Helicobacter pylori Seropositivity in Patients with Type 2 Diabetes Mellitus in South-East of Iran

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Abstract- It has been also reported that that *H. pylori* infection may be responsible for some endocrine disorders, such as autoimmune thyroid diseases, diabetes mellitus and primary hyperparathyroidism. *H. pylori* which express cytotoxin-associated gene A (CagA) may be more virulent than those that do not. The aim was to evaluate the seroprevalence of anti-*H. pylori* IgG and anti-CagA antibodies in patients with type 2 diabetes mellitus (type 2 DM) and healthy individuals from Rafsanjan city (Iran). A total of 100 patients with type 2 diabetes and 100 age-matched healthy individuals were enrolled to study. A blood sample was collected from each participant. The type 2 DM established according to the fasting blood glucose level of 126 mg/dl. The sera were tested for the presence of anti-*H. pylori* IgG antibodies and antibody to CagA by use of enzyme linked immunosorbent assay. The seroprevalence of anti-*H. pylori* antibodies in diabetic patients (76%) was similar to that observed in healthy subjects (75%). The mean titer of anti-*H. pylori* IgG in healthy control group (131.63±11.68 U/ml) was significantly higher than diabetic group (54.43±4.50 U/ml; *P*<0.0001). The prevalence of serum anti-CagA IgG antibodies was 78.9% in infected diabetic patients and 77.3% in healthy control group with mean titer of 75.02±4.54 U/ml and 84.34±5.85 U/ml, respectively. No significant differences were observed between diabetic and healthy control groups regarding the prevalence and the mean titer of anti-CagA IgG antibodies. In the diabetic group, the seropositive rate of anti-*H. pylori* IgG was higher in women as compared to men, but the difference was not statistically significant. These results show that *H. pylori* seropositivity rate was similar in type 2 DM patients and non-diabetics control group. No association was also found between CagA-positive strains of *H. pylori* and type 2 DM.

Introduction

H. pylori is one of the most common chronic bacterial infections in humans. It occurs throughout the world and causes several gastroduodenal diseases including gastric ulcer, duodenal ulcer, gastric MALT lymphoma, and distal gastric cancer (1). *H. pylori* strains are genetically diverse. *H. pylori* strains may be divided into at least two subgroups based on the expression (type I) or non-expression (type II) of CagA and the vacuolating cytotoxin. The cytotoxin-associated gene A (*cagA*) has been identified as major virulence factor of *H. pylori* (2,3).

It has been reported that the CagA-positive *H. pylori* strains induce more severe gastric mucosal inflammation than CagA-negative strains and are associated with higher risks of peptic ulcer disease and gastric cancer (3). We have previously reported that the seroprevalence of *H. pylori* in healthy Iranian adults and children was 67.5% and 46.6% and the prevalence anti-CagA antibody was 72.8% and 67.4% in infected subjects, respectively (4).

In addition to the gastrointestinal symptoms, some extragastric diseases such as cardiovascular diseases, lung diseases, hematologic diseases, eye and skin diseases, hepatobiliary diseases, and neurological disorders have

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been also linked to *H. pylori* infection (5,6). It has been reported that that *H. pylori* infection may be also responsible for some endocrine disorders, such as autoimmune thyroid diseases, diabetes mellitus and primary hyperparathyroidism (7).

The prevalence of diabetes mellitus in Iran has been reported to be 24% in individuals with age > 40 years and 5.5% in those aged 15-65 years (8,9). Recently, it has been also reported the seroposivity for *H. pylori* infection was associated with an increased incident of diabetes in a prospective cohort study. If *H. pylori* plays an etiological role in the development of type 2 diabetes, preventive strategies such as increased hygiene and treatments of *H. pylori* with antibiotics can be consider for high risk diabetic communities. The objective of the present study was to evaluate the seroprevalence of anti-*H. pylori* IgG and anti-CagA antibodies in type 2 DM patients and healthy individuals to clarify any association.

