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Research Report

DETECTION OF *HELICOBACTER PYLORI* INFECTION IN CHRONIC GASTRITIS BIOPSY SPECIMEN USING WARTHIN-STARRY AND MODIFIED GIEMSA STAIN IN DR SOETOMO HOSPITAL SURABAYA

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ABSTRACT

Helicobacter pylori is a bacteria that commonly cause chronic gastritis. Identification of its infection is essential for eradication treatment. Detection of H.pylori bacteria in gastric biopsy specimen by histology method is a diagnostic tool that widely accepted because it is superior to serology examination. Although the bacteria can be seen in routinely Hematoxylin-Eosin staining, Modified-Giemsa and Whartin-Starry stain was commonly used to identify the bacteria more clearly. Whartin-Starry stain gives more contrast to the bacteria but modified-Giemsa stain is preferable at many centres because it is a cheaper and simple method. This study aim is to explore the differences of two stain method to identify H.pylori. Paraffin blocks from gastric biopsy patients with chronic gastritis in the year 2017 were retrieved from Anatomic Pathology Laboratory Dr.Soetomo Hospital Surabaya. Thirty paraffin blocks were taken randomly and were made into microscopic slides for staining with Warthin-Starry and modified-Giemsa stain concomitantly. Specimen with Whartin-Starry stain found 19 out of 30 were positive for H.pylori while in modified-Giemsa stain only 16 out of 30 specimen were positive for H.pylori. Detection of H.pylori Warthin-Starry stain give more chance to obtain positive result because it use silver technique that coat the bacteria making it is more clearly visible in microscopic examination.

Keywords: *H.pylori detection, H.pylori infection, Warthin-starry, modified Giemsa, chronic gastritis.*

ABSTRAK

Helicobacter pylori adalah bakteri penyebab gastritis khronis yang umum dijumpai. Identifikasi infeksi H.pylori diperlukan untuk acuan terapi eradikasi. Deteksi H.pylori pada spesimen biopsi gaster dengan metode histopatologi merupakan teknik diagnostik yang diterima secara umum mengingat teknik ini lebih akurat dibandingkan dengan pemeriksaan serologi. Walaupun bakteri H.pylori dapat terlihat pada pengecatan rutin Hematoxylin-Eosin, akan tetapi umumnya digunakan pengecatan tambahan modified-Giemsa atau Whartin-Starry untuk melihat bakteri dengan jelas. Pengecatan modified-Giemsa lebih disukai di banyak sentra laboratorium oleh karena lebih murah dan lebih mudah dikerjakan akan tetapi pengecatan Whartin-Starry dapat melihat bakteri lebih jelas dengan kontras yang lebih baik. Penelitian ini bertujuan untuk membandingkan adanya perbedaan hasil identifikasi H.pylori pada kedua jenis pengecatan tersebut. Blok parafin biopsi lambung diambil dari pasien dengan gastritis kronis yang diperiksa di Laboratorium Patologi Anatomik Rumah Sakit Dr.Soetomo Surabaya pada tahun 2017. Tiga puluh blok parafin diambil secara acak untuk dibuat slide mikroskopis dan dilakukan 2 jenis pewarnaan yakni Warthin-Starry dan modified-Giemsa. Pada pengecatan Whartin-Starry bakteri H.pylori terdeteksi pada 19 dari 30 spesimen sedangkan pada pengecatan modified-Giemsa hanya 16 dari 30 spesimen yang menunjukkan adanya H.pylori. Deteksi infeksi H,pylori dengan pengecatan Whartin-Starry memberikan hasil positif yang lebih banyak dibandingkan dengan pengecatan modified-Giemsa. Pereaksi perak pada reagen Whartin-Starry dapat membuat bakteri menjadi terlihat lebih jelas pada pengamatan mikroskopis.

