

Insulin resistance in *H pylori* infection and its association with oxidative stress

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of adding antioxidant vitamins to *H pylori* eradication therapy on insulin resistance during *H pylori* infection.

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Abstract

AIM: To determine the insulin resistance (IR) and oxidative status in *H pylori* infection and to find out if there is any relationship between these parameters and insulin resistance.

METHODS: Fifty-five *H pylori* positive and 48 *H pylori* negative patients were enrolled. The homeostasis model assessment (HOMA) was used to assess insulin resistance. Serum total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) were determined in all subjects.

RESULTS: The total antioxidant capacity was significantly lower in *H pylori* positive group than in *H pylori* negative group (1.36 ± 0.33 and 1.70 ± 0.50 , respectively; $P < 0.001$), while the total oxidant status and oxidative stress index were significantly higher in *H pylori* positive group than in *H pylori* negative group (6.79 ± 3.40 and 5.08 ± 0.95 , and 5.42 ± 3.40 and 3.10 ± 0.92 , respectively; $P < 0.001$). Insulin resistance was significantly higher in *H pylori* positive group than in *H pylori* negative group (6.92 ± 3.86 and 3.61 ± 1.67 , respectively; $P < 0.001$). Insulin resistance was found to be significantly correlated with total antioxidant capacity ($r = -0.251$, $P < 0.05$), total oxidant status ($r = 0.365$, $P < 0.05$), and oxidative stress index ($r = 0.267$, $P < 0.05$).

CONCLUSION: Insulin resistance seems to be associated with increased oxidative stress in *H pylori* infection. Further studies are needed to clarify the mechanisms underlying this association and elucidate the effect

INTRODUCTION

H pylori is a noninvasive, microaerophile, nonspore-forming, and spiral-shaped microorganism. *H pylori* is associated with severe gastric pathologies, including chronic active gastritis, peptic ulcer, gastric adenocarcinoma and type B low-grade mucosa-associated lymphoid tissue lymphoma^[1].

H pylori infection causes inflammation, accumulation of reactive oxygen species (ROS), and oxidative DNA damage in gastric mucosa^[1]. *H pylori* induces infiltration and activation of neutrophils and macrophages^[2]. One characteristic event in inflammation is the infiltration of the affected tissue by neutrophils, which produce large amounts of ROS in host defence reactions. Enhanced ROS levels due to neutrophil infiltration and increased oxidative DNA damage have been reported in *H pylori*-infected patients^[3-6]. The increased level of pro-oxidative factors and decreased level of antioxidants in severe oxidative stress can modulate many processes in gastric epithelium^[2].

Although *H pylori* seems to be a cause for gastric focal inflammation, it can invade and colonize human stomach, and directly interact with gastric epithelial cells. Moreover, it is associated with non-gastrointestinal tract conditions such as atherosclerosis, insulin resistance, diabetes mellitus and some autoimmune diseases^[1,7-9]. The association of *H pylori* infection with insulin resistance has been reported^[10-12]. However, to our knowledge, the association between insulin resistance and oxidative status has not been previously investigated in *H pylori* infection.

The present study was, therefore, to determine the insulin resistance, systemic parameters of oxidative stress

and antioxidative system in *H pylori* infection and to find out if there is any relationship between oxidative status and insulin resistance in *H pylori* infection.

MATERIALS AND METHODS

Subjects

One hundred and three patients who underwent upper gastrointestinal endoscopy for evaluation of dyspeptic complaints and diagnosed as non ulcer dyspepsia were included in the present study. The patients were divided into two groups according to the presence of *H pylori* infection. Fifty-five patients were *H pylori* positive and 48 patients were *H pylori* negative. The study protocol was carried out in accordance with the Helsinki Declaration revised in 1989. All subjects were informed about the study protocol and written consents were obtained from all participants.

