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Full Length Research Paper

Evaluation of glycemic control, gastric juice nitric oxide and oxidative stress in diabetic patients infected by Helicobacter pylori

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Recently, diabetes mellitus has been known as one of the main cause of upper gastrointestinal symptoms. It has also been suggested that delayed gastric emptying may lead to bacterial overgrowth in the upper gastrointestinal tract. Since high prevalence of *Helicobacter pylori* (*H. pylori*) in diabetic patients has been reported. The aim of this study was to evaluate the relationship between dyspepsia, the level of gastric juice Nitric Oxide oxidative stress and hyperglycemic control in diabetic *H. pylori* infected patients. Sixty *H. pylori* infected diabetic patients (27 males and 33 females) with mean age of 39.5 ± 12 years, 60 diabetic patients without *H. pylori* infection (28 males and 32 females) with mean age of 34 ± 15 years and 60 healthy individuals (28 males and 32 females) with mean age of 41 ± 8 referred to endoscopy Department were selected as Case, Control-2 and Control-1 groups respectively. 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 level of NO° in gastric juice was measured calorimetrically and the activities of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) in gastric biopsy were determined using standard methods. The percentage of blood level of glycated Hemoglobin (HbA1C) was measured by ion exchange chromatography. Comparing with the two control groups significant elevation in the mean level of HbA1C was noticed in the case group ($p<0.0001$ in the both cases). The mean level of NO° in gastric juice was meaningfully higher than those in the control 1 and 2 groups ($p<0.0001$ in the both cases). The mean activities of SOD and GPX in the gastric mucosa were markedly higher than those of the both control groups ($p<0.0001$ in all cases). In patients with metabolically uncontrolled diabetes mellitus the prevalence of *H. pylori* infection is high and the bacteria colonization occurs at the antrum of stomach. After eradication therapy of *H. pylori* the control of the glyceamia will be useful. Increased levels of HbA1C in the case group in comparison with those in the uninfected subjects confirm the finding. On the other hand the treatment of the *H. pylori* infection improves the level of NO° in the gastric juice and reduces cellular damage resulting from acute oxidative and nitrosative stress produced by reaction between superoxide radicals of *H. pylori* and NO° of the gastric juice.

Key words: Diabetes mellitus, *Helicobacter pylori* infection, glycated Hb, nitric oxide, Oxidative stress.

INTRODUCTION

Recently diabetes mellitus was introduced as main cause of upper gastrointestinal symptoms, attributed to abnormal motility of stomach, gallbladder or small bowel (Hamed et al., 2008). Increased prevalence of

Helicobacter pylori (*H. pylori*) infection was reported in diabetic patients (Candelli et al., 2003). Delayed gastric emptying and reduced function of antrum are important consequences of diabetes mellitus. Hyperglycemia may stimulate *H. pylori* contamination or convert silent contamination to an over and active from, with dyspepsia symptoms (Block et al., 2002).

Although some studies concerning coexistence of *H. pylori* and diabetes mellitus exist, but the results are conflicting. There are several reports in relation to the prevalence of *H. pylori* in diabetic patients and its possible role in metabolic control (Candelli et al., 2003; Sargyn et al., 2003; Saluja et al., 2002). Some of the scientific evidences could not show any differences between control of diabetes in patient with and without *H. pylori* infection (Xia et al., 2001). In contrast some investigators reported complexity in control of diabetes in *H. pylori* contaminated patients (Candelli et al., 2003; Block et al., 2002; Sargyn et al., 2003; Saluja et al., 2002). The levels of HbA1C in diabetic patients with *H. pylori* infection are higher than those of diabetic subjects without the infection (Block et al., 2002). The eradication therapy of the infection reduces the level of HbA1C in the patients and this may improve the production and secretion of Nitric Oxide (NO°) (Bayraktutan, 2002). The symptoms and lesions resulting from the reaction between Superoxide radicals (O_2°) and NO° disappear following the treatment (Inoue et al., 1999; Takahara et al., 1999). NO° is a multifunction gas which attach with high affinity to iron and copper. The radical is produced by the vascular endothelium, neurons, neutrophiles and macrophages (Ansari et al., 2006; Brown et al., 1992; Calatayud et al., 2001). All the isozymes involved in the synthesis of NO° are present in the gastric mucosa. In gastric juice NO° is also produced through non-enzymatic reduction of salivary and food nitrites. Physiologically the level of NO° in gastric juice is relatively high (~ 5 µM) (Park et al., 2003). *H. pylori* produce large amounts of superoxide radicals which reacting with gastric juice NO° reduce its bactericidal effect. By this growth and colonization of the bacteria at the Antrum of the stomach occurs (Ansari et al., 2006; Nagata et al., 1998; Nakamura et al., 2000). The effect of *H. pylori* infection on the treatment and control of diabetes is not fully understood. Further studies are required to demonstrate the effect of the infection on the control of diabetes and its metabolic consequence. On the other hand there is little study about relationship between alternations in glucose metabolism in *H. pylori* infected patients and chemical changes in gastric juice including oxidative stress.

