

# COMPARISON OF LEVELS OF NITRIC OXIDE, SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE OF GASTRIC JUICE IN INFECTED AND NON-INFECTED PATIENTS WITH *HELICOBACTER PYLORI*

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**Abstract-** *Helicobacter Pylori* infection leads to different clinical and pathological outcomes in humans, including chronic gastritis and gastric neoplasia. It has been demonstrated that oxidative stress associated with inflammation plays an important role in gastric carcinogenesis. To evaluate the oxidative stress in *H. Pylori* infection we studied the gastric juice levels of nitric oxide and the activities of superoxide dismutase and glutathione peroxidase. A total of 43 patients suffering from *H. Pylori* infection were selected and 43 non-infected individuals were chosen as control group. Compared to control group, significant reduction in the mean levels of nitric oxide in the gastric juice of the patients was noticed ( $P = 0.0001$ ). In the patients activities of superoxide dismutase and glutathione peroxidase in gastric juice were markedly higher than those of control group ( $P = 0.0001$  and  $P = 0.0001$ , respectively). A reverse and meaningful relationship was observed between the levels of nitric oxide and the activities of superoxide dismutase in the gastric juice of patients ( $r = -0.35$ ,  $P = 0.023$ ). The results of this study confirm that *H. Pylori* has developed various mechanisms to escape the effect of immune system. *H. Pylori* have also developed strategies for defense against oxidative stress.

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**Key words:** *Helicobacter pylori*, nitric oxide, oxidative stress, superoxide dismutase, glutathione peroxidase

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## INTRODUCTION

*Helicobacter Pylori* infection is a chronic and widespread infection, which affects the social life of

population living in underdeveloped regions.

Although treatable, *H. Pylori* infects approximately one half of the world's population (1, 2). It never attacks underlying tissues of the stomach and blood vessel tissues but colonizes on the surface of stomach mucosa and can induce gastric inflammation. This gastritis increases the risk for gastric and duodenal ulceration, distal gastric adenocarcinoma and gastric mucosal lymphoproliferative diseases.

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Fractions of pathogens derived from food and oral cavity are effectively killed in the stomach by the high concentrations of hydrogen ion and nitric oxide (NO) in gastric juice of normal individuals; (3, 4) the latter is a multifunctional gaseous radical that binds to iron and copper containing proteins with high affinity (5). NO can be produced from the acidic juice through the nonenzymatic reduction of nitrate derived from saliva and food and by three types of NO synthases which is seen in various cells, such as the vascular endothelial cells, neurons, neutrophils, and macrophages. All these mechanisms and the isozymes of the three types of synthases are present in the gastric mucosa. Due to this, the physiological concentration of NO in gastric juice is relatively high (~5  $\mu\text{M}$ ) (3, 6, 7).

Although gastric NO exhibits potent bactericidal activity against several aerobic bacteria, *H. Pylori* is resistant to it. The potent bactericidal activity of NO is due to its strong interaction with terminal oxidases of many aerobic bacteria. Yet, the sensitivity of *H. Pylori* respiration to NO is markedly lower than that of other bacteria. It also generates the superoxide radical, during the transformation of from bacillary to coccoid form, which is extremely reactive with NO (8-11). From reaction between NO and superoxide, peroxyxynitrite is generated which is also toxic for many organisms and causes oxidative stress in the host's tissue (12, 13).

Formation of high levels of superoxide radical by *H. Pylori* will increase the activity of superoxide dismutase (SOD) in gastric mucosa. SOD catalysis the dismutation of the superoxide free radical and anion to hydrogen peroxide and oxygen. This enzyme is very important in protection of tissues from oxidative stress injury and maintenance of tissue homeostasis (12, 14). Therefore the assessment of the activity of SOD may be an important factor in evaluation of *H. Pylori* infection (15).

Glutathione (GSH) values are significantly lower in patients infected with *H. Pylori* than in patients who are *H. Pylori* negative. It has been shown that *H. Pylori* directly reduces intracellular GSH and vitamin E and impairs GSH metabolism of gastric epithelial cells, which in turn causes increased formation of free radicals (16, 17). Determination of

glutathione peroxidase (GPX), an enzyme involved in GSH metabolism, in gastric mucosa or gastric juice as a complementary parameter may be helpful in evaluation of *H. Pylori* infection.

## MATERIALS AND METHODS

The subjects were recruited from patients undergoing gastrointestinal endoscopy at the gastroenterology outpatient clinic, Imam Khomani Hospital, Tehran. The following patients were excluded from the present study: patients receiving drugs affecting free radical scavenging such as Vitamin C, E and A, and bismuth and other drugs which could affect the results, patients suffering from any concurrent conditions such as inflammatory disease of gastrointestinal tract and cancer, which could elevate free radical production, and smokers. We obtained informed consent from all participants.

