



## DIFFERENCE BETWEEN HSP60 IN POSITIVE AND NEGATIVE H.PYLORI GASTRITIS PATIENTS AND ITS CORRELATION WITH NEUTROPHIL AND LYMPHOCYTE RATIO

Ananda Rahmat Putra\*<sup>1</sup>, Gontar Alamsyah Siregar<sup>2</sup> & Imelda Rey<sup>2</sup>

\*<sup>1</sup>Resident of the Department of Internal Medicine, Faculty of Medicine, University of Sumatera Utara

<sup>2</sup>Staff Department of Internal Medicine, Faculty of Medicine, University of Sumatera Utara

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### Abstract

**Background:** Gastritis is a disease characterized by an inflammatory process in both the mucosal and submucosal lining of the stomach. *Helicobacter pylori* (*H.pylori*) binds to gastric and mucin epithelial cells via HSP60, and the adaptive immune system against HSP60 is induced in patients infected with *H. Pylori*.

**Objective:** To determine the comparison of serum HSP60 levels between positive and negative *H. pylori* gastritis patients and their correlation with neutrophil-lymphocyte ratio.

**Research Method:** Cross-sectional design. Sampling was carried out at the Endoscopy Unit of Adam Malik General Hospital Medan. The study sample was taken as many as 60 subjects consecutively. The independent variable of the study was *H. pylori* infection and the dependent variable was HSP60 and neutrophil-lymphocyte ratio. Data analysis used the Mann Whitney U test and the Spearman correlation test.

**Results:** The demographic characteristics of the study subjects were 36 male subjects (60%), the median value of age 47.7 (25-66) years, the highest educational history was 34 subject high schools (56,7%), the most work history was private employees 24 subject (40%). There was a difference in HSP60 in patients with positive and negative *H.pylori* gastritis ( $p < 0,000$ ). There was no correlation between HSP60 and neutrophil-lymphocyte ratio in patients with positive and negative *H. pylori* gastritis ( $p < 0.121$ ).

**Conclusion:** There are differences in HSP60 in patients with positive and negative *H.pylori* gastritis.

**Keywords:** *H. pylori*, HSP60, neutrophil-lymphocyte ratio.

### Introduction

Gastritis is a disease characterized by an inflammatory process in both the mucosal and submucosal layers of the stomach.<sup>1</sup> Based on the health profile in Indonesia in 2012, gastritis is one of the diseases in the 10 most diseases in hospitalized patients in hospitals in Indonesia with 30,154 cases (4 cases), 9%). From a study conducted by the Indonesian Ministry of Health in 2013 the incidence of gastritis in several cities in Indonesia was high reaching 91.6%, namely in Medan City, then in several other cities such as Jakarta 50.0%, Denpasar 46.0%, Palembang 35.5%, Bandung 32.5%, Aceh 31.7%, Surabaya 31.2% and Pontianak 31.1%.<sup>2,3</sup>

Gastritis can be caused by several factors, including transmission agent factors, chemical, physical, related to immunity and idiopathic.<sup>4</sup> Gastritis caused by disease factors is most often caused by colonization of *Helicobacter pylori* in the upper gastrointestinal tract - specifically the stomach, which is about 50% of the world's population.<sup>5</sup> Prevalence of *H. pylori* colonization is around 30% in the United States and other developed countries compared to 80% in many developing countries.<sup>6</sup>

The barrier system that lines the gastric mucosa consists of mucus-bicarbonate, the mucosal barrier formed by a continuous surface epithelial cell layer, which secretes mucus and bicarbonate and produces PG, HSP, TFFs, and cathelicidin.<sup>7</sup> large hetero-oligomeric protein.<sup>8</sup> *H. pylori* binds to gastric and mucin epithelial cells via HSP60, and the adaptive immune system against HSP60 is induced in patients infected with *H. Pylori*.<sup>9</sup>



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**Methods****Research sample**

The study sample was taken from patients with gastritis who came to H. Adam Malik General Hospital Medan and network hospital with consecutive sampling techniques. Research subjects were 60 gastritis patients consisting of 30 patients with H. pylori positive and 30 patients with H. pylori negative

**Study design**

This study is a cross-sectional design. The patient will undergo an endoscopy and biopsy to diagnose gastritis. To detect H. pylori serological examination (CLO) is performed. Examination of HSP60 levels by ELISA method. Examination of neutrophil and lymphocyte ratio uses the value calculated by the comparison of absolute neutrophils with absolute lymphocyte counts from blood tests.

