Helicobacter Pylori Detection In Gastric Biopsy: Immunohistochemistry And Toluidine Blue Comparison

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Helicobacter pylori detection in gastric biopsy: immunohistochemistry and toluidine blue comparison

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ABSTRACT

Background: *Helicobacter pylori* is a bacterium often found in the stomach and can cause several diseases, including gastritis, gastric ulcers, MALT-lymphoma, and gastric carcinoma. An invasive and direct gastric biopsy is a more appropriate way to see the presence of these bacteria. Microscopy of *Helicobacter pylori* on gastric biopsy specimens can be seen using several tissue stains. This study aimed to analyze the concordance of immunohistochemistry and toluidine blue tissue stain results on gastric biopsies in detecting *Helicobacter pylori* at Dr. Soetomo Hospital.

Methods: This study was a retrospective study with a cross-sectional design to detect *Helicobacter pylori* in paraffin-block of gastric biopsy specimens at Dr. Soetomo Hospital, Surabaya. Each sample would be stained by immunohistochemistry and toluidine blue. Data were analyzed using SPSS version 25 for Windows.

Results: *Helicobacter pylori* have been found in all 45 samples by immunocytochemical staining and in 66.67% of them by toluidine blue staining. The Kappa test indicated a significant difference between immunohistochemical staining and toluidine blue ($p=0.000$; $p<0.05$).

Conclusion: There are significant differences between the two methods for detecting *Helicobacter pylori* in gastric biopsy. Their concordance is at 66,67%. Some factors should be considered for the future routine process.

Keywords: *Helicobacter pylori*, Immunohistochemistry, Toluidine Blue.
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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, flagellated spiral-shaped bacterium.[1] *H. pylori* infects the gastric mucosal surface and can be easily detected in the gastric mucosal surface's gastric pit.[2] *H. pylori* is a pathogen that causes various gastroduodenal diseases, including gastritis, peptic ulcers, gastric cancer, and MALT (Mucosa-Associated Lymphoid Tissue) lymphoma.[3] More than half of the world's population is still thought to have *H. pylori* infection, according to global prevalence statistics from 62 nations. Africa (79.1%), Latin America and the Caribbean (63.4%), and Asia (54.7%) have the greatest prevalence rates.[4] Asian nations were classified as high-risk (Japan, Korea, China), medium-risk (Vietnam), or low-risk (Thailand and Indonesia) for gastric cancer based on age-standardized incidence rates.[5]

Numerous ethnic groups comprise Indonesia, which also has a low frequency of *H. pylori* infection (between 2% and 68%).[6,7] Based on data from the WHO International Agency for Research on Cancer, Indonesia has a relatively low-risk stomach cancer (ASR: 1.3 out of 100,000) with an overall prevalence of *H. pylori* infection in five of the largest islands in Indonesia at 22.1%.[8] The prevalence rate considers geographic location, age, and social and economic status.[9] In Indonesia, dyspepsia is the fifth and sixth most prevalent condition among inpatients and outpatients, respectively, although few facilities offer gastrointestinal endoscopy services there.[8]

Both direct and indirect *H. pylori* infection detection methods have been developed. Direct assays include histopathological analysis, immunohistochemistry, bacterial culture, a fast urease test, and polymerase chain reaction (PCR). Comparatively, indirect diagnostics include the detection of bacterial antigens in feces, urea breath testing, and serological antibody detection.[10] Several variables affect how accurately *H. pylori* may be found in stomach biopsy samples. The degree of infection, previous antibiotic use that resolved the infection or decreased the number of bacteria, the use of proton pump inhibitors, the

type of diagnostic technique, the location of the biopsy, the technique used to process the sample, as well as the intensity and types of inflammatory changes in the tissue.[11–13] Not all patients are willing to undergo endoscopy to diagnose *H. pylori*. As a result, many people with *H. pylori* infection cannot be detected.[6] Continuous efforts to systematically monitor *H. pylori* prevalence and disease burden are critical because they will change the health system.[4]

With this much information on *H. pylori*, it is logical to assume Indonesia's screening procedure or diagnostic services are dispersed unevenly. In some hospitals, immunohistochemistry services are not offered regularly. A referral hospital in Surabaya, RSUD Dr. Soetomo offers endoscopic and gastric biopsy treatments. H&E and Diff-Quik stains are still used in the detection process, though. To assess the adequacy of the two and determine the prevalence of the pathogen, this study will begin efforts to detect *H. pylori* in stomach biopsies by detecting tissue antigens using immunohistochemistry and morphology and staining qualities using Toluidine Blue.