All subjects underwent endoscopy. The presence of chronic active gastritis was studied in gastric mucosa and gastric biopsies were also checked with rapid urease test for presence of *H. Pylori*. The patients were divided into two groups; 43 patients (18 males and 25 females) with mean age of  $44 \pm 1.65$  years suffering from *H. Pylori* infection and 43 non-infected individual (19 males and 24 females) with mean age of  $43 \pm 1.47$  years. The second group was used as control group.

From each participant, a gastric juice sample and a biopsy sample were collected. A smear from homogenized biopsy samples was prepared and stained with Gram method. Then, the levels of NO in gastric juice were measured colorimetrically by Griess method (18). In this method, NO undergoes a series of reactions with several molecules present in biological fluids including:  $\text{O}_2^-$ ,  $\text{O}_2$  and  $\text{NO}_2$ . The final products of NO *in vivo* are nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ). The relative proportion of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  is variable and cannot be predicted with certainty. Thus the best index of total NO production is the sum of both  $\text{NO}_2^-$  and  $\text{NO}_3^-$ . The method used in this study provides an accurate and convenient measurement of nitrate/nitrite concentration in a simple two-step process. The first step is the

conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Griess reagents, which convert nitrite into deep purple azo compound. Colorimetric measurement of the absorbance due to this azo chromophore accurately determines nitrite concentration.

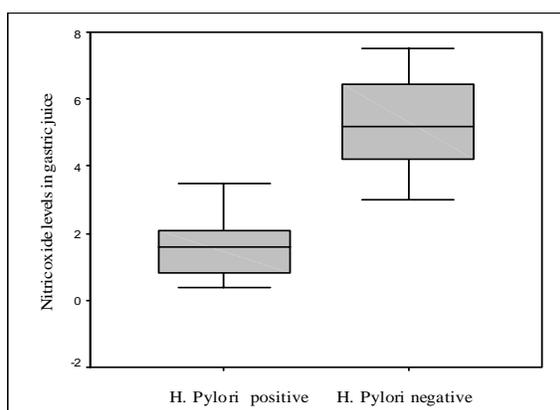
The activities of SOD and GPX were determined by colorimeter using Randox Kits (15, 19). The concentrations of protein in gastric juice were measured colorimetrically by Lowry method (20). The results of the enzyme analysis were reported as U/mg protein.

SPSS 12 for window was used to perform statistical analysis. All data are expressed as the mean  $\pm$  SEM and statistical significance was set at  $P < 0.05$ . The  $t$  test was performed to compare data, and regression analysis was calculated for correlation between parameters.

## RESULTS

A total of 86 patients were included in the present study of which 50% were *H. Pylori* infected (case) and the rest were non-infected (control). Statistical analysis showed no significant differences in sex and age in the *H. Pylori* infected and non-infected groups ( $P > 0.05$ ).

As shown in figure 1, marked difference was



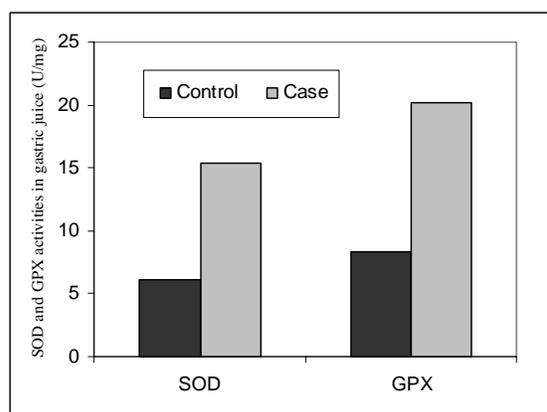
**Fig. 1.** Comparison of the mean values of gastric juice nitric oxide in case and control groups, showing marked difference in two groups.

observed in the levels of NO in the gastric juice obtained from *H. Pylori* infected and non-infected individuals, with a significant reduction in the infected group ( $P < 0.05$ ).

Measuring the activities of SOD and GPX in gastric juice of the two groups showed significant ( $P < 0.05$ ) elevation in the activities of both enzymes in the *H. Pylori* infected group (Table 1 and Fig. 2).

