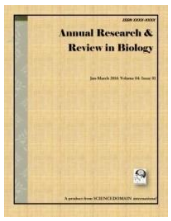

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Association of *Helicobacter pylori* IgG Antibody with Microvascular Complications in Type II Diabetic Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author JV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MB managed the analyses of the study and the literature searches. Author VD designed the study and managed the literature searches. Author MP wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to investigate the association between HP infection and microvascular complications of type two diabetes mellitus (T2DM).
Study Design: Cross-sectional study.
Place and Duration of Study: Shahid Beheshti Hospital of Qom, Iran, between March 2012 and March 2013.
Methodology: In this cross-sectional study 211 T2DM patients have been examined. Subjects were divided into two groups (HP+ and HP-) based on HP infection (diagnosed with IgG serology), and the association between these groups and microvascular complications of T2DM including

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nephropathy (based on protein excretion in 24-hour urine collection), retinopathy (based on examination by an ophthalmologist) and neuropathy (diapason and monofilament examination) has been evaluated.
Results: Of the 211 subjects studied, 125 (59.24%) were HP+. The mean diabetes duration was not significantly different in both groups. A significant association was found between HP infection and diabetic neuropathy (p=0.04), but there was no correlation between HP infection and diabetic nephropathy and retinopathy (p=0.2 and p=0.43, respectively). Conclusion: Infection with *H. pylori* increases the risk of diabetic neuropathy and is considered as a possible risk factor diabetic neuropathy.

Keywords: Diabetes mellitus; diabetes complications; Helicobacter pylori.

1. INTRODUCTION

Many organs are affected by the chronic complications of type two diabetes mellitus (T2DM) and it causes the majority of morbidity and mortality associated with it [1]. T2DM is the leading cause of blindness between the ages of 20 and 74 in the United States. This problem is highlighted by the finding that diabetic patients are 25 times more likely to become legally blind than non-diabetics. Moreover, diabetic nephropathy is one of the leading causes of chronic kidney failure [2]. Also, it is the most common complication of diabetes and many studies have shown that more than half of the diabetic patients develop diabetic nephropathy, and as much as 30% of those manifestations are painful [3].

Although chronic hyperglycemia is an important etiologic factor for diabetic complications, the mechanisms by which it leads to such diverse cellular and organ dysfunctions is unknown. Inflammation plays an essential role in the progression of diabetic microvascular complications. Proinflammatory cytokines, C-Reactive Protein (CRP), tumor necrosis factor (TNF)-α, and interleukin (IL)-6 increase in T2DM [4,5].

Recent studies have emphasized the importance of targeting oxidative stress and inflammation in the treatment of diabetic nephropathy [3,6]. Ross has proposed that dysfunction of the vascular endothelium and chronic systemic low-grade inflammations are the key features in the pathophysiology of atherothrombosis and microalbuminuria [7]. In addition, patients with chronic complications of diabetes, progress the stages more rapidly in the presence of inflammatory markers. For example in Nath et al.'s study, diabetic nephropathy patients with higher CRP and lower albumin levels were less likely to progress to hemodialysis [8].

Chronic infections may cause the inflammatory condition in diabetic patients. Various infectious diseases such as *Helicobacter pylori* (HP) infection may be listed among the etiologic factors related with this vascular endothelial damage and consequently developing atherosclerosis [9-11]. HP infection is the most common infection, particularly in the developing countries. Its prevalence in diabetic patients is higher than non-diabetic patients and is associated with diabetes duration [12-14]. In addition, it has been shown that HP infection increases inflammatory factors like IL-8 and TNF-α in diabetic patients [15]. It is also associated with many extra gastrointestinal manifestations like hematological diseases such as idiopathic thrombocytopenic purpura (ITP) and unexplained iron deficiency anemia; neurological disorders like stroke, Parkinson and Alzheimer's disease; obesity and skin disorders. High quality studies show the improvement of iron deficiency anemia and ITP after HP eradication [16-18].

The association between HP infection and vascular events such as atherosclerosis was confirmed in Vafaeimanesh et al.'s study [19]. It has been demonstrated that persistent systemic inflammatory response related with HP increases the vascular injury in diabetic patients and predisposes them to pulmonary, cardiovascular and cerebral diseases [20-22]. We performed a cross-sectional case control study to investigate a possible association between microvascular complication of T2DM and HP infection diabetic patients.