Kata kunci: *deteksi H.pylori, infeski H.pylori, pewarnaan Warthin-starry, pewarnaan modified giemsa, gastritis kronis.*

INTRODUCTION

H.pylori is a bacteria that closely related with chronic gastritis and dyspepsia. Chronic gastritis case and dyspepsia are commonly found in daily practice. Beside causing chronic gastritis, *H.pylori* infection plays some important role in the presence of gastric malignancy either in the form of gastric carcinoma or gastric lymphoma.[1,2] According to the American College of Gastroenterologists guidelines on management of *H.pylori* infection[3], the diagnostic method H.pylori infection detection comprises of urea breath test and gastric endoscopic biopsy. Serology method should be avoided. Nevertheless if the serologic test gave positive results, it should be confirmed with a test that identify an active infection such as the urea breath test or stool antigen test. Urea breath test, stool antigen test, histology examination with special staining for *H.pylori* organisms, and bacteria culture are considered to be the gold standard tests for diagnosis of *H.pylori* infection.[4,5]

Culture is not routinely used for initial diagnosis of *H.pylori* infection but is required for antibiotic susceptibility testing if physicians suspect antibiotic resistance in patients who have previously failed therapy.[5] For stool antigen test, Mayo Medical Laboratories utilizes the POCone Infrared Spectrophotometer. Performance characteristics for this instrument have not been established for persons under age 3. For patients 3 to 17 years, age, weight and height must be included in test request for appropriate result interpretation.[6]

The gold standard diagnostic test for *H.pylori* infection is to find the bacteria through direct smear or *H.pylori* culture.[4] Cross reaction with other antigens often encountered in serology test with posibly to obtain false positive result. Several staining methods of biopsy specimen are proposed to detect *H.pylori* infection, usually start with routinely stain hematoxylin-eosin to more specific stain using monoclonal antibody for immunohistochemistry testing.[7] Whartin-Starry stain is used mainly to detect Spirochetes bacteria with silver impregnation methods. Whartin starry has some advantage compare to other methods because it gives more contrast color (bacteria stained black in yellow background).[8] This method can improve sensitivity of its detection. On the other hand, Giemsa stain is a common method for examining blood smear. In tissue, Giemsa stain can detect microorganism that appears dark blue in pink-pale blue background. A modification of Giemsa stain was proposed to reduced the staining time and it was known as modified-Giemsa technique.[9] Modified-Giemsa stain is a non-silver stain that commonly use to detect *H pylori* infection in many health centre including Dr.Soetomo general hospital. The staining methods are quite simple and the reagent is easy to obtain and cheap. Sometimes it fails to detect microorganism due to lack of contrast in dirty background. It must be used by experienced experts. There are still a debate which stain should be used to detect *H. pylory* as a routine procedure.[10] This study aim is to compare the result of these two staining methods for detection of *H.pylori* infection in gastric biopsy specimens and to find out if there is difference result of H.pylori identification in gastric biopsy tissue using Whartin–Starry stain compared to modified-Giemsa stain.

MATERIAL AND METHOD

Thirty paraffin blocks from patients who diagnosed clinically as chronic gastritis were retrieved from Anatomic Pathology laboratory archives at Dr Soetomo hospital Surabaya in the year 2017. Two microscopic slides were made from each paraffin blocks by slicing the tissue 6 µm thick and placed on object glass. The first slide was stained with modified-Giemsa technique according to Bancroft[9] which have done routinely in the laboratory while the other slides stained with Warthin-Starry for spirochetes (Bio-optica cat no.04-040903). In modified-Giemsa stain, the *H.pylori* bacteria were identified as a reddish purple microorganism in the background of other cells that was stained blue and pale-blue. Warthin-Starry stain give more contrast colour. The bacteria were stained black while the background cells are stained yellow. The two microscopic slides were assessed by one pathologist and *H.pylori* infection scoring was made according to updated Sidney classification system.[11] *H.pylori* infection in gastric tissue biopsy were score as follows: score +1 when there were sparse bacteria found in specimen (mild density of bacteria), score +2 when there were some bacteria (moderate density) and score +3 when there were many bacteria in tissue (marked bacteria were found).[12] This study has been approved by Health-Research Ethical Commission in Dr. Soetomo General Hospital.

RESULT AND DISCUSSION

On examination of 30 gastric biopsy specimens with Warthin-Starry stain, 19 tissue biopsies were found positive for *H.pylori* bacteria while specimens with modified-Giemsa stain only 16 biopsies tissue were found positive for *H.pylori* (Table 1).