The relationships between the levels of NO and the activities of the SOD and GPX in gastric juice of both groups were studied performing correlation coefficient test. As shown in table 2 and figure 3, in control group a reverse but not meaningful correlation was noticed between NO levels and the activities of SOD ( $r = -0.269$ ,  $P = 0.089$ ) and that of GPX ( $r = -0.125$ ,  $P = 0.437$ ). A reverse and significant relationship was found between the levels of NO and the activities of SOD in gastric juice of *H. Pylori* case group ( $r = -0.355$ ,  $P = 0.023$ ) but in the case of GPX the relationship was direct and not meaningful ( $r = 0.264$ ,  $P = 0.096$ ) (Table 2).

As shown in figure 4 a direct and significant relationship between the activities of SOD and GPX was observed in the gastric juice obtained from control group ( $r = 0.702$ ,  $P = 0.0001$ ) but the relation in those of case group was not meaningful ( $r = -0.028$ ,  $P = 0.859$ ).



**Fig. 2.** Comparison of the mean values of gastric juice superoxide dismutase and glutathione peroxidase in case and control groups.

**Table 1.** The mean  $\pm$  SEM activities of SOD and GPX in the gastric juice of in case and control groups

Enzyme	Number	Mean $\pm$ SEM (U/mg protein)	<i>t</i>	<i>P</i> value	CI 95%
<b>SOD</b>					
Case	43	15.37 $\pm$ 0.46	13.94	0.0001	7.9-10.5
Control	43	6.12 $\pm$ 0.47			
<b>GPX</b>					
Case	43	20.18 $\pm$ 1.2	7.971	0.0001	8.88-14.81
Control	43	8.33 $\pm$ 0.77			

Abbreviations: SOD, superoxide dismutase; GPX, glutathione peroxidase; CI, confidence interval.

## DISCUSSION

*H. Pylori* induces gastric inflammation which increases the risk for gastric and duodenal ulceration, gastric adenocarcinoma, and gastric mucosal lymphoproliferative diseases. More than 50% of population in developing countries and about 40% of those living in developed countries are carriers of *H. Pylori* (21).

Many studies published in the last few years have demonstrated that isolated *H. Pylori* possesses substantial phenotypic and genotypic diversity, which may cause differential host inflammatory responses and these differences eventually influence clinical outcome (22). These recent investigations have described the mechanisms of *H. Pylori* pathogenesis, which will ultimately help to define colonized individuals bearing the highest risk for disease, and enable physicians to appropriately focus on diagnostic testing and eradication therapy.

NO produced by the NO synthase (NOS) is a critical component of host defenses against different pathogens (23). The compound is a central component of innate immunity and an effective antimicrobial agent. The survival of *H. Pylori* despite marked induction of inducible NOS synthase in macrophages and gastric tissues suggests that the bacterium has developed mechanisms to avoid NO-

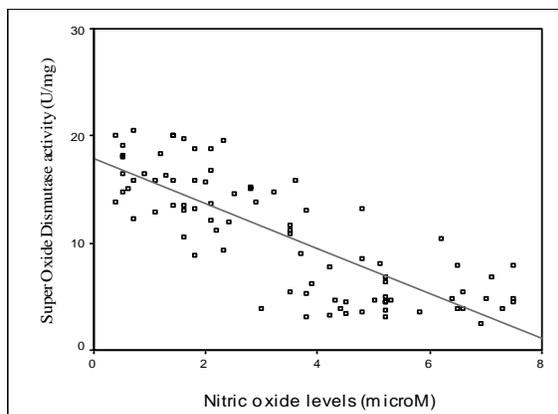
dependent killing. Gobert *et al.* suggested that mammalian arginase compete with NOS for the common substrate L-Arginine which is an essential amino acid in these microorganisms supplied from the extracellular store and hydrolyzing it to urea and L-Ornithine (23-27). Therefore, arginase can regulate cellular NO production and counteract the biological effect of NO. The arginase is active in wild-type strains of *H. Pylori* and inactive in other strains so the wild-type strains lead to gastroduodenal diseases. In this study low level of NO in gastric juice of *H. Pylori* infected patients confirms the results reported by Gobert *et al.* (23) and Shiotani *et al.* (24). In 2002 they also reported that in activated macrophages by *H. Pylori*, the Arginase II is stimulated and the enzyme in turn inhibits NOS and reduces the NO release in gastric juice. Ornithine decarboxylase that metabolizes L-Ornithine to polyamines is also induced in *H. Pylori* stimulated macrophages. Apoptosis is abolished by inhibition of ornithine decarboxylase and restored by polyamines, spermidine and spermine (25).

Our results suggest that *H. Pylori* may reduce the levels of NO in gastric juice to escape from host immunity response and to colonize successfully in human stomach mucosa. Furthermore, according to previous studies, bacterial arginase has also an important role in down regulating eukaryotic NO production.

