrophic body gastritis (type A gastritis).
* $p < 0.05$; ** $p < 0.01$.
Hp: H. pylori*; HE: hematoxilin-eosin; M: male; F: female; Hp+:* H. pylori positive*; Hp-:* H. pylori *negative.*

TABLE 2 – Sensitivity, specificity, PPV and NPV of HE stain in relation to Giemsa stain for the identification of Hp in chronic gastritis with mild and patent inflammatory activity

Activity of gastritis	Sensitivity	Specificity	PPV	NPV
Mild	0.67*	1	1	0.8*
Moderate/severe	0.83*	1	1	0.4*

PPV: positive predictive value; NPV: negative predictive value; HE: hematoxilin-eosin; Hp: H. pylori.
* $p < 0.05$.

DISCUSSION

In many countries, because of the great availability of the non-invasive methods for the diagnosis of Hp infection, such as serology and urea breath test, which are more rapid and affordable, the histopathological tests have been less performed in these locations. In addition, little difference of the results has been reported between invasive and non-invasive tests for Hp diagnosis, as urea breath test, culture, rapid urease test, and histology. The results of the comparative study among these tests showed little differences in their specificities, whereas the histology using the Giemsa method for staining Hp has showed to be significantly more sensitive than the other special stains[8, 9].

Among the methods first used to demonstrate Hp in tissue sections it should be mention the Warthin-Starry and the Carbol-fuchsin. The first one was used in the early discovery of Hp in gastric mucosa by Marshall and Warren, and the latter one by Rocha and colleagues, who adapted the carbol-fuchsin method, frequently used in the laboratories of bacteriology, for application in tissue sections[10, 11]. The Warthin-Starry and carbol-fuchsin methods gradually were replaced by new techniques, simpler while also effective. Carbol-fuchsin method is inexpensive and easy to perform, and give Hp significant contrast when lying free on the gastric mucus. However, the major disadvantage of this method is to hide the morphology of the gastric mucosa.

The application of immunohistochemistry for Hp identification was first proposed in 1988. Endoscopic biopsies from antral mucosa of dyspeptic patients were used to evaluate Hp culture as gold standard, and by the peroxidase-antiperoxidase (PAP) method applied in histological sections of formaldehyde-fixed biopsy specimens. Comparing cultures results, the sensitivity and PPV of PAP method for Hp identification (at that time, named *Campylobacter pylori*) were respectively, 95% and 100%[12]. In the following years, several immunohistochemical methods have been applied for Hp identification and in general, all of them proved to be highly specific and with low inter-observer variation. However, this methodology have not been recommended for routine because it is expensive, and in most cases of gastritis with patent inflammatory activity, other easier and cheaper methods could have similar levels of accuracy. Nevertheless, the immunohistochemical methods would be reserved for inconclusive cases of gastritis, and cases in which Hp does not induce strong inflammatory reaction, as frequently occur in oxyntic mucosa or in previously treated patients with possible low microorganism density[4, 6, 7, 13].

For some researches, HE stain should be considered adequate only for the initial assessment of the presence of Hp in gastric biopsies of dyspeptic patients[2]. An institutional quality assurance review has compared HE with Giemsa, Warthin-Starry, and with immunohistochemical staining methods for identifying Hp infection in tissue sections. HE sections presented a very good index of positivity: 83% of the cases[1]. In the present study, among 224 patients with chronic gastritis with variable degree of activity, we found 154 (68.7%) positive results for Hp by Giemsa stain and 122 (54.5%) by the routine HE stain. Therefore, in relation to Giemsa stain, HE stained sections presented sensitivity of 67% in gastritis with mild inflammatory reaction cases, and 83% in gastritis with severe infiltration of PMNs cases (Tables 1 and 2).

Therefore, considering only infection with patent inflammatory activity cases, the positivity levels increased significantly in both methods Giemsa and HE, respectively 90.2% and 75.6%. Otherwise, in the cases of gastritis with mild activity, the positive rates of both staining methods were much lower, respectively, 42.6% and 28.7% (Table 1). This result is consistent with some reports indicating HE as the staining method of choice for the chronic gastritis with patent inflammatory activity cases, while the auxiliary stains would be used in cases suspected of low bacterial density as in previously treated patients.

Except for the 20 patients with ABG, the prevalence of Hp infection index in our sample of dyspeptic patients (n = 370) was 41%, which is, apparently, a very low prevalence for adult Brazilian patients enrolled in the National Health Service (SUS) in Belo Horizonte city. Twenty years ago, the seroprevalence of Hp among adults asymptomatic blood donors of the same institution was found to be 62.1%[14]. At that time the prevalence of Hp infection in most South American countries were believed to range from 70% to 90%[15]. The apparently low prevalence of 41% we found in the present work could have several explanations. Among them, it should be consider that an unknown number of these patients could have performed endoscopy as a control for Hp treatment, which is nowadays a common procedure in our place. Nearly 20 years has gone since the prevalence of Hp in Belo Horizonte city had been calculated by Rocha, Castro *et al*., and it is possible that part of this apparent discrepancy between the prevalence of 62% versus 41% could be result of improvement of the public health of our community.

ACKNOWLEDGMENT

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Distrito Federal, Brasil, and by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Belo Horizonte, Brasil. The authors thank Ms. Luciene S. P. Faria, from the IAG Laboratory of Gastrointestinal Histopathology, for technical assistance.

RESUMO

Introdução: *Diversos métodos de coloração especial estão disponíveis para a identificação da bactéria* H. pylori *(Hp) em cortes histológicos. Entretanto, questiona-se a utilidade desses métodos na rotina diária dos laboratórios de patologia diagnóstica.* ***Objetivo:*** *Comparar a utilidade da coloração por hematoxilina-eosina (HE) com a do Giemsa para diagnóstico histopatológico do Hp.* ***Métodos:*** *Foram revistos os cortes histológicos de 390 pacientes consecutivos cadastrados no Laboratório de Histopatologia do Instituto Alfa de Gastroenterologia, Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil. Os pacientes foram divididos em quatro grupos: Grupo I – mucosa gástrica apresentando morfologia normal (*n = *146); Grupo II – gastrite crônica (GC) com atividade inflamatória discreta (*n = *101); Grupo III – CG com atividade inflamatória patente (*n = *123); Grupo IV – pacientes com gastrite atrófica corpo (*n = *20). Todos os cortes histológicos foram cuidadosamente avaliados por dois examinadores no aumento microscópico de imersão (1000×).* ***Resultados:*** *A identificação do Hp foi positiva pelo Giemsa e pela HE em, respectivamente, 111 (90,2%) e 93 (75,6%) pacientes do Grupo III (*$p < 0,01$*) e em 43 (42,6%) e 29 (28,7%) pacientes (*$p < 0,05$*) do Grupo II, e negativa nos grupos I e IV.* ***Conclusão:*** *Os resultados mostram que a coloração pelo Giemsa é superior à pelo HE para identificação do Hp em cortes histológicos. Conclui-se que o Hp pode ser visualizado pelo HE na maioria dos casos de GC, contudo, um número significativo de pacientes infectados pode ser negligenciado, independentemente da intensidade da resposta inflamatória.*

Unitermos: *gastrite crônica;* Helicobacter pylori*; Giemsa; hematoxilina-eosina; mucosa gástrica; estômago.*

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