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RESEARCH ARTICLE

Comparative Evaluation of Modified Giemsa and Warthin-Starry Stains for Helicobacter pylori Detection and Density Grading Using the Updated Sydney System

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Abstract

Objective: Helicobacter pylori is one of the main causes of chronic gastritis, gastric ulcers, and stomach cancer. Accurate diagnosis of *H. pylori* infection is essential for effective treatment and prevention. **Methods:** This research is an analytical observational study with a cross-sectional retrospective design that aims to compare histochemical special stains, Modified Giemsa, and Warthin-Starry for *H. pylori* Detection and Density Grading using the Updated Sydney System in 150 gastric biopsies of Chronic Gastritis at the Department of Anatomical Pathology, Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia. **Result:** There was a significant difference in detection of *H. pylori* (p=0.001), Warthin-Starry detected more *H. pylori* positive 111 samples (74.00%) than Modified Giemsa positive 95 samples (63.30%) and there was a significant difference in Density Grading using the Updated Sydney System (p=0.001), Warthin-Starry staining showed a higher density score of Grade 2 (moderate) with 63 samples (42.00%), compared to Modified Giemsa, the most dominant density score of Grade 1 (mild) with 61 samples (40.70%). **Conclusion:** Warthin-Starry detects more *H. pylori* compared to Modified Giemsa. Additionally, the Warthin-Starry yields higher Density Grading using the Updated Sydney System for Grade 2 (Moderate) compared to the Modified Giemsa, with a dominant grade at Grade 1 (Mild) in chronic gastritis. This highlights the importance of selecting the most appropriate histochemical staining method for accurate early diagnosis, where resource limitations restrict the use of advanced diagnostic modalities that may not be routinely available.

Keywords: Modified Giemsa- Warthin-Starry- Detection- Density- Updated Sydney System

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Introduction

Gastritis is a disease characterized by inflammation of the stomach mucosa. The etiological components are the *Helicobacter pylori* (*H. pylori*) agent. In cases of chronic gastritis, changes to the mucosa are most commonly associated with *H. pylori*-induced gastritis. The discovery of *H. pylori* demonstrated that gastric ulcers and chronic gastritis are caused by this bacterium [1]. *H. pylori* is a gram-negative, spiral-shaped bacterium, and a major etiological agent of chronic gastritis, peptic ulcers, and stomach cancer [2]. Accurate diagnosis of *H. pylori* infection is essential for effective treatment and prevention strategies [3], and this organism is also associated with stomach carcinoma and malignant lymphoma [4].

Although *H. pylori* can be visualized using hematoxylin & eosin (H&E) staining, its detection sensitivity and specificity are enhanced by special staining methods [5] such as Modified Giemsa, Warthin-Starry, and Immunohistochemistry (IHC) [6, 7], staining using H&E provides a basic visualization of gastric histology; however, due to limited contrast, it may fail to detect

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H. pylori in mild or patchy infections [8]. Special stains such as Modified Giemsa, Genta, Warthin-Starry silver, and IHC are more specific and sensitive [9, 10], and Modified Giemsa is a Romanowsky stain containing eosin, methylene blue II, and methylene azure II. The staining works by exploiting the chemical interaction between basic dyes and acidic cellular components, such as RNA & polysaccharides in the bacterial cell wall, resulting in a purplish-blue appearance of the bacteria. This staining method is simple, widely used, and cost-effective [9, 10]. The Warthin-Starry staining technique is a silver impregnation method that uses silver nitrate and formic acid. Silver interacts with parts of the bacterial cell wall, particularly peptidoglycan, causing silver metal to accumulate and making the bacteria look bigger and easier to see when viewed under a microscope, usually appearing black against a yellow or light brown background [9, 10].

The Updated Sydney System is generally used to classify gastritis based on histopathology. This system combines morphological, etiological, and topographical aspects. The assessment is semi-quantitative, covering atrophy, inflammation, *H. pylori* density, and intestinal metaplasia [11]. This is widely used worldwide, particularly in developing countries; however, it is not commonly used for routine examinations, including in Indonesia. In Indonesia, specifically at Dr. Wahidin Sudirohusodo Hospital in Makassar, one of the largest hospitals in Eastern Indonesia, the Anatomic Pathology Laboratory plays a crucial role in diagnosing gastritis. In 2016, a study at the hospital analyzed 162 stomach tissue samples; only 10 of them, or about 6.2% showed the presence of *H. pylori* [12]. This low occurrence might be because of difficulties in diagnosis, such as depending on hematoxylin & eosin (H&E) staining alone and the lack of routine use of more sensitive techniques like Modified Giemsa or IHC. Research on the detection of *H. pylori* infection with Warthin-Starry compared to Modified-Giemsa of paraffin blocks of gastric biopsies in 2017 has been done [10], while comparative research on special histochemical staining of Modified Giemsa and Warthin-Starry for *H. pylori* Detection and Density Grading using the Updated Sydney System in chronic gastritis in Makassar City, South Sulawesi Province, Indonesia has never been done. This research highlights the importance of selecting the most appropriate histochemical staining method for accurate early diagnosis, in clinical decision-making related to effective treatment of chronic gastritis [13], to more severe conditions, including strategies for preventing stomach carcinoma [14, 15], especially in resource-limited areas where advanced diagnostic techniques may not be routinely available. Therefore, accuracy for *H. pylori* Detection and Density Grading using the Updated Sydney System is crucial.

Aim of the Study

A comparative study of Modified Giemsa and Warthin-Starry stains for *H. pylori* detection and density grading using the Updated Sydney System in chronic gastritis.

