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Effect of *Helicobacter pylori* infection on oxidative stresses in patients with chronic gastritis

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The *Helicobacter pylori* infection is currently endemic worldwide with high prevalence in developing regions. The infection causes chronic gastritis, gastric and gastric adenoid carcinoma. Infection by *H. pylori* may act as a risk factor for proteins, lipids and DNA damages. In this study, gastric biopsies were obtained in patients with a chronic gastric to investigate effects of *H. pylori* infection on oxidative stress in patients with chronic gastritis. Urease test and polymerase chain reaction (PCR) were performed on endoscopic gastric biopsy obtained from 150 subjects. The result showed that the presence of a 492-bp DNA fragment was indicative of a positive reaction, also fresh samples had a positive urease test for *H. pylori.* Malondialdehyde (MDA) in patients with gastritis as a result of *H. pylori* were significantly increased as compared to the control groups ($p<0.05$). Mean ± SE of reduced glutathione (GSH) and total antioxidant capacity (TAC) in patients were decreased significantly ($p<0.05$). The result shows mean ± SE of oxidized glutathione (GSSG) in patients showed significant increased as compared to the control groups. These finding show that *H. pylori* infection can cause oxidative stress disorders that may act as risk factors.

Key words: Malondialdehyde, reduced glutathione, oxidized glutathione, total antioxidant capacity, *Helicobacter pylori*.

INTRODUCTION

Helicobacter pylorus is one of the most infectious human pathogens, as over half of the world's population is infected with this Gram-negative bacteria (Kusters et al., 2006). *H. pylori* possesses an unusual characteristic of urea hydrolysis, forming carbon dioxide and ammonia (Xia et al., 1994). The major etiologic association of chronic gastritis is chronic infection by *H. pylori* (Adisa et al., 2011).

Environmental and genetic factors appear to be important in the progression of *H. pylori* on initiated gastritis to more serious outcomes (Brown, 2000).The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage (Demir et al., 2003). The body possesses an antioxidant defense system that removes peroxides, free radicals and superoxide generated within the mucosa. Reduced glutathione plays an important role against tissue oxidative damage, its depletion results in the accumulation of free radicals which initiate bio-

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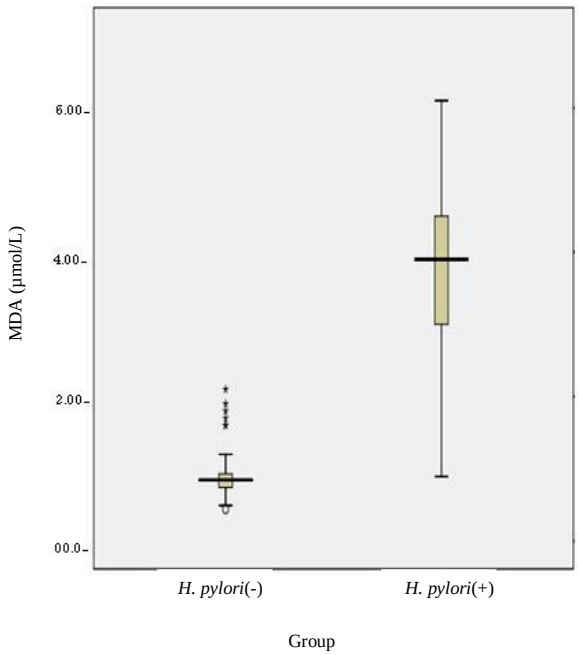

Figure 1. Malondialdehyde (MDA) in gastritis patients with and without *H. pylori* infection.

chemical damage by covalent binding to macromolecules and by lipid peroxidation (Santra et al., 2000). When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction (Kusano, 2008) then, the maintenance of adequate antioxidant levels is essential to prevent or even manage a great number of disease conditions (Kusano, 2008).

On the other hand, the use of total antioxidant capacity test, is as a biomarker of disease in biochemistry, medicine, food and nutritional sciences (Kusano, 2008). The changes of parameters such as malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG) and total antioxidant capacity (TAC) are a causative agent for chronic and acute gastritis and the study in this field is attractive for scientists.

The aim of this study was to investigate effects of *H. pylori* infection on MDA, GSH, GSSG and TAC in patients with chronic gastritis. This is a new subject of study in *H. pylori* infection in antrum of gastritis and oxidant and anti oxidant status.