In this study effect of *H. pylori* infection on the control of glycemia by measuring HbA1C levels in diabetic patients with *H. pylori* infection was evaluated. Also possible role of alterations in glucose metabolism that may have an effect on promoting *H. pylori* colonization, due to chemical changes in gastric juice, was assessed by measuring reductive enzymes and oxidative stress. The other aim of the study was the evaluation of mechanisms of the reactions between hosts cells and *H. pylori*, which may help to find the best way of *H. pylori* treatment. So the level of nitric oxide of gastric juice and gastric mucosa oxidative stress were simultaneously evaluated in diabetic patients with and without *H. pylori* infection.

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MATERIALS AND METHODS

The subjects were recruited from patients undergoing gastrointestinal endoscopy in Imam Khomeini Hospital referred from endocrinology and metabolism Department, Sina Hospital, Tabriz-Iran. The following patients were excluded from the study: patients receiving drugs effecting free radicals 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. In this study 60 *H. pylori* infected diabetic patients (27 males and 33 females) with mean age of 39 ± 12 years, 60 diabetic patients without *H. pylori* infection (28 males and 32 females) with mean age of 34 ± 15 years and 60 healthy individuals (28 males and 32 females) with mean age of 41 ± 8 years were selected as case, control 2 and control 2 group respectively. From each participant a gastric juice sample and biopsy sample were collected. A smear from homogenized biopsy sample was prepared and stained with Gram method. Then the levels of NO° in gastric juice were measured colorimetrically by Griess method (Ansari et al., 2006; Green et al., 1982; Sun et al., 2003; Nims et al., 1995). The activities of SOD and GPX were determined in Cobas Mira autoanalyzer using Randox kits (Brown et al., 1992; Gotz et al., 1996; Paglia and Valentine, 1967). The levels of protein in the samples were measured colorimetrically by Lowry method (Ansari et al., 2006; Lowry et al., 1951; Bensadoun and Weinstein, 1976). The results of the enzyme analysis were reported as IU/mgProtein. To detect the presence of chronic active gastritis and *H. pylori* the biopsy sample were also sent to pathology department.

Continuous variables were reported as the mean±SE. All statistical analysis was performed using SPSS 17 for widows and a $p<0.05$ was considered statistically significant. For correlation between parameters the One-Way ANOVA test and multiple comparisons (Tukey HSD) were used.

RESULTS

The clinical characteristics of the case group and the two control groups are shown in Table 1.

As shown in Figure 1 significant differences were observed in the mean levels of gastric juice NO° in the three groups. The mean±SE in the control-1, control-2 and case groups were 5.22 ± 0.175, 4.21 ± 0.175 and 1.45 ± 0.175µmol/Liter respectively and p.values were less than 0.0001 in all cases (Figure 1).

The percentage of HbA1C in the diabetic patients with *H. pylori* infection (Case group) was higher than those of

Table 1. The clinical characteristics of the case and control groups.