Materials and Methods

1. Study design

The methodology is an analytical observational study with a cross-sectional retrospective design.

2. Population and Sample

The research population was 150 samples diagnosed by anatomic pathologists as chronic gastritis with positive and negative *H. pylori* results based on Hematoxylin Eosin and Giemsa staining, obtained from paraffin blocks of gastric biopsy tissues sent to the Anatomical Pathology Laboratory of Dr. Wahidin Sudirohosodo Hospital, Makassar, Indonesia, between January 2020 to December 2024.

Data Collection Techniques: 1). Collect and categorize all eligible samples based on histopathological examination diagnosed by anatomic pathologists as chronic gastritis with *H. pylori* positive and negative using Hematoxylin Eosin staining and Giemsa staining taken from angulus, antrum, antrum and corpus, antrum and angulus, antrum, angulus and corpus, antrum and cardia, and corpus sites from biopsies sent by clinicians. 2). Collect all eligible samples for paraffin blocks in order by registration number, then perform special histochemical staining examination to compare the evaluation of Modified Giemsa and Warthin-Starry for *H. pylori* detection and density grading using the Updated Sydney System.

3. Procedures

This research uses a special histochemically modified Giemsa stain; the detection of *H. pylori* will appear as bacterial colonies stained blue in chronic gastritis tissue, which is declared positive or negative through observation of the preparation using a light microscope and oil immersion. Objective criteria: (Nominal data scale) - Positive: there are blue-stained bacterial colonies in chronic gastritis tissue; - Negative: There are no blue-stained bacterial colonies in chronic gastritis tissue.

The detection of *H. pylori* using a special histochemical Warthin-Starry stain will appear as bacterial colonies stained black on a brown background and yellow to dark yellow in chronic gastritis tissue, which is declared as positive or negative through observation of the preparation using a light microscope with oil immersion. Objective criteria: (Nominal data scale) - Positive: there are bacterial colonies stained black with brown and yellow to dark yellow background in chronic gastritis tissue; - Negative: no bacterial colonies stained black with brown and yellow to dark yellow background in chronic gastritis tissue.

The Updated Sydney System is a combination of endoscopic and histologic findings. The density grading using the Updated Sydney System detection was compared between Modified Giemsa and Warthin-Starry in chronic gastritis expressed as descriptive: Grade 0 for None/Normal, Grade 1 for Mild, Grade 2 for Moderate, and Grade 3 for Severe/Marked, through observation of preparations using a light microscope using oil immersion. Objective criteria: (Ordinal data scale) - Grade 0 for None/Normal: No *H. pylori* present anywhere; - Grade

1 for Mild: Only a few *H. pylori* in single or multiple foci; - Grade 2 for Moderate: Many *H. pylori* are seen in separate foci; - Grade 3 for Severe/Marked : >50% of the surface area is covered with *H. pylori*.

4. Statistical analyses

All data obtained from the research results were recorded, and then statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, NY, USA). Categorical variables were described as numbers and percentages, and continuous variables were described as means with standard deviations or medians. 1). Univariate analysis, used to describe the characteristics of the data obtained, namely in the form of frequency distribution, ranges, and mean values presented in tabular form. 2). Bivariate analysis, consisting of: a). The Wilcoxon test is a non-parametric statistical test used to compare two groups of the same nominal data. In this study, the detection of *H. pylori* was expressed as positive and negative between the two groups of Modified Giemsa and Warthin-Starry. b). The Wilcoxon test is a non-parametric statistical test used to compare two groups of equal ordinal data. In this study, the density grading using the Updated Sydney System was assessed in the assessment of *H. pylori* density grading between two groups of Modified Giemsa and Warthin-Starry. All tests were two-tailed, and p-values <0.05 were considered statistically significant.

Results

1. General Characteristics of the Sample

The total number of samples was 150 Table 1 based on age category, with 84 samples (56.00%) in the >50 years age category and 66 samples (44.00%) in the <50 years age category. Based on gender, there were 84 male samples (56.0%) and 66 female samples (44.0%). Meanwhile,

Table 1. General Characteristics of the Sample (n=150)

Characteristics	Univariate analysis	
	Sample Quantity (n=150)	Percentage (%)
Age		
> 50 Years	84	56
< 50 Years	66	44
Gender		
Male	84	56
Female	66	44
Location		
Angulus	1	0.7
Antrum	11	7.3
Antrum, corpus	30	20
Antrum, angulus	61	40.7
Antrum, angulus, corpus	41	27.3
Antrum, cardia	2	1.3
Corpus	4	2.7

Source: Primary Data, 2024

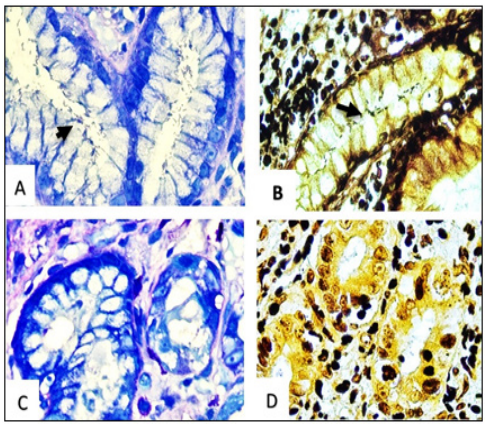

Figure 1. Comparison Modified Giemsa and Warthin-Starry Stains for Detection of *H. pylori* Magnification (100x). a) Modified Giemsa: Positive Bacteria, b) Warthin-Starry: Positive Bacteria, c) Modified Giemsa: Negative Bacteria, d) Warthin-Starry: Negative Bacteria. Source: Primary Data, 2024.

based on the biopsy locations sent by clinicians, there were from the angulus 1 samples (0.70%), antrum 11 samples (7.30%), antrum and corpus 30 samples (20.00%), antrum and angulus 61 samples (40.70%), antrum, angulus, and corpus 41 samples (27.30%), antrum and cardia 2 samples (1.30%), and corpus 4 samples (2.70%).