Table 1. Comparison of *H.pylori* bacteria examination with Warthin-Starry and modified-Giemsa stain

	Warthin-Starry with positive bacteria	**Warthin-Starry with negative bacteria**
Modified-Giemsa with positive bacteria	16	0
Modified-Giemsa with negative bacteria	3	11

Detection of H.pylori in gastric biopsy specimen has been accepted worldwide to diagnose H.pylori infection. Culture method is gold standard for infection detection but H.pylori culture did not routinely used due to its complexity and need a longer period to obtain the result.[4] Until this date, majority of microbiologic laboratory in the world are not equipped to perform H.pylori culture. Instead of culture, PCR technique for detection of H.pylori may be considered as a gold standard, but PCR technique also has a limitation due to genetic sharing of other microbes which can gives false positive result and the presence of PCR inhibitors can give false negative result in specimen with low bacterial count.[13]

Histological examination from gastric biopsy specimen has been a method of choice for identifies H.pylori infection. There are several staining methods that can be used to identify the presence of the bacteria which can be classified into two groups: silver-based stain and common histochemistry stain. Silver-based stain such as Whartin-Starry stain has an advantage over other stain since it can detect H.pylori bacteria even if its morphology has been altered by proton pump inhibitor and antibiotic administration, which can alter the bacteria morphology to cocoid and short bacillary forms. Proton pump inhibitor administration can cause *H.pylori* migrate into deeper portion of oxyntic glands, making its detection is imposible without a silver-based or immunohistochemical stain.[14]

Modified-Giemsa stain is a histochemistry stain that has been performed in lots of laboratory because it is cheap and simple method. The lack of contrast is a disadvantage of this technique. Silver-based stain such as H.pylori silver stain and Whartin-Starry stain give more clearly visible bacteria. So it can be detected easily on histological examination. The disadvantage of this technique it is more expensive than modified-Giemsa stain and it takes longer period to do the staining protocol.[15]

Immunohistochemistry with specific antibody has been proposed to be used as a gold standard to detec H.pylori infection.[8] But according to the study of Patel, et al. there is no difference result for H.pylori detection by immunohistochemistry technique compared with modified-Giemsa stain. The limitation of immunohistochemistry technique is more expensive than any other stain including Whartin-Starry and it took longer period than any other stain. It also needs a control specimen to be done with every slide making it is more complicated.[13]

Table 1 is showed that there were 3 cases of *H.pylori* infection detected by Warthin-Starry stain which did not detected by modified Giemsa stain. The discordance occurs due to lack of contrast between the color of micro-organism and background color. Using Whartin-Starry stain one can easily direct to get the presence of the bacteria because it provides black color of bacteria in yellow background. Furthermore, the silver reagent in Whartin-Starry stain gives a good result for detecting H.pylori because the organism are coated with the silver stain and therefore look larger, making their identification easier.[15]

Assessment of *H.pylori* infection in gastric biopsy specimen has performed according to Updated Sidney System scoring. The method is to detect *H.pylori* bacteria throughout entirely biopsy specimen and making the score by counting the number of bacteria in one high power field which give most number of bacteria.[11] The result of *H.pylori* scoring according to Updated Sidney System was presented in Figure 1.

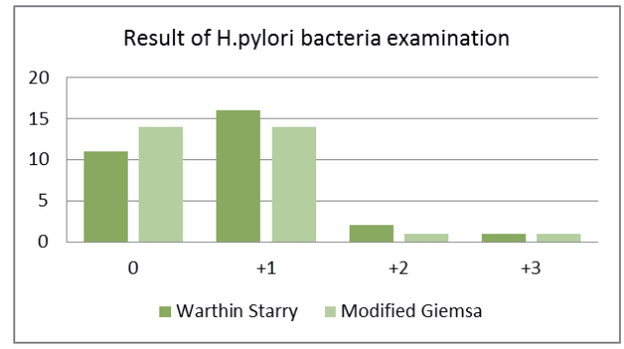

Figure 1. Result of *H.pylori* bacteria examination with Warthin-starry dan modified Giemsa stain.
Score 0 = no bacteria detected, score +1 = only sparse bacteria detected, score +2 = moderate bacteria count detected, score +3 = many bacteria detected.

Figure 1 is showed that Warthin-starry stain gives more results of identified bacteria compared to modified-Giemsa stain. Three cases show *H.pylori* score 0 on modified Giemsa staining (no bacteria detected) while these cases show sparse *H.pylori* bacteria (score +1) on Warthin-starry staining. There were also 1 case which gives *H.pylori* score +1 with modified Giemsa staining while it gives score +2 with Warthin-starry staining.

H.pylori infection can be easily detected with Whartin-starry stain compare to that of modified Giemsa stain either in low density or high density bacteria as shown at Figure 2 and Figure 3.