METHODS

This was a retrospective cross-sectional investigation to detect *Helicobacter pylori* in paraffin-block stomach biopsy tissues from Dr. Soetomo Hospital in Surabaya during study period using consecutive sampling. The last two years of stomach biopsies, excluding poor paraffin blocks, will be randomly collected according to the inclusion and exclusion criteria. The inclusion criteria included complete medical records of patients with gastritis as the chief complaints. Immunohistochemistry (Biocare Medical's antibody concentrate CM 383AC) and routine toluidine blue would be used to re-stain samples. Two independent pathologists and microbiologists analyzed the blinded samples. The Kappa test will then analyze the data with SPSS version 25 for Windows to determine the concordance.

RESULTS

Of the 45 samples tested for *H. pylori* with a total of 100%, positive immunohistochemical staining was obtained, whereas, in Toluidine Blue staining, 30 samples (66.67%) were positive, as seen in Table 1. Based on the result above, statistical analysis was conducted to determine the suitability between Immunohistochemical staining and Toluidine Blue using the Kappa test. The test results showed a significant difference between Immunohistochemical staining and Toluidine Blue (p=0.000; p<0.05).

By using Sydney Scale, we observe the density of *H.pylori*. Most *H.pylori* detected by immunohistochemistry are in marked condition (38 samples), while toluidine blue could vary in moderate and marked scales. Immunohistochemistry found the bacterial density in grades 3 (84.44%) and 2 (15.56%). Toluidine Blue found the bacteria in grades 3 (35.56%) and 2 (31.11%). It is less sensitive than in previous immunohistochemistry, as seen in Table 2.

On histopathological examination, thirty-five cases were diagnosed with inactive chronic gastritis (77.78%) and 10 with active chronic gastritis (22.22%).

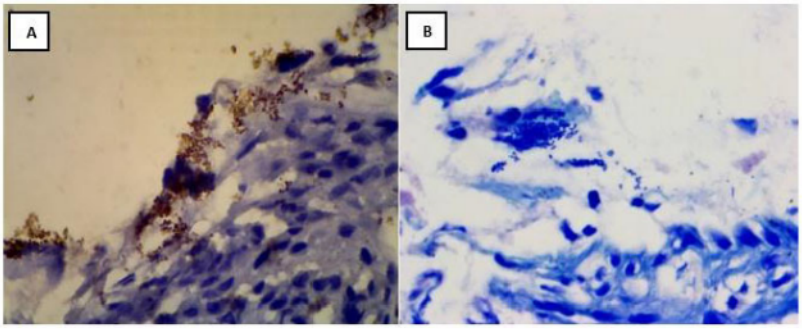

Figure 1. *H. pylori* was detected by immunohistochemistry (A) and by toluidine blue (B).

Table 1. Immunohistochemical and Toluidine Blue staining results for *H. pylori* on Gastric Biopsy.