2. General Characteristics of the H. pylori Detection

The results Table 2 of the *H. pylori* detection using Modified Giemsa was positive for 95 samples (63.30%) and negative for 55 samples (36.70%) while Warthin-Starry was positive for 111 samples (74.00%) and negative for 39 samples (26.00%) in chronic gastritis with a description of Histopathology results can be seen in Figure 1.

3. General Characteristics of the H. pylori Density Grading using the Updated Sydney System

The results Table 3 of *H. pylori* density grading using the Updated Sydney System criteria between Modified Giemsa obtained Grade 0 (None/Normal) group as many as 55 samples (36.70%), Grade 1 (Mild) as many as 61 samples (40.70%), Grade 2 (Moderate) as many as 25 samples (16.70%) and Grade 3 (Severe/Marked) as many as 9 samples (6.00%) while Warthin-Starry obtained Grade 0 (None/Normal) group as many as 39 sample (26.00%), Grade 1 (Mild) as many as 14 samples (9.30%), Grade 2 (Moderate) as many as 63 samples (42.00%) and Grade 3 (Severe/Marked) as many as 34 samples (22.70%) in chronic gastritis with a description of Histopathology results can be seen in Figure 2.

4. Comparison of the detection of H. pylori between Modified Giemsa and Warthin-Starry stains

Comparison of detection of *H. pylori* between Modified Giemsa and Warthin-Starry stains in chronic gastritis, the statistical results showed a significant mean difference of p=0.001 (p<0.05), which indicates that there is a significant difference. Where Warthin-Starry is more effective in detecting *H. pylori*, it obtained positive

Table 2. General Characteristics and Comparison of Modified Giemsa and Warthin-Starry stains for Detection of *H. pylori* (n=150)

Detection *H. pylori*	Univariate analysis				Bivariate analysis
	Modified-Giemsa		Warthin Starry		p-value
	Sample Quantity (n=150)	Percentage (%)	Sampel Quantity (n=150)	Percentage (%)	
Positive bacteria	95	63.3	111	74,00	*0.001
Negative bacteria	55	36.7	39	26,00	

* Wilcoxon test, statistically significant if the p value is less than 0.05.

Table 3. General Characteristics and Comparison of Modified Giemsa and Warthin-Starry stains for *H. pylori* Density Grading using the Updated Sydney System (n=150)

H. pylori Density Grading using the Updated Sydney System	Univariate analysis				Bivariate analysis
	Modified-Giemsa		Warthin Starry		p-value
	Sample Quantity (n=150)	Percentage (%)	Sample Quantity (n=150)	Percentage (%)	
Grade 0 (None/Normal)	55	36.7	39	26	*0.001
Grade 1 (Mild)	61	40.7	14	9.3	
Grade 2 (Moderate)	25	16.7	63	42	
Grade 3 (Severe/Marked)	9	6	34	22.7	

* Wilcoxon test, statistically significant if the p value is less than 0.05.

results in as many as 111 samples (74.00%) compared to Modified Giemsa, which obtained positive results in as many as 95 samples (63.30%) in chronic gastritis biopsies, with the results can be seen in Table 2.

5. Comparison of Modified Giemsa and Warthin-Starry stains for H. pylori density grading using Sydney Grading System

Comparison of Modified Giemsa and Warthin-Starry for *H. pylori* density grading using the Updated Sydney System in chronic gastritis, the statistical results showed a significant difference of p=0.001 (p<0.05), which indicates that there is a significant difference. Where Warthin-Starry produced a higher Density Score of the Updated Sydney System of Grade 2 (Moderate) as many as 63 samples (42.0%), compared to Modified Giemsa produced the most dominant Density Score of the Updated Sydney System of Grade 1 (Mild) as many as 61 samples (40.70%) in chronic gastritis biopsies with the results can be seen in Table 3.

Discussion

A total of 150 samples Table 1, the characteristics based on age category> 50 years were found the most, namely 84 samples (56.00%). These results are in line with the research of Breckan et al. (2016), who reported a population-based study involving all age groups that reported persistent *H. pylori* infection and suggested intrafamilial and environmental transmission routes. The results of this study support the implementation of community-level interventions [16], Everhart et al. (2000), noted that seroepidemiological studies in the US show ethnic disparities in the prevalence of *H. pylori*, with higher rates in certain minority groups, and social-environmental determinants likely contributing to the risk [3]. Meanwhile, in a study by Kamada et al. (2015), over 40 years in Japan, the prevalence of *H. pylori* and atrophic gastritis decreased with improvements in hygiene and living conditions, highlighting strong cohort and environmental effects [17]. In a study by Nakajima et al. (2010), over 17 years in Japan, the prevalence of *H. pylori* decreased alongside changes in gastrointestinal disease patterns, reflecting cohort effects and improvements in public health [18].

In addition, based on gender, 84 samples (56.00%) were male. These results are in line with the research of meta-analysis by Ibrahim et al. (2017), of 244 studies, which found differences in the prevalence of *H. pylori* according to gender in child and adult populations, suggesting biological and behavioral factors in the acquisition of infection [19]. A clinical study by Chuang et al. (2009) reinforced these findings chronic *H. pylori* infection is associated with differences in ghrelin and leptin levels according to gender. Hormonal changes may mediate the metabolic effects of long-term infection [20]. A study by Almashhadany, D.A., et al. (2023) reported a high prevalence of *H. pylori* with significant differences according to age and gender. The findings emphasize the need for context-appropriate screening and prevention strategies [21], and a study by Zamani et al. (2018) estimated that the prevalence of *H. pylori* is close to half of the world's population, with significant regional heterogeneity, emphasizing the need for targeted prevention and control [22].

