Study of Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) of gastric mucosa in diabetics infected with *Helicobacter Pylori*

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**ABSTRACT**

**Introduction and objectives:** *Helicobacter Pylori* Infection and diabetes mellitus are considered the major concerns and priorities of public health in modern and developed communities. The prevalence of *H. Pylori* infection has been reported increasing in diabetic patients. The aim of this study was to investigate peroxisome proliferator-activated receptor gamma (PPAR-GAMA), oxidative stress and nitrosative of stomach tissue in diabetics infected with *H. Pylori* in Tabriz city.

**Materials and Methods:** In this study, three gastric antrum biopsy specimens, one gastric juice biopsy specimen and a total blood sample in vials containing the anticoagulant were taken from 60 diabetic patients infected with *H. Pylori*. As well, 60 non-diabetic patients uninfected with *H. Pylori* were sampled as a control group. One gastric biopsy specimen of the three was sent to a pathology laboratory for urease examination and another for pathological evaluation. The third biopsy was tested to measure total antioxidant capacity through total antioxidant assay kit made in England, and the PPAR-γ was measured by ELISA Kit (ChemiLuminescence) manufactured by Signosis. Hemoglobin A1C (HbA1c) was assessed using the ion exchange chromatography method by commercial kit (Biosystem, Spain), and gastric juice of patients was used to measure the NO radical with Grace Colorimetric method.

**Results:** The mean age of the study group was 39/52±12/95 and of the control group was 34/87±15/33 years, who were not statistically significantly different (p=0.15). A significant increase in PPAR-γ receiver mean in gastric mucosa cell nucleus was observed in the case group in comparison with that of the control group (p=0.007). The mean of nitric oxide (NO) in gastric juice in the control group was significantly different from that of the control group, being much lower than in the control group (p=0.0001). The percentage of glycated hemoglobin was significantly higher in the group than in the control group and showed a significant increase as the p-value was less than 0.0001. Gastric tissue total antioxidant capacity in the case group was significant from that of the control group, and the decreased level revealed that the p-value equaled to 0.012.

**Conclusions:** The results showed that firstly, *H. Pylori* infection in diabetic people controls hyperglycemia very badly, and may cause the problems of blood glucose increase more complex in these patients. Secondly, the increase in oxidative stress and nitrosative, and also the increased level of nuclear PPAR-γ receptors in stomach tissue increase the risk of cancer in stomach tissue in diabetic patients infected with *H. Pylori*.

**KEY WORDS:** *Helicobacter Pylori*, diabetes mellitus, Peroxisome proliferator-activated receptor gamma (PPAR-GAMA), Oxidative and Nitrosative stresses

**INTRODUCTION**

*Helicobacter Pylori* is a gram-negative bacteria, single-pole, multi flagella, Micro-aerophilic curved bacillus that causes acute and chronic gastritis, duodenal ulcer and gastric cancer in humans. *H. Pylori* infection is considered a major cause of peptic ulcers and other gastrointestinal disorders (1). Gastritis induced by *H. Pylori* infection has relatively high prevalence and about 10 percent of people in their lifetime suffer from gastritis diseases. Its prevalence is slightly higher in men than in women (2). There are several hypotheses about the causes of duodenal ulcer (3). Infection with *H. Pylori* and diabetes mellitus are addressed main concerns and priorities of public health in modern and developed communities, both of which trigger the risk of cardiovascular diseases(4), cancer (5) and may increase metabolic abnormalities(6). In recent years, diabetes mellitus has been mentioned as the root cause of disorders and abnormalities in gastrointestinal tract activities (1). Since *H. Pylori* may also bring about many cases of dyspepsia, the high prevalence of *H. Pylori* has been
reported in diabetic patients (2). Delay in gastric emptying and decrease in gastric antral part function are considered the serious problems of dyspepsia in patients with diabetes. The role of H. Pylori infection in diabetic dyspepsia is originally assigned to hyperglycemia. Hyperglycemia may stimulate H. Pylori infection or reactivate a mild infection -without obvious symptom-, and give rise to dyspepsia in diabetes. Infection with H. Pylori in diabetic patients which has not been controlled is the common infection and H. Pylori are colonized in the gastric antrum of the patients (3).

