

Study of Association between *Helicobacter pylori* Infection and Microalbuminuria in Type-2 Diabetic Patients

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Helicobacter pylori is a prevalent infectious agent in community which could cause gastrointestinal infections. In diabetic patients, microalbuminuria is a significant problem which could threaten their health. Bacterial infections cause renal failure and proteinuria. This study was conducted to investigate the association between *H. pylori* infection and microalbuminuria in patients with type 2 diabetic. In this case-control study, 86 patients with type 2 diabetic were investigated. The patients were divided into two groups of *H. pylori*-infected (n: 40) and non *H. pylori*-infected (n: 46) based on two simultaneous positive tests, rapid urease test (RUT) and ELISA. The patients in the two groups were examined and compared for proteinuria and other blood-urine indices. Of the patients with type 2 diabetes, 40 (46.51%) were *H. pylori*-infected. The infected type 2 diabetic patients had a significantly higher leakage of protein compared to non-infected diabetic patients ($P<0.05$). Further, infected patients had a significantly high level of inflammatory indices of C-reactive protein, lipoprotein a, and increase in blood leukocytes ($P<0.05$). *H. pylori* infection could increase inflammation in the body and contribute to inducing systemic inflammation and renal disorders. This study also showed that *H. pylori* infection was significantly associated with microalbuminuria and increase in blood inflammatory markers.

Key words: *Helicobacter pylori*, type 2 diabetes mellitus, microalbuminuria.

Helicobacter pylori is a gram-negative, spiral microorganism with flagella, which could accumulate and colonize in human gastric. Currently the rate of *H. pylori* infection is very high in developing countries, so that approximately 50% of their population is *H. pylori*-infected¹⁻³. *H. pylori* infection could increase the risk of gastritis and gastric cancer, which is one of the most common reasons for cancer-associated mortality worldwide. In the development process of gastritis, *H. pylori* is able to colonize in gastric and hence cause induction of inflammation in gastric mucosa

and gastrointestinal tract through involving immune system⁴⁻⁶. Type 2 diabetes or adult diabetes is developed because of the body's inability to produce adequate insulin or to use the produced insulin efficiently. Type 2 diabetes is frequently seen in the individuals who are over 40 years of age and obese, and have family history of diabetes, but the incidence rate has recently increased in younger people due to today's lifestyle⁷. Microalbuminuria is a significant index of nephropathy in diabetic patients. Microalbuminuria could be due to endothelial damage to glomerular vessels. Microalbuminuria is referred to leakage of 30-300 mg albumin per 24 h, or 20-200 μ g albumin per one mg creatine in urine. However, pseudo-positive cases should be excluded, including transient hyperglycemia, severe

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physical activity, urinary tract infections, blood hypertension, heart failure, and acute febrile disease⁸⁻¹⁰. Regarding high rate of *H. pylori* infection and number of patients with the type 2 diabetes, it is necessary to investigate threat effects of *H. pylori* on gastrointestinal and urinary tract. Several diseases and agents could damage glomerulus and renal artery, which contributes to progressing renal disorders. Bacterial infections, such as Chlamydia and *H. pylori* infections, and viral infections, including Epstein-Barr Virus infection and hepatitis, could cause these renal and cardiovascular diseases¹¹⁻¹⁴. This study is conducted to determine and investigate the association between *H. pylori* infection and proteinuria, especially microalbuminuria in patients with type 2 diabetes.

MATERIALS AND METHODS

A total of 86 patients with type 2 diabetes and gastrointestinal disorder referring in HAJAR hospital and Pasteur Laboratory, Shahr-e-kord, IRAN, were enrolled and after being informed and data was recorded and process was approved by the medical ethics committee. None of them had received anticoagulants and nonsteroidal anti-inflammatory drugs (NSAIDs) for 1 month before specimens collection and none of them had treatment for *H. Pylori* infection. The patients who were under diet therapy or insulin therapy and above 40 years of age were selected. The parametrics which were investigated to confirm the diagnosis of type 2 diabetes and considered as inclusion criteria were age of over 40 years, at least 3 years since the development of diabetes, 24-h proteinuria \geq 500 mg, the serum creatinine level $<$ 1.5 mg/dl, triglyceride level $<$ 400 mg/dl, and negative urine culture samples. The patients were assigned to two groups, *H. pylori*-infected (n: 40) and non *H. pylori*-infected (n: 46), based on two simultaneously positive or negative tests, rapid urease test (RUT) and ELISA IgM and IgG antibody against bacteria. Samples was taken as biopsies for determining *H. pylori* infection by RUT test and blood sample for antibody against bacteria. The exclusion criteria consisted of history of treatment for *H. pylori* infection, acquisition of other infectious diseases, use of antibiotics, and under treatment for renal disorders. The investigated