**Statistic analysis**

Data from the study were statistically analyzed using the SPSS computer program version 22.0. To find out the comparison of total HSP60 levels between positive and negative H.pylori gastritis patients using the Mann Whitney U test. Correlation of HSP60 to neutrophil-lymphocyte ratio using the Spearman test.

**Results**

Based on the characteristics of 60 research subjects, the age of all research subjects has a median of 47.7 (25 - 66) years. Research subjects were 36 male subjects (60%) and 24 female subjects (40%). The education level of the research subjects was divided into 4 groups where 34 subjects (56.7%) high schools, 14 subjects (23.3%) junior high, 9 subjects (15%) university and 5 subjects (3.2%) elementary school. Most tribes in the study subjects were 35 Batak (58.3%).

*Table 1. Overview of Characteristics of Research Subjects*

Characteristics of respondents	Median	n (60)	Percentage (%)
Age	47,7 (25-66)		
Gender			
• Male		36	60
• Female		24	40
Education			
• Elementary School		3	5
• Junior High School		14	23,3
• Senior High School		34	56,7
• University		9	15
Etnics			
• Batak		35	58,3
• Jawa		20	33,3
• Aceh		5	8,3
Employment history			
• Private employe		24	40
• Housewife		14	23,3
• Enterpreneur		18	30
• Civil servants		4	6,7

Characteristics of positive and negative H. pylori gastritis patients in 60 study subjects who participated in this study, there were 30 subjects (50%) with positive H. pylori results and 30 subjects (50%) with negative H. pylori results. In this study HSP60 levels in all subjects had a median value of 10.3 with a minimum grade of 1.6



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and a maximum level of 3.6. The ratio of neutrophils and lymphocytes in all subjects of this study has a median value of 2.77 (1.4-10.2).

**Table 2. Characteristics Age, BMI, HSP60 and as neutrophil and lymphocyte ratio**

Characteristics (n=60)	Median
Age	47.71 (25-66)
Body Mass Index	22.63 (16.94-33.59)
HSP60 (ng/mL)	10.30 (1.6-36)
Neutrophil and lymphocyte ratio	2.77 (1.4-10.2)

Based on the statistical analysis of the Mann Whitney U test on 60 study samples, it was found that there were significant differences in HSP60 levels between the positive and negative H. pylori groups with a p value of <0.001. Where the median value in the positive H. pylori group was 16 and the median value in the negative H. pylori group was 3.2 so the difference between the two groups was 12.8.

**Table 3. Differences in HSP60 levels between positive and negative groups of H. pylori gastritis patients**

	<i>Helicobacter pylori</i>		P
	Positive	Negative	
HSP60 (ng/mL)	16	3.2	0,000

Uji UMan whitney

Based on the statistical analysis of the Spearman test on 60 study samples, it was found that there was no significant relationship between HSP60 levels and the Lymphocyte Neutrophil Ratio in patients with positive and negative H. pylori gastritis with a p value of > 0.121.

**Table 4. Correlation of HSP60 levels with Neutrophil Lymphocyte Ratio**

	HSP60 (ng/mL)
<b>Neutrophil Lymphocyte Ratio</b>	P : 0.121 r : 0,020

uji Spearman

## Discussion

In this study the characteristics of the median age of gastritis patients as a whole were 47.7 (25-66) years. Age characteristics of gastritis patients are almost the same found in previous studies<sup>10</sup> conducted by Ghoshal et al (2011) who summarized reports of dyspeptic events in Asia where the age range suffering from dyspepsia is at the age of 18-80 years with a median value of 45.<sup>11</sup> The frequency of dyspeptic events is very different in different populations. This difference can be caused by differences in the criteria used in diagnosing and the degree of accuracy in excluding other organic causes.<sup>10</sup> The frequency of unexplored dyspepsia and functional dyspepsia ranges between 8% -30% and 8% -23%, especially in parts of Asia.

In this study it was found that the most sex characteristics of gastritis patients were 36 male subjects (60%). The results of this study are relevant to previous research conducted by Patel, et al (2014) in a study of the incidence of helicobacter infections in gastritis cases where researchers found 64% of all cases of male sex and 60% of cases with positive helicobacter infection also of male sex male.<sup>12</sup> Other relevant research conducted by De



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Martel (2006) using a meta-analysis method confirms the predominance of men from *H. pylori* infection in adults as a global and homogeneous phenomenon.<sup>13</sup>