2. METHODOLOGY

2.1 Patients

In this cross-sectional study, the subjects were selected from patients attending the outpatient department during March 2012 to 2013 in Shahid Beheshti Hospital of Qom, Iran. A population of

211 diabetic outpatients was analyzed through a review of clinical records and personal interview. Patients with T2DM diagnosed according to the report of the Expert Committee for the Diagnosis and Classification of T2DM were included in the study [23].

Inclusion criteria were the following parameters: age of diabetes onset >35 years, serum creatinine level <1.5 mg/dL, serum triglyceride level <400 mg/dL, negative urine culture, and the absence of the other exclusion criteria. The subjects were divided into two groups according to HP infection as Group 1 (HP+) and Group 2 (HP-).

2.2 Ethics

All individuals signed informed consent prior to their enrolment in the study. Also, the study was planned according to the ethical guidelines following the Declaration of Helsinki and Ethics Committee of Qom University of Medical Sciences approved it.

2.3 Exclusion Criteria

Those previously diagnosed to have HP infection or had undergone or were currently undergoing HP eradication during one year ago, those receiving anti-ulcer treatment in the last three months and still receiving proton-pump inhibitors (PPI) or H2 receptor blockers, those who had vascular or inflammatory disease or had to continue antibiotic treatment for various reasons, and those suspected for or diagnosed as rheumatoid or immunological disease, diabetic patients with periodontal disease diagnosed by a dentist and requiring intensive treatment and those with poor oral hygiene, smokers, those not providing consent for the study, and those with poor socioeconomic level unable to return for follow-ups regularly were excluded from the study.

2.4 Parameters and Measurement Methods Used throughout the Study

Demographic and clinical parameters of the study were age (years), gender (male or female), anti-diabetic treatment, body mass index (BMI, kg/m2), duration of T2DM (years) and systolic and diastolic blood pressure (mmHg). Laboratory parameters were total cholesterol (mg/dL) measured by Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran, with intra- and inter-assay coefficients of variation (CV) of 0.95 and 1.09, blood glucose (mg/dL) measured by Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran, with Intra and intra-assay CV of 1.50 and 0.90, triglyceride (mg/dL) measured by Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran, with Intra and intra-assay CV of 1.60 and 1.23, high-density lipoprotein-cholesterol (HDL-C, mg/dL) measured by Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran, with Intra and intra-assay CV of 0.78 and 1.8, low-density lipoprotein-cholesterol (LDL-C, mg/dL) was determined by Friedwald formula: LDL-cholesterol=TC-(HDL+TG/5) and glycated hemoglobin (HbA1c, %) was measured by Chromatography. The presence of HP infection was also assessed. GFR was calculated based on Cockcroft-Gault equation.

2.5 Outcome Data and Assays

Body mass index was calculated by using the Quetelet index ($weight_{(kg)}/height_{(m^2)}$) [24]. Plasma glucose (hexokinase method), total cholesterol (enzymatic method), triglyceride (enzymatic method without glycerol blocking), and HDL-C (dextran sulfate- MgCl2 precipitation) were measured on an automated analyzer using reagent kits supplied by the manufacturer of the analyzer. LDL-C was calculated by the Friedewald equation [24]. HbA1c was measured by an automated ion-exchange chromatographic method, and the reference range was 5.1-6.4%. The micro-vascular complications included recording of retinopathy (ophthalmology visit), nephropathy, (24 hour urine analysis), and neuropathy (monofilament and the diapason therapeutic examination). Peripheral neuropathy was assessed by determining vibration sensation and pressure sensation (monofilament). Vibration testing was conducted with a 128-Hz tuning fork applied to the dorsum of the interphalangeal joint of the right hallux. The test conducted twice on each great toe. The vibrating tuning fork was put on the interphalangeal joint and when nothing was felt, the score was 2 points. When something was felt, the still vibrating tuning fork was immediately placed at the dorsal wrist. When it was felt the same at that location the score was 0 points, when it felt stronger the score was 1 point [25].

For pressure sensation, a 5.07(10-g) monofilament was placed at a right angle to the skin on the plantar surface of the foot; pressure was then increased until the filament buckled. Four sites (1st, 3rd and 5th metatarsal heads and plantar surface of distal hallux) were tested on

each foot. Areas of callus, ulcer, scar and necrotic tissue were avoided in testing. The patient was asked if he or she felt the pressure. Loss of the ability to detect this pressure at one or more sites on the plantar surface of the foot was considered as neuropathy [26]. Those patients without these complications were considered as HP- without microvascular complications.