demographic characteristics were: age, Gender, anti diabetic therapies (diet and/or insulin), body mass index (BMI), and length of diabetes development. Moreover, the examined clinical parameters consisted of total cholesterol, glycemia, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), lipoprotein a, glycated hemoglobin (HbA1c), number of leukocytes, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and microalbumin (Malb). Further, the presence of infection with *H. pylori* were investigated by RUT and ELISA based on change at the RUT band color, from yellow to red and IgM and IgG antibodies against *H. pylori*, respectively (with 95% sensitivity and 97% specificity)^{15,16}. BMI was measured in Kg/m². Systolic and diastolic pressure was measured by voices heard by the manometer (Omron) after 10 minutes of seating, and diastolic pressure above 85 mmHg and systolic pressure above 120 mmHg were considered as hypertension. Blood sugar was measured by Cobas Integra (hexokinase method), triglycerides by enzymatic method, and HDL, LDL, HbA1c, and CRP by kits manufacturer's instructions. Lipid a (LPa) was measured by enzyme Immunoassay (Boehringer Kit). Diagnosis of coronary heart disease was confirmed by electrocardiography. Leakage of 24-h urine albumin over 300mg and clearance rate of creatinine under 70 ml/min in patients were considered as nephropathy. Leakage of urine microalbumin was carried out by nephelometry, so that 24-h urine was collected and measured every four weeks for 12 weeks and 30-300 mg microalbumin was considered as microalbuminuria^{17,18}. However, the patients with 30-300 mg microalbumin that did heavy exercise and/or suffered from urinary tract infections were not considered as microalbuminuria patients. To investigate *H. pylori* infection, gastric antrum biopsies taken in hospital following the patient's referring with his/her written consent were used for RUT and blood samples was used to examine the presence of *H. pylori* antibody. If both tests were positive, the patient was considered as *H. pylori*-infected, with 95-99% sensitivity.

Statistical analysis

For all the data, mean (standard deviation) was used per the statistician's advice. The distribution of data was examined by Kolmogorov-Smirnov test and t-test. Non parametric variables

were investigated by Mann-Whitney test. The association between albuminuria and other variables was examined by Spearman correlation coefficient and regression. A statistician analyzed the data using SPSS and P<0.05 was considered significant (95% CI).

Findings

Table 1 shows demographic characteristics and clinical data for diabetic patient by *H. pylori*-infected and non *H. pylori*-infected patients.

Table 2 shows the data on nutrition and therapeutic regimen in the patients.

The existence of *H. pylori* was confirmed in 40 (46.51%) samples of hepatitis patients. No significant increase in microalbuminuria was seen in *H. pylori*-infected patients compared to the non *H. pylori*-infected patients. Further, the number of leukocytes, serum ESR, CRP, and lipoprotein-a was significantly higher in *H. pylori*-infected patients

than non *H. pylori*-infected patients. Endoscopy was done for all the patients and 30 *H. pylori*-infected patients and 16 non *H. pylori*-infected patients had abnormal endoscopic findings (P<0.001). Microalbuminuria level was associated with *H. pylori* infection, independent of the measured parameters such as LDL and HbA1C, and gender.

