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REVIEW

Histological Diagnostic Methods in Stomach *Helicobacter Pylori* Infectio

Mide Helicobacter Pylori Enfeksiyonlarında Histolojik Tanı Yöntemleri

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Abstract

Helicobacter pylori (*Hp*) is a type of bacteria that causes gram-negative, spiral-shaped, motile, flagellated epithelial damage that can lead to gastrointestinal disorders, gastritis, peptic ulcer, andgastric malignancies. The diagnosis of this microorganism, which has infected almost half of the world's population, is important. Among the diagnostic methods, non-invasive methods include urea breath test and serological examinations, while bacteria should be demonstrated in the final diagnosis in endoscopic biopsy and eradication therapy should be performed after diagnosis. Cases should be re-evaluated by biopsy in case of failure to eradicate due to reinfection or inadequate treatment. In this article, it is aimed to create a summary source by defining histopathological diagnosis methods of gastric biopsies used in Hp diagnosis.

Keywords: Biopsy; *Helicobacter pylori*; Histology; Stomach

Helicobacter pylori (*Hp*) are a mobile, spiral-shaped, microaerophilic gram-negative rod that settles on the gastric mucosa. Despite the acidic environment and thick mucin layer in the stomach, *Hp* is colonized in the antrum and corpus in the stomach and in the duodenum thanks to its urease enzyme and various adhesins. As a result of this colonization, *Hp* specifically causes gastritis, gastric and duodenal ulcers, gastric cancer, and MALT lymphoma, and has been classified as a class I carcinogen by the International Cancer Research Agency.[1,2]

Accurate diagnosis and treatment are important in patients infected with *Hp*. Many diagnostic methods have been developed since the discovery of *Hp*. These are divided into the following two: non-invasive and invasive tests. In non-invasive methods, serology, urea breath, and stool antigen tests are used. In invasive methods, urease test, culture, and histological examinations of gastric biopsies are used.[3,4] In this article, the histopathological diagnostic methods used in the diagnosis of *Hp* are reviewed.

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Stomach Anatomy and Histology

The stomach consists of the cardia, fundus, corpus, and antrum regions. The distal part starts from the gastro-esophageal border; the proximal part covers the fundus. The point where the stomach begins to narrow just before the border of the duodenum is called the incisura angularis in the corner in the small curvature. The distal of this region is considered the antrum. It ends with the stomach pyloric sphincter. The stomach wall consists of mucosa, submucosa, muscularis propria and serosa.[4]

Histologically, the mucosal surface is covered with foveolar epithelium cells characterized by long columnar cells with a basal nucleus defined as gastric pit and apical de neutral mucin. The deepest cells of the gastric pit or foveolar epithelium are mucus neck cells. These cells, which are progenitors of surface epithelial cells and gland epithelial cells, form proliferative zone. There are glands deeper in the mucosa than in the foveolar neck region. These are in different structures according to the anatomical regions.[1,3] Cardia glands; covered with cells that secrete mucus. The glands contain parietal cells that secrete acid and intrinsic factors, main cells that secrete pepsinogen, and neuroendocrine (enterochromaffin-like) cells.[4] Antral/pyloric type glands; it is characterized by vacuoles with cytoplasm, cells secreting mucus, and neuroendocrine cells known as gastrin-secreting G cells. In the pylorus, these deep mucus glands are surrounded by muscularis mucosa fibrils.[5]

Dishing Mechanism

Fecal *Hp* is transmitted orally. The bacteria can live in stagnant water for 10 days at 4°C. It can be transmitted from person to person. Inadequate hygiene conditions (not washing fruits and vegetables properly, use of common cutlery, use of common materials in tooth brushing, etc.) are the most important sources of contamination.[6]

When the *Hp* reaches the stomach, the acid in it cannot survive due to the environment and must be placed in the mucus layer suitable for it. The stomach mucous layer is surrounded by sulfated polysaccharides and acts as a buffer against stomach acid.[7–9] Thanks to the rapid movement of bacteria, it reaches the mucous layer by producing the urease enzyme. Stomach epithelium cells release bicarbonate and urea. The bacteria break this urea with the urease enzyme that it produces and forms ammonia in the environment. Ammonia provides a suitable pH balance for bacteria. Outer membrane proteins contribute to the attachment of bacteria to this epithelium layer. Bacteria that damage these proteins while attaching cause inflammation in this area. Therefore, polymorphic leukocytes are abundant in the submucosal tissue in histological examinations.[7,10–12]

