

# Glutathione peroxidase level in patients with *Helicobacter pylori*-associated gastritis

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**Abstract.** *Helicobacter pylori* (*H. pylori*) associated with the generation of reactive oxygen species (ROS), with leads to oxidative stress in the gastric mucosa. GPX is one of human antioxidative defense system allows the elimination of excess ROS. A cross-sectional study was in 80 consecutive gastritis patients who came to the endoscopic unit of Adam Malik General Hospital and PermataBunda Hospital in Medan, Indonesia, from May–September 2017, to determine the difference of GPX serum level between positive and negative infected *H. pylori*. the diagnosis of gastritis used Histopathology. Rapid urease test for diagnosis of *H. pylori* infection. Serum samples were obtained to determined circulating GPX. It used Univariate and bivariate analysis (Mann Whitney U test). There were 50 patients (62.5%) infected with *H. pylori*. GPX levels in patients with positive *H. pylori* gastritis were lower than those of negative *H. pylori* but did not differ significantly. In conclusion, there were no significant differences in GPX level between positive and negative infected *H. pylori* patients.

## 1. Introduction

*Helicobacter pylori* (*H. pylori*) is a bacterium that infects almost 50% of world's population.[1] *H. pylori* infection is a major cause of gastritis, peptic ulcer disease, and gastric cancer, and associated with neutrophils, macrophages, and lymphocytes infiltration in the gastric mucosa. If host immune response cannot fully control the infections, it will cause persistent inflammation in gastric mucosa. In other words, *H. pylori* infection can cause chronic inflammation, accumulation of reactive oxygen species (ROS), and DNA damage.[2]

*H. pylori* have a connection with a generation of ROS, with leads to oxidative stress in the gastrointestinal mucosa. Oxidative stress is a condition in which free radicals are more dominant than antioxidants defenses.[3] *H. pylori* stimulate the expression of Interleukin 8 (IL-8) which plays a potent neutrophil chemotactic. Neutrophil infiltration of cellular lipid membranes produces superoxide anions that are part of ROS.[4]

The ROS triggers oxidative stress may increase as the concentration of endogenous antioxidants decreases. GPX is one of human antioxidative defense system allows the elimination of excess ROS. [5,6] Reports on effects of *H. pylori* on activities of GPX in the gastric mucosa were conflicting.[7]



Previous studies have reported different results. The purpose of this study was to determine the difference of GPX serum level between positive and negative infected *H. pylori* patients.

## 2. Methods

### 2.1. Patient Selection

This study was a cross sectional study conducted on 80 research subjects who came to endoscopy unit at Adam Malik General Hospital, Medan Indonesia from June until August 2017. All patients gave informed consent, and the study has an agreement from the local ethical committee. Exclusion criteria were patients who had taken antibiotics, H<sub>2</sub> antagonists, proton pump inhibitors, bismuth, immunomodulators within one month before endoscopy. Patients with systemic disease and malignancy were also an exclusion.

### 2.2. Diagnosis of Gastritis

Gastritis is diagnosed based on histopathological examination. Gastric mucosal tissues were from the gastric antrum and corpus during endoscopy. These tissues were then stained using Hematoxylin-Eosin. All specimens were examined by one same pathologist at the Anatomical Pathology Laboratory of Sumatera Utara University.

### 2.3. Diagnosis of *H. pylori*

Positive results of rapid urease test were considered *H. pylori* positive. The result of the rapid urease test was read within 24 hours after being taken.

### 2.4. Detection of GPX Level

The sample used was venous blood mixed with heparin as an anticoagulant. Reagent kit used was Ransel Glutathione Peroxidase Cat RS505 (Randox Laboratories Ltd., United Kingdom). Measurement using an Advia 1800 instrument (Siemens Healthcare GmbH, Germany). The reference range was 27.5 – 73.6 U/g Hb. Processing steps followed instruction kit.<sup>8</sup> This work was at the Prodia Research and Esoteric Laboratory.

### 2.5. Statistical Methods

Data were analyzed univariate and bivariate using SPSS version 22 (SPSS Inc., Chicago) with 95% confidence interval. The analysis was carried out using Mann Whitney U-test with significance level  $p < 0.05$ .

## 3. Result

### 3.1. Baseline characteristics of subjects

A total of 54 patients (67.5%) were men with an average age of 49.4 years old. Majority of subjects ethnic was Batak (57.5%). Two major occupations of subjects were the entrepreneur (42.5%), followed by housewives (30%). Mean of subject's BMI was 22.18 kg/m<sup>2</sup>. Median GPX level was 121 U/g HGB. (Table 1).

