RELATIONSHIP BETWEEN THE ACTIVITIES OF SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXYDASE, MALONDIALDEHYDE AND TOTAL ANTIOXIDANT STATUS IN PLASMA WITH *H. PYLORI* INFECTION IN CHRONIC GASTRITIS PATIENTS

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SUMMARY

Objectives: To study the relationship between activities of superoxide dismutase, glutathione peroxydase, malondialdehyde, and total antioxidant status in plasma with H. pylori infection in chronic gastritis patients. Subjects and methods: A descriptive cross-sectional study on 136 chronic gastritis patients. Results:

- Superoxide dismutase activity in plasma in chronic gastritis patients with H. pylori - positive was 217.78 ± 142.9 ng/mL, lower than that of H. pylori - negative patients with 512.54 ± 576.70 ng/mL (p = 0.001).

- Glutathione peroxydase activity in plasma in chronic gastritis patients with H. pylori - positive was 116.65 ± 77.21 pg/mL, lower than that of H. pylori - negative group, 264.93 ± 279.1 pg/mL (p = 0.003).

- Malondialdehyde activity in plasma in chronic gastritis patients with H. pylori - positive was 3.25 ± 2.6 mmol/L lower than that of H. pylori - negative group with 8.0 ± 8.52 mmol/L (p < 0.001).

- Total antioxidant status activity in plasma in chronic gastritis patients with H. pylori - positive was 0.82 ± 0.42 U/mL lower than the group with H. pylori - negative was 2.99 ± 4.40 U/mL (p = 0.03).

Conclusion: Activities of superoxide dismutase, glutathione peroxydase, malondialdehyde and total antioxidant status in plasma in chronic gastritis patients with H. pylori - positive (217.78 ± 142.9 ng/mL; 116.65 ± 77.21 pg/mL; 3.25 ± 2.6 mmol/L; 0.82 ± 0.42 U/mL, respectively lower with significant statistics versus H. pylori - negative group (512.54 ± 576.70 ng/mL; 264.93 ± 279.1 pg/mL; 8.0 ± 8.52 mmol/L; 2.99 ± 4.40 U/mL respectively) with p < 0.05, respectively.

** Keywords: Chronic gastritis; Oxidative stress; H. pylori; Glutathione peroxidase; Superoxide dismutase; Malondialdehyde.*

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Date received: 18/02/2019

Date accepted: 19/04/2019

INTRODUCTION

Gastritis is a complex biochemical protective reaction to fight cell/tissue level injury. Injury causing ischemia - reperfusion is an important pathological process in gastric mucositis. When an ischemia/ reperfusion occurs again, the production of large amounts of ROS increases [12]. When ROS production occurs in an uncontrolled way, resulting in excessive cell/tissue damage, chronic inflammation and destruction of normal tissues. There is an evidence that *H. pylori* infection and oral NSAID use are important causal factors in the pathogenesis of gastric mucosal injury in humans due to oxidative stress state [8]. In response to *H. pylori* infection or NSAID, neutrophils are focused on the affected area, producing active ROS and nitrogen radicals (RNS) [8]. However, neutrophils are unable to kill bacteria that live in stomach mucus and free radicals produced by neutrophils can harm normal tissues. The formation of free superoxide by *H. pylori* will increase the activity of superoxide dismutase (SOD) in the stomach lining. Glutathione (GSH) is significantly lower in patients with *H. pylori* infection than in non - *H. pylori* infected patients. In addition, many authors argue that *H. pylori* eradication treatment reduces oxidative stress in the gastric mucosa [10].

These issues have not been deep studied and evaluated in Vietnam yet, due to the fact that we have carried out this project aiming: *To research the relationship between the activities of SOD, GPx, TAS and MDA in plasma with H. pylori infection in patients with chronic gastritis.*

SUBJECTS AND METHODS

1. Subjects.

136 patients who went to Internal Gastrointestinal Clinic of General Hospital of Medical Examination from September 2015 to December 2017.

** Standard selection:*

- Chronic gastritis diagnosed by Sydney standards.

- Patient ≥ 16 years old.

- Regardless of gender, occupation.

- The patients neither used antibiotics before 1 - month endoscopy nor H_2 antagonists and proton pump inhibitors.

- *H. pylori* infection determined by urease test and histopathology.

- Patients agreed to participate in the study.

** Exclusion criteria:*

- Patients did not meet the above criteria.

- Patients were taking medication for chronic gastritis; children, pregnant women.