Microbiological Features

Hp spiral structure, microaerophilic, Gram-negative bacteria, 3 micrometers long, and 0.5 micrometers in diameter. *HP*'s outer membrane contains lipopolysaccharides and phospholipids. It has 4–6 flagella. It breathes oxygen. However, in order to reproduce it, it needs a low amount of oxygen environment.[10,12] Oxygen in the atmosphere is too much for this bacterium. Therefore, these are called microaerophilic bacteria that live with less than 1% oxygen. Apart from this, with the enzyme urease, it enters the mucous layer inside the gastric mucosa and creates a suitable living condition here. In addition, catalase is a bacterium with oxidase enzymes.[13–15]

Histopathological Diagnostic Methods

The parts taken by endoscopy are evaluated together with the routine dye Hematoxylin Eosin (H&E) and additional histochemical examinations and *Hp* diagnosis is made. One of the main features of this bacterium is that it does not enter the tissue and triggers the immune system by triggering the immune system (although it also has mechanisms that directly damage the epithelium).[16] Due to this pathogenetic nature, *Hp* organisms can only be seen in the surface mucus layer. The presence of *Hp* in biopsies can be demonstrated in a good H&E section.[12,14] One of the pitfalls here is that the search for the organism not only in the superficial foveolar epithelium but also in the neck and isthmus parts of the fovea is often skipped. Additional auxiliary methods are *Hp* immunohistochemistry by May Grunwald Giemsa (MGG), Warthin-Starry, and Toluidine Blue methods. In the first two methods, it is important not to confuse different types of bacteria (cocci and shorter and smaller bacilli than *Hp*) with *Hp*, which contaminate the biopsy or chemicals used.[16]

H&E Method

It is the main staining technique used routinely in histology, it is the most commonly used dye method in medicine that distinguishes nucleus and cytoplasm. Sections of 4 µm thickness prepared from paraffin blocks are kept in an oven at 70°C for 15 minutes and then H&E staining is performed by passing the following processes, respectively (Table 1).

Table 1. Hematoxylin Eosin (H&E) method

1.	Xylol	5 minutes
2.	Xylol	5 minutes
3.	90% Ethanol	2 minutes
4.	80% Ethano	1 minutes
5.	60% Ethanol	1 minutes
6.	Distilled water	1 minutes
7.	Harris Hematoxylin	5 minutes
8.	Distilled water	1 minutes
9.	1% acid alcohol (HCl)	30 seconds
10.	Distilled water	1 minutes
11.	1% ammonia water	30 seconds
12.	Distilled water	1 minutes
13.	70% Ethanol	1 minutes
14.	Eosin	3 minutes
15.	80% Ethanol	1 minutes
16.	95% Ethanol	1 minutes
17.	96% Ethanol	1 minutes
18.	Xylol	5 minutes
19.	Xylol	5 minutes
20.	Xylol	5 minutes
21.	Mounting in D.P.X	

Table 2. May–Grunwald Giemsa (MGG) method

1.	Xylol	5 minutes
2.	Xylol	5 minutes
3.	90% Ethanol	2 minutes
4.	80% Ethano	1 minutes
5.	60% Ethanol	1 minutes
6.	Distilled water	1 minutes
7.	May-Grunwald	5 minutes
8.	Distilled water	5 minutes
9.	Giemsa	30 minutes
10.	Distilled water	10 minutes
11.	70% Ethanol	1 minutes
12.	Air drying	
13.	Mounting in D.P.X	

***Hp* H&E Microscopy:** It is a small spiral rod-shaped gram-negative bacterium found in micro-aerobic conditions in the neutral myco-environment between *Hp* mucus and superficial columnar mucosa cells. The organisms here are painted pale pink with H&E staining. These organisms do not invade the mucosa and do not directly damage it, but by changing the microenvironment, they prepare the ground for mucosal damage. *Hp* organisms contain urease and produce a protective ammonia cloud to be affected by stomach acid (Fig. 1).[16]