**Table 1.** Basic characteristics of the subjects.

Characteristics	n = 80
Sex, n(%)	
Male	54 (67.5%)
Female	26 (32.5%)
Age (years) <sup>a</sup>	49.4 ± 12.15
Ethnic	
Batak	46 (57.5%)

Javanese	24 (30%)
Acehnese	10 (12.5%)
Occupation, n (%)	
Entrepreneur	34 (42.5%)
Housewife	24 (30%)
Employee	20 (25%)
Farmer	2 (2.5%)
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	22.18 ± 3.27
<i>H. pylori</i> , n(%)	1.43 ± 0.31
Positive	50 (62.5%)
Negative	30 (37.5%)
Serum GPX (U/g HGB) <sup>b</sup>	121 (86 – 192)

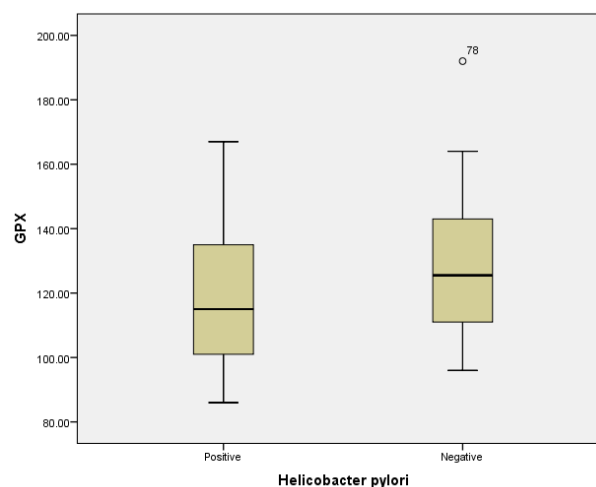
n = total number of subjects  
<sup>a</sup>mean ± SD  
<sup>b</sup>median (min-max)

### 3.2. Serum GPX level in *H. pylori* infection

Serum GPX level was lower in *H. pylori* positive than *H. pylori* negative, but not different significantly ( $p > 0.05$ ). (Table 2)

**Table 2.** Comparison of serum GPX level between *H. pylori* positive and negative.

<i>H. pylori</i>	Serum GPX	P
Positive	115 (86 – 167)	0.062*
Negative	125.5 (96 – 192)	



**Figure 1.** GPX serum level in *H. pylori* positive and negative.

## 4. Discussion

*Helicobacter pylori* induce gastric inflammation which increases the risk of gastric and duodenal ulceration, gastric adenocarcinoma, and Mucosa-Associated Lymphoid Tissue (MALT) lymphoma. *H. pylori* induce proinflammatory cytokines, such as IL-1 $\beta$ , IL-6, Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), IL-8 via Nuclear Factor- Kappa Beta (NF- $\kappa$ B) activation. The inflammatory response that occurs causes cell T regulatory (Treg) to secrete an immunosuppressive cytokine that can retain *H. pylori* in the gastric mucosa, leads to chronic inflammation caused by *H. pylori*. Chronic inflammation due to *H. pylori* invasion causes a buildup of ROS that exceeds the capacity of antioxidants to neutralize free radicals, causing further cell damage. Also, *H. pylori* with cytotoxin-associated gene A (CagA) (+) positively associated with peptic ulcers and gastric cancers that lead to

lower levels of antioxidants.[9-10]*H. pylori* will cause oxidative stress that affects basal membrane damage and DNA mutagenesis. Under normal circumstances, free radicals are produced in small amounts that can be neutralized by enzymatic and non-enzymatic antioxidants. GPX is one the most important free radical scavengers.[5,11]

Verhulst et al. found that glutathione level was significantly lower in patients infected with *H. pylori* positive than negative.[12] Shirin et al. measured the impact of *H. pylori* on reduced glutathione levels, an endogenous antioxidant, in gastric epithelial cells both in vivo and in vitro. Reduced glutathione levels in the gastric mucosa were significantly lower in *H. pylori*-infected patients than in control group. These levels were correlated inversely with a concentration of inflammatory cells. They suggested low levels of reduced glutathione in patients infected with *H. pylori* due to direct effects of *H. pylori* invasion and also antioxidant consumption due to high ROS.[13]

Although mean GPX activities in the *H. pylori*-infected gastritis patients were not significantly different than those in the *H. pylori*(-) patients, they tended to decrease. A limitation of the study was that the study population was relatively small (n = 80). In this study was not evaluated the degree of gastritis severity, whereas those severities can affect GPX level and there is no evaluation *H. pylori* virulence status in which *H. pylori* with CagA(+) and active VacA protein will lead to more severe tissue damage that will further influence levels of endogenous antioxidants.

## 5. Conclusion

There were no significant differences in GPX level between positive and negative infected *H. pylori* patients.

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