〈Brief Note〉

Usefulness of Helicobacter pylori detection using a kit

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Summary Warrem and Marshal succeeded in isolating Helicobacter pylori (H. pylori) from the stomach under a highly acidic condition in 1983, and the relationship between pathological changes in the stomach or duodenum and H. pylori have been investigated.

Epidemiologically, the progression of inflammation was related to chronic atrophic gastritis (leading to a precancerous condition and stomach ulcer or cancer). The Warthin-Starry method is superior in detecting H. pylori, and PCR and ISH have also recently been attracting attention.

Compared to other staining methods, H. pylori detection using a kit (Muto Pure Chemicals Co., Ltd.) may be simple and very useful with regard to saving time and cost-reduction.

Introduction of the kit may facilitate the observation of H. pylori and subsequently lead to the elucidation of many roles.

Key words: Helicobacter pylori, Chronic gastritis, Kit stain, Immunohisotochemical stain

1. Introduction

Helicobacter pylori (H. pylori) is a gram-negative spiral pathogen re-discovered by Warrem and Marshal. It has potent urease activity, produces ammonia, and inhabits the mucous gel layer of the epithelium lining the stomach[1), 2)]. The association of H. pylori with MALT lymphoma[2)], chronic active gastritis, hyperplastic polyp, and gastric cancer has been suggested. H. pylori in stomach biopsy specimens can be observed by Hematoxylin-eosin (HE) staining.

In addition, Giemsa, Warthin-Starry, Genta staining[3)], and PCR[4)] have been used, and immunostaining is also recommended.

However, there are advantages and disadvantages to these special and immunohistochemical staining methods with regard to the techniques, time required for staining, and cost.

We investigated the use of a kit (Muto Pure Chemicals Co., Ltd.), which has recently been attracting attention. The kit method is simple and takes only several minutes.

The kit method improves the simplicity of H. pylori detection with regard to the cost and technique, such as shortening of the staining time, and facilitates markedly clear identification of the pathogen, H. pylori.

This kit may be introduced into various fields,

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such as elucidation of the pathological diagnoses and evaluation of treatment outcomes.

2. Materials and Methods

Written informed consent was obtained from all volunteers. The study was performed in conformity with the regulations concerning human studies of our institution. Samples were collected from 12 chronic gastritis patients.

After formalin fixation, 3 μ m paraffin-embedded sections were prepared and subjected to analysis with the kit (Muto Pure Chemicals Co., Ltd.), HE, Giemsa, and immunostaining. Giemsa and HE staining were performed employing the standard methods.

1. The principle of the kit staining is as follows:

The dyes used are methylene blue and eosin, and these are basic and acidic, respectively. It further binds to eosin. Azure is present as a neutral dye.

Composition

Helico A:

Eosin 0.6%

Methylene blue 0.4%

Methanol 99%

Helico B:

Azure II 0.5 g

Methylene blue 1.0 g

Potassium dihydrogen phosphate 6.0 g

Sodium dihydrogen phosphate 2.0 g

Distilled water 1,000ml

2. Staining Procedure:

1) Deparaffinize and rehydrate sections appropriately
2) Wash with water (10 seconds)
3) Place slides in Helico stain A solution (2 minutes)
4) Helico stain B solution (15 seconds)
5) Distilled water (10 seconds)
6) 1% hydrochloric acid alcohol (15 seconds)
7) Dehydration (70% alcohol: 10 seconds)
8) Dehydration (80% alcohol: 10 seconds)
9) Dehydration (90% alcohol: 10 seconds)
10) Dehydration (100% alcohol: 10 seconds)
11) Xylene (10 seconds)
12) Xylene (10 seconds)
13) Clarification with xylene
14) Mounting

3. Immunohistochemistry

Rabbit polyclonal anti-Helicobacter pylori antibodies (1:50, DAKO) were used. Immunohistochemical staining was performed employing the standard avidin-biotin-peroxidase complex (ABC) method.

For color development of the bound antibodies, the slides were reacted with 3,3'-diamino-benzidine

Table 1 Summary of details for the cases studied

Case	Age/Sex	Pathological diagnosis	Kit	Giemesa	HE	Immuno stain
1	46/F	Chronic gastritis	+		+	
2	52/F	Chronic gastritis		+		+
3	47/M	Chronic gastritis	+	+		+
4	58/M	Chronic gastritis	+		+	+
5	39/F	Chronic gastritis		+		
6	60/M	Chronic gastritis		+	+	
7	57/M	Chronic gastritis	+			+
8	49/M	Chronic gastritis	+	+		
9	43/F	Chronic gastritis	+	+		+
10	51/M	Chronic gastritis	+		+	+
11	44/F	Chronic gastritis				+
12	65/M	Chronic gastritis		+		

Kit: Muto Pure Chemicals Co., LTD
Immuno stain: Immunohistochemical stain

tetrahydrochloride (DAB) dissolved in Tris-HCl buffer (pH 7.6) for 3-10 minutes, and the reaction was stopped with running water.

The nuclei were stained with Mayer's hematoxylin for 30 seconds, followed by dehydration, clarification, and mounting.

3. Results

The results are shown in the Table 1.