- Patients had other gastric diseases: gastric ulcer, stomach cancer, gastroesophageal reflux, functional stomach disease...

- Patients had other acute and chronic conditions accompanied.

- Patient was alcoholism, cigarette addiction, exposure to toxic chemicals.

- Patients did not cooperate in research.

2. Methods.

- Prospective study, patient selection and analysis of results according to cross-sectional descriptive statistics.

- Blood samples to determine enzyme activity of SOD, GPx and TAS were

separated plasma immediately after being sent to Department of Pathophysiology, Military Medical University, was then stored in refrigerator at -80°C.

- Determining SOD, GPx, MDA and TAS in plasma:

+ Using the ELISA kit of Melsin (Human ELISA kit from China).

+ Determination of activity (concentration) by Sandwich-ELISA method.

+ Principle: Specific antibodies were pre-coated on 96-well plates. These antibodies were then combined with added antigens (from standard solutions or samples). After incubation and washing, only the antigen complex - antibodies stick to the bottom of the well. Antibodies were then added. After incubation, wash, add the substrate and read the optical density on the spectrophotometer.

Optical density measurement at Department of Pathophysiology, Military Medical University with Diaglostic Automation, Inc ELISA DAR 800 reader (USA). Develop standard curves and calculate results by Microsoft Excel 2013 software.

Detection threshold: SOD: 6 ng/mL; GPx: 3 pg/mL; TAS: 50.05 U/mL; MDA 0.01 mmol/L.

Collect and process data using SPSS 20.0 software.

RESULTS

** H. pylori test results by histopathology:*

Negative: 65 patients (47.8%); positive (+): 39 patients (28.7%); positive (++): 27 patients (19.8%); positive (+++): 5 patients (3.7%).

52.2% of patients had *H. pylori* - positive at different levels.

** Quantitative rate of SOD, GPx, TAS and MDA:* 100% of patients had GPx and TAS activity above the detection threshold; 98.5% of patients had SOD and MDA activity above the detection threshold.

Table 1: SOD activity (ng/mL) in chronic gastritis patients related to *H. pylori*.

Enzyme activity	Criteria of compare	*H. pylori* - negative (n = 64)	*H. pylori* - positive (n = 70)	p value
SOD	X ± SD	512.54 ± 576.70	217.78 ± 142.9	$p^* = 0.001$
	Median	335.88	137.19	
	Minimum	80.65	12.47	
	Maximum	3041.72	608.07	

(p: Mann - Whitney test)*

As can be seen from the table, the SOD activity in the group of patients with *H. pylori* - positive chronic gastritis was lower than the group with negative *H. pylori* (p = 0.001).

Table 2: GPx activity (pg/mL) in patients with chronic gastritis related to *H. pylori.*

Enzyme activity	Criteria of compare	*H. pylori* - negative (n = 65)	*H. pylori* - positive (n = 71)	p value
GPx	$\overline{X}$ ± SD	264.93 ± 279.1	116.65 ± 77.21	$p^* = 0.001$
	Median	175.04	88.17	
	Minimum	42.84	16.0	
	Maximum	1052.78	321.57	

(*p*: Mann - Whitney test)*

The GPx activity in the group of *H. pylori* - positive chronic gastritis was lower than that in the group with negative *H. pylori* (p = 0.001).

Table 3: TAS activity (U/mL) in patients with chronic gastritis related to *H. pylori.*

Enzyme activity	Criteria of compare	*H. pylori* - negative (n = 65)	*H. pylori* - positive (n = 71)	p value
TAS	$\overline{X}$ ± SD	2.99 ± 4.40	0.82 ± 0.42	$p^* = 0.01$
	Median	0.79	0.68	
	Minimum	0.28	0.26	
	Maximum	20.64	2.15	

(p: Mann - Whitney test)*

The TAS activity in the group of *H. pylori* - positive chronic gastritis was lower than the group with negative *H. pylori* (p = 0.01).

Table 4: MDA activity (mmol/L) in patients with chronic gastritis related to *H. pylori.*

Enzyme activity	Criteria of compare	*H. pylori* - negative (n = 64)	*H. pylori* - positive (n = 70)	p value
MDA	$\overline{X}$ ± SD	8.0 ± 8.52	3.25 ± 2.6	$p^* < 0,001$
	Median	5.96	1.39	
	Minimum	0.97	0.27	
	Maximum	43.08	9.6	

(p^*: Mann - Whitney test)

The MDA activity in the group of patients with *H. pylori* - positive chronic gastritis was lower than the group with *H. pylori* - negative (p < 0.001).