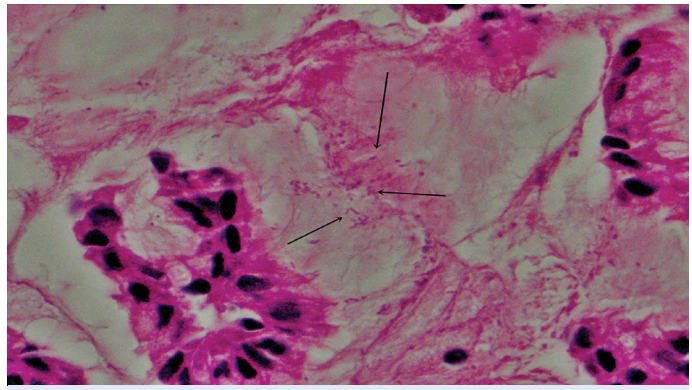

Figure 1. Hp cholines determined by H&E painting method (H&E, x1000).

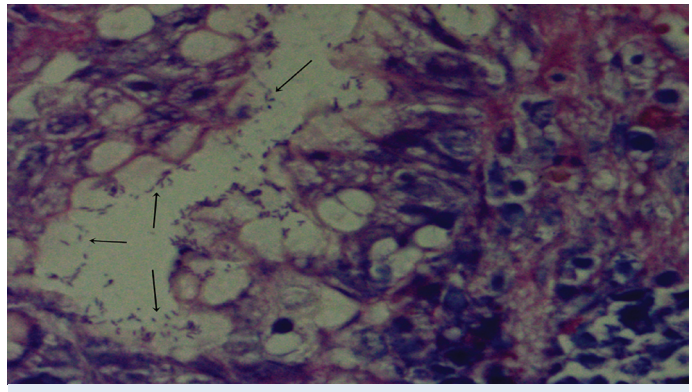

Figure 2. *Hp* cholines determined by MGG staining method (MGG, x1000).

MGG Method

4 µm thick sections prepared from paraffin blocks are kept in a 70°C oven for 15 minutes and then MGG staining is performed by passing the following processes, respectively (Table 2).

***Hp* MGG Microscopy:** *Hp* organisms stimulate the cytokine production of epithelial cells, enabling the collection and activation of immune and inflammatory cells in the lower lamina propria. Most patients with active gastritis have *Hp* in their mucus on the surface. Organisms can be represented here with methylene blue dye. With this staining, the bacilli appear in blue color (Fig. 2).[16]

Toluidine Blue Method

4 µm thick sections prepared from paraffin blocks are kept in a 70°C oven for 15 minutes, and then the following processes are applied and the Toluidin Blue is stained (Table 3).

***Hp* Toluidine Blue Microscopy:** In this staining, microorganisms appear in pale blue color (Fig. 3).

Warthin-Starry Method

After waiting for 15 minutes in 70°C oven in sections of

Table 3. Toluidine Blue Method

1.	Xylol	5 minutes
2.	Xylol	5 minutes
3.	90% Ethanol	2 minutes
4.	80% Ethano	1 minutes
5.	60% Ethanol	1 minutes
6.	Distilled water	1 minutes
7.	Toluidune Blue	5 minutes
8.	Distilled water	5 minutes
9.	70% Ethanol	1 minutes
10.	Air drying	
11.	Mounting in D.P.X	

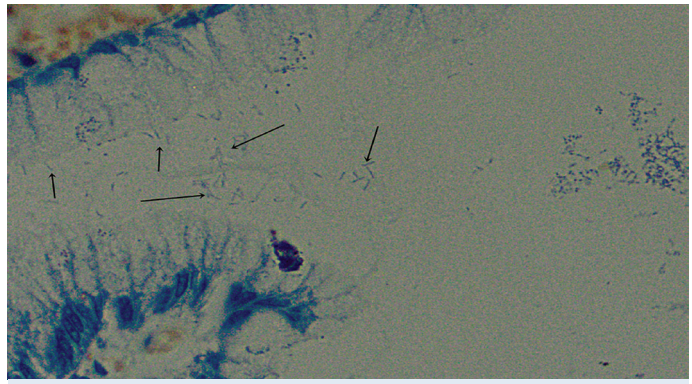

Figure 3. *Hp* cholines detected by Toluidine Blue staining method (TB, x1000).

