

ORIGINAL ARTICLE

## Gastric histopathological features after the administration of omeprazole, amoxicillin, and clarithromycin in gastritis *Helicobacter pylori* rat model

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### ABSTRACT

**Objective:** This research work aimed to assess the histopathological features and degree of gastritis severity in a rat model, induced by *Helicobacter pylori* infection after administering omeprazole, amoxicillin, and clarithromycin as the standard first-line eradication regimen.

**Material and Methods:** Twenty-one male rats were adapted for 7 days and randomly divided into three equal groups. Group 1 was considered a negative control. Group 2 and Group 3 were treated as *H. pylori*-inoculated groups. Group 2 was set as a positive control. Group 3 was administered omeprazole, amoxicillin, and clarithromycin as a first-line eradication regimen. Gastric histopathological examination was conducted. The difference in the severity of gastritis among the groups was examined using the one-way analysis of variance test. The significance was determined to be  $p < 0.05$ .

**Results:** Gastritis was found in all inoculated groups. The severity of gastritis was highest in Group 2 ( $p < 0.05$ ). We could see a refinement in gastritis severity after administering omeprazole, amoxicillin, and clarithromycin as a first-line eradication regimen (Group 3 vs. Group 2;  $p < 0.05$ ).

**Conclusion:** Gastritis, induced by the *H. pylori* rat model, was found in all inoculated groups. There was a refinement in the degree of gastritis severity after the administration of omeprazole, amoxicillin, and clarithromycin as a first-line eradication regimen.

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### Introduction

The integrity of the gastric mucosa is maintained through a balance between defensive and aggressive factors. Defense factors include mucus, gastric epithelium, bicarbonate, and gastric mucosal bloodstream. On the contrary, aggressive factors include pepsin, biliary reflux, acid, nicotine (cigarettes), alcohol, non-steroid anti-inflammatory drugs, corticosteroids, free radicals, and *Helicobacter pylori* [1]. The disruption of the balance of these two groups of factors, predominating the aggressive factors, promotes gastroduodenal pathologic lesions. Clinically, the lesions vary depending on the severity of gastroduodenal damage [2]. In day-to-day clinical practice, gastritis, an inflammation of the gastric mucosa, and gastroduodenal infections are assessed to be over 80%, brought about by *H. pylori* [3].

*Helicobacter pylori* is a pathogenic bacteria that cause gastric mucosal damage in the form of acute gastritis, chronic gastritis, peptic ulcer disease, and gastric malignancy [4,5]. Nowadays, gastritis prevalence due to *H. pylori* in non-industrial countries is more than in industrial countries. Although in general, in any event, the greater part of the total population has been exposed to and infected with *H. pylori*. Gastritis by *H. pylori* is inseparable from the distribution pattern, the environment, the level of sanitation, the social economy, and bacterial resistance to antibacterial agents [6,7].

The clinical manifestations of patients with gastritis by *H. pylori* vary, ranging from asymptomatic to severe dyspepsia [8,9]. The invasive diagnosis modalities include endoscopic examination, biopsy with a rapid urease test,

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and histopathological examination. Gastric tissue biopsy and histopathology are currently the gold standards in assessing the severity of gastritis by scoring, according to the updated Sydney system (USS) [10,11].

Managements of gastritis *H. pylori* are currently developing to obtain an optimal eradication success rate [12]. Treatment of gastritis by *H. pylori* is recommended considering several factors, namely the pattern of bacterial resistance and antibacterial agents' combination as an eradication regimen [13,14]. The current antibiotic resistance issue is severe and primarily related to the lack of data on antibacterial resistance patterns [7]. At present, globally, the standard regimen for eradication of *H. pylori* known as proton pump inhibitors (PPIs)-based triple therapy [15]. The standard first-line eradication regimen consists of a PPI and two types of antibacterial agents, namely amoxicillin and clarithromycin, for 7–14 days [14].