DISCUSSION

One of the most important reasons for physiological defect in renal function is diabetic nephropathy, associated with hypertension and urinary protein excretion. Nephropathy occurs at various and sequential degrees, including an increase in glomerular filtration rate and then microalbuminuria and macroproteinuria²¹. Microalbuminuria could be a simple and sensitive symptom of development of renal failure in the

Table 1. Demographic characteristics and clinical data for *Helicobacter pylori*-infected and non *H. pylori*-infected patients

The investigated variables	Non <i>Helicobacter pylori</i> -infected patients	<i>Helicobacter pylori</i> -infected patients	P Value
Number of patients	46	40	Total (n: 86)
Gender (male/female)	26/20	33/7	Total (n: 86)
Age (yr)	53±5.2	54±7.8	-
Total cholesterol	189±10	178±12	0.05<
High density lipoprotein (mg/dl)	30±5.8	22±4.4	< .0.5
Low density lipoprotein (mg/dl)	124±3.3	127±4.5	0.05 <
(mg/dl)Lipoprotein a	40±2.5	49±4.4	0.05 >
Triglyceride (mg/dl)	200±4.2	198±2.5	0.05 <
Fasting blood sugar (mg/dl)	124.4±3.3	121±4.8	0.05 <
HbA1c (%)	5.9±0.8	7±1	0.05 <
Body mass index (Kg/m ²)	30±2	29±1.6	0.05 <
Proteinuria (mg/24 h)	133±8.8	161±10	0.05 >
C-reactive protein (mg/dl)	28±2	39±10	0.05 >
Leukocytes count (10 ³ /mm ³)	9±1	13±2.3	0.05 >

Table 2. The data on nutrition and therapeutic regimen in the *Helicobacter pylori*-infected and non *H. pylori*-infected patients

	Non <i>Helicobacter pylori</i> -infected patients	<i>Helicobacter pylori</i> -infected patients	P Value
Administered with diet (male/female)	12/10	16/4	-
Administered with oral insulin (male/female)	7/8	8/6	-
Administered with parenteral insulin	3/6	4/2	-

future. Therefore, study of potentially associated factors is necessary to find out useful approaches to preventing worse consequences of nephropathy²². Microalbuminuria level was obtained higher in the *H. pylori*-infected patients than the non *H. pylori*-infected. Prevalence of *H. pylori* infection in the patients with hepatitis has been reported approximately 40-80%²⁴⁻²⁵. Similarly, the prevalence of *H. pylori* infection was derived approximately 46.51% in the patients in the present study, which is consistent with other studies. Microalbuminuria is closely related to endothelial inflammation, so that different studies have shown that Persistent and chronic inflammatory responses of the immune system to the presence of *H. pylori* cause increase in and escalation of inflammation and vascular endothelial damage in the patients with diabetes²⁶. As demonstrated in some studies, microalbuminuria level was significantly higher in *H. pylori*-infected patients with hepatitis than the non *H. pylori*-infected, and *H. pylori* elimination in diabetes patients led to microalbuminuria level amounting to the level in the non infected patients, which is inconsistent with some studies^{27,28}. Further, some studies, inconsistent with the present study, have demonstrated a significant association between fasting blood sugar in *H. pylori*-infected and non *H. pylori*-infected patients with diabetes. Consistent with the present study, no significant association was seen between HbA1c in *H. pylori*-infected and non *H. pylori*-infected patients with diabetes²⁷. Some studies have indicated a significant association between *H. pylori*-infection and serum levels of lipoprotein a, HDL, LDL, and inflammatory cytokines such as interleukin 1 and interleukin 6. In this study serum lipoprotein was significantly associated with inflammatory factors such as CRP, ESR, and the number of leukocytes in *H. pylori*-infected hepatitis patients. Similarly, another study found a significant association between hepatitis and the bacterial infection, but not HbA1C level²⁴. As with the present study, some studies have obtained a significant association between other variables such as BMI, smoking, hypertension, and duration of disease in *H. pylori*-infected and non *H. pylori*-infected patients²⁶. It is noteworthy that in a study in Italy, a significant association between gender and the bacterial infection was seen²⁹, which is not consistent with our study. In view of the inflammation in the

infected patients with hepatitis, the level of microalbuminuria was significantly higher in the infected patients than non infected patients. In few studies, no significant association of nephropathy and bacterial infection with microalbuminuria level was seen, as well²⁶. As a result, *H. pylori* infection in hepatitis patients could affect systemic inflammation and renal function failure, causing increase in leakage of some proteins in kidney and urinary tract. However, further studies with larger sample size and investigation of other factors and inflammatory markers are needed for more definite conclusion.

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