The results Table 2 of *H. pylori* detection between Warthin-Starry and Modified Giemsa showed that in detecting the presence of *H. pylori*, Warthin-Starry was

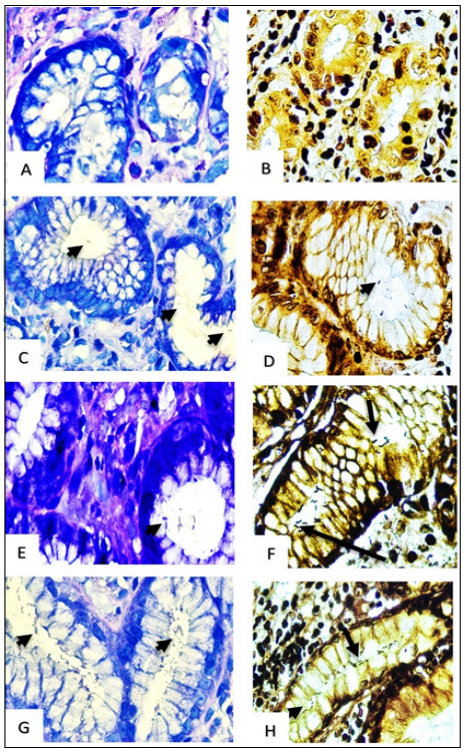

Figure 2. Comparison Modified Giemsa and Warthin-Starry stains for *H. pylori* density grading using the Updated Sydney System magnification (100x). a) Modified Giemsa: Grade 0 (None/Normal), b) Warthin-Starry: Grade 0 (None/Normal), c) Modified Giemsa: Grade 1 (Mild), d) Warthin-Starry: Grade 1 (Mild), e) Modified Giemsa: Grade 2 (Moderate), f) Warthin-Starry: Grade 2 (Moderate), g) Modified Giemsa: Grade 3 (Severe/Marked), h) Warthin-Starry: Grade 3 (Severe/Marked). Source: Primary Data, 2024

positive as many as 111 samples (74.00%), while Modified Giemsa was positive as many as 95 samples (63.30%) in chronic gastritis biopsies, with Histopathology results picture can be seen in Figure 1. These results align with the study by Farouk et al. (2018), which reported that among histochemical stains, modified Giemsa balances sensitivity and ease of interpretation, while Warthin–Starry aids detection in biopsies with few bacteria but is more technically demanding [9], Krogfelt el al. (2005), the diagnostic testing for *H. pylori* emphasizes context-appropriate selection and combination of methods to improve accuracy; noninvasive tests require careful validation [23], Taha et al. (2018), where Immunohistochemistry shows higher sensitivity than conventional histochemical staining for *H. pylori*; the authors recommend immunohistochemistry as a supplement to diagnostically challenging biopsies [24], Pandya et al. (2013), the polymerase chain reaction test surpasses conventional staining in detecting *H. pylori*, identifying infections in several biopsies that are negative by conventional staining. This molecular test is applicable for confirmation and research [25]. Akeel et al. (2021) describe the evaluation of the diagnostic yield of immunohistochemistry (IHC) for *H. pylori* in patients in Saudi Arabia with minimal or atypical infection. IHC is superior to routine histochemical staining, especially in cases of low bacterial density, supporting its role as a confirmatory test [26]. Gowsik et al. (2019) explain that comparative research shows immunohistochemistry to be the most sensitive method for *H. pylori*; Giemsa remains a pragmatic option in resource-limited settings. The choice of method must balance accuracy and feasibility [6], the study by Kusters et al. (2006), elucidating bacterial virulence and host responses underlying gastritis, ulceration, and carcinogenesis, providing a mechanistic basis for targeted interventions [27], despite their importance and the study of Rokkas, T., et al. (2010), on his study showing that first-degree relatives of gastric cancer patients have a higher prevalence of *H. pylori* and poor gastric histology, targeted screening of high-risk families may be necessary [28].

The results Table 3 of *H. pylori* density grading using the Updated Sydney System using Warthin-Starry staining obtained Grade 2 (Moderate) in 63 samples (42.00%), while Modified Giemsa obtained Grade 1 (Mild) in 61 samples (40.70%). A description of the Histopathology results can be seen in Figure 2. These results are in line with research conducted by Sandhika et al. (2019), who concluded in the Indonesian cohort that Warthin–Starry and modified Giemsa stains are suitable for routine detection of *H. pylori*, although sensitivity varies; careful technique improves performance [10], Yadav et al. (2022), who found that Modified Giemsa is superior to some routine stains for screening, with IHK recognized as the benchmark for accuracy [5], Almeida et al. (2015), found in their study that certain *H. pylori* genotypes are associated with more severe gastric histopathology in Southern Europe. Genotyping can help classify the risk of mucosal damage [29]. The study by de Almeida et al. (2021) noted that variations in NOD2 in the host are associated with susceptibility to *H. pylori* infection, highlighting the role of innate immunity in disease risk. These findings support the integration of host genetics into epidemiological risk models [30], as a consideration of factors influencing the assessment of *H. pylori* density scores. Histological assessment was performed based on the latest Sydney system mentioned in the study by Miftahussurur et al. (2016), to rigorously validate indirect tests (serology, fecal antigen) in order to avoid biased prevalence estimates and recommend algorithms appropriate to the context [31]. These results align with research conducted by Misra et al. (2000), which states that the density and distribution of *H. pylori* vary topographically in the stomach and correlate with the degree of gastritis; multi-site sampling improves detection and assessment of the disease [32], and Jung et al. (2017), research, a meta-analysis in Korea showing that bismuth-based regimens and some non-bismuth quadruple regimens achieve better eradication rates than the old triple therapy regimen. Selection should reflect local resistance patterns [33], and Oling et al. (2015) study in a tertiary hospital with limited resources showed that dyspepsia patients have a high prevalence of *H. pylori*, confirming the need for easily accessible diagnostics and appropriate management protocols [34].