Variables		Toluidine Blue (N=45)		Total
		Positive (N=30)	Negative (N=15)	
IHC	Positive, n (%)	30 (66.67)	15 (33.33)	45 (100.00)
	Negative, n (%)	0 (0.00)	0 (0.00)	0 (0.00)

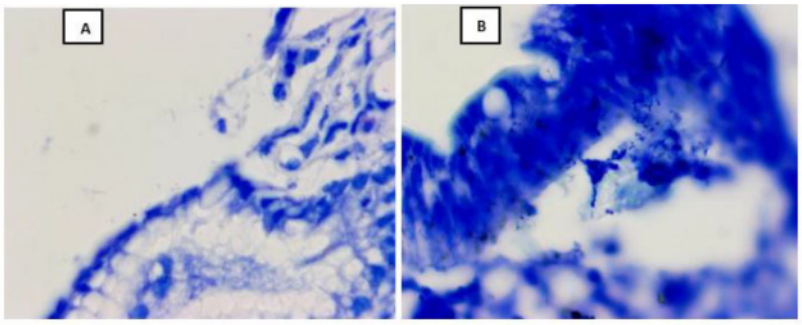

Figure 2. Sydney scale of *H. pylori* detected by toluidine blue, moderate (A) and marked (B).

Table 2. Sample distribution by Sydney Scale.

Variables	Normal=0	Mild=1	Moderated=2	Marked=3
IHC, n (%)	0 (0.00)	0 (0.00)	7 (15.56)	38 (84.44)
Toluidine Blue, n (%)	15 (33.33)	0 (0.00)	14 (31.11)	16 (35.56)

Immunohistochemistry mainly detects *H. pylori* on a marked scale, even in inactive gastritis (64.44%). In comparison, toluidine blue only detects *H. pylori* in 13 of 35 samples of inactive gastritis, as seen in Table 3.

DISCUSSION

An additional invasive procedure called a stomach biopsy tests patients with *Helicobacter pylori* infection. Patients who exhibit dyspeptic symptoms are typically the ones who undergo this procedure.[14] According to estimates, 25% of Indonesia's population suffers from dyspepsia. Our population develops dyspepsia for various reasons, such as *H. pylori* infection, stress, metabolic disorders, medications, and functional dyspepsia. It has been established that dyspepsia lowers the standard of living in Indonesia. Additionally, several variables, including feminine gender, advanced age, and more severe dyspeptic symptoms, can affect a person's quality of life.

Gastrointestinal endoscopy is not readily available for the majority of Java residents. Because it is not fully covered by health insurance in Indonesia, invasive *H. pylori* infection detection is regarded as being unprofitable.[15]

Forty-five samples were used in this investigation, and more men (53.33%) than women (46.67%) were present. According to the age group, the age group of 41 to 50 years had the greatest percentage (22.22%), followed by the age group of 51 to 60 years with 20%. Each sample came from the corpus and antrum. The detection rate of extra corporal biopsies rose by up to 6% in a prior study by Miftahussurur M and Yamaoka Y in 2016 compared to antral region biopsies alone.

The antrum appears to be a better area to live for survival, given that *H. pylori* have the potential to infiltrate both the corpus and antrum. Based on its histological component, the antrum has more foveolar cells than parietal cells. Foveolar cells can contribute to the presentation of mucus for life in physiological settings. The ability of *H. pylori's* flagella to travel and thrive in a slimy environment is another benefit.[9] The lumen, mucus, epithelial cell surface, gastric pH, amino acid nutrition, peptides, and other metal ions are only a few of the variables that are thought to be involved in *H. pylori* infection.[16]

There are a few restrictions on the ability to detect *H. pylori* using standard histopathology stains. Long processing times, expert dependency, intraobserver variability, antibiotic treatment or eradication, and proton pump inhibitors (PPI), which alter the bacterial shape and make it less visible with conventional procedures before, are the limitations.[16] In this investigation, toluidine blue stained just 66.67% of the samples, whereas immunohistochemistry stained 100% of them positively for *H. pylori*. These results support the idea that immunohistochemistry is the best method for detecting *H. pylori*. Immunohistochemistry should be contrasted with the other staining technique. Toluidine blue staining's low sensitivity can be related to the method's failure to identify *H. pylori* adhering to the glandular epithelium, especially in situations where colonization is only modest. Toluidine staining makes the glandular epithelium and *H. pylori* both appear blue. Other histochemistry methods that stain *H. pylori* in blue, like Modified-Giemsa, have been discovered to have the same issue.[17] Under extreme circumstances, such as antibiotic therapy, *H. pylori* also changes its shape from a spiral to an atypical form. Histochemistry staining cannot distinguish these unusual bacteria, which may explain the limited sensitivity. Additionally, toluidine blue staining shows a minor decrease in the contrast between the tissue and the organism, as was reported in a prior work by Tajalli R et al. However, compared to H&E and Giemsa staining, toluidine blue is less expensive and more illustrative.[18]

