International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Inoculation of Helicobacter Pylori Bali03isolate as a Risk Factor in Increasingthe Severity of Gastritis Compared to ATCC 43504 Isolatein Balb/C Mice

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Abstract: ***Gastritis is a common gastrointestinal health problem, where H. pylori infects nearly 50% of the world's population. The prevalence of H. pylori infection in developing countries is higher compared to developed countries. H. pylori causes gastric inflammation in all infected patients. The aim of this study was to find out the mean difference of gastritis degree and prove the degree of gastritis is more severe in BALB/c mice inoculated with H. pylori Bali 03 isolate compared to ATCC 43504 isolate.This study was an experimental study with a randomized posttest only control group design, in 36 male BALB / c mice which were divided into 2 groups : group I was inoculated with H. pylori ATCC 43504isolate, while group II was inoculated with Bali 03 isolates. The study was conducted at the Department of Anatomical Pathology, Sanglah Hospital, Denpasar, and the Biomedical Laboratory of NTB Province Hospital, Mataram, from February to April 2019.In the eighth week, all samples were euthanized, then a gastric resection is performed to assess the gastritis degrees according to revised Sydney system by conventional histopathology and immunohistochemistry.Mice inoculated by H. pylori ATCC 43504 isolate had the incidence of mild and moderate gastritis, 16 (88.8%)and 2 (11.1%) respectively, while mice inoculated by H. pylori Bali 03 isolate had the incidence of mild and moderate gastritis 7 (38.8%)and 11 (61.1%) respectively. The mean difference of gastritis degree showed that the average gastritis score in mice inoculated by H. pylori ATCC 43504 isolate was 3.88; while mice inoculated by H. pylori Bali 03 isolate was 6.22, with p values: 0,000. The difference in the proportion of gastritis degrees withx^2 test showed that H. pylori Bali 03 isolate caused severergastritisdegree by 5.5 times compared to H. pylori ATCC 43504 isolate (RR 5.5; 95% IK 1.41-21.37).***

Keywords: H. pylori, ATCC 43504, Bali 03, Gastritis

Inoculation of Bali03 helicobacter pylori isolate increased the risk more severe degrees of gastritis in male Balb/ C compared to atcc 43504 h pylori isolate

1. Background

Gastritis is one of gastrointestinal health issues that often occur. Helicobacter pylori (H. pylori) infects almost 50% of the world population.[1]The prevalence of H. pylori infection in developing countries is higher compared with developed countries. The prevalence in developed countries is around 30-40%, while in developing countries 80-90%. [2]African countries such as Ethiopia, Gambia, Nigeria, more than 90% of adult population is infected *with H. pylori.* Gambia, 95% of children aged less than 5 years old infected with *H pylori*. Latin American countries, such as Chile and Mexico, approximately 70% of adults infected with *H. pylori*, whereas in children only 47%. The high prevalence of*H. pylori* is also happening in India, in adults is about 88%. Research in Bangladesh to get the prevalence *of H. pylori* by 42% in children aged 2 years and rapidly increased to 67% in children aged 10 years. [2,3]The prevalence of H. *pylori* infection in Indonesia is still controversial. Research by Syam AF, et.al., in patients with dyspepsia in Java, Papua, Sulawesi, Borneo and Sumatra, found that the prevalence of *H. pylori* infection was 22.1%. Ethnic Papua, Batak and Bugis has a risk of infection *with H. pylori* is greater than the Javanese, Dayak and Chinese. [4]

H. pylori is a gram-negative bacterium that can survive in the acid environment in the stomach or duodenum, curved or *S-shaped* with a length of about 3 micrometers, 0.5 micrometers in diameter, have one or more flagella at one end. *H. pylori* colonization is not a disease in itself, but it will affect the risk of various diseases and upper gastrointestinal tract may also hepatobiliary.[5] Wirawan , et. al., reported and proved that the bacterium H. pylori isolates Bali 03 may cause precancerous lesions in BALB/c. Glandular atrophy and intestinal metaplasia found only in 12 weeks.[6] No data yet that compared the degrees of gastritis on Balb/c inoculated with H. pylori Bali isolate 03 compare with H. pylori ATCC 43504. In this study we compared the degrees of gastritis in Balb C inoculated by H.pylori Bali isolat 03 comared with H. pylori ATCC 43504.

2. Material and Method

Balb/c were randomly divided into two groups. Eighteen (18) Balb/c inoculated by H. pylori ATCC 43504 (group I) and 18 Balb/c inoculated by H. pylori Bali 03 isolate. After 8 weeks, 36 Balb/c were euthanazied by cervical dislocation, and all gasters samples were processed and stained with hematoxillin-eosin stain and immunohisthochemistry method. Degrees of gastritis were compared using modified Sydney system criteria.

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3. Results

Gastritis occurred in all Balb/c gastric samples (36; 100%). Acute inflammatory cell infiltrations such as neutrophils and chronic inflammatory cells such as lymphocytes and plasma cells were found in both treatment groups. Simultaneous presence of inflammatory neutrophils and lymphoplasmacytic cells in the gastric mucosa is a characteristic of chronic active gastritis caused by H. pylori bacteria (Yamaoka, 2013).