Chronic gastritis was confirmed in stomach biopsy preparations in the 12 patients. H. pylori was morphologically identified as a spiral organism.

H. pylori was also identified in sections by the kit, Giemsa, and immunohistochemical staining in 7 of the 12 patients.

On the kit staining (Fig. 1), H. pylori was clearly stained blue. It was also stained blue by Giemsa staining (Fig. 2), and dark brown by immunohistochemical staining (Fig. 3).

H. pylori was identified in sections by HE staining in 4/12. It was weakly stained red by HE staining (Fig. 4).

4. Discussion

Gimenez staining was developed by modifying Macchiavello staining for rickettsia, and became a useful method to detect legionella bacteria[5]. Giemsa, Warthin-Starry, and Genta staining[3] were previously recommended, but these have recently been rarely used.

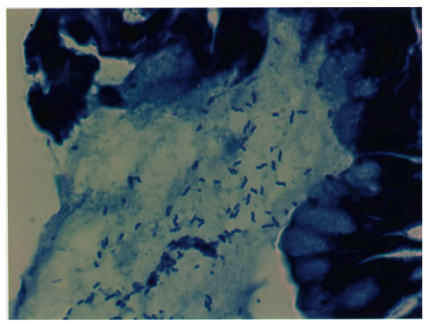

Fig. 1 (Case 1)
Gastric mucosa with numerous bacilli in the lumen of the gland.
Kit Stain x100

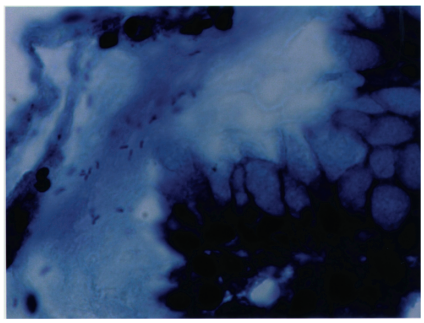

Fig. 2 (case 3)
Gastric mucosa with numerous bacilli in the lumen of the gland.
Giemsa Stain x100

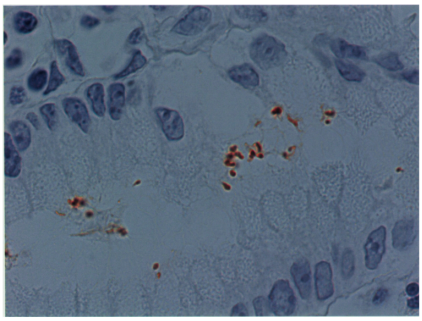

Fig. 3 (Case 10)
Gastric mucosa with numerous bacilli in the lumen of the glan.
Immunohistochemical Stain x100

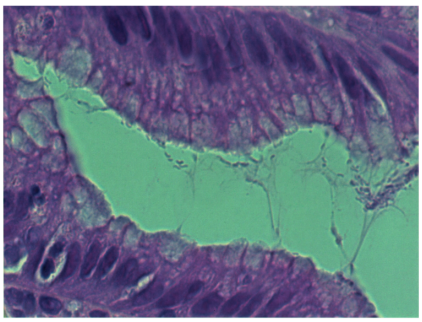

Fig. 4 (Case 6)
H. pylori adhering to superficial mucus could be most easily observed.
HE Stain x100

H. Pylori is described with regard to infection. It is also stated in the guidelines for internal medical treatment that the combination of HE and Giemsa staining is desirable[6)-12)].

On the other hand, Sugimoto et al.[13)] modified Giemsa staining to detect this pathogen and developed a rapid staining method.

While many studies on H. pylori detection have been reported, we performed this study using the kit sold by Muto Pure Chemicals Co., Ltd.

Immunohistochemical staining[14)] and the ISH method[15)] have recently been introduced, but the results of our staining method can be judged within 5 minutes, as described above.

Warthin-Starry, immunohistochemical, HE, and Giemsa staining take about 1, 5, 2, and 12 hours, respectively.

Considering these staining times, the kit staining is markedly useful. In addition, the cost of the kit staining is as low as those of HE and Giemsa staining, and immunohistochemical staining is expensive. Some institutions use only HE staining to detect H. pylori.

Warthin-Starry staining was previously recommended to demonstrate H. pylori, but its procedure is technically as complex as that of immunohistochemical staining[14)].

Both H. pylori and Campylobacter are gram-negative spirilla. The target tissue of H. pylori detection is the stomach, whereas the targets of Campylobacter are the feces and blood. Therefore, although H. pylori and Campylobacter are morphologically similar, they are detected in different tissues.

Jonkers D et al.[8)] reported that immunohistochemical staining or analysis of coccoid morphology is useful to differentiate H. pylori from other bacteria[11)].

We did not investigate the accuracy of the diagnoses because some biopsy specimens were destroyed and the number of specimens varied (for example, 2, 3, and 6 or partial).

The sensitivity and specificity of HE staining are 47-99 and 72-100%, respectively, and those of Giemsa staining are 87-96 and 79-99%[18, 16-23, 24)], respectively. Therefore, if the kit staining becomes routine in histological examination of stomach biopsy, its usefulness may increase with regard to the time, technique, and cost, which may contribute to not only the early discovery of cancer, but also deciding on a therapeutic policy.

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