Our research has shown that toluidine blue does not generate as many bacteria as immunohistochemistry. Immunohistochemistry was deemed to be the gold standard for evaluating the sensitivity of culture methods in a prior study conducted by Miftahussurur M and Yamaoka Y in Bhutan, Myanmar, and Indonesia in 2016.[10] Compared to other standard staining methods; immunohistochemistry is a reliable method for determining the presence of *H. pylori* because it is simple to identify the bacterial antigens that are present in the lamina

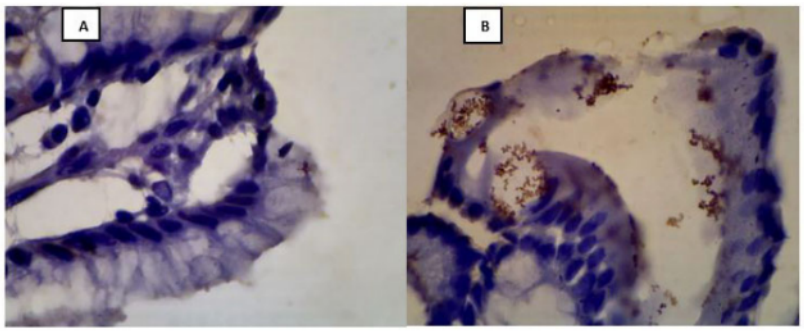

Figure 3. Sydney scale of *H. pylori* detected by immunohistochemistry, moderate (A) and marked (B).

Table 3. The density of *H. pylori* by Sydney Scale according to histopathological diagnosis.

Variables	Normal=0	Mild=1	Moderated=2	Marked=3	Total
IHC					
Inactive gastritis, n (%)	0 (0.00)	0 (0.00)	6 (13.33)	29 (64.44)	35 (77.78)
Active gastritis, n (%)	0 (0.00)	0 (0.00)	1 (2.22)	9 (20.00)	10 (22.22)
Toluidine Blue					
Inactive gastritis, n (%)	14 (31.10)	0 (0.00)	8 (17.78)	13 (28.89)	35 (77.78)
Active gastritis, n (%)	1 (2.22)	0 (0.00)	6 (13.33)	3 (6.67)	10 (22.22)

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propria and beneath the epithelium[18-20]. Immunohistochemistry is also superior to conventional staining because it can detect inflammation while missing *H. pylori*. The main causes include hypoxia and other stressful circumstances.[10,21] The limitation of immunohistochemistry for a routine diagnostic process is its high cost.[17] So, when the infection is mild, immunohistochemistry is advised.[22]

CONCLUSION

There is no difference in the treatment algorithm based on the results of the density of *H. pylori* bacteria. Eliminating *H. pylori* is necessary to reduce the risk of developing neoplasms, enhance the function of stomach mucus, avoid mucosal damage, combat inflammation, and rebuild the mechanism. Inactive chronic gastritis is not always a sign that *H. pylori* bacteria are not present. Therefore, *H. pylori* identification will continue to be important in the near future. Immunohistochemistry, a pricey but sensitive technique, could be utilized as a last choice following the prior screening technique. Toluidine blue might be an appropriate choice. A comparison of sensitivity for a more effective screening method requires further investigation.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

ETHICAL CLEARANCE

The Health Research Ethics Committee at RSUD Dr. Soetomo Hospital approved this study based on a certificate of ethical conduct No. 0530/KEPK/XI/2022 dated November 24, 2022.

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AUTHOR CONTRIBUTIONS

All authors contributed by preparing the proposal, data collection, data analysis, writing and revising the manuscript, and approving the final version for publication.

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