The Difference of Glutathione Peroxidase Levels among Chronic Atrophic Gastritis, Intestinal Metaplasia, and Dysplasia in Patients with *Helicobacter pylori*-associated Gastritis

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Abstract: *Helicobacter pylori* infection is the main etiology of chronic gastritis. Chronic mucosal inflammation can lead to chronic atrophic gastritis, intestinal metaplasia, and gastric dysplasia. Oxidative stress plays a role in inflammatory and malignancy process. Glutathione peroxidase (GPX) levels will decrease due to oxidative stress. This study was conducted to evaluate the difference of GPX serum levels among chronic atrophic gastritis, intestinal metaplasia, and dysplasia in *H. pylori*-associated gastritis patients. A cross-sectional study on 70 consecutive gastritis patients who came to the endoscopic unit of Adam Malik General Hospital and Permata Bunda Hospital in Medan, Indonesia, from April – June 2018. The diagnosis of gastritis was derived histopathologically. Rapid urease test for diagnosis of *H. pylori* infection. Serum samples were obtained to determined circulating GPX. Univariate and bivariate (Kruskal Wallis test) analysis were performed with SPSS version 22. There were 21 patients (30%) with chronic atrophic gastritis, 15 patients (21.4%) with intestinal metaplasia, and 8 patients (11.4%) with dysplasia. There were significant differences in GPX levels among chronic atrophic gastritis, intestinal metaplasia, and dysplasia ($p = 0.037$). GPX levels were significantly lower in patients with dysplasia than chronic atrophic gastritis. There were no significant difference in GPX levels between patients with intestinal metaplasia and chronic atrophic gastritis or dysplasia.

1 INTRODUCTION

Gastritis is an inflammatory process in mucosa and submucosa stomach as response to injuries that can be acute or chronic (El-Zimaity, 2007). It is found that *Helicobacter pylori* (*H. pylori*) is the main etiology of chronic gastritis, gastric premalignant lesion, and gastric cancer. *H. pylori* is a type 1 carcinogen according to International Agency for Research on Cancer (IARC). Nearly 50% of the world's population was estimated to be infected with *H. pylori*, about 70-90% occur in developing countries and only 40-50% in industrial Countries (Chekhonin, 2013). Reactive oxygen species (ROS) can be generated by *H. pylori. H. pylori* infection can cause chronic inflammation resulting in accumulation of ROS (Suzuki, 2012; White, 2015). The human body has a protective mechanism that neutralizes free radicals, with the presence of *superoxide dismutase* (SOD), *catalase*, and *glutathione peroxidase* (GPX) enzymes (Birben, 2012). GPX is an enzyme that acts to catalyze hydrogen peroxide (H_2O_2) and organic hydroperoxide to prevent lipid peroxidation of cell membranes. GPX can be found in mitochondria, cytosol or extracellular (Lubos, 2012). In certain conditions, free radicals can exceed the body's defense system, this condition is called as oxidative stres (Ayala, 2014).

ROS that exceeds the capacity of antioxidants to neutralize free radicals, causing further cell damage.

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ROS will cause mucosal damage by causing basal epithelial membrane degradation so that there will be changes in cell metabolism and damage to DNA (Mahmood, 2009). Tissue damage and DNA lesions can cause dysregulation of cellular homeostasis in gastric mucosa that plays a role in gastric carcinogenesis due to *H. pylori* from chronic gastritis, gastric premalignant lesions (atrophic gastritis, intestinal metaplasia, and dysplasia), to gastric cancer (Kalisperati, 2017; Dinis-Ribeiro, 2012).

Research on antioxidant level in gastric premalignant lesion were still limited. The purpose of this study was to determine the difference of glutathione peroxidase levels among chronic atrophic gastritis, metaplasia intestinal, and dysplasia in patients with *H. pylori*-associated gastritis.

2 METHODS

2.1 Patient Selection

This study was a cross sectional study conducted on 70 subjects who came to endoscopy unit at Adam Malik General Hospital, Medan Indonesia from April until June 2018. Exclusion criteria were patients who refuse to participate, patients with systemic disease like diabetes mellitus, hypertension, liver disease, kidney disease, heart disease and malignancy. All patients gave informed consent, This study was approved by the Institutional Review Board of Universitas Sumatera Utara.