Studies with human subjects are widely conducted, but the presence of confounding factors along with a high-enough dropout rate often produces inconsistent results, low validity, and very little research that discusses the effect of a standard first-line eradication regimen on gastric histopathological features in the management of gastritis by *H. pylori* [16,17]. This work aimed to assess the histopathological features and the severity of gastritis in a rat model inoculated with *H. pylori*, after treatment with omeprazole, amoxicillin, and clarithromycin as the standard first-line eradication regimen.

## Materials and Methods

### Ethical clearance and study design

We carried out all the research procedures based on the ethical guidelines from the Animal Research Ethics Committee, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia, with reference no. 0448/KEPH-FMIPA/2019. This research was conducted from March until May 2020 at Biomedical Research Unit, West Nusa Tenggara General Hospital, Indonesia. We made a posttest-only with the control group and simple random sampling as the design of the study.

### Bacterial preparation

We cultured and reproduced *H. pylori* from human isolates, previously stored in the Microbiology Laboratory, Biomedical Research Unit, West Nusa Tenggara Province General Hospital. These isolates were derived from a biopsy specimen of duodenal ulcer patients. *Helicobacter pylori* was then cultured using Trypticase Soy Agar media enhanced with 10% fresh sheep blood, 2/500 ml Dent, and 10/500 ml Vitox supplement (Oxoid™, Thermo Scientific™,

Hampshire, UK). A CO<sub>2</sub> incubator was utilized for the incubation process with O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub> in concentrations of 5%, 10%, and 85%, respectively, for 72 h at 37°C under microaerophilic atmospheric conditions [18]. We further confirmed the presence of *H. pylori* based on colony characteristics, microscopic images through Gram stain, and biochemical analysis.

### Experimental animals

There were 21 male albino rats (*Rattus norvegicus*) used in this study. The animals' terms and conditions were the mean age and body weight (BW) of rats were 10 weeks and 295 gm, respectively, wellbeing, dynamic movement, and a great appetite. They were accommodated at room temperature around 27°C; 70%–80% humidity, in cages under 12 h light/dim cycle, and the cages cleaned routinely. All rats were fed standard commercial rodent food and full access to water. They were adapted for a week in a laboratory facility, then grouped randomly into three groups, seven in each group, namely (1) control negative, without *H. pylori* inoculation; (2) control positive (*H. pylori* inoculation); and (3) *H. pylori* inoculation + aqueous solution of a first-line eradication standard regimen (amoxicillin 50 mg/kg BW + clarithromycin 25 mg/kg BW + omeprazole 20 mg/kg BW). The subjects in Group 2 and Group 3 were inoculated with suspension comprising *H. pylori*  $5 \times 10^8$ – $5 \times 10^{10}$  colony-forming unit/ml equal to 2.0 McFarland Standard in 0.9% (w/v) sodium chloride (0.9% NaCl) at 1 ml/rat.

### Standard eradication regimen

The standard eradication regimen comprised an aqueous solution of omeprazole, amoxicillin, and clarithromycin (PT. Indofarma, Indonesia). All drugs were crushed in a dose of 50, 25, and 20 mg/kg BW separately, in 0.5% (w/v) carboxymethyl cellulose (0.5% CMC). All agents were obtained from a commercial medical store.

### Experimental procedure

All rats in Group 1 took routinely drinking water. Simultaneously, Group 2 and Group 3 were pretreated streptomycin (PT. Indofarma, Indonesia) crushed in faucet water (5 mg/ml) for 72 h before inoculation of *H. pylori* to the stomach. This protocol of the experiment was followed after a week of the adaptation period. Streptomycin was obtained from a commercial medical store. All animals within the following day were fasted for 24 h. Group 1 animals were augmented with a liquid of 0.9% NaCl at 1 ml/rat orally by a feeding tube twice a day at a lapse of 4 h for three subsequent days. Group 2 and Group 3 were inoculated water with *H. pylori* suspension at 1 ml/rat orally by a feeding tube twice a day at a lapse of 4 h for three

subsequent days. Group 2 and Group 3 animals received oral omeprazole (PT. Indofarma, Indonesia) at 400 µmol/kg BW crushed in 0.5% CMC, at 1 ml/rat once every day orally by a feeding tube 3 h before the first *H. pylori* inoculation and during the accompanying 6 days. At the same time, rats in Group 1 were given 0.5% CMC suspension in the same protocol.