Diagnosis of *H pylori* infection

During upper gastrointestinal endoscopy, 2 antral biopsy samples were taken for rapid urease test (CLO test) and histopathologic examination. *H pylori* was considered to be present when the rapid urease test and histological examination were positive. Biopsy samples were stained with hematoxylin and eosin for histopathological examination and evaluated according to the updated Sydney System^[13]. The patient was considered to be *H pylori* negative if both rapid urease test and histological examination were negative. The diagnosis of *H pylori* infection was confirmed if both the urease test and histological examination were positive.

Exclusion criteria

Exclusion criteria included recent gastrointestinal by-pass surgery, pregnancy, usage of supplemental vitamins several months prior to the study, *H pylori* eradication therapy, H₂ receptor antagonist or proton pump inhibitor within the last 4 wk or nonsteroidal antiinflammatory drugs (NSAIDs) within the last 2 wk prior to study, existence of diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, cerebrovascular disease, rheumatoid arthritis, renal disease, smoking, cancer, systemic or local infection.

Samples

Blood samples were obtained following an overnight fasting. Samples were withdrawn from a cubital vein into blood tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 r/min for 10 min.

Laboratory methods

Serum glucose concentration was measured using commercial kits (Abbott®) in an autoanalyser (Aeroset®, Germany). Serum insulin levels were measured using an automated chemiluminescence autoanalyzer (Roche®).

Measurement of serum total antioxidant capacity

Total antioxidant capacity (TAC) of serum was determined

using a novel automated measurement method as previously described^[14]. In brief, hydroxyl radical which is the most potent biological radical was produced. In the assay, ferrous ion solution which is present in the reagent 1 was mixed with hydrogen peroxide which is present in reagent 2. The sequential-produced radicals, such as brown-colored dianisidiny radical cation produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample on the potent free radical reactions initiated by the produced hydroxyl radical, was determined. The assay achieved excellent precision values lower than 3%. The results were expressed as mmol Trolox equivalent/L.

Measurement of total oxidant status

Total oxidant status (TOS) of serum was determined using a novel automated measurement method as previously described^[15]. Oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂ equivalent/L).

Determination of oxidative stress index

The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following formula^[16]: OSI (arbitrary unit) = TOS (μmol H₂O₂ equivalent/L)/TAC (mmol Trolox equivalent/L).

Insulin resistance

The insulin resistance index was calculated on the basis of fasting values for glycaemia and insulinemia, according to the homeostasis model assessment (HOMA)^[17]: insulin resistance (HOMA-IR) = fasting insulinaemia (μU/mL) × fasting glycaemia (mmol/L)/22.5.

Statistical analysis

All data were expressed as mean ± SD. Comparisons of parameters were performed with Student's *t* test and correlation analyses were performed using Pearson correlation test. *P* < 0.05 was considered statistically significant. Statistical analyses were performed by SPSS 11 statistical package.

RESULTS

Demographic characteristic of the subjects are shown in Table 1. There were no statistically significant differences between the two groups with regard to age, gender, and body mass index (BMI) and glucose level (*P* > 0.05) (Table 1).

Insulin and insulin resistance levels were significantly

Table 1 Demographic and clinical data of *H pylori* positive and negative groups (mean \pm SD)

Parameters	<i>H pylori</i> negative (n = 48)	<i>H pylori</i> positive (n = 55)	P
Age (yr)	35 \pm 15	37 \pm 12	NS
Sex (Female/Male)	28/20	29/26	NS
Body mass index (kg/m ²)	22.5 \pm 3.1	23.5 \pm 1.6	NS
Glucose (mg/dL)	98.15 \pm 14.97	97.71 \pm 14.39	NS
Insulin (μ U/mL)	3.61 \pm 1.67	6.92 \pm 3.86	< 0.001
HOMA-IR	0.89 \pm 0.47	1.67 \pm 0.99	< 0.001

IR: Insulin resistance; NS: Not significant.

higher in *H pylori* positive group than in *H pylori* negative group ($P < 0.001$) (Table 1).