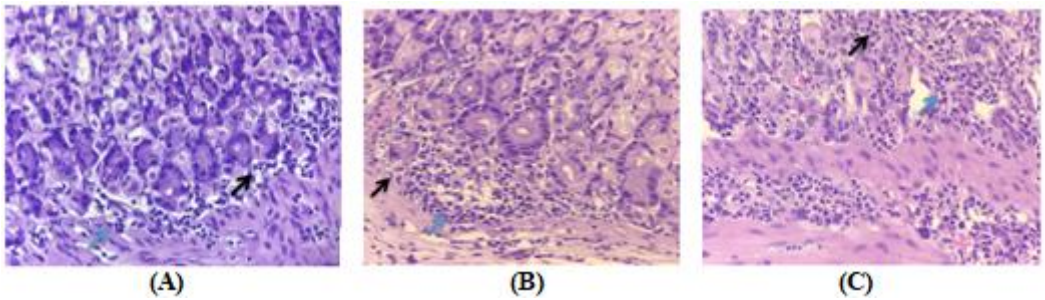

Figure 1: Infiltration of mononuclear (blue arrow) and polymorphonuclear (black arrow) cell in mucosa and submucosa gaster group I.A. Mild infiltration, B. Moderate infiltration, C. Severe infiltration (400x magnification)

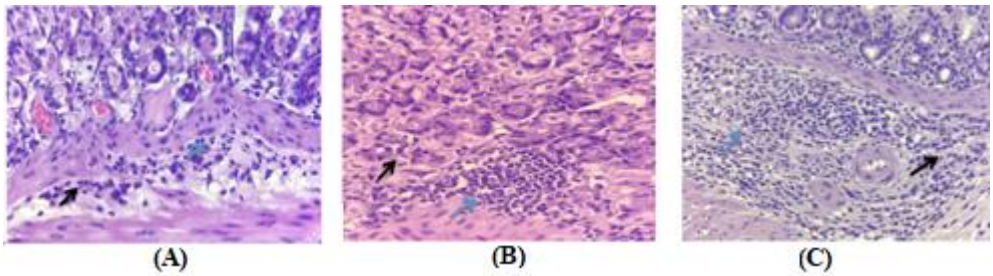

Figure 2: Infiltration of mononuclear (blue arrow) and polymorphonuclear (black arrow) cell in mucosa and submucosa gaster group II. A. Mild infiltration; B. Moderate infiltration; C. Severe infiltration (400x magnification)

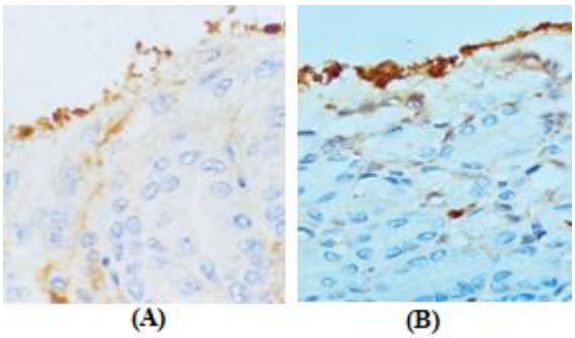

Figure 3: H. pylori were stained as brown colour by immunohisthochemistry. A: group I; B: Group II (1000x magnification)

The mean difference of gastritis degrees between two groupswas measured by independent sample t-test.The mean gastritis score in mice inoculated H. pylori ATCC 43504 was 3.88; while mice inoculated with H. pylori Bali 03 was 6.22. The p value was 0.000 ($p < 0.05$ considered as statistically significant result) (Table 1).

Table 1: Independent t-test to measured the differential mean of gastritis degrees (score) in both groups

Hp isolate	Sample	Mean ± SD	pvalue
ATCC 43504	18	3,88 ± 1.49	0.000
Bali 03	18	6,22 ± 1.89	

The difference of gastritis degrees proportionin the two groups was measuredby Chi square test.The p value was 0,002 ($p < 0.05$ considered as statistically significant result) (Table 2).

Table 2: Chi-square result to measure the difference of proportion in both groups

Hp isolate	Gastritis degree		RR CI 95%	p value
	Mild	Moderate		
ATCC 43504	16 (88.8%)	2 (11.1%)	5.5	0.002
Bali 03	7 (38.8%)	11 (61.1%)	1.41- 21.37	

Mice that inoculated by H. pylori ATCC 43504 isolatehad the incidence of mild and moderate gastritis were 16 and 2 (88.8% and 11.1% respectively), while micethat inoculated by H. pylori Bali 03isolate had the incidence of mild and moderate gastritis were 7 and 11 (38.8% and 61.1% respectively). The Chi square test found that the group inoculated with H. pylori Bali 03 isolate had increased the risk of more severe gastritis 5.5 times than group inoculated by H. pylori ATCC 43504 isolate (RR 5.55; 95% CI 1.41 - 21.37). In other words, H. pylori Bali03 isolates have the ability to lead the more severegastritis by 5.5 times compared to H. pylori ATCC 43504isolate.