Serum HP-specific IgG antibody titer (titer >30 AU/mL was interpreted as positive according to the manufacturer's instructions) was measured as follows: blood samples were drawn after an overnight fast and were centrifuged within 15 minutes of drawing. The levels were measured by the enzyme linked immunosorbent assay (ELISA) method by means of a standard kit made by Padtan Elm Co, Iran.

2.6 Statistical Evaluation

Basic statistical description of the study population adopted percentage proportions for categorical parameters while the continuous variables were expressed as mean and 95% confidence interval (CI). Significant difference among groups was tested with one-way ANOVA and Bonferroni post hoc test for continuous variables and λ^2 test for categorical variables. Predictors in logistic regression were described by their odds ratio and CI. Lemeshow test was used for statistical significance of the whole logistic models. The analyze was performed using SPSS version 16.0, and a p value less than 0.05 was considered as being statistically significant.

3. RESULTS AND DISCUSSION

Totally, 211 patients were analyzed and 125 patients (59.24%) were HP+ and 86 patients (40.56%) were HP-. The mean HP IgG titer in HP+ and HP- patients were 39.58±4.21 and 20.16±3.54 U/mL, respectively.

As shown in Table 1, there was no statistically difference in blood glucose, HbA1c, BMI and T2DM duration between both groups. Also, cholesterol and TG level were not different in both groups but HDL-cholesterol level had a statistically significant difference in both groups and was lower in HP+ group (60.6±2.43 vs. 68.75±3.03 mg/dL).

In this study 86 patients had diabetic nephropathy so that in HP+ group, 44%(55 ones) and in HP- group, 36%(31 ones) had diabetic nephropathy which was not statistically difference between both groups (*P*= .2) (Table 2). Also, no statistically difference was found between both groups for retinopathy (*P*= .34) (Table 2). But diabetic neuropathy was statistically different in both groups so that in HP+ groups, 70 ones (60.8%) and in HP- group, 39 ones (45.4%) had diabetic neuropathy (*P*= .04).

Table 1. The demographic, clinical and laboratory characteristics of the patients

Parameters	HP+ (n=125)	HP- (n=86)	P value	CI 95%
Patients N(%)	125(59.24)	86(40.56)		
Male/Female (n)	62/63	39/47		
HP IgG (U/mL)	39.58±4.21	20.16±3.54		
Blood glucose (mg/dL)	182.1±5.9	171.56±6.3	0.23	-28.1/6.9
HbA1c (%)	8.1±0.15	8.01±0.14	0.48	-0.58/0.27
Total cholesterol (mg/dL)	205.3±6.02	208.5±6.9	0.72	-15.02/21.5
Triglyceride (mg/dL)	113.1±10.1	105.03±11.3	0.73	-35.5/25.5
HDL-cholesterol (mg/dL)	60.6±2.43	68.75±3.03	0.026	1.07/16.3
LDL-cholesterol (mg/dL)	116.06±4.8	108.45±4.5	0.27	-21.2/5.94
Body mass index (kg/m^2)	28.45±0.43	29.74±0.53	0.062	-0.06/2.6
Diabetic duration (year)	8.92±0.4	8.37±0.43	0.36	-1.74/0.64
Systolic BP (mmHg)	122.0±10.0	126.0±14.0	0.30	-16.61/15.59
Diastolic BP (mmHg)	76.5±6.0	78.0±8.0	0.71	-12.4/18.4
AST (mg/dL)	23.8±15.0	21.0±9.3	0.24	-2.69/0.6
ALT (mg/dL)	23.0±14.5	22.0±7.8	0.21	-2.52/0.5
GFR (mL/min/1.73m^2)	91.0±13.0	89.0±6.3	0.74	-25.5/21.5

±SEM

Table 2. The prevalence of microvascular complications of the patients

Parameters	HP+	HP-	OR	P value	CI
Diabetic Nephropathy N(%)	55(44)	31(36)	1.87	0.027	1.07-3.25
Diabetic Retinopathy N(%)	45(36)	28(32.6)	1.4	0.25	0.79-2.45
Diabetic Neuropathy N(%)	76(60.8)	39(45.4)	1.1	0.61	0.65-2.1

In this study 59.24% of diabetic patients had HP infection. In previous studies, the prevalence of HP infection in diabetic patients was from 30 to 80% [20,21,27-34]. The prevalence of our study is close to Demir et al.'s study which was 61.7% [27].