	Groups		
	Case	**Control-1**	**Control-2**
Number	60	60	60
Male/Female	27/33	28/32	28/32
Age (years)	39.5 ± 13.9	41.2 ± 8.6	34.8 ± 15.3

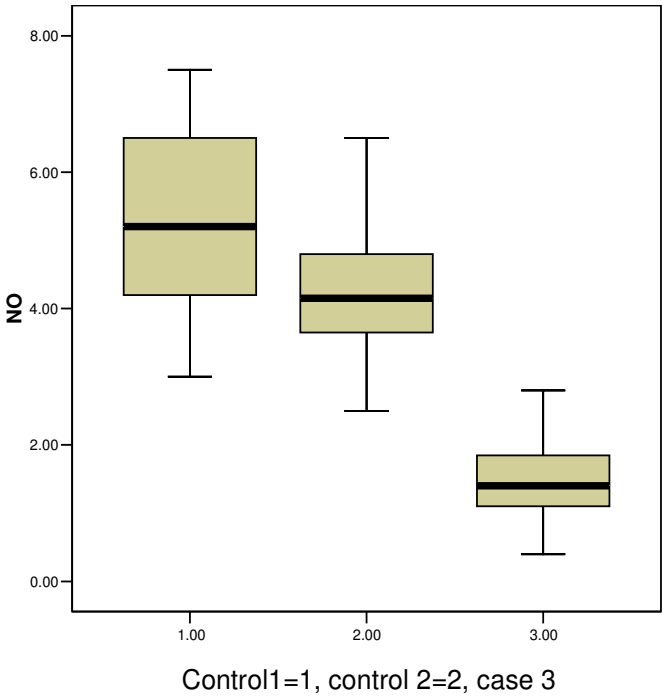

Figure 1. Comparison the mean levels of gastric juice NO° in the control-1, control-2 and case groups.

the two control groups. The mean±SE percentage of HbA1C in control-1, control-2 and case 5.25 ± 0.21, 7.26 ± 0.25 and 10.58 ± 0.29 respectively ($p<0.0001$ in all cases). The results are summarized in Figure 2. As shown in Figure 3 the mean activity of SOD in the case group was significantly higher than those of the two control groups, but no meaningful difference was noticed between the two control groups.

The mean levels of SOD in the gastric mucosa of the control-1, control-2 and case groups were 6.07 ± 0.53, 6.27 ± 0.56 and 15.03 ± 0.58 respectively ($p<0.0001$ in both cases).

In the diabetic patients with *H. pylori* infection the activity of GPX in the gastric mucosa was markedly higher than those in the two control groups. As presented in Figure 4. The mean activities of the enzyme in the control-1, control-2 and cases groups were 8.40±1.15, 7.71±1.13 and 19.79±1.11 IU/mgProtein ($p<0.0001$ in the all cases).

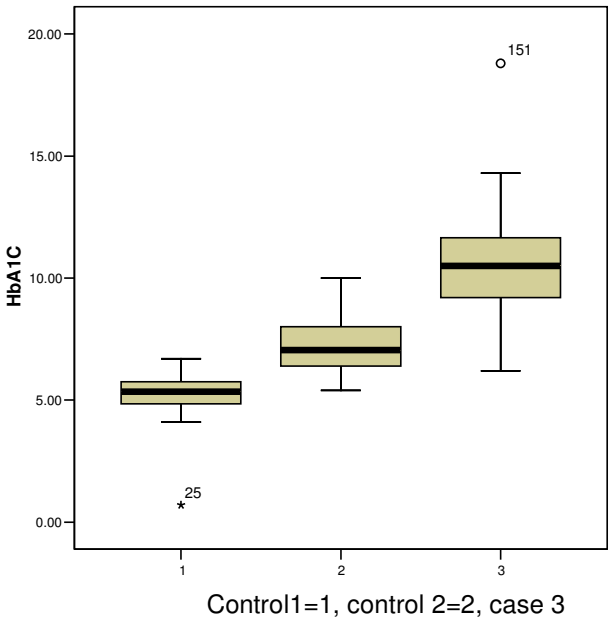

Figure 2. Comparison of mean percentage of HbA1C in the control-1, control-2 and case groups.