Table 4. Warthin–Starry method

1.	Xylol	5 minutes
2.	Xylol	5 minutes
3.	90% Ethanol	2 minutes
4.	80% Ethano	1 minutes
5.	60% Ethanol	1 minutes
6.	Distilled water	1 minutes
7.	Put 4.5 mL of concentrated buffer solution in 13 mL distilled water. Then 20 drops of silver nitrate concentrate solution are placed and shaken until homogeneous. It is incubated for 90 minutes in a 6070°C oven by dripping onto the tissue	
8.	The tissue removed from the oven is allowed to cool for 5 minutes	
9.	It is heated for 10 minutes in an oven of 50°C in the amount that I will use from C and D solutions. Then solution E is dropped and shaken	
10.	Drip on the tissue; incubate for 10 minutes in a 50°C oven	
12.	Distilled water	2 minutes
13.	70% Ethanol	1 minutes
14.	Eosin	1 minutes
15.	80% Ethanol	1 minutes
16.	95% Ethanol	1 minutes
17.	96% Ethanol	1 minutes
18.	Xylol	5 minutes
19.	Xylol	5 minutes
20.	Xylol	5 minutes
21.	Mounting in D.P.X	

4 µm thickness prepared from paraffin blocks, Warthin–Starry staining is applied (Table 4).

***Hp* Warthin-Starry Microscopy:** In this staining, microorganisms appear in black color (Fig. 4).

Histopathological Reporting

Hp gastritis is a chronic inflammation; patients may not have complaints or may overlap with the complaints and findings of other diseases when they exist. Morphologically, the key component is the filtration of lympho plasmocyte. Rarely, lymphocytes can be seen in the stomach. In fact, small lymphoid aggregates can be seen in a normal biopsy in other aspects, especially on the background of the mucosa; in these cases, it is not correct to diagnose chronic gastritis.

Normally, plasma cells can be seen individually and very few in the stomach, but plasma cells that form groups (at least three are recommended as threshold values) are sufficient for the diagnosis of chronic gastritis. Generally, there is no possibility of the presence of the *Hp* organism in a biopsy without any lymphocyte or plasma cell infiltration.

Neutrophils are prominent inflammatory cells in the acute

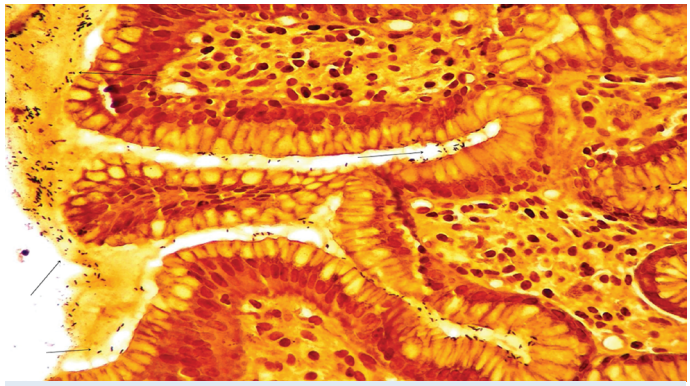

Figure 4. *Hp* cholines detected by Warthin Starry staining method (WS, ×60).

period or in newly colonized mucous areas. Shortly after, intense infiltration of lymphocytes and plasma cells appears on the mucosa. Secondary lymphoid follicles are formed. Epithelial cell destruction is slower than in acid-related injury, so epithelial cell loss/erosion and ulceration will not be a common finding. However, since the loss of cells in the surface epithelium due to *Hp* and immune response will cause gastric acid to contact lamina propria, it should be expected that the properties seen in reactive gastropathy / erosive gastritis morphology at any time of *Hp* gastritis. Moreover, in addition to *Hp* chronic gastritis, reactive gastropathy/erosive gastritis processes may be present simultaneously in a patient. It will be useful to state in the report that these are seen.

Conclusion

Histological staining is a frequently used method to detect *Hp* and provides additional information about the gastric mucosa. H&E and MGG staining are frequently used in routine and are generally considered sufficient. We think that this review article will be a summary resource for clinicians and pathologists and will facilitate differential diagnosis.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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