However, several studies have investigated the prevalence of H. Pylori in diabetic patients and possible role of H. Pylori in metabolic control (3-1). Some have rejected the association between H. Pylori infection and diabetes, and did not support the relationship between H. Pylori infection and metabolic control (7). While other studies have proved H. Pylori infection in patients with diabetes and glycemic control in patients with type-I diabetes in comparison with non-diabetic patients. Furthermore, a relationship was revealed between H. Pylori, insulin and serum glucose level (8-11).

According to the conducted research, it turned out that the glycemic control may be useful in diabetic patients for a while to eradicate H. Pylori. This has been realized when glycosylated hemoglobin level reduced to about the same level of the group who have never been infected with H. Pylori (11). In other words, individuals suffering diabetes and infected with H. Pylori have higher level of glycosylated hemoglobin in comparison with the uninfected individuals with H. Pylori, and eradicating infection may reduce glycosylated hemoglobin level in them. This may rectify NO° secretion and production disorders. On the other hand, it is actively involved in withdrawal of symptoms caused by oxidative stress which are produced from metabolites of reaction between radical superoxide and nitric oxide.

Radical nitric oxide is a multifunctional gas that binds firmly with proteins containing copper and iron (12-13). This radical produced from different cells, such as vein endothelial cells, neurons, neutrophils and macrophages. Additionally, all necessary isoymes exist in gastric mucosa for NO° synthesis (14-15). Furthermore, NO° in gastric juice appears through non-enzymatic reduction of nitrite produced by saliva and food. So, NO° physiological concentrations in gastric juice are relatively high (° 5μM) (11). It has been reported that H. Pylori produces a large amount of superoxide radical (O°−) which reacts with gastric juice NO°. This reaction may take part in the mechanism which enables H. Pylori to resist gastric juice NO° and actually to change bactericial function of gastric juice NO°. Through this way, the possibility is provided for the growth and colonization of this bacterium in stomach antrum area (14-17). It is necessary to note that super oxide radical is produced during the transformation of H. Pylori from bacillus form to coccoid form. As a consequent of the reaction between O°− and NO°, the two O°− metabolites and proxy nitrite are produced which are very toxic to different organisms, and render the oxidative stress reactions to related tissue (18-19).

Peroxisome proliferator-activated receptor gammas (PPARs) are members of the nuclear receptor super family that act as ligand-dependent transcription factors. These receptors are activated by ligand and induce the expression of certain genes. Three subtypes of PPAR receptors called α, δ and γ have been identified. They have been shown to control lipid followed by the gene expression activation involved in metabolism (20). PPARγ is mainly found in adipose tissue, two isoforms of which has been recognized. PPARγ1 is expressed in many tissues, like white and brown adipose tissue, skeletal muscles, liver, pancreatic β-cells, macrophages, colon, bone and placenta. While PPARγ2 expression is limited to fatty brown and white tissues under certain circumstances. The recent receptors are involved in lipid poisoning and resistance to insulin, and have been observed in liver and adipose tissue, skeletal muscle (21-22). It is known that the activation of these receptors leads to healing and a sense of relief in many tissue disorders. However, they are also activated by fatty acids, prostanoids and thiazolidinediones which are introduced a series of anti-diabetic factors in recent years (23, 25).

On the basis of the findings presented in recent years, it can be inferred that the PPAR-γ level and its increase could have anti-diabetic properties. Therefore, the aim of this study was to study the peroxisome proliferator-activated receptor gamma (PPAR-γ) level of stomach tissue in diabetic patients infected with H. Pylori in Tabriz City to obtain new information in relation with the receptor in diabetic patients infected with H. Pylori in this city and offer suggestions about controlling the disease.