Comparison of Modified Giemsa and Warthin-Starry for *H. pylori* detection in chronic gastritis, the statistical results showed a significant mean difference of p=0.001

($p<0.05$), which indicates that there is a significant difference in *H. pylori* detection between Modified Giemsa and Warthin-Starry stains in chronic gastritis. Where Warthin-Starry is more effective in detecting the presence of *H. pylori*, it obtained positive results in as many as 111 samples (74.00%) compared to Modified Giemsa, which obtained positive results in as many as 95 samples (63.30%) in chronic gastritis biopsies, with the results can be seen in Table 2 and Figure 1. These results are in line with research conducted by Mujtaba et al. (2025), which explains the comparison of current diagnostic techniques supporting the integrated use of invasive and non-invasive tests to maximize detection and guide effective therapy [35], Farouk et al. (2018), his study among histochemical stains, modified Giemsa balances sensitivity and ease of interpretation, while Warthin–Starry aids detection in biopsies with few bacteria but is more technically demanding [9], Krogfelt et al. (2005), the diagnostic testing for *H. pylori* emphasizes context-appropriate selection and combination of methods to improve accuracy; noninvasive tests require careful validation [23]. This is attributed to Warthin-Starry being an argentaffin (silver-based) staining method, which allows staining of *H. pylori* into a solid black color against a pale yellow background making it easier to detect, especially in cases with low bacterial density, whereas Modified Giemsa uses aniline-based dyes (azure B and eosin), which rely more on the color contrast of the cytoplasm and bacteria. As a result, it is easier to miss in cases of mild infection or at low densities. The advantages of Warthin-Starry detection over Modified Giemsa in chronic gastritis are associated with high sensitivity in detecting *H. pylori*, visualization ability in low-density cases, and high accuracy in histopathological confirmation, although this method requires specialized techniques and is more expensive. Modified Giemsa remains relevant for rapid and practical screening, but is less accurate as a single method in cases of mild infection or advanced chronicity.

Comparison of Modified Giemsa and Warthin-Starry stains for *H. pylori* density grading using the Updated Sydney System in chronic gastritis, statistical results showed a significant difference of $p=0.001$ ($p<0.05$). Where Warthin-Starry produced a higher density Score of the Updated Sydney System of Grade 2 (moderate) as many as 63 samples (42.00%) compared to Modified Giemsa produced the most dominant Density Score of the Updated Sydney System of Grade 1 (mild) as many as 61 samples (40.70%) in chronic gastritis biopsies with the results can be seen in Table 3 and Figure 2. These results are consistent with research conducted by Kocsmár, É., et al. (2017), in which Giemsa sensitivity to *H. pylori* decreased in low inflammatory activity, while IHC and FISH maintained higher detection rates. Additional testing is recommended when inflammation is minimal [36], as explained by Yakoob, J., et al. (2005), who noted that combining rapid urease testing with histopathology enhances the reliability of *H. pylori* diagnosis in developing countries, mitigating the limitations of using a single test alone [8]. In a study by Although in a study by Nurdin et al. (2016), IHC was superior to Giemsa in detecting *H. pylori* and correlated with morphological changes in active chronic gastritis [37], and Laheij et al (2000), demonstrated that without a single gold standard, latent class analysis showed that histology, culture, and rapid urease testing were accurate, with the best performance when combined. A multi-test strategy minimizes classification errors [38]. This was attributed to Warthin-Starry using silver staining and high contrast methods, which enabled the visualization of even small, weakly staining forms, or cocci, making it superior in detecting very low densities. In contrast, Modified Giemsa is effective for the detection of spiral forms of *H. pylori* at moderate to high densities, but fails to recognize cocci or very low-density forms that often appear in chronic infection or are on partial treatment.

Emphasis on the importance of choosing the most appropriate histochemical staining method for accurate early diagnosis in clinical decision-making. In addition to sensitivity, the selection of staining techniques for routine use must consider cost efficiency and technical feasibility, especially in resource-limited areas where advanced diagnostic techniques may not be readily available, particularly in regions such as Makassar, Indonesia. Sandhika et al. (2019); Taha et al. (2018), in their study, found that although Warthin-Starry showed higher sensitivity, this method is more expensive, more technically complex, and time-consuming compared to Modified Giemsa staining, which is simpler and more cost-effective. This makes Modified Giemsa a practical and adequate option for initial screening. However, in cases with high clinical suspicion of *H. pylori* infection but negative or unclear results on Modified Giemsa staining, or for accurate density assessment in research settings, Warthin-Starry is highly sensitive, although immunohistochemistry (IHC) is considered the gold standard for detection [10, 24]. Similarly, Kocsmár, É., et al. (2017) found in their study that Giemsa staining was less sensitive for detecting *H. pylori* than immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH), and that many cases of *H. pylori* were not detected when using Giemsa alone [36].