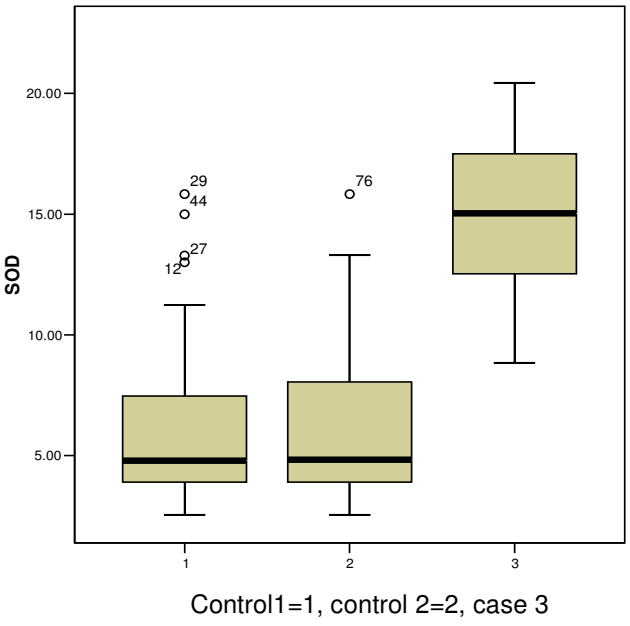

Figure 3. Comparison of the mean activities of superoxide dismutase (SOD) in the gastric mucosa of the three groups.

DISCUSSION

H. pylori is a common cause of chronic bacterial infection in the world. In this study it was assumed that contamination with H. pylori may affect the control of glycemia in diabetic patients possibly through oxidative stress. In metabolically uncontrolled diabetic patients high

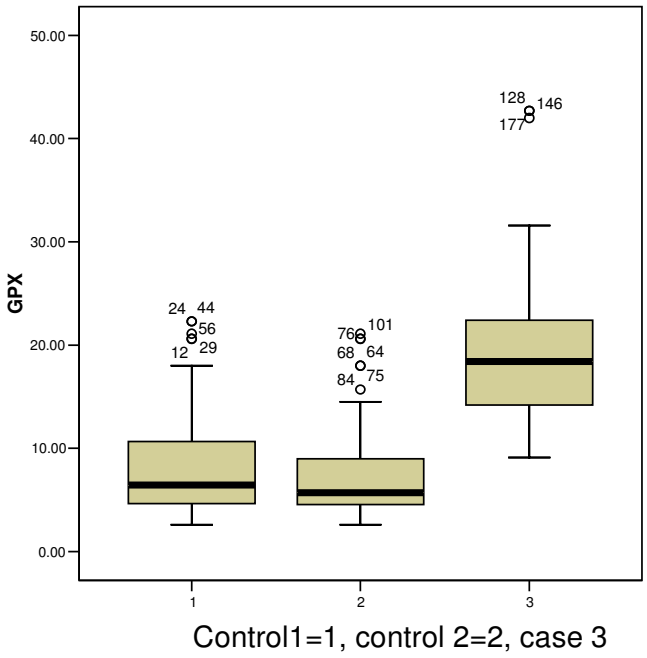

Figure 4. The mean activities of glutathione peroxidase (GPX) in the gastric mucosa of the control-1, Control-2 and case groups.

prevalence of *H. pylori* infection has been reported (Candelli et al., 2003; Sargyn et al., 2003). Some investigators studying the relationship between *H. pylori* infection and diabetes mellitus have published contradictory data (Candelli et al., 2003; Block et al., 2002; Sargyn et al., 2003; Saluja et al., 2002). Sargyn et al. (2003) confirmed the relationship, on the other hand for example Xia et al. (2001) could not find any correlation between the *H. pylori* infection and control of hyperglycemia.

In this study significant differences between HbA1C levels of diabetic patients with *H. pylori* and the two control groups were observed ($p<0.0001$ in all case). The findings suggest that control of hyperglycemia in *H. pylori* infection is not as easy as non-infected patients. Our results are in agreement with those reported by Candelli, et al. (2003).

H. pylori infection is the cause of about 90% of gastrointestinal diseases. In all cases, it can induce gastric inflammation and the gastritis increases the risk for gastric and duodenal ulceration, distal gastric adenocarcinoma and gastric mucosal lymphoproliferative diseases. So the diseases should be treated immediately after the diagnosis (Dunn et al., 1997; Kidd and Modlin, 1998).