Of the microscopic complications we evaluated, only HP infection and diabetic neuropathy had statistically significant association and no statistically significant association was found between HP and diabetic nephropathy and retinopathy. It was found in Demir et al.'s study which had a similar HP infection prevalence [27]. It was confirmed in Gulcelik et al.'s study too. They found a positive association between HP infection and diabetic neuropathy but no association was found between HP infection and diabetic nephropathy and retinopathy. By the way, the HP prevalence in this study was higher than our study and 75.6% of the patients were affected by this microorganism [20]. The association between HP and diabetic neuropathy was confirmed in other studies [14,21]. On the other side, de Luis and colleagues did not confirm it in their study [14,21,35].

Similar to our study, Gulcelik et al. and de Luis et al. [20,34] found no association between HP infection and diabetic nephropathy but in some large studies like Wang and colleagues' meta analysis [36] including 8 studies, a significant association was found and HP was associated with an increase risk of nephropathy and neuropathy (relative risk [RR]: 1.35, 95% CI: 1.06-1.73, p=0.45 and RR: 1.20, 95% CI: 1.03-1.40, p=0.29). They also discovered significant associations between bacterial infection and nephropathy risk in oriental people (RR: 1.73, 95% CI: 1.19-2.50, p=0.82) and in type 2 diabetic patients (RR: 1.50, 95% CI: 1.11-2.02, p=0.29). Also, a large meta analysis in 2013 on 39 studies showed an association between HP and diabetic nephropathy [37]. Duration of diabetes mellitus, type and degree of diabetes control and duration of diabetes may affect these findings [27].

Also, we found no association between HP infection and retinopathy. Like us, Gulcelik et al. and de Luis et al. found no association but Agrawal et al. found opposite findings [14,20,34]. With regard to the issues raised by our findings and other researches, we found that HP infection increases the incidence of diabetic neuropathy. Specific risk factors for the occurrence of diabetic neuropathy include body mass index, duration of T2DM, smoking, elevated triglyceride levels and given that the subjects of both HP+ and HP- groups in our study had no statistically significant difference in these factors (Table 1), and smoking was the exclusion criteria, it seems that seropositivity for HP is a risk factor for diabetic neuropathy.

Helicobacter pylori is a human pathogen infecting the gastric mucosa. It causes inflammatory process increasing in peptic ulcer, chronic gastritis, adenocarcinoma and gastric lymphoma of mucosa-associated lymphoid tissue [38]. HP infection is reported to be associated with many extra gastrointestinal manifestations. During the last years, many studies have been performed on the relationship between HP infection and vascular diseases [19,39]. Gastric infection with HP may also induce the synthesis of acute phase reactants and activate immune mechanisms due to cross-reacting antibodies to HP and heat shock protein (HSP 60/65) with endothelial derived HSP 60/65 [40,41].

The inflammatory response to HP includes recruitment of neutrophils, lymphocytes (T and B), macrophages, and plasma cells. The pathogen causes local injury by binding to class II MHC molecules expressed on gastric epithelial cells, leading to cell death (apoptosis). Moreover, bacterial strains that encode Cag-PAI can introduce CagA into the host cells and cause further cell injury and activation of cellular pathways involved in cytokine production. In the gastric epithelium of HP–infected individuals high concentrations of multiple cytokines including interleukin (IL) 1α/β, IL-2, IL-6, IL-8, tumor necrosis factor (TNF-α) and interferon (IFN-γ) are found [42].

Inflammation has an important role in development of diabetic microvascular complications. Proinflammatory cytokines including C-reactive protein (CRP), interleukin

(IL)-6 and tumor necrosis factor (TNF)-α increase in diabetes [4]. On the other side, it is shown that inflammatory cytokines play a role in diabetic neuropathy and there is an elevation in inflammatory mediators including the pro-inflammatory cytokines (TNF-α, interleukin-1, 6, 13 and 17), chemokines (MIP 1 and 3, RANTES, Fractalkine) and cell adhesion molecule sICAM that are associated with neuropathy. So it seems that HP infection causes diabetic neuropathy via increasing the inflammatory cytokines.

4. CONCLUSION

Helicobacter pylori infection is associated with some of the vascular complications of T2DM so that diabetic neuropathy rate was significantly higher in diabetic patients with H. pylori infection. The exact mechanisms by which H. pylori increases the chances of developing neuropathy are not clear and additional studies on the etiology of this association is necessary.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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