MATERIALS AND METHODS

Endoscopic gastric biopsy were obtained from one hundred and fifty patients (males/females) aged between 30 to 50 years old with gastritis resulting from *H. pylori* (Sacco et al., 2007). Fraction of biopsy specimens 3 mm maintained from antrum was placed in 0.9% NaCl for PCR test. At the time of biopsy, five milliliters of blood were drawn from each of the patients and placed on ice. Serum was separated in refrigerated centrifuge at 6000 *g* and kept at -80°C until they were tested.

Urease test

One fraction from the biopsy specimen was used for urease testing for *H. pylori*. The change in colour of the broth from pale yellow to deep pink was taken as a positive test. On urease positive samples (Berry,2006), PCR test were carried out .

Sixty eight gastritis patients were *H. pylori* positive by PCR and urease positive were chosen as patient groups, 68 gastritis patients without *H. pylori* infection were chosen as a control groups

Helicobacter pylori PCR detection

H. pylori PCR detection was carried out by DNA extraction using CinnaGen DNP (#DN8116C) and amplification of samples were carried out by CinnaGen *H.pylori* PCR detection kit (CinnaGen Molecular Biology and Diagnostic,Tehran,Iran), and then electrophoresis was performed by using 100 bp ladder as a marker in 1.5% agarose gel, and positive samples show a single band at 492 bp DNA fragment.

Measurement of oxidized and reduced glutathione GSSG/GSH, malondialdehyde, total antioxidant capacity

GSSG/GSH was assayed using instruction for factory kit (Oxford Bio Medical Research USA). For measurement of GSSG, 30 µL of scavenger from GSSG kit was added to 100 µl of serum and then all serum samples were kept at -80°C until they were tested. MDA levels in samples were spectrophotometrically determined (Khanzode et al., 2003).

Total antioxidant capacity test was performed by randox kit (Randox laboratories, UK) and samples were measured automatically with BT-3000 (Biotechnical Instruments, Italy). The amount of total antioxidant was reported (mmol/L). Statistical calculations were done by SPSS 16 and P value less than 0. 05 were accepted as significant.

RESULTS

In this study, MDA, GSH, GSSG and TAC concentrations in serum samples from chronic gastritis patients with *H. pylori* and without *H. pylori* infection were studied. Results show that MDA, GSH and GSSG and TAC levels in patients were different from that of the controls.

MDA levels and mean values with standard errors in patient groups and control groups were 3.75±0.15 and 0.92±0.04 µmol/L, respectively ($p<0.05$) (Figure 1). The GSH values of the patient groups were 0.59±0.03 µmol and the control group were 2.14±0.08 µmol, statistical comparisons revealed significant differences between the two groups ($p<0.05$), results indicate a decrease in glutathione levels in the patient group than the control group (Figure 2).

Oxidized glutathione in patient groups and control groups were 0.26±0.01 and 0.05±0.03 µmol, respectively. Levels of GSSG were significantly increased ($p <0.05$) (Figure 3). The results of the TAC show that in the patient groups it was 0.92±0.04 mmol/L and the control groups it was 1.70±0.06 mmol/L ($p <0.05$) (Figure 4). The results suggest a correlation between GSSG, GSH and GSSG with MDA present in patient groups.