Figures 2 and Figure 3 are showed various *H.pylori* score in gastric biopsy specimen. This study was showed that there were some cases when the presence of *H.pylori* infection is either undetectable or detectable in smaller amounts in modified-Giemsa stain compare to Whartin-Starry stain. In Warthin-starry stain the spirochete bacteria wall were react with silver nitrate impregnation so that the bacteria will appear black within a yellow background. According to Glickman, *H.pylori* bacteria can not be detected if it present in a small number.[16]

The gold standard diagnosis of *H.pylori* infection in gastritis is made by finding *H.pylori* bacteria in gastric tissue. Gastric tissue specimens are generally obtained by endoscopic biopsy techniques. This technique is a minimally invasive but it can see directly at the morphology of the gastric tissue followed by tissue endoscopic biopsy for pathology examination.[17]

The presence of *H.pylori* bacteria in gastric biopsy can be detected by culture, PCR technique and *H.pylori*

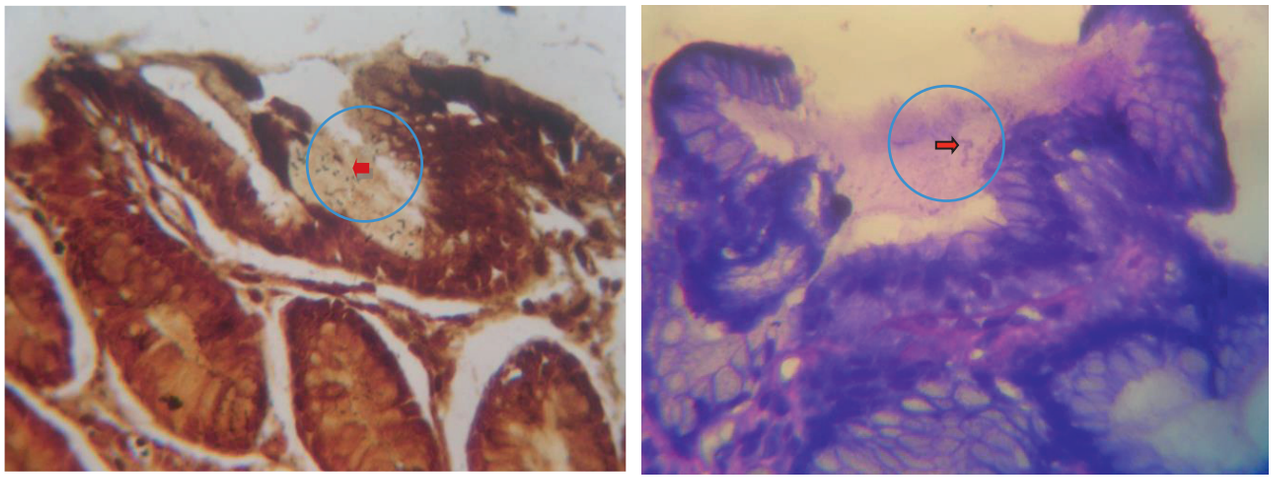

Figure 2. Gastric biopsy specimen with high density of H.pylori bacteria (+3). Specimen stianed with Whartin-starry (left) and modified Giemsa (right). Thebacteria were found on gastric mucous layer (➡) (400 X magnification).