MATERIALS AND METHODS

Diabetic patients referred to the endocrinology and metabolism clinic, Sina hospital, Tabriz University of Medical Sciences, who have been reported with gastrointestinal symptoms, were identified by an endocrinologist, subspecialty, and were referred to undergo endoscopy to the Endoscopy section of Imam Reza educational, healthcare and research center. In present study, the diabetic patients were separately examined in the two groups, but in the format of one group: patients with type I diabetes and patients with type-II diabetes. And each group was matched with their control groups, in terms of age, gender, history of disease, income level and etc., so that the present study can be generalized to all the diabetic people. Being referred to hospital, endoscopy was performed by gastroenterologist on the patients, and of each patient three biopsy specimens were taken from the gastric antrum area, 2 cm away from the pylorus; one biopsy specimen, from gastric juice; and
one blood sample in vials containing the anticoagulant. Non-diabetic patients and uninfected with *H. Pylori*, which referred to the above-mentioned endoscopy section with dyspepsia symptoms, were sampled and tested as the control group as well. A biopsy of the three biopsy specimens was sent to the pathology laboratory in order to rapid urease test, and another one, to examine pathologically the presence of chronic active gastritis and the presence or absence of *H. Pylori*. Infection with *H. Pylori* was considered positive, provided that all the three tests (urease, the presence of bacteria in pathological samples, and the presence of chronic active gastritis) were positive. According to the references available, considering that the use of biopsy for definitive diagnosis of dyspepsia and *H. Pylori* infection (i.e. urease rapid test, the presence of chronic active gastritis and bacteria in the gastric antrum) is regarded as a gold standard technique, so this method was used. On the other hand, the cases which might be interfering in our study were also detected and removed from the study. Additionally, due to the existing references, there might be no immune response in diabetic patients, therefore serological methods were found not be used for detection, and also, the individuals might have antibodies against *H. Pylori*, but without dyspeptic symptoms or vice versa. Such subjects were excluded from the study. As mentioned before, the infected with *H. Pylori* case group were selected if only their three test results showed to be positive (urease test + chronic active gastritis + the presence of bacteria). This point should also be made that these processes were performed only on patients in group 1 (case group). While only the rapid urease test was performed on the other patients-the one that is normally done in endoscopic procedures and doesn't incur a high cost as serologic procedures do.

The third biopsies were utilized to measure the total antioxidant capacity and PPAR-γ. The total blood sample was used for HbA1C test, and gastric juice of the subjects for NO radical level. PPAR-γ was measured through ELISA Kit (Chemiluminescence) manufactured by Signosis with TE-0010 product number. The total antioxidant capacity of the stomach tissue was assessed by Total Antioxidant Kit RONDOX England (RANSOD). The percentage of glycosylated hemoglobin A1c was calculated using the ion exchange chromatography method by commercial kit (Biosystem, Spain), and gastric juice levels of nitric oxide was measured using grace colorimetric method.

Data obtained were statistically analyzed by using SPSS (22nd version) and statistical Independent Sample t-Test. Differences were considered significant when P<0.05. The data were presented as mean ±standard deviation.

RESULTS

Comparison of age mean in the three groups:
A total of 120 patients participated in this study: 60 subjects were infected with *H. Pylori* and diabetics (case group); 60 non-infected with *H. Pylori* and non-diabetic group (control group). The case group contained 27 male participants (45%) and 33 female participants (55%). The control group included 28 male subjects (46.6%) and 32 females (53.4%). Among the case group participants, the minimum age was 17 years, the maximum age was 59 years and the average age was 39.52±12.95. Among the control group, 15 years was the minimum age, the maximum age was 65 years, and the average one was 34.87±15.33. Using t-test showed that the mean age of control group was not statistically significant from that of the case groups (p=0.15). In other words, the study group and control group have been matched well:

<table>
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<th>Age (Year)</th>
<th>n</th>
<th>Mean ± SD</th>
<th>t</th>
<th>P value</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Control Group</td>
<td>60</td>
<td>34.87±15.33</td>
<td>-1.795</td>
<td>0.15</td>
<td>-9.78 to 0.48</td>
</tr>
<tr>
<td>Case Group</td>
<td>60</td>
<td>39.52±12.95</td>
<td></td>
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</tbody>
</table>