TAC level was significantly lower in the patients *H pylori* positive group than in *H pylori* negative group ($P < 0.001$), while TOS level and OSI value were significantly higher in *H pylori* positive group than in *H pylori* negative group ($P < 0.001$) (Table 2).

In Pearson correlation analysis, IR was found to be significantly correlated with TAC ($r = -0.251$, $P < 0.05$), TOS ($r = 0.365$, $P < 0.05$), and OSI ($r = 0.267$, $P < 0.05$).

DISCUSSION

Information about the association of insulin resistance with *H pylori* infection is scarcely available^[10,11]. Aydemir *et al*^[10] reported that insulin resistance is significantly related with *H pylori* infection. However, Park *et al*^[11] reported that no improvement in the metabolic parameters including insulin resistance could be observed following eradication of *H pylori*. In term of increased insulin resistance during *H pylori* infection, our results are in consistent with those of Aydemir *et al*^[10]. As we did not investigate the effect of *H pylori* eradication on insulin resistance, we were not able to compare our results with those of Park *et al*^[11].

To our knowledge, the association of oxidative stress with insulin resistance in *H pylori* infection has not been investigated previously. We found a significant association between increased oxidative stress and insulin resistance in *H pylori* infection. It has been reported that *H pylori* infection is associated with increased tissue and systemic oxidative stress^[18]. Moreover, oxidative stress has been proposed as the root cause for the development of insulin resistance, B-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus^[19]. In addition, various antioxidants such as vitamin E, alpha-lipoic acid, and N-acetylcysteine have been shown to have improving impact on insulin resistance^[20-22]. Thus, the association of insulin resistance with *H pylori* infection observed in our study seems to be due to oxidative stress induced by *H pylori*.

Many studies indicate that there are evident alterations in gastrointestinal hormone levels in *H pylori* infection^[10,23-27]. *H pylori* infection has been found to decrease the expression of antral somatostatin and to increase the release of acid-stimulating hormone

Table 2 Oxidative and antioxidative parameters in *H pylori* positive and negative groups (mean \pm SD)

	<i>H pylori</i> negative (n = 48)	<i>H pylori</i> positive (n = 55)	P
TAC (mmol Trolox eq./L)	1.70 \pm 0.50	1.36 \pm 0.33	< 0.001
TOS (μ mol H ₂ O ₂ equiv./L)	5.08 \pm 0.95	6.79 \pm 3.40	< 0.001
OSI (arbitrary unit)	3.10 \pm 0.92	5.42 \pm 3.40	< 0.001

TAC: Total antioxidant capacity; TOS: Total oxidant status; OSI: Oxidative stress index.

gastrin^[23]. Gastrin can inhibit glucose absorption in the small intestine^[24] and amplify glucose-stimulated insulin release^[25]. A link between *H pylori* infection, serum gastrin, insulin and serum glucose concentrations has been demonstrated in dyspeptic patients^[26]. During oral glucose ingestion, gastrin probably contributes very little to the insulin release. Gastrin may significantly stimulate the insulin secretion after protein-rich meals. Ordinary meal could stimulate immediate release of endogenous gastrin. The rise in serum gastrin is acute, preceding the increase in insulin concentrations^[25]. Somatostatin regulates pancreatic insulin secretion and has an inhibitory effect on insulin release^[10,26,27]. Decreased somatostatin and increased gastrin hormone levels in patients with *H pylori* infection may play a role in the development of insulin resistance. However, in the present study, since we did not investigate gut hormones except for insulin, we could not provide any information related to this topic.

In conclusion, our findings suggest that insulin resistance seems to be associated with increased oxidative stress in *H pylori* infection. Further studies are needed to clarify the mechanisms underlying this association and elucidate the effect of adding antioxidant vitamins to *H pylori* eradication therapy on insulin resistance during *H pylori* infection.

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