**Table 2.** Relationship between the levels of nitric oxide and the enzymes, superoxide dismutase and glutathione peroxidase, in the case and control groups

Enzymes	Nitric Oxide (Case)		Nitric Oxide (Control)	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
SOD	-0.269	0.089	-0.355	0.023
GPX	-0.125	0.437	0.264	0.096

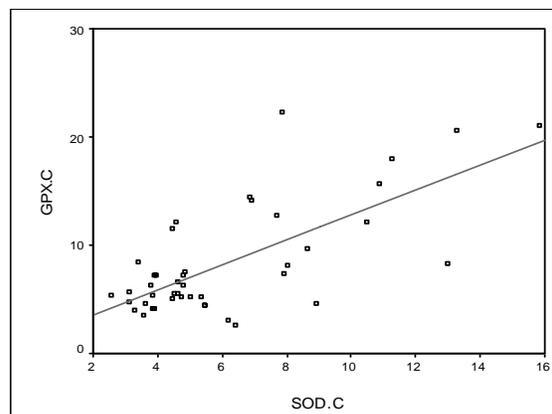
Abbreviations: SOD, superoxide dismutase; GPX, glutathione peroxidase.



**Fig. 3.** The regression correlation between the levels of gastric juice NO and the activity of SOD in case group.

Modulating effect of L-Arginine availability on local NO production and parasite killing in experimental trypanosomiasis has been studied (27). It was reported that L-Arginine depletion, induced by arginase and parasites has a role in modulating the L-Arginine-NO pathway under pathophysiological conditions. Although gastric epithelial apoptosis is a programmed physiological event in the superficial aspect of mucosa and is important for healthy cell turnover, *H. Pylori* infection reportedly promotes such a cell death sequence. Reduced level of apoptosis could contribute to the generation of gastric cancer (28). Some investigators examining the mechanisms by which *H. Pylori* induces gastric mucosal cell injury concluded that it induces apoptosis in gastric epithelial cells through inducible NO, which plays an important role in gastric cell injury (29).

Although *H. Pylori* has been shown to be an important pathogen of gastric and duodenal inflammation, its pathogenic mechanisms are not well defined. It has been reported that *H. Pylori* infection plays a pathological role in many gastrointestinal diseases through excessive mucosal reactive oxygen species production, pronounced membrane damage and the depletion of gastric antioxidants (16). One of the potential toxic factors involving *H. Pylori* induced gastric injury are oxygen radicals, which are released from activated neutrophils which have a chemotactic activity for *H. Pylori* (30).



**Fig. 4.** The regression correlation between the activities of gastric juice SOD and GPX in control group.

The local inflammation caused by *H. Pylori* invasion may generate an amount of reactive oxygen species (ROS) that exceeds the anti-oxidants capacity to remove them, resulting in further cell damage (31, 32). *H. Pylori* generates large amounts of superoxide radicals to reduce the levels of NO in gastric juice that rapidly react with NO and generate peroxynitrite (8, 33). These two mechanisms of free radical generation by bacteria and host cause high oxidative pressure (15, 16, 26, 34). Additionally antioxidant activity may increase the tissue damage. In normal subjects free radicals are removed by enzymatic and nonenzymatic antioxidant systems. Most important of the former are SOD (35, 36) and GPX (37, 38). In this study marked increase in the activities of the both enzymes in gastric juice of *H. Pylori* infected group was noticed. The possible biological significance of SOD in the *H. Pylori* positive and *H. Pylori* negative gastric mucosa obtained from endoscopy has been studied and suggested that Mn-SOD plays an important role as an anti-oxidant against ROS generated in *H. Pylori* infected gastric mucosa and, subsequently, in the maintenance of cell turnover in gastric mucosa (15). Reports on effects of *H. Pylori* on activities of SOD and GPX in the gastric mucosa are conflicting (37-39), although this lack of agreement might be explained by differences in species or durations of infection.

In this study elevated activities of the enzymes in gastric juice of infected individual may be due to the enzymes expression caused by ROS derived from

activated macrophages in *H. Pylori* infected mucosa (40) or release of the enzyme from the damaged mucosal cells. GPX is considered to be complementary to SOD (39) and in this study direct correlation between the two enzymes activities in gastric juice of uninfected group and indirect correlation between those in the infected group may suggest the presence of oxidative stress in *H. Pylori* infection. Medical eradication treatment to reduce the oxidative stress in *H. Pylori* infected gastric mucosa may be considered important in the treatment of gastroduodenal diseases and more importantly in the prevention of the reactive oxygen species accumulation and cellular damage in epithelial cells.

#### Conflict of interests

The authors declare that they have no competing interests.

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