Nitric Oxide produced by nitric oxide synthase is a critical component of host defenses against *H. pylori*. The compound is a central component of innate immunity and effective antimicrobial agent (Ansari et al., 2006; Gobert et al., 2001). The bacterium has developed mechanisms to avoid NO° dependent killing. Gobert et al. (2001) suggested that mammalian arginase compete with NO° synthase for the common substrate L-arginine, which is an essential amino acid in these microorganisms supplied from the extra cellular store and hydrolyzing it to urea and L-ornithine. Therefore, arginase can regulate cellular nitric oxide production and counteract the biological effect of NO°. In our study low levels of nitric oxide in gastric juice of *H. pylori* infected patients, especially those suffering from diabetes mellitus (case group), confirms the results reported by others (Bayraktutan, 2002; Gobert et al., 2000, 2001).

Diabetes mellitus in human and animal models causes endothelial damage and vascular dysfunction (Hamed et al., 2008). Hypertension may be due to lack of regulation of gene coding to endothelial NO° synthase which cause further reduction in the synthesis of NO° radical in diabetic patients. In this study finding very low levels of NO° in the gastric juice of case group confirms the suggestion of Gobert et al. (2000), Candelli et al. (2003), Gonzalez et al. (1997). In 2002 they also reported that in activated macrophages by *H. Pylori*, the arginase II is stimulated and the enzyme in turn inhibits nitric oxide synthase and reduces the NO° release in gastric juice (Gobert et al., 2002). The results of our study and others suggest that *H. pylori* may reduce the levels of nitric oxide in gastric juice to escape from host immunity response and to colonize successfully in human stomach mucosa. The very low concentration of NO° in the gastric juice of diabetic patients with *H. pylori* infection may make the condition more favorable to the bacteria.

One of the potential toxic factors involving *H. pylori* induced gastric injury are oxygen radicals, which are released from activated neutrophils which have a chemo tactic activity for *H. pylori* (Naito and Yoshikawa, 2002). The local inflammation caused by *H. pylori* invasion may generate an amount of reactive oxygen species (ROS) that exceeds the antioxidants capacity to remove them, resulting in further cell damage (Suzuki et al., 1996; Olczak et al., 2002). *H. pylori* generate large amounts of superoxide radicals to reduce the levels of NO° in gastric juice that rapidly react with NO°and generate peroxynitrite (Nagata et al., 1998). Normally free radicals are removed by enzymatic and non-enzymatic antioxidant systems. The most important enzymatic system are SOD (Rumley and Paterson, 1998) and GPX (Shirin et al., 2001). In this study high activities of the both enzymes were observed in the gastric mucosa of the both groups of patients, and elevation in group with diabetes was higher than those without. The results suggest that SOD and GPX play an important role as an antioxidant against ROS and the generation of ROS in infected diabetics is higher than those non-diabetics. Reports on effects of *H. pylori* on the activities of SOD and GPX in gastric mucosa are conflicting (Olczak et al., 2002; Rumley and Paterson, 1998; Shirin et al., 2001), although this lack of agreement might be explained by

differences in species or durations of infection. In our study increased activities of the enzymes in gastric mucosa of infected individual may be due to the enzymes expression caused by ROS derived from activated macrophages in *H. pylori* infected mucosa (Olczak et al., 2002) or release of enzyme from the damaged mucosal cells. GPX is considered to be complementary to SOD (Ueda et al., 1998). In this study simultaneous increase in the activities of the both enzymes in the patients groups are agreements with those of others (Ansari et al., 2006; Noguchi et al., 2002; Jung et al., 2001; Felley et al., 2002). The higher activities of the antioxidant enzymes in the gastric juice of diabetic patients with *H. pylori* infection may suggest more production of ROS in this group of patients because of the infection.

In conclusion, results of this study show high levels of HbA1C in *H. pylori* infection in diabetes mellitus patients compared to non-*H. pylori* control group. The higher activities of antioxidant enzymes in the case group may indicate more production of ROS and sever oxidative stress in the case group. Therefore, to reduce oxidative stress and to monitor the glycemia by HbA1C measurement medical eradication treatment of *H. pylori* treatment is very important.

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