4. Discussion

Gastritis occurred in both of groups, group I (18 individuals; 100%) and group II (18 individuals; 100%). This is in accordance with previous studies conducted by Wirawan et al., who examined gastric inflammation in BALB/c mice by inoculating H. pylori isolate Bali 03, where all mice also experienced gastritis. In contrast to Wirawan et al., there were no premalignant lesions (glandular atrophy or intestinal metaplasia) was found. It is possible that the factor of giving a low iron diet in the study of Wirawan et al. was considered

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as the cause of the occurrence of premalignant lesions faster, affecting the inflammatory mechanism through proliferation, activation and differentiation of T cells.[6] While in this study both groups were given normal feed content with normal amounts of iron, so that it was thought to have a good immune response in eliminating the presence of H. pylori agents so that premalignant lesions did not occur.Cellular and adaptive immune responses due to H. pylori infection in mice are almost the same as those that occur in humans. A distinctive feature of the immune response in H. pylori infection is the presence of gastric mucosal infiltration by T cells, plasma cells, and neutrophil cells in the gastric mucosa simultaneously. [8,9] Clinical and histopathological studies in human gastric and mice show T cell subset TCD4 $^{+}$, TCD8 $^{+}$ are involved in an immune response against H. pylori infection. This is in accordance with the results of this study. All gastric mice histopathological results 36 (100%) shown an inflammation that were in accordance to of gastric inflammation characteristic caused by H. pylori.The degree of gastritis is assessed based on the five components in updated Sydney system including: the density of H. pylori bacteria, as acute inflammatory cells neutrophil, lymphocytes and plasma cells, gland atrophy and intestinal metaplasia. There are differences in the degree of gastritis in the two study groups. The degree of gastritis was found more severe in mice that inoculated by H. pylori Bali 03 isolate than in mice that inoculated by H. pylori ATCC 43504 isolate, and statistically significant with *p* value: 0.002 ($p< 0.05$). The same diet with the same level of iron adequacy in the two groups, assuming that the immune response that arises due to H. pylori infection will be just as good. The difference in the degree of gastritis that arises in this study is suspected by the different strains of H. pylori in both of groups.H. pylori Bali03 contains East Asian CagA^{+} virulence factors, while H. pylori ATCC 43504 also contains CagA^{+} virulence factors but different types was Western type. Yuan et al., found that gastritis due to H. pylori correlates with the presence of the cagA $^{+}$ gene. The results shown that gastric mucosal inflammatory cell infiltration was significantly higher in patients infected with East Asian CagA compared to Western type CagA gene ($p <0.05$). [10,11]H. pylori strains with the East Asian type cagA gene are closely associated with high IL-8 secretion in vitro and in vivo compared with Western type H. pylori CagA strains ($p <0.01$). [11,12,13] H. pylori strains with the East Asian type cagPAI gene can translocate cagA into host cells very strongly. These results indicate that H. pylori strains with the East Asian type cagPAI gene are more virulent than the cagPAI gene Western type. [11,14]The East Asian type CagA has a higher affinity for Src-2 homologues containing phosphatase 2 (SHP2) which causes a higher risk for gastric ulcer and / or gastric cancer than Western type cagA. Miftahuzurrur et al., found that subjects infected by strains with the EPIYT sequence had higher inflammation, compared with strains that had the EPIYA sequence.[15]This is in accordance with the results of this study where the degree of gastritis was found to be more severe in the group inoculated with H. pylori Bali03 containing cagA $^{+}$ East Asian type.[6]H. pylori adhere to epithelial cells through various components of the bacterial surface. Adhesin molecul is Bab A, an outer membrane protein that is bound to the Lewis blood antigen group. Some other proteins family Hop protein (outer membrane protein) is also a component of adhesin in epithelial cells. Evidence shown that adhesin is related to H. pylori related diseases and can affect the severity of the disease.[1] Other virulence factor such as adhesin has not explained in H. pylori Bali isolates 03 yet. H. pylori ATCC 43504 has a virulence factor vacA + which is the main toxin protein secreted by H. pylori. 88 kDa vacA toxin (sub unit p33 and p55) secreted by bacteria and inserted into the host cell through a type IV secretion system, giving rise to "vacuolization" which is characterized by the accumulation of large vesicles from endosomes and lysosomes. The development of "vacuole" has been associated with the formation of selective anion vacA channels in epithelial cell membranes.[12,14,15] Regardless of the effect of its vacuole, recent research has suggested that vacA also directly affects mitochondrial function. Previous studies have shown that the p33 subunit can enter the mitochondria to modulate organelle function. There are variations in vacuolation activity from different H. pylori strains, mainly due to differences in the vacA gene structure in the signal region (s), namely s1 and s2 and the middle region (m), namely m1 and m2. In vitro experiments showed that the s1 / m1 strain was the most cytotoxic strain such as the vacA gene possessed by H. pylori ATCC 45304. VacA s1 / m1 gene toxicity ability was followed by s1 / m2 strain, then s2 / m1 strain, and s2 / m2 which is not cytotoxic.[1]The vacA virulence factor and it's subtype in H. pylori Bali 03 not yet known because there's no study for this matter yet.

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