Comparison of Stomach tissue PPAR-γ average levels in the two groups:
As can be seen in Figure-1, using analysis of bilateral variance, a significant difference was observed in PPAR-γ receptor mean of nucleus of gastric mucosa in the two groups. PPAR-γ receptor level in the case group compared with that of the control group showed a statistically significant increase. The results have been expressed as mean±SD values (p = 0.007).
Comparison of gastric juice nitric oxide average level in the two groups:
As it can be observed in Figure-2, the average of gastric juice nitric acid in the control group was statistically significant from that of the case groups, much lower than in the control group. The results have been expressed as mean±SD values ($p = 0.0001$).

Comparison of glycated hemoglobin percentage in the two groups:
According to the Figure-3, the percentage of glycated hemoglobin in diabetic patients infected with *H. Pylori* (the case group) differed significantly from that of the control group (non-diabetics and uninfected with *H. Pylori*), showing a high rise. The results are displayed mean±SD values: the mean glycated hemoglobin percentages were 10.35±0.56 percentage and 3.32±0.27 percentage in the case and control groups, respectively, and the p-value was less than 0.0001.
Conclusion: *H. Pylori* infection and diabetes mellitus raised issues of concern about public health for modern and advanced communities to be taken into consideration, both of which increase the risk of cardiovascular disease(4), cancer (5)and metabolic abnormalities(6). Recently, diabetes mellitus has been expressed as the main cause of abnormalities in gastrointestinal tract activities (1). Since *H. Pylori* trigger many cases of dyspepsia, an increased prevalence of *H. Pylori* infection has been reported in diabetic patients (1-3).
In this study, the attempts were made to determine some part of the mechanisms and interactions between host cells and Helicobacter pylori in people with diabetes, so that a small step to be taken, no matter how small or big it is, towards clarifying how to deal with microbial contamination be taken of the globe. Several studies published in recent years have shown that H. Pylori strains isolated from gastric mucosa contain phenotypic and genotypic changes that may cause a variety of inflammatory reactions, and thus impact on clinical outcomes (26). The results of further research have identified the mechanisms and virulence factors of H. Pylori, and clinical and laboratory findings are inconsistent on this issue, requiring further research.

In recent study, it has been demonstrated that firstly, H. Pylori in diabetic patients reduces gastric juice nitric oxide level, which is one of the most important components of the host innate immunity and includes a strong bactericidal activity against aerobic bacteria. Secondly, it decreases total antioxidant capacity of stomach mucous tissue resulting from an increase in free radicals, such as superoxide, proxy nitrite and so on. The latter shows the presence of oxidative stress in this tissue. Thirdly, it increases the PPAR-γ at the nucleus of gastric mucosa tissue cells which may serve as a marker for increasing risk of gastric cancer in these patients. And fourthly, it may disrupt hyperglycemia control in these patients which was determined by a high percentage of glycated hemoglobin.

The nitric oxide synthesized by nitric oxide synthase is a key molecule affecting on innate immune response against different pathogens. A significant finding determined in Gobert's et al study (2001) (27) was that H. Pylori is an extracellular pathogens, and effectively killed by released nitric oxide form activated macrophages. They also showed arginase enzyme of this bacteria compete with nitric oxide synthase for their common substrate that is L-Arginine, as a result, prevent nitric oxide production by host cells. Arginase enzyme is active only in wild strains of H. Pylori and inactive in other strains of this enzyme. And it is the wild strains which render gastrointestinal diseases. This theory is independent of the effects of nitric oxide synthase translation increase by H. Pylori in culture mediums which has been expressed by some researchers. And in fact, the results of this study confirm Gobert and his colleagues' findings, in which they described a unique way of escaping from host natural defenses, by H. Pylori (27).