The Warth-Starry stain highlights the crucial role of accurate histopathological diagnosis as the primary line of defense in identifying patients at high risk for *H. pylori*-associated sequelae, including stomach cancer. This aligns with the ongoing global effort to improve early cancer detection. More sensitive *H. pylori* detection is a crucial step in identifying individuals at high risk for stomach cancer, the goal that aligns with recent findings by Noto et al. (2012) and de Brito et al. (2018) on the molecular pathogenesis of *H. pylori*-induced carcinogenesis [39, 40]. The recent research by Wang et al. (2025) focusing on the discovery of novel molecular biomarkers [41], and the study by Díaz et al. (2024), Matsuoka et al. (2023), and Mirzaei et al. (2024) on the development of advanced biosensing platforms utilizing innovative biomaterials for ultra-sensitive detection [42, 43, 44]. However, these advanced technologies are often not routinely available in resource-limited areas such as Makassar City, South Sulawesi Province, Indonesia. Therefore, optimizing

and validating affordable and readily available methods, such as the comparative evaluation of histochemical dyes presented in this research, remains a pragmatic and essential strategy to bridge this technological gap.

The accurate grading of *H. pylori* density not only guides immediate clinical management but also helps stratify patients for more intensive surveillance, potentially bridging the gap until more advanced molecular or point-of-care tests become accessible and affordable in these regions. Regarding effective treatment of chronic gastritis and more severe conditions, including strategies for preventing stomach carcinoma, Malfertheiner, P., et al. (2022) found in their study that Maastricht IV/ Florence 2012 confirmed the eradication of *H. pylori* as the primary strategy for preventing stomach ulcers and stomach carcinoma [14], the 2015 Kyoto Report by Sugano, K., et al. (2015) emphasized that *H. pylori* gastritis is a disease that requires treatment and introduced the Kyoto Classification, emphasizing that staging gastritis through the Updated Sydney System to assess the risk of stomach carcinoma, which is the basis for a global strategy for stomach carcinoma prevention through *H. pylori* eradication [15]. Rugge, M., et al. (2007). The OLGA staging system is a practical method for assessing the risk of stomach carcinoma based on the degree and location of gastritis atrophy. This system can provide clinical guidance for monitoring patients, especially after *H. pylori* eradication [13].

In conclusion, warthin-Starry detects more *H. pylori* compared to Modified Giemsa. Additionally, the Warthin-Starry yields higher Density Grading using the Updated Sydney System for Grade 2 (moderate) compared to the Modified Giemsa, with a dominant grade at Grade 1 (mild) in chronic gastritis. This highlights the importance of selecting the most appropriate histochemical staining method for accurate early diagnosis, where resource limitations restrict the use of advanced diagnostic modalities may not be routinely available.

Declarations

Funding Statement

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Clinical trial registration

Not applicable.

Conflict of Interest/Competing interests

The authors declare no conflicts of interest.

Code availability

Not applicable.

Authors' contributions

R.F. contributed to data collection and primary drafting of the manuscript. U.A.M., M.H.C. contributed to the manuscript's conception, study design, and final drafting, and supervised the research. U.A.M. and M.H.C. contributed to data interpretation and manuscript editing. U.A.M. and M.H.C. contributed to histopathological evaluation and analysis. S.W. and J. supervised the research. S.T. contributed to statistical analysis and interpretation. A.Y. contributed to the Anatomical Pathology Laboratories at Dr. Wahidin Sudirohusodo Hospital. All authors reviewed and approved the final version of the manuscript.

Ethical Approval

The research has obtained ethical approval and was conducted in accordance with the Declaration of Helsinki. The confidentiality of the research data was guaranteed, and the research procedures and protocols were approved by the Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Approval Number: 726/UN4.6.4.5.31-/PP36/2024).

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References

1. Sgouras DN, Trang TTH, Yamaoka Y. Pathogenesis of *Helicobacter pylori* Infection. Helicobacter. 2015 09;20 Suppl 1(0 1):8-16. https://doi.org/10.1111/hel.12251
2. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet (London, England). 1983 06 04;1(8336):1273-1275.
3. Everhart J, Kruszon-Moran D, Perez-Perez G, Tralka T, McQuillan G. Seroprevalence and Ethnic Differences in Helicobacter pylori Infection among Adults in the United States. The Journal of Infectious Diseases. 2000 04;181(4):1359-1363. https://doi.org/10.1086/315384
4. Francesco VD. Helicobacter pylori therapy: Present and future. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2012;3(4):68. https://doi.org/10.4292/wjgpt.v3.i4.68
5. Yadav R, Sagar M. Comparison of Different Histological Staining Methods for Detection of Helicobacter pylori Infection in Gastric Biopsy. Cureus. 2022 07 26;. https://