2.2 Diagnosis of Gastritis

Diagnosis of gastritis is based on histopathological examination. During endoscopy, tissue samples were taken from antrum and corpus gaster. These tissues were then stained with Hematoxylin-Eosin stain. Histopathological examinations were examined under the same pathologist of Pathology Anatomy Laboratory of Universitas Sumatera Utara.

2.3 Diagnosis of *H.pylori*

Positive results of CLO test would indicate presence of *H. pylori* bacteria. A rapid urease test was performed within 24 hours after the collection of the sample.

2.4 Diagnosis of GPX Level

The sample used was venous blood mixed with heparin as an anticoagulant. The Reagent kit used was Ransel Glutathione Peroxidase Cat RS505 (Randox Laboratories Ltd., United Kingdom). The Instrument for measurement was Advia 1800 instrument (Siemens Healthcare GmbH, Germany) with a reference range of 27.5 – 73.6 U/g Hb. Processing steps followed instruction kit (Mahmood, 2009). The examination was conducted at Prodia Research and Esoteric Laboratory.

2.5 Statistical Method

Statistical data composed of univariate and bivariate were analyzed using SPSS version 22 (SPSS Inc., Chicago) with 95% confidence interval. The analysis was carried out using Kruskal Wallis test with significance level $p<0.05$.

3 RESULT

3.1 Baseline Characteristics of Subjects

A total of 40 patients (57.1%) were men with an average age of 51 years old. Majority of subjects were Batak ethnicity (68.6%). Two major occupations of subjects were the private employee (38.6%), followed by enterpreneur (31.4%). Mean of subject's BMI was 23.4 kg/m^2 (Table 1).

Table 1: Basic characteristics of subjects.

Variable	n = 70
Sex	
Male	40 (57.1%) [a]
Female	30 (42.9%)
Age, years	51 ± 8.9 [b]
BMI, kg/m^2	23.4 ± 4.5 [b]
Ethnic	
Batak	48 (68.6%) [a]
Javanese	15 (21.4%)
Acehnese	7 (10%)
Occupation	
Private Employee	27 (38.6%) [a]
Enterpreneur	22 (31.4%)
Housewife	16 (22.9%)
Student	5 (7.1%)

n = total number of subjects

[a]Percentage

[b]Mean ± SD

3.2 Prevalence of Chronic Atrophic Gastritis, Intestinal Metaplasia, and Dysplasia

Through the histopathological examination, a total of 21 patients (30%) were diagnosed with chronic atrophic gastritis, 15 patients (21.4%) were diagnosed with intestinal metaplasia, and 8 patients (11.4%) were diagnosed with dysplasia (Table 2).

Table 2: Prevalence of chronic atrophic gastritis, intestinal metaplasia, and dysplasia.

Gastritis *H. pylori*	n = 70
Chronic atrophic gastritis	21 (30%)
Intestinal metaplasia	15 (21.4%)
Dysplasia	8 (11.4%)

3.3 The Difference of GPX Levels among Chronic Atrophic Gastritis, Intestinal Metaplasia, and Dysplasia in Patients with *Helicobacter pylori*-associated Gastritis

There were significant differences in GPX levels among chronic atrophic gastritis, intestinal metaplasia, and dysplasia (p = 0.037). GPX levels were significantly lower in patients with dysplasia than chronic atrophic gastritis. There were no significant difference in GPX levels between patients with intestinal metaplasia and chronic atrophic gastritis or dysplasia (Table 3).