Table 5: Relationship between activities of SOD, GPx, TAS and MDA with level of *H. pylori* - positive on histopathology.

Enzyme activity		n	$\overline{X}$ ± SD	Median	p value
SOD (ng/mL)	HP (+)[(a)]	39	199.6 ± 142.78	104.46	$p^{**} = 0.32$ $p^{(a,b)*} = 0.16$
	HP (++)[(b)]	26	238.5 ± 145.01	200.53	
	HP (+++)	5	251.83 ± 140.35	333.21	
GPx (pg/mL)	HP (+)[(a)]	39	109.59 ± 76.12	62.46	$p^{**} = 0.58$ $p^{(a,b)*} = 0.35$
	HP (++)[(b)]	27	125.54 ± 81.38	116.87	
	HP (+++)	5	123.76 ± 71.81	129.82	
TAS (U/mL)	HP (+)[(a)]	39	0.79 ± 0.39	0.69	$p^{**} = 0.77$ $p^{(a,b)*} = 0.95$
	HP (++)[(b)]	27	0.83 ± 0.45	0.68	
	HP (+++)	5	1.05 ± 0.52	0.77	
MDA	HP (+)[(a)]	38	3.01 ± 2.51	1.36	$p^{**} = 0.81$ $p^{(a,b)*} = 0.57$
	HP (++)[(b)]	27	3.52 ± 2.78	1.99	
	HP (+++)	5	3.54 ± 2.55	3.23	

*(p**: Kruskal Wallis; p*: Mann - Whitney test)*

There was no correlation between the activities of enzymes SOD, GPx, TAS and MDA with the positive level of *H. pylori* bacteria on histopathology.

DISCUSSION

In our study, 52.2% of patients had *H. pylori* - positive at different levels. According to Ray-Offor E et al (2018) [11], the rate of positive *H. pylori* in patients with chronic gastritis detected through endoscopic method accounted for 38.5%. Atayan Y et al (2017) [3] studied 171 cases of chronic gastritis, showing that rate of *H. pylori* infection through biopsy method was 81.4%. Zhang C et al (2005) [16] studied 4,102 patients with chronic gastritis showed that the rate of *H. pylori* infection was 55.0%. The reason for these differences was that the prevalence of *H. pylori* infection has an influence on the study site and socio-economic conditions. Many studies have demonstrated that in areas with poor sanitation, water and food are initial important spread source of *H. pylori* [1, 6].

To avoid being killed by oxidants, *H. pylori* prevents NADPH oxidase from phagocytosis by producing NADP+ and a large amount of superoxide anions into the extracellular environment. The addition of prescribed doses of vitamin E and C with antibiotics increases the effectiveness of *H. pylori* treatment. Recent research has demonstrated that increased levels of ROS was produced in *H. pylori* infected gastric epithelial cells and this may be a mechanism leading to process of cell death follow program related to infected [4]. Besides, chronic gastritis easily leads to poor absorption of iron. The change in

intracellular iron balance may affect the enhancement of pathogens associated with oxidative stress [15].

In this study, we found that 2/136 cases equivalent to 1.5% had very low SOD activity, less than the detection threshold of the Elisa kit (detection threshold ≥ 6 ng/mL). The SOD activity in patients with *H. pylori* - positive chronic gastritis was 217.78 ± 142.9 ng/mL, lower than that of *H. pylori* - negative patients, 512.54 ± 576.70 ng/mL (p = 0.001).

The study by Noguchi K et al (2002) [10] conducted the measurement of SOD concentration in gastric mucosa in 74 patients with chronic gastritis, of which 46 patients were positive with *H. pylori* and 28 patients with *H. pylori* - negative. The results showed that mucosal SOD concentrations in the *H. pylori* - positive group were 15.5 ± 7.0 U/mg, higher than the *H. pylori* - negative group (9.2 ± 10.6 U/mg protein) and this concentration significantly reduced after *H. pylori* treatment (8.2 ± 4.2 U/mg protein).

Ansari M et al (2006) [2] studied 43 patients with *H. pylori* - positive chronic gastritis and 43 individuals with *H. pylori* - free chronic gastritis. The results showed that the SOD concentration in the gastric fluid was significantly higher in the *H. pylori* infected group compared with the *H. pylori* - non infected group (p = 0.0001).