All rats were not fed at night before the treatment, 14 days after the last day of inoculation of *H. pylori*. To assess *H. pylori* infection, rats from each group were haphazardly chosen and victimized under diethyl ether sedation in a box. A laparotomy procedure was performed on a chosen rat from all groups. To identify *H. pylori* in gastric tissue, the stomach was taken out, and the gastric antral area (4 mm<sup>2</sup>) was selected and made a urease test (Pronto Dry®, Gastrex, France). A positive urease test in all rats from Group 2 and Group 3 was found from this procedure. On the following day, the leftover animals from groups were managed based on the protocol. All subjects from Group 1 and Group 2 were treated with a 0.5% CMC suspension once orally by feeding tube for 7 days.

On the contrary, rats in Group 3 were medicated with a standard eradication regimen at a lapse of 6 h orally 1 ml/rat once every day by a feeding tube for 7 days. On day 28 after complete treatment, all subjects were victimized under diethyl ether sedation in a box. The laparotomy was carried out to make a rapid urease test and histopathological examination. In this period, we found all inoculated groups completely infected with *H. pylori*. Later on, gastric antral sections were prepared for histopathological examination.

### Histopathological examination

A little part of the gastric antral section was selected, and fixation with a 10% buffered neutral formalin solution (pH 7.4, 24 h) was carried out. At that point, preparation and instilling of the gastric antral tissue were carried out on the paraffin block. A thickness of 5 µm from the paraffin block was made and presented on the slides before the staining mode. The setting was prolonged using the hematoxylin and eosin (H&E) staining mode. The degree of gastritis severity and *H. pylori* density were examined microscopically, scored, and categorized as normal, mild, moderate,

or severe gastritis using the USS in assessing neutrophil activity, lymphocyte infiltration, glands atrophy, intestinal metaplasia and *H. pylori* existence [10]. The results of the examination were reported and blindly analyzed by a pathologist using the Olympus® CX22 LED binocular light microscope and Vivo® 9 camera.

### Statistical analysis

For assessing the normality of the quantitative variable, we carried out a Shapiro–Wilk test. For normal and non-normally distributed data, the results were displayed as mean ± standard deviation (SD) and as median (minimum–maximum), respectively. To compare and analyze the mean of quantitative variables with normal and non-normal distribution, a one-way analysis of variance (ANOVA) with *post hoc* Tamhane and Kruskal–Wallis test was carried out separately. Significance was determined at  $p < 0.05$ .

### Results

A positive urease test was recorded to monitor the *H. pylori* infection process, and even each rat representing *H. pylori* inoculated groups. The degree of gastritis severity in this study was assessed by histopathology, based on the USS method (Table 1). The severity of gastritis was higher in Group 2 (ANOVA test,  $p < 0.05$ ). We could see an improvement in gastritis after *H. pylori* eradication by administering the first-line eradication regimen, as shown in Table 1 ( $p < 0.05$ ). The comparison of the total USS score in each group was carried out with *post-hoc* analysis. This showed differences in the degree of severity of gastritis between groups, which can be seen in Table 2 ( $p < 0.05$ ). Histopathological representation of the rat's stomach tissue of each group is shown in Figure 1. The microscopic assessment results based on the histopathological examination indicated no rat classified as severe gastritis. We found as many as 22.2% of rats without gastritis, 44.4% with mild gastritis, and moderate gastritis in 33.3%.