Materials and Methods

Subjects

From August 2011 to December 2011, a cross-sectional seroprevalence study was carried out in Rafsanjan (a city that located in South-East of Iran). 100 type 2 DM patients (mean age: 42.86±6.42 years; range: 24 to 54 years; 69 men and 31 women) and 100 healthy subjects (mean age: 41.75±7.19 years; range: 26 to 57 years; 63 men and 37 women) were included in the study. The healthy control group was recruited among blood donors of Rafsanjan Blood Transfusion Center. The healthy controls did not undergo endoscopy and were basically health, with no acute or chronic illnesses. The criteria for enrolment included no history of peptic ulcer disease, no abdominal surgery, no history of therapy for *H. pylori* infection, and no symptoms of upper gastrointestinal disease such as indigestion, nausea, vomiting and epigastric burning pain. The type 2 DM patients had disease, according to the physician's diagnosis and fasting blood glucose level of 126 mg/dl as a major criteria defined by the American Diabetes Association. A blood sample was obtained from each participant and the sera were separated and stored at -20°C until analysis.

This study was evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences. Moreover, patients were recruited if they agreed for blood sampling.

Determination of *H. pylori*-specific antibodies in serum

The serum levels of anti-*H. pylori* immunoglobulin G (IgG) were measured by using the commercial enzyme-linked immunosorbent assay (Trinity Biotec, Ireland). According to manufacturer guideline the results were obtained as U/ml and the value of 5 U/ml used to discriminate the negative from positive samples. Serum levels of anti-CagA IgG were also assayed by ELISA method using commercial kits (Diagnostic Bioprobes, Italy). The serum concentrations of anti-CagA antibodies were expressed in arbitrary units per milliliter (U/ml) as no International Standard is available. According to the manufacturer's guidelines the value of 5 U/ml used to discriminate the negative from positive samples. Moreover, in each group the serum concentrations of anti-*H. pylori* and anti-CagA antibodies expressed as mean±SEM.

Statistical analysis

Differences in variables were analyzed using t-test and Chi-square test as appropriate and *P*-values of less than 0.05 were considered significant.

Results

Anti-*H. pylori* IgG seropositivity

The overall seroprevalence of anti-*H. pylori* IgG antibodies in diabetic patients (76%) was similar to that observed in healthy subjects (75%). As demonstrated in Table 1, in the diabetic group, the seropositivity of anti-*H. pylori* IgG was significantly higher in females as compared to males but the difference was not significant (83.9% vs. 72.5%; *P*<0.1). The mean titer of anti-*H. pylori* IgG antibodies in healthy control group (131.63±11.68 U/ml) was significantly higher than diabetic group (54.43±4.50 U/ml; *P*<0.0001) (Table 1).

Anti-*Cag*A seropositivity

The prevalence of serum anti-CagA IgG antibodies was 78.9% in infected diabetic patients and 77.3% in healthy control group with mean titer of 75.02±4.54 U/ml and 84.34±5.85 U/ml, respectively (Table 1). No significant differences were observed between diabetic and healthy control groups regarding the prevalence and the mean titer of anti-CagA IgG antibodies.

Table 1. Seroprevalence of anti-*Helicobacter pylori* and anti-*CagA* antibodies in type 2 diabetic patients and healthy control group and according to their gender.

Group	Sex	No.	Anti-*H.pylori* IgG rate	Anti-*CagA* rate	Anti-*H.pylori* IgG Concentration (U/ml)	Anti-cagA concentration (U/ml)
Healthy control	Male	63	47 (74.6%)	37 (78.7%)	108.62±11.28*	84.86±7.74*
	Female	37	28 (75.7%)	21 (75%)	170.25±23.47	83.42±8.89
	Total	100	75 (75%)	58 (77.3%)	131.63±11.68	84.34±5.85
Diabetic patients	Male	69	50 (72.5%)	38 (76%)	57.13±5.53	75.89±5.55
	Female	31	26 (83.9%)	22 (84.6%)	49.24±7.78	74.00±8.01
	Total	100	76 (76%)	60 (78.9%)	54.43±4.50	75.02±4.54

* represent that the serum concentration of immunoglobulins expressed as mean±SEM

Table 2. The association of the *H. pylori* with DM in different studies.

Country	*H. pylori* seropositivity		P. value	References
	DM	Control		
Pakistan	73%	51.4 %	0.001	(10)
Turkey	75.6%	46%	0.05	(11)
Greece	37.3%	35.2%	NS	(13)
Romania	70%	73%	NS	(14)
United Arab Emirates	76.7%	64.8%	0.009	(12)
Czech Republic	27%	51%	0.001	(28)
Australia	33%	32%	NS	(15)
I.R. Iran	76%	75%	NS	Present study

In both DM and healthy control groups, no significant differences were observed between men and women with respect to the prevalence and the mean titer of serum anti-CagA IgG antibodies.