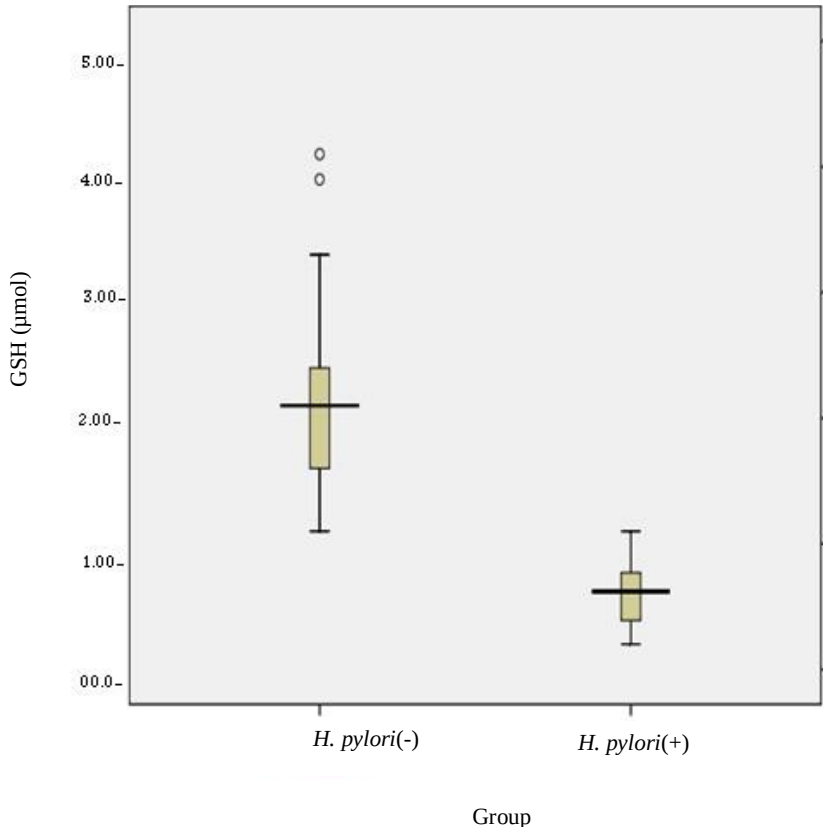

Figure 2. Reduced glutathione (GSH) in gastritis patients with and without *H. pylori* infection.

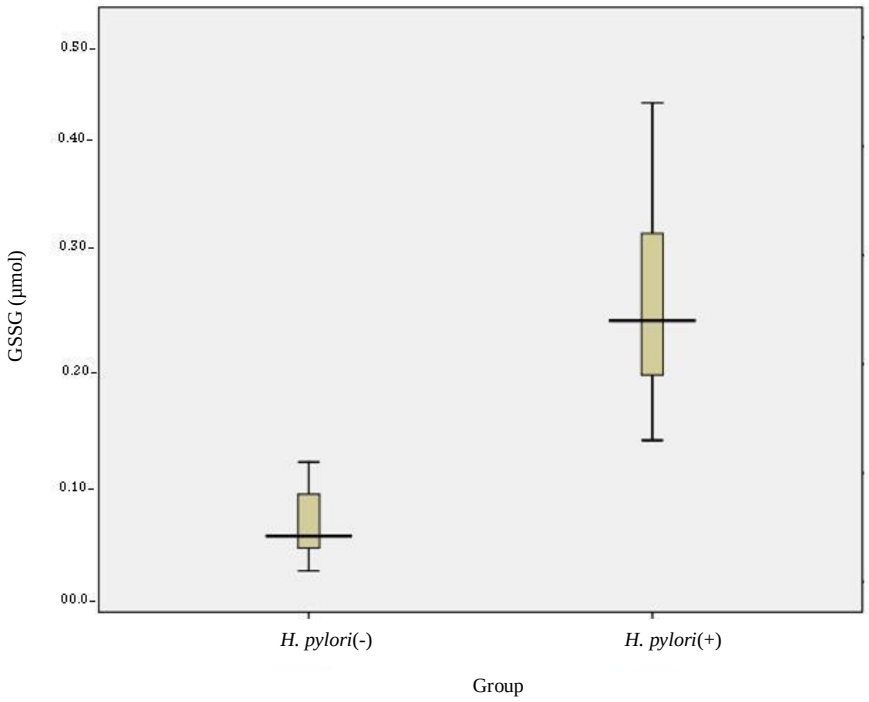

Figure 3. Oxidized glutathione (GSSG) in gastritis patients with and without *H. pylori* infection.