Based on statistical analysis of the U Man whitney test, it was found that there were significant differences in HSP60 levels between groups h. positive and negative pylori with a p value of <0.001. Where is the median value in group h. positive pylori 16 and median values in group h. pylori negative 3.2 thus the difference between the two groups is 12.8. A recent study showed a correlation between *H. pylori* adhesion with human gastric carcinoma cells and HSP60 expression on cell surfaces. The results showed that *H. pylori* adhesion to human gastric mucosal cells might be related to HSP60, and also showed the potential role of HSP60 as one of the virulence factors responsible for induction of gastritis. However, the immunological nature of *H. pylori* HSP60 is currently unknown.<sup>14,15</sup>

*H. pylori* induces an immune response to HSP60 in about 50% of patients infected by bacteria. HSP60 *H. pylori* cannot be correlated with certain clinical conditions of the disease; However, given the high degree of similarity between HSP60 *H. pylori* and its homologues in humans, this allows these antibodies to cross-react with HSP60 in humans. This cross reactivity plays a role in tissue damage that occurs in gastritis caused by *H. pylori*, and can be another pathogenic mechanism involved in gastric mucosal infections by this bacterium.<sup>16</sup>

*H. pylori* binds to gastric epithelial cells and mucin through HSP60, and the adaptive immune system against HSP60 is induced in patients infected with *H. pylori*.<sup>9</sup> The IgG response to HSP60 is closely related to the inflammatory process in the stomach. In vitro studies by Yamaguchi et al., In 1999 showed that HSP60 induces IL8 from gastric epithelial cells and IL6 from monocytes / macrophages.<sup>17</sup>

Based on the statistical analysis of the Spearman test, it was found that there was no significant relationship between HSP60 levels and Lymphocyte Neutrophil Ratio in gastritis patients h. positive and negative pylori with a p value of > 0.121. Non-specific inflammatory parameters such as leukocytes, absolute neutrophil count (ANC), C-reactive protein (CRP), and blood sedimentation rate (LED) indicate the degree of inflammatory reaction in the acute phase and are often used for shows bacterial infection.<sup>18</sup> White blood cell populations (lymphocytes and neutrophils) play an important role in the systemic inflammatory response to severe infections, trauma, and shock. The immune response to endotoxemia is characterized by an increase in circulating neutrophils in the blood and a low lymphocyte count.<sup>19</sup> A single protein is pure and identified by spectrometry as a *H. pylori* 60 heat shock protein (HSP60). Consistent with the activity that induces IL-6 observed from *H. pylori*. HSP60 stimulates IL-6 production in macrophages and NF- $\kappa$ B activation.<sup>20</sup>

Interleukin-6 (IL-6) and its receptor (sIL-6Ra) regulate the leukocyte recruitment transition, through a shift in chemokine production. During acute inflammation, IL-6 supports the resolution of neutrophilic infiltrate and the initiation of the immune response. Whereas in chronic inflammation, IL-6 increases mononuclear cell infiltrate and participates in the pathogenesis of the disease. In studies conducted by Kaplanski et al it is known that the IL6-sIL6Ra complex supports the transition from neutrophils to monocytes in inflammation. the occurrence of neutrophilia, through rapid mobilization of neutrophils into the circulation, followed by accelerated neutrophil release from the bone marrow.<sup>22,23</sup> IL-6 also along with TNF and IL-1 promotes subsequent neutrophil recruitment by inducing the production of chemokines in surrounding cells and by increasing the expression of ICAM-1 in endothelium.<sup>24</sup>

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) regulates a large number of genes involved in various immune processes and inflammatory responses.<sup>1</sup> NF- $\kappa$ B activation involves two main signaling pathways, the canonical and non-canonical (or alternative) pathways, both of which are important in regulating immunity body and inflammatory response.<sup>25,26</sup> Innate immune cells, including macrophages, dendritic cells and neutrophils, are important players of innate immunity and inflammation. These cells express PRR which detects various microbial components. Inflammation also involves adaptive immune components, specifically CD4 + T-helper (Th) lymphocyte cells.<sup>27</sup> Naive T cell activation is initiated based on TCR involvement by specific antigens presented on cells that present antigens, mostly by dendritic cells. NF- $\kappa$ B promotes differentiation of Th1 lymphocyte cells by regulating TCR signaling and functions to mediate induction of cytokines, such as IL-12, which promotes Th1



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differentiation.<sup>28</sup> So it can be concluded that HSP60 stimulates IL-6 production in macrophages thereby causing an increase in neutrophils in circulation and also HSP60 stimulates NF- $\kappa$ B activation which triggers an inflammatory response with the end result being an increase in neutrophil and lymphocyte counts.

### Conclusion

HSP60 differences in positive and negative *H.pylori* gastritis patients ( $p < 0.001$ )

### Suggestion

Further analysis was carried out on the factors that could influence HSP60 differences in the positive and negative *H. pylori* groups.

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