### Discussion

This study used rats as animal experiments because of their physiological similarities with humans [19]. Monkeys, guinea pigs, pigs, cats, ferrets, mice, gerbils, and

**Table 1.** Gastric histopathological examination results based on the USS.

| Groups  | Variable    |             |                |                       |                          | USStotal score | p-value |
|---------|-------------|-------------|----------------|-----------------------|--------------------------|----------------|---------|
|         | Lymphocyte  | Neutrophil  | Glands atrophy | Intestinal metaplasia | <i>H. pylori</i> density |                |         |
| Group-1 | 0.00 ± 0.00 | 0.33 ± 0.52 | 0.00 ± 0.00    | 0.00 ± 0.00           | 0.00 ± 0.00              | 0.33 ± 0.52    | <0.001* |
| Group-2 | 1.17 ± 0.41 | 2.17 ± 0.41 | 1.00 ± 0.00    | 1.00 ± 0.00           | 1.50 ± 0.55              | 6.83 ± 0.41    |         |
| Group-3 | 1.00 ± 0.00 | 1.83 ± 0.41 | 0.67 ± 0.52    | 0.17 ± 0.41           | 0.33 ± 0.52              | 4.00 ± 0.63    |         |

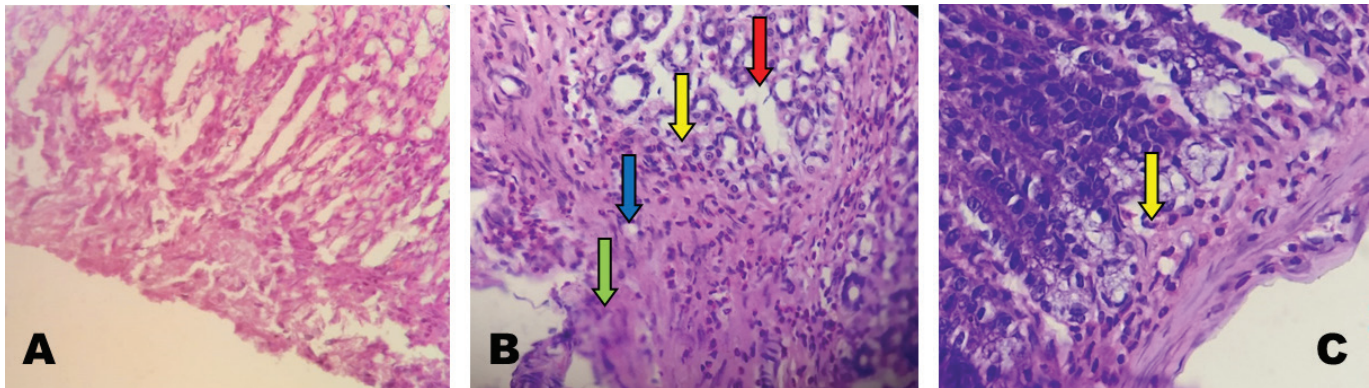
Data stated as mean ± SD. Statistical test using one-way ANOVA. USS = Updated Sydney System. The number of rats in each group was 6. \* $p < 0.05$ .

**Table 2.** Post-hoc analysis comparison of the USS total score in each group.

| Group comparison   | Mean difference | 95% CI  |         | p-value |
|--------------------|-----------------|---------|---------|---------|
|                    |                 | Minimum | Maximum |         |
| Group 1 vs Group-2 | -6.500          | -7.28   | -5.72   | <0.001* |
| Group 1 vs Group-3 | -3.667          | -4.63   | -2.71   | <0.001* |
| Group 2 vs Group-3 | 2.833           | 1.92    | 3.74    | <0.001* |

Post-hoc Tamhane. The number of rats in each group was 6.

\* $p < 0.05$ .



**Figure 1.** Photomicrograph of histopathological features; representation of the rat's gastric tissue (hematoxylin and eosin staining). (A) Group 1; (B) Group 2; (C) Group 3. Red arrows, yellow arrows, blue arrows, and green arrows indicate *H. pylori* bacteria, PMN cells, edema, and vasodilation, respectively. (Olympus CX22 LED microscope with a magnification of 400× and Vivo 9 camera.)

rats as animal models of *H. pylori*-induced infection have been used for a long time until now [20]. The technique for the induction of *H. pylori* infection to rat's stomach in this current study was consistent with our previous work, and we found that the success rate arrives at 100%, proved by rapid urease test [18]. The fundamental rule of this technique is to create a milieu that supports the accretion of *H. pylori* in the stomach. This procedure begins with streptomycin and omeprazole's initial administration to prevent other bacteria's accretion and degrade gastric acidity separately [19,21].