GPx activity in patients with *H. pylori* - positive chronic gastritis was 116.65 ± 77.21 pg/mL, lower than that in *H. pylori* - negative group, 264.93 ± 279.1 pg/mL (p = 0.003).

Glutathione is a substrate of many enzymes involved in cell detoxification and defence mechanisms. Optimal maintenance of the rate of glutathione/oxidative glutathione (GSH/GSSG) in cells is important for survival. GSH deficiency puts cells at risk of oxidative damage. Tatemichi et al investigated the association between glutathione S-transferases and the level of titre of gamma immunoglobin in plasma against *H. pylori* in healthy *H. pylori* - positive individuals, suggesting that glutathione S-transferases may be involved in protection against mucosal atrophy caused by *H. pylori* [14].

According to Haim Shirina H et al (2001) [7], GPx concentration was significantly lower in gastric biopsy samples in 19 patients with *H. pylori* chronic gastritis compared with 38 patients with chronic gastritis uninfected *H. pylori*.

Ansari M et al (2006) [2] studied 43 patients with *H. pylori* - positive chronic gastritis and 43 patients with *H. pylori* - free chronic gastritis. The results showed that GPx concentration in gastric fluid was significantly higher in *H. pylori* infected group compared with non-infected group (p = 0.0001).

Tala Z.Z et al (2017) [13] studied 80 chronic gastritis patients, of whom 50 patients were positive with *H. pylori* (62.5%). Results of GPx quantitative in erythrocytes showed that the average GPx activity in positive *H. pylori* group was 115 U/g Hb lower than the negative *H. pylori* group of 125.5 U/g Hb.

TAS activity in chronic gastritis patients with *H. pylori* - positive were 0.82 ±

0.42 U/mL, lower than the group with *H. pylori* - negative were 2.99 ± 4.40 U/mL (p = 0.03).

Dulger A.C et al (2011) [5] conducted a study on 67 patients with chronic gastritis, of whom 42 patients were positive with *H. pylori*, 25 patients with *H. pylori* - negative. The total plasma oxidation (TAS) status in the *H. pylori* - positive group was 1.61 ± 0.29 mmol/L lower in the negative *H. pylori* - group of 1.78 ± 0.36 mmol/L (p < 0.05). Positive *H. pylori* - group was treated with LAC regimen for 2 weeks, after treatment, TAS concentrations in plasma was 1.94 ± 0.47 mmol/L higher than before treatment (p = 0.001)

The MDA activity in the group of *H. pylori* - positive chronic gastritis was 3.25 ± 2.6 mmol/L lower than the *H. pylori* - negative group with 8.0 ± 8.52 mmol/L (p < 0.001). There were even 2/136 cases making up 1.5% with low MDA activity, smaller than the detection threshold of ELISA kit. Our research results were different from Navvabi A et al's findings (2013) [9]. The author studied 136 chronic gastritis patients, of whom 68 patients were positive with *H. pylori* and 68 patients with *H. pylori* - negative. Mean MDA concentration in patients and control groups was 3.75 ± 0.15 and 0.92 ± 0.04 mmol/L, respectively (p < 0.05). The explanation for this abnormality may be due to the usual compensatory mechanism in the early stages of the disease. In the early stages of the disease, the antioxidants in the body are enough to neutralize free radicals, which help to protect cells, reduce the products of lipid peroxosis, thus MDA concentrations in plasma in the *H. pylori* infection group decreased. However, in our opinion, this is only a temporary decrease, it will increase when the disease becomes worse.

These comparisons show that there were differences in the evaluation of antioxidant activity results in patients with chronic gastritis. Conflicting results in assessing antioxidant activity in patients with chronic gastritis may be partly explained by the concept of "offset gap", which is the stage of the disease, in which antioxidant level/activity increases due to the compensatory mechanism. When the ability to compensate is no longer available, the antioxidant activity starts to fall below normal levels and each antioxidant may have a different offset distance. In addition, other factors such as methodology, sample size, statistical analysis, sampling location, history of the disease, research group, manufacturer test kit, etc… cause conflicting results.

Moreover, in our study, there was no correlation between the activity of enzymes SOD, GPx, TAS and MDA with the positive level of *H. pylori*. This may be due to the relatively small sample size (n = 71). Additionally, this study did not evaluate the virulence status of *H. pylori* (through CagA (+) and VacA (+)) where CagA (+) and VacA (+) would damage tissue aggravated and therefore will affect the level of activity of endogenous antioxidants. Therefore, the results may not reflect the effects of *H. pylori* on endogenous antioxidants.