doi.org/10.7759/cureus.27316
6. Gowsik DK, V. DA. Comparison of various histochemical staining methods for identification of helicobacter pylori. Tropical Journal of Pathology and Microbiology. 2019 09 30;5(9):692-695. https://doi.org/10.17511/jopm.2019.i09.12
7. Khan H, Rauf F, Muhammad N, Javaid M, Alam S, Nasir S. Comparison of special stains (Giemsa stain and Modified Toluidine Blue stain) with immunohistochemistry as gold standard for the detection of H. pylori in gastric biopsies. Arab Journal of Gastroenterology. 2022 05;23(2):75-81. https://doi.org/10.1016/j.ajg.2021.12.005
8. Yakoob J, Jafri W, Abid S, Jafri N, Abbas Z, Hamid S, Islam M, et al. Role of rapid urease test and histopathology in the diagnosis of Helicobacter pylori infection in a developing country. BMC Gastroenterology. 2005 Dec;5(1):38. https://doi.org/10.1186/1471-230X-5-38
9. Farouk WI, Hassan NH, Ismail TR, Daud IS, Mohammed F. Warthin-Starry Staining for the Detection of Helicobacter pylori in Gastric Biopsies. Malaysian Journal of Medical Sciences. 2018;25(4):92-99. https://doi.org/10.21315/mjms2018.25.4.9
10. Sandhika W. Detection Of Helicobacter Pylori Infection In Chronic Gastritis Biopsy Specimen Using Warthin-Starry And Modified Giemsa Stain In Dr Soetomo Hospital Surabaya. Indonesian Journal of Tropical and Infectious Disease. 2019 Oct 09;7(6):150. https://doi.org/10.20473/ijtid.v7i6.8404
11. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and Grading of Gastritis: The Updated Sydney System. The American Journal of Surgical Pathology. 1996 Oct;20(10):1161-1181. https://doi.org/10.1097/00000478-199610000-00001
12. Tammasse I. Analisis gambaran histopatologi mukosa lambung dan bakteri Helicobacter pylori pada pasien gastritis di RSUP Dr. Wahidin Sudirohusodo tahun 2016 [skripsi]. Makassar (Indonesia): Universitas Hasanuddin; 2016. Available from: Https://Www.Researchgate.Net/Publication/375592887_Analisis_Gambaran_Histopatologi_Mukosa_Lambungdan_Bakteri_Helicobacter_Pylori_Pada_Pasien_Gastritis_Di_Rsup_Dr_Wahidin_Sudirohusodo_Tahun_2016.
13. Rugge M, Meggio A, Pennelli G, Piscioli F, Giacomelli L, De Pretis G, Graham DY. Gastritis staging in clinical practice: the OLGA staging system. Gut. 2007 05 01;56(5):631-636. https://doi.org/10.1136/gut.2006.106666
14. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou J, Schulz C, Gasbarrini A, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut. 2022 09;71(9):1724-1762. https://doi.org/10.1136/gutjnl-2022-327745
15. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, Haruma K, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015 09;64(9):1353-1367. https://doi.org/10.1136/gutjnl-2015-309252
16. Breckan RK, Paulssen EJ, Asfeldt AM, Kvamme J, Straume B, Florholmen J. The All-Age Prevalence of Helicobacter pylori Infection and Potential Transmission Routes. Helicobacter. 2016 Dec;21(6):586-595. https://doi.org/10.1111/hel.12316
17. Kamada T, Haruma K, Ito M, Inoue K, Manabe N, Matsumoto H, Kusunoki H, et al. Time Trends in Helicobacter pylori Infection and Atrophic Gastritis Over 40 Years in Japan. Helicobacter. 2015 06;20(3):192-198. https://doi.org/10.1111/hel.12193
18. Nakajima S, Nishiyama Y, Yamaoka M, Yasuoka T, Cho E. Changes in the prevalence of Helicobacter pylori infection and gastrointestinal diseases in the past 17 years. Journal of Gastroenterology and Hepatology. 2010 05;25(s1). https://doi.org/10.1111/j.1440-1746.2009.06214.x
19. Ibrahim A, Morais S, Ferro A, Lunet N, Peleteiro B. Sex-differences in the prevalence of Helicobacter pylori infection in pediatric and adult populations: Systematic review and meta-analysis of 244 studies. Digestive and Liver Disease. 2017 07;49(7):742-749. https://doi.org/10.1016/j.dld.2017.03.019
20. Chuang C, Sheu B, Yang H, Lee S, Kao A, Cheng H, Chang W, Yao W. Gender Difference of Circulating Ghrelin and Leptin Concentrations in Chronic Helicobacter pylori Infection. Helicobacter. 2009 02;14(1):54-60. https://doi.org/10.1111/j.1523-5378.2009.00653.x
21. Almashhadany DA, Mayas SM, Mohammed HI, Hassan AA, Khan IUH. Population- and Gender-Based Investigation for Prevalence of Helicobacter pylori in Dhamar, Yemen. Canadian Journal of Gastroenterology and Hepatology. 2023 01 25;2023:1-10. https://doi.org/10.1155/2023/3800810
22. Zamani M, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, Derakhshan MH. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. Alimentary Pharmacology & Therapeutics. 2018 04;47(7):868-876. https://doi.org/10.1111/apt.14561
23. Krogfelt KA, Lehours P, Mégraud F. Diagnosis of Helicobacter pylori Infection. Helicobacter. 2005 09;10(s1):5-13. https://doi.org/10.1111/j.1523-5378.2005.00341.x
24. Taha Ali E, Al-Shamma S, Al-Araj E, Al-Zubaidi A, Hamza A. Comparison Between Immunohistochemical Marker and Conventional Histochemical Stains in Detecting Helicobacter pylori [Internet]. EC Microbiology. 2018. Available from: https://www.researchgate.net/publication/326262763..
25. Pandya HB, Patel JS, Agravat HH, Patel SB, Thakkar MC. Identification of Helicobacter pylori by different conventional staining techniques and its comparison with polymerase chain reaction. Saudi Medical Journal. 2013 09;34(9):942-948.
26. Akeel M, Elhafey A, Shehata A, Elmakki E, Aboshouk T, Ageely H, Mahfouz MS. Efficacy of immunohistochemical staining in detecting Helicobacter pylori in Saudi patients with minimal and atypical infection. European Journal of Histochemistry. 2021 07 20;65(3). https://doi.org/10.4081/ejh.2021.3222
27. Kusters JG, Van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori Infection. Clinical Microbiology Reviews. 2006 07;19(3):449-490. https://doi.org/10.1128/CMR.00054-05
28. Rokkas T, Sechopoulos P, Pistiolas D, Margantinis G, Koukoulis G. Helicobacter pylori infection and gastric histology in first-degree relatives of gastric cancer patients: a meta-analysis. European Journal of Gastroenterology & Hepatology. 2010 09;22(9):1128-1133. https://doi.org/10.1097/MEG.0b013e3283398d37
29. Almeida N, Donato MM, Romãozinho JM, Luxo C, Cardoso O, Cipriano MA, Marinho C, Fernandes A, Sofia C. Correlation of Helicobacter pylori Genotypes with Gastric Histopathology in the Central Region of a South-European Country. Digestive Diseases and Sciences. 2015 01;60(1):74-85. https://doi.org/10.1007/s10620-014-3319-8
30. De Almeida NM, Fernandes A, Romãozinho JM, Freire P, Donato MM, Cardoso O, Luxo C, et al. Correlation of NOD2 genotypes with Helicobacter pylori infection in a South-European country. Advances in Digestive Medicine. 2021 09;8(3):163-171. https://doi.org/10.1002/aid2.13210
31. Miftahussurur M, Yamaoka Y. Diagnostic Methods of Helicobacter pylori Infection for Epidemiological Studies: Critical Importance of Indirect Test Validation. BioMed Research International. 2016;2016:1-14. https://doi.