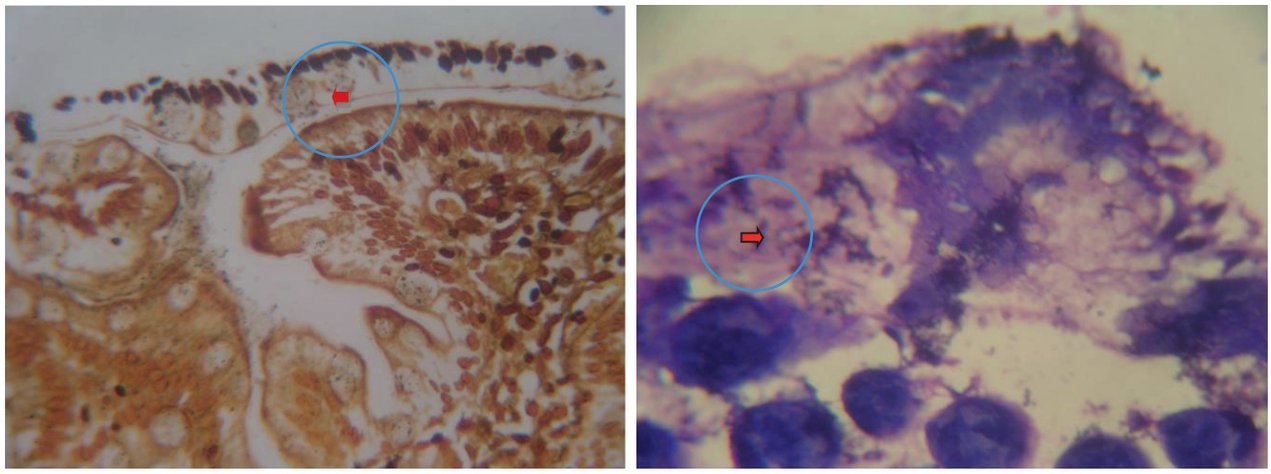

Figure 3. Gastric biopsy specimen with low density of H.pylori bacteria (+1). Specimen stianed with Whartin-starry (left) and modified Giemsa (right). Thebacteria were found on gastric mucous layer (➡) (400 X magnification).

visualization techniques with histochemical stain to indentify directly *H.pylori* by light microscope.[7] The histochemical technique of gastric biopsies has an advantage over culture methods because it can directly see *H.pylori* in a relatively simple and faster way. Therefore, culture methods are not commonly used for *H.pylori* detection. Histochemical staining techniques are also easier to perform and have less cost than PCR molecular techniques. The histochemical technique has become the standard diagnostic of *H.pylori* infection in many health centers.[4]

Routine hematoxyllin-eosin staining has been used in *H. pylory* examination but in several cases the bacteria can not be visualized without special stain.[18] Histochemical special stain for *H.pylori* detection include Gimenez, Toluidine blue, Romanowski, Genta and Giemsa stain which can be used for visualization of with various modifications (modified-Giemsa) for a more rapid work-up time.[4] Silver-based stain such as Warthin-Starry stain has advantage compare to common histochemistry stain since it uses silver impregnation technique to give black color to *H.pylori* bacteria. In Warthin-starry, *H.pylori* bacteria will appear dark brown, making them easier to see and can increase the sensitivity of *H.pylori* detection in gastric biopsy tissue.[14] This study was performed on gastric endoscopic biopsy specimens in patients with chronic gastritis. In this study, 19 samples (63.33%) were detected as *H.pylori* positive on Warthin-Starry stain from 30 biopsy specimens of chronic gastritis patients. Adlekha, et al reported *H.pylori* infection in 329 of 530 (62%) chronic gastritis patients in Kerala India.[19] The positivity of *H.pylori* from India is similar to this study. Adlekha took a gastric biopsy specimen from patients with dyspepsia complaints as many as 530 patients in 2010 - 2012. On microscopic slides biopsy material performed Hematoxylin-Eosin and modified-Giemsa staining. *H.pylori* examination was performed by a Pathology specialist and the results were

presented in grading (mild, moderate and severe) infections in accordance with the updated Sydney grading system.[11]

Immunohistochemistry technique using monoclonal antibody anti-H.pylori can gives more specific result. However, this technique rarely be done in many Pathology laboratory due to higher cost of antibody-based detection.[20] According to Rodger Haggit recommendation: immunohistochemistry stain should not be use as a routine procedure. It was only performed in gastric biopsy specimen with chronic inflammation with negaive finding on *H.pylori*.[21] Detection of *H.pylori* bacteria by special stain has been served as a routinely *H.pylori* detection in tissue specimen.[13]

The results of this research showed difference of *H.pylori* detection due to increased sensitivity detection by Warthin-Starry stain compared to modified-Giemsa stain. Warthin-starry was found more specimens with positive result than modified-Giemsa stain in 3 out of 30 cases. The presence of *H.pylori* that is undetected by the observer is largely due to lack of color contrast in modified-Giemsa stain. Bacteria are missed from observation in *H.pylori* infection with mild intensity due to the unclear color. In infections with moderate to severe intensity, this result found 100% concordance result of *H.pylori* stained with modified-Giemsa compared to Warthin-Starry.

CONCLUSION

A study has been conducted to find the difference of *Helicobacter Pylori* detection in gastric biopsy tissue with Warthin-starry and modified Giemsa staining. Detection of H.pylori Warthin-starry stain give more possibility to obtain positive result because it use silver technique that coat the bacteria making it is more clearly visible in microscopic examination.

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Conflict of interest. There is no conflict of interest for this research. The manuscript has not been published previously.

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