CONCLUSION

Activities of SOD, GPx, MDA and TAS in plasma in chronic gastritis patients with *H. pylori* - positive (217.78 ± 142.9 ng/mlL;

116.65 ± 77.21 pg/mL; 3.25 ± 2.6 mmolL; 0.82 ± 0.42 U/mL, respectively) lower and significant versus *H. pylori* - negative group (512.54 ± 576.70 ng/mL; 264.93 ± 279.1 pg/mL; 8.0 ± 8.52 mmol/L; 2.99 ± 4.40 U/mL, respectively) with $p < 0.05$, respectively.

REFERENCES

1. Agbor N.E, Esemu S.N, Ndip L.M et al. Helicobacter pylori in patients with gastritis in West Cameroon: Prevalence and risk factors for infection. BMC Res Notes. 2018, 11 (559).

2. Ansari M, Nobar M.R, Dolatkhah H et al. Comparison of levels of nitric oxide, superoxide dismutase and glutathione peroxidase of gastric fluid in infected and non-infected patients with *Helicobacter pylori.* Acta Medica Iranica. 2006, 44 (3), pp.159-166.

3. Atayan Y and Hacisalihoglu P. The correlation between tissue *Helicobacter pylori* severity and the increase in serum neutrophil/lymphocyte ratio in patients with active chronic gastritis. Research Article - Biomedical Research. 2017, 28 (11).

4. Ding S.Z, Minohara Y, Fan X.J et al. Helicobacter pylori infection induces oxidative stress and programmed cell death in human gastric epithelial cells. American Society for Microbiology. 2007, 75 (8), pp.4030-4039.

5. Dulger A.C, Aslan M, Nazligul Y et al. Peripheral lymphocyte DNA damage and oxidative status after eradication therapy in patients infected with *Helicobacter pylori.* POLSKIE ARCHIWUM MEDYCYNY WEWNĘTRZNEJ. 2011,121 (12), pp.428-431.

6. Graham D.Y. History of *Helicobacter pylori*, duodenal ulcer, gastric ulcer and gastric cancer. World J Gastroenterol. 2014, 20 (18), pp.5191-204.

7. Haim Shirina H, Pintob J.T, Liuc L.U et al. Helicobacter pylori decreases gastric mucosal glutathione. Cancer Letters. 20011, 64, pp.127-133.

8. Kim Y.J, Kim E.H, Hahm K.B. Oxidative stress in inflammation-based gastrointestinal tract diseases: Challenges and opportunities. Journal of Gastroenterology and Hepatology. 2012, 27 (6), pp.1004-1010.

9. Navvabi A, Ansari M.H.K, Navvabi N et al. Effect of *Helicobacter pylori* infection on oxidative stresses in patients with chronic gastritis. African Journal of Microbiology Research. 2013, 7 (50), pp.5632-5636.

10. Noguchi K, Kato K, Moriya T et al. Analysis of cell damage in *Helicobacter pylori*-associated gastritis. Pathol Int. 2002, 52 (2).

11. Ray-Offor E, Obiorah C.C. *Helicobacter pylori* and precancerous lesions of the stomach in a Nigerian Metropolis: A cohort study. Niger J Clin Pract. 2018, 21 (3), pp.375-379.

12. Sasaki M, Joh T. Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant. Protective Agents. J Clin Biochem Nutr. 2007, 40, pp.1-12.

13. Tala Z.Z, Siregar G.A, Siregar G.P. Glutathione peroxidase level in patients with *Helicobacter pylori*-associated gastritis. Earth and Environmental Science. 2017, 125 (012058).

14. Tatemichi M, Iwasaki M, Sasazuki S et al. Association between polymorphisms in glutathione S-transferase Mu3 and IgG titer levels in serum against *Helicobacter pylori*. J Hum Genet. 2009, 54 (10), pp.557-63.

15. Yokoyama H, Fujii S. Structures and metal-binding properties of *Helicobacter pylori* neutrophil-activating protein with a Di-Nuclear Ferroxidase Center Biomolecules. 2014, 4, pp.600-615.

16. Zhang C, Yamada N, Wu Y.L et al. Comparison of *Helicobacter pylori* infection and gastric mucosal histological features of gastric ulcer patients with chronic gastritis patients. World J Gastroenterol. 2005, 11 (7), pp.976-981.