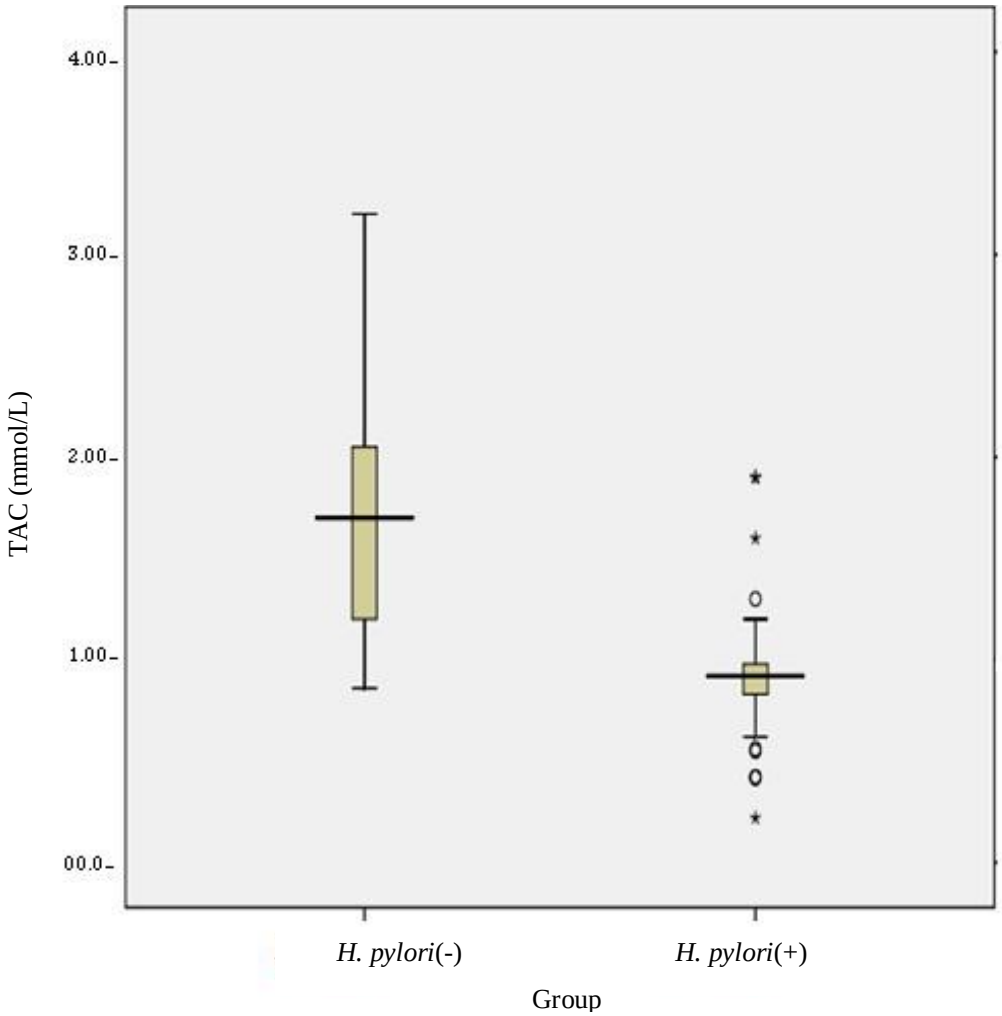

Figure 4. Total antioxidant capacity (TAC) in gastritis patients with *H. pylori* infection (case) and gastritis patients without *H. pylori* infection (control).

DISCUSSION

H. pylori is one of the most infectious human pathogens, as over half of the world's population is infected with this Gram-negative bacteria (Kusters et al., 2006).The prevalence of *H. pylori* infection appears to be higher in developing countries (Adisa et al., 2011). Reactive oxygen species (ROS) produced by this bacteria may be one of the crucial factors whereby oxidative stress can play a role in the pathogenesis of ulcer disease (Arend et al., 2005).

In the present study, *H. pylori* effects on oxidative stress including MDA, GSH and GSSG and TAC were tested. Our findings with the aim of studying *H. pylori* effects on oxidative stress in chronic gastritis patients showed that the amount of malondialdehyde and oxidized glutathione were increased in patients, levels of total antioxidant and reduced glutathione were decreased in these patients. Significant increase in serum superoxide dismutase and serum malondialdehyde was observed in *H. pylori* gastritis and gastric cancer patients as compared to control subjects (Khanzode et al., 2003). Our findings are concordant with these observations. It is known that radical scavengers play a significant role in protecting membranes from oxidative damage (Demir et al., 2003).

Depletion of gastric mucosal GSH may result in the accumulation of free radicals that can initiate membrane damage by lipid peroxidation (Demir et al., 2003). A deficiency of GSH puts the cell at risk for oxidative damage (Demir et al., 2003). *H. pylori* directly decrease cellular glutathione. A decreased level of GSH has been detected in the gastric mucosa of patients with *H. pylori* infection (Beil et al., 2000). In *H. pylori*-positive subjects, the sera values of total antioxidative status were significantly lower as compared to *H. pylori* m negative while the ratio between oxidized and reduced glutathione decreased in *H. pylori*-positive subjects (Hutt et al.,

2008). The results are in agreement with our findings.

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