It is noteworthy that production of nitric oxide from macrophage by wild strains of H. Pylori is only inhibited at L-argnine physiological concentrations (28).And it is evident that in the culture medium without this condition, the bacteria strains may show opposite features, and may act as shown in some studies, in Watanabe et al(2000) and others' studies for instances (29-31), that H. Pylori induces an increase in nitric oxide synthase enzyme activity in culture medium. In fact, the finding proved the role of nitric oxide synthase in NO\textsuperscript{+} production on H. Pylori infection and in condition outside of the body. For this reason, some researchers have repeatedly have presented that in human gastritis caused by H. Pylori infection, nitric oxide synthase appears to be regulated or inhibited, in other words (32). H. Pylori are located in close proximity to gastric mucosal epithelial cells, right at the advent of nitric oxide synthase enzyme in lamina layers. As a result, it is expected that H. Pylori arginase may directly inhibit the production of NO\textsuperscript{+} level through nitric oxide synthase, and in this place, the bacteria can easily react with nitric oxide synthase enzyme-containing cells (32). Furthermore, in the study conducted by Gobert et al (2002) (33), it has been stated that arginase enzyme II is stimulated and activated in macrophages which are activated by H. Pylori stimuliates. Accordingly, an increased activity of this enzyme may inhibit the nitric oxide synthase, reducing nitric oxide synthesis in the gastric juice on the one hand. On the other hand, as a result of produced products, such as polyamines, spermidine and spermine produced from L-Ornithine metabolized by the ornithine decarboxylase enzyme, it may cause apoptosis in gastric mucosa macrophages (33).

We have also demonstrated that PPAR-γ, nuclear level shows a significant increase in diabetic patients infected with H. Pylori. In recently done studies, it has been found that PPAR-γ is expressed in humans in stomach cancer as well as in colorectal cancer. The activation of PPAR-γ promotes apoptosis and improves the growth of epithelial cells of the gastric tissue. H. Pylori known as the main cause for gastric increases the volume of gastric epithelial cells, and infection with this organism for a long time is associated with an increased risk of gastric cancer. The study by Sun et al (2007) (34) has suggested that as a result of infection with H. Pylori, the gene expression level and subsequently, mRNA level of PPAR-γ receptor in gastric tissue shows a significant increase, but it did not specify whether the increased expression of the gene, may cause an increase in the receptor. So in this study, we determined that H. Pylori infection in people with diabetes can enhance the PPAR-γ receptor protein which can effectively be gone up by an increased gene expression.

However, in this study we have revealed that the amount of glycosylated hemoglobin in diabetic patients infected with H. Pylori are significantly higher than that of diabetics' non-infected volunteers with H. Pylori. That is, controlling hyperglycemia in individuals infected with H. Pylori is hardly done. And to improve hyperglycemia control in diabetics infected with H. Pylori, the bacteria should be eradicated as soon as possible; otherwise, long-term or short-term effects of an increase in blood glucose would be irreparable in these patients. In a study, Candelli et al(2003) (8) as well in another one, Dolatkah et al(2011) (1) have reported that diabetic and H. Pylori -infected individuals showed to have higher levels of HbA1C than non-infected volunteers. In these studies, it has also been reported that eradication of H. Pylori infection may lower HbA1C levels in these patients, the results of which correspond to the results of the recent study.
Although several studies have been conducted on *H. Pylori* infection and diabetes mellitus done, the results are controversial. In a comprehensive study by Xia et al (7) performed in 2001, the prevalence of *H. Pylori* infection was not statistically significantly different between diabetes patients and non-diabetic participants, the finding of which was inconsistent with our results. However, Sargyn et al study (2003) (9)indicated that *H. pylori* eradication results may vary in diabetics with type-II and non-diabetic controls, carrying out the study based on determining eradication level of Helicobacter pylori infection in patients with type-II diabetes, which can be consistent with the results of this study.

**Conclusion:** The results suggest that first, *H. Pylori* infection causes uncontrolled or poorly controlled hyperglycemia in diabetic patients, and may make the problems of increasing blood glucose in the patients more complex. Second, an increase in oxidative stress and nitrosative as well as an increase in PPAR-γ nuclear receptor of stomach tissue may trigger stomach tissue cancer in diabetic patients infected with *H. Pylori*.

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