There are several factors associated with *H. pylori* infection, including impaired gastric motility, gastric acidity, and biofilm formation [22–25]. The condition of impaired gastric motility and gastric acidity develops due to disproportionate activities and increased somatostatin and gastrin [22,23]. Bacterial biofilm formation is one of the leading factors that cause resistance to antibacterial agents [26]. Hence, the above-mentioned factors support the agent or drug's outcome in eradicating *H. pylori*. This study did not assess impaired gastric motility, gastric acidity, and biofilm formation because we focused more on the histopathological changes associated with *H. pylori* infection.

Gastric injury or infection induces inflammation of the gastric mucosa. *Helicobacter pylori* transmission in the stomach promotes a prolonged and lasting inflammation

by activating mediators adjusting the movement of neutrophils, macrophages, and other leukocytes, which in turn cause pathologic lesions of the gastric mucosa [13,27]. Histopathological diagnosis is a gold standard that specifies the degree of gastritis severity according to the USS. Gastritis due to *H. pylori* is portrayed by an enhanced polymorphonuclear activation and density of mononuclear cells, generally associated with glands atrophy, intestinal metaplasia, and *H. pylori* density in gastric mucosa [11]. The high density of *H. pylori* will be appropriately related to the degree of gastritis severity [10]. In this current research, gastritis was obtained in the inoculated groups. Several studies noted that eradicating *H. pylori* with the first-line eradication regimen diminishes the inflammation and improves gastritis severity [28,29]. The same result was found in this study.

Omeprazole, amoxicillin, and clarithromycin are the first-line eradication regimen. The existence of omeprazole degrades gastric luminal acidity by lowering the pH inclination and creating a chemotactic bias to *H. pylori*. The outcome of antibacterial action becomes more ideal [15]. Amoxicillin blocked the bacterial cell wall and its membrane synthesis. The concentration of amoxicillin in the stomach increased beyond the minimum inhibitory concentration due to reduced gastric acidity, following omeprazole administration. Therefore, the combination of

omeprazole and amoxicillin promotes the ability of *H. pylori* eradication [13,14]. The transcription process, the translation into the formation of *H. pylori* protein, is inhibited by clarithromycin, an antibacterial macrolide. Clarithromycin works by binding to the 50s bacterial ribosomal subunit. A widespread study found that the combination of clarithromycin and amoxicillin increases the eradication rate by more than 70% [30,31]. We found in this work that the administration of a first-line eradication regimen delivers a perfect eradication rate. We also found some limitations in this study, including the absence of data regarding bacterial resistance to antibacterial agents and the unknown type of *H. pylori* strain.

## Conclusion

In this study, the induction of gastritis with *H. pylori* inoculation by our method can produce a high percentage of success; it can be seen that gastritis *H. pylori* was found in all inoculated groups. There was a refinement in the degree of gastritis severity after the administration of omeprazole, amoxicillin, and clarithromycin as a first-line eradication regimen.

## List of abbreviations

PPIs: Proton pump inhibitors; BW: Body weight; USS: Updated Sydney system; CMC: Carboxymethyl cellulose; SD: Standard deviation; ANOVA: Analysis of variance

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## Conflict of interest

The authors declare no potential conflict of interest related to the study, preparing, and publication of this manuscript.

## Authors' contribution

All authors participated and contributed to this manuscript. OKY planned, designed, managed the research, interpreted the data, and drafted the manuscript. SBT contributed to preparing, critical checking, and wrote the manuscript. OKI analyzed data, statistical examination, and wrote the manuscript. ZM did laboratory works, collected data, and preparation of the manuscript. All authors read and approved the final manuscript.

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