org/10.1155/2016/4819423
32. Misra V, Misra S, Dwivedi M, Singh ÜPA, Bhargava V, Gupta SC. A topographic study of Helicobacter pylori density, distribution and associated gastritis. Journal of Gastroenterology and Hepatology. 2000 07;15(7):737-743. https://doi.org/10.1046/j.1440-1746.2000.02240.x
33. Jung YS, Park CH, Park JH, Nam E, Lee HL. Efficacy of Helicobacter pylori eradication therapies in Korea: A systematic review and network meta-analysis. Helicobacter. 2017 08;22(4):e12389. https://doi.org/10.1111/hel.12389
34. Oling M, Odongo J, Kituuka O, Galukande M. Prevalence of Helicobacter pylori in dyspeptic patients at a tertiary hospital in a low resource setting. BMC Research Notes. 2015 Dec;8(1):256. https://doi.org/10.1186/s13104-015-1184-y
35. Mujtaba A, Ibrahim MS, Parveen S, Sarwar N, Alsagaby SA, Raza MA, Abdelgawad MA, et al. Comparative Analysis of Diagnostic Techniques for Helicobacter pylori Infection: Insights for Effective Therapy. Journal of Cellular and Molecular Medicine. 2025 03;29(6):e70487. https://doi.org/10.1111/jcmm.70487
36. Kocsmár É, Szirtes I, Kramer Z, Szijártó A, Bene L, Buzás GM, Kenessey I, et al. Sensitivity of Helicobacter pylori detection by Giemsa staining is poor in comparison with immunohistochemistry and fluorescent in situ hybridization and strongly depends on inflammatory activity. Helicobacter. 2017 08;22(4):e12387. https://doi.org/10.1111/hel.12387
37. Nurdin W, Krisnuhoni E. Comparison of Helicobacter pylori Detection Using Immunohistochemistry and Giemsa and Its Association with Morphological Changes in Active Chronic Gastritis. 2016. https://doi.org/10.1111/jcmm.70487.
38. Laheij RJ, De Boer WA, Jansen JB, Van Lier HJ, Sneeberger PM, Verbeek AL. Diagnostic performance of biopsy-based methods for determination of Helicobacter pylori infection without a reference standard. Journal of Clinical Epidemiology. 2000 07;53(7):742-746. https://doi.org/10.1016/S0895-4356(99)00222-X
39. Noto JM, Peek RM. The Role of microRNAs in Helicobacter pylori Pathogenesis and Gastric Carcinogenesis. Frontiers in Cellular and Infection Microbiology. 2012;1. https://doi.org/10.3389/fcimb.2011.00021
40. Brito BBD, Silva FAFD, Melo FFD. Role of polymorphisms in genes that encode cytokines and Helicobacter pylori virulence factors in gastric carcinogenesis. World Journal of Clinical Oncology. 2018 09 14;9(5):83-89. https://doi.org/10.5306/wjco.v9.i5.83
41. Wang J, Wang X, Luo H, Xie Y, Cao H, Mao L, Liu T, Yue Y, Qian H. Extracellular vesicles in Helicobacter pylori-mediated diseases: mechanisms and therapeutic potential. Cell Communication and Signaling. 2025 02 11;23(1):79. https://doi.org/10.1186/s12964-025-02074-6
42. Díaz Del Arco C, Fernández Aceñero MJ, Ortega Medina L. Liquid biopsy for gastric cancer: Techniques, applications, and future directions. World Journal of Gastroenterology. 2024 03 28;30(12):1680-1705. https://doi.org/10.3748/wjg.v30.i12.1680
43. Matsuoka T, Yashiro M. Novel biomarkers for early detection of gastric cancer. World Journal of Gastroenterology. 2023 05 07;29(17):2515-2533. https://doi.org/10.3748/wjg.v29.i17.2515
44. Saber Mirzaei S, Mehrdadi N, Nabi Bidhendi G, Pourmadadi M, Ahmadi M, Meknatkhah S. Novel detection of H.pylori using ultrasensitive electrochemical aptasensor based on surface modified graphene oxide doped gold nanoparticles conjugated polythiophene. Microchemical Journal. 2024 05;200:110279. https://doi.org/10.1016/j.microc.2024.110279