Table 3: The differences of GPX levels among chronic atrophic gastritis, intestinal metaplasia, and dysplasia in patients with *Helicobacter pylori*-associated gastritis**.**

Diagnosis	GPX (U/g HGB)	p
Chronic atrophic gastritis	101.5 (86 – 167)	0.037*
Intestinal metaplasia	97.5 (70 – 124)	
Dysplasia	86 (70 – 92)[#]	

*p<0.05, [#]there was a significant difference with chronic atrophic gastritis

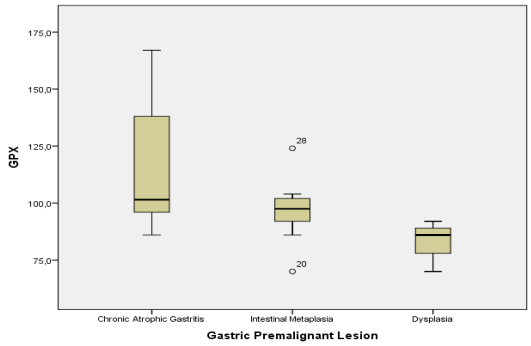

Figure 1. Serum GPX levels in chronic atrophic gastritis, intestinal metaplasia, and dysplasia.

4 DISCUSSION

Gastric carcinogenesis is a multistep and multifactorial process. Gastric cancer is preceded by a cascade of precancerous lesions, such as chronic atrophic gastritis, intestinal metaplasia and dysplasia. The following step is invasive carcinoma, which is thought to be associated with degradation of the intercellular matrix. Besides environmental, diet and genetic factors, gastric cancer is closely associated with *H. pylori* infection (Correa, 2012; Park, 2015).

Infection of *H. pylori* is one of the thoroughly studied risk factors of gastric cancer. A primary factor that is important in the events that lead to the progression of the inflammation-to-carcinoma is oxidative DNA damage induced by *H. pylori* infection (Farinati, 1998), which is probably due to infiltrating neutrophils, and also direct effects of *H. pylori* (Obst, 2000). Under normal circumstances, free radicals are produced in low quantity. However, this is not pathological because free radicals will be suppresed by the elevated amount of endogenous antioxidants (GPX, SOD, and catalase) as a compensatory mechanism to prevent further tissue damage (Li, 2015). But in certain conditions, free radicals can exceed the body's defense system, this condition is called as oxidative stres (Mahmood, 2009). Recruitment of phagocytes in gastritis will induce an increase in free radicals. Anion superoxide radicals (O_2^-) are generated by neutrophil infiltration reactions to cellular lipid membranes that lead to lipid peroxidation formation (Li, 2015). These lipid peroxidation reactions damage the cell membranes, eventually causing the release of intracellular components such as lysosomal enzymes, which will further tissue damage, degradation of epithelial

basement membrane, disrupt cell metabolism, and MDA reactions with DNA will form mutagenic MDA deoxyguanosine (Mi-dG) (Choi, 1999; Drake, 1998).

Production of ROS in the *H. pylori*-infected gastric epithelium is linked to the presence of cagPAI and contribute to the oxidative stress response in gastric epithelial cells (Ding, 2007). It is well known that *H. pylori* infection causes elevated level of polyamines, in particular spermine and this is associated with an induction of spermine oxidase (Cheng, 2009). Action of spermine oxidase on spermine leads to the production of elevated levels of hydrogen peroxide, which is a powerful oxidizing agent and also contributes to the production of free radicals such as hydroxyl radial (Xu, 2004). Additionally, *H. pylori* will activate macrophages which will result in a significant upregulation of spermine oxidase, contributing to oxidative stress and damage to the gastric epithelial cells (Chaturvedi, 2004).

In a previous study, Subha et al reported that there were statistically significant decrease of mean GPX levels in cancer patients compared to control groups. Cancer patients showed a lower mean of GPX levels than control group. Research on GPX levels in patients with premalignant lesions of gastric is still limited. In this study there were significant difference in GPX levels among chronic atrophic gastritis, intestinal metaplasia, and dysplasia. GPX levels were significantly lower in patients with dysplasia than chronic atrophic gastritis and there were no significant difference in GPX levels between patients with intestinal metaplasia and chronic atrophic gastritis or dysplasia. Lower GPX levels are associated with the progression of precancerous lesions, which is supported by GPX levels that were significantly lower in dysplasia than chronic atrophic gastritis. This study showed that antioxidant supplements may be considered in patients with gastric premalignant lesions. A further study is needed to evaluate the antioxidant options and their role in the improvement of oxidative stress in patients with gastric premalignant lesion.

5 CONCLUSION

GPX levels were significantly lower in patients with dysplasia than chronic atrophic gastritis.

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