Discussion

The results of the some studies regarding the association of the *H. pylori* with type 2 DM have been summarized in table 2. As demonstrated in this table, the relationship between DM and *H. pylori* infection is controversial. The results of the some studies were showed that there is a higher prevalence of *H. pylori* infection in patients type 2 DM in comparison to control group (10-12). In contrast, other studies showed that *H. pylori* infection is not associated with DM, so that there is no significant difference in the prevalence of *H. pylori* infection between diabetics and non-diabetic control groups (13-15). The relationship between gastrointestinal diseases and *H. pylori* infection in DM is also controversial. The results of

the some studies were showed that there is no significant difference between diabetic and non-diabetic groups regarding the prevalence of *H. pylori*-related gastrointestinal symptoms (13). On the other hand, the higher prevalence of peptic ulcer has been reported in *H. pylori*-positive patients with DM (11), suggesting that the diabetic patients should be considered as high risk group for *H. pylori* infection and suitable candidates for treatment. This discrepancy may be attributing largely to differences in the age, gender, socio-economic status, job, race and ethnic background of participants. However, different inclusion criteria of patients and controls used in the various studies, different diagnostic methods used for *H. pylori* infection and genetic heterogeneities of *H. pylori* may be account for some differences. Accordingly, the results of the one study may not necessarily apply to other populations.

The results of the present study also showed that the mean titer of anti-*H. pylori* IgG antibodies in type 2 DM group was significantly lower than healthy control group.

This phenomenon may be largely attributed to the weaker immune responses of DM patients or micro-angiopathy in patients with DM. The presence of micro-angiopathy in patients with DM has been reported as a negative factor for *H. pylori* colonization, because micro-vascular alterations in the gastric mucosa may produce an unfavorable condition for the colonization or persistence of *H. pylori* (16). Interestingly, the results of one investigation even showed a lower *H. pylori* seropositivity in patients with DM, in comparison to healthy control group (17). Moreover, in another study showed a significantly lower incidence of *H. pylori* infection in diabetic patients with active duodenal ulceration in comparison to non-diabetic group (18).

It is well demonstrated that serum testing for presence of specific IgG antibodies against the CagA is a sensitive method for detection of the CagA-positive strains (19). The results of the present study also showed that there are no significant differences between diabetic and healthy control groups regarding the prevalence and the mean titer of anti-CagA IgG antibodies. Consistent with the results of the present study, we have recently reported that the serum concentrations of hs-CRP were not affected by the expression of bacterial CagA virulence factor (20). Moreover, in another our study, it has been observed that the prevalence of anti-CagA antibody was similar in patients with ischemic heart disease (IHD) and healthy control group (21). However, in our previous study in same population a strong association was observed between infection with CagA-positive *H. pylori* strains and peptic ulcer (22). Furthermore, we have observed higher levels of serum inflammatory cytokines including IL-17 and IL-18 in patients with peptic ulcer patients infected with CagA-positive strains (23,24). These observations represent that the CagA-positive strains may have differential role in peptic ulcer and diabetes or IHD pathogenesis.

The results of some studies showed that there is no relationship between *H. pylori* infection and diabetic complications while in other investigations the CagA-positive strains of *H. pylori* are associated with diabetic complications such as macro-angiopathy, neuropathy and micro-albuminuria in type 2 diabetic patients. Although the results of many studies have been shown the association of CagA with a more severe inflammatory response and more severe pathological changes (2,3), the results of some investigations failed to demonstrated any association between the expression of inflammatory parameters and *H. pylori* virulence factors such as CagA (25,26). It should be noted that the wide geographical variations have been reported in the genotype of CagA-positive strains (27). Accordingly, both host and bacterial factors should be considered in order to understand the *H. pylori*-associated inflammatory responses.

In conclusion, the results of the present study showed that *H. pylori* seropositivity rate was similar in type 2 diabetic patients and healthy non-diabetic control group. No association was also found between CagA-positive strains of *H. pylori* and type 2 DM.

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