Original Article

Prevalence of Helicobacter Pylori in Diabetes Mellitus Patients with Non Ulcerative Dyspeptic Symptoms and its Relationship to Glycemic Control and Microalbuminuria.

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Abstract:

Background: Helicobacter pylori (HP) infection is an issue which is contentious and deserves further investigation. The relationship between DM and HP infection is controversial. **Objective:** The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria. **Patients and methods:** This study included two groups of patients, **Group I:** Included 30 patients, non diabetic with dyspeptic symptoms and **Group II:** Included 30 patients, with type 2 diabetes with dyspeptic symptoms. All patients provided written informed consent before enrollment in the study. All patients were subjected to history taking, thorough clinical examination included neurological examination, laboratory investigation included CBC, fasting and postprandial plasma glucose, renal functions and liver function tests, HbAlc and microalbuminuria in 24 h collected urine and upper gastrointestinal endoscopy with biopsy specimens obtained from gastric antrum and corpus. H. pylori status was evaluated in each patient by both the rapid urease (CLO test) test and histopathological examination. **Result:** There were no statistical significant difference in the prevalence of H. pylori between the two groups by both histopathology and by CLO test (p=0.284 &0.118 respectively).Among the thirty diabetic patients, 17 patients were infected by H. pylori and 13 patients were not infected as shown by histopathological study. The mean fasting blood sugar (FBS), the prevalence of HbA1c > 7 , The prevalence of Microalbuminuria & the mean 24 hours protein were statistically significantly lower in H pylori negative patients than in H pylori positive patients (p=0.013,0.007 ,0.004&0,002 respectively). **Conclusion:** No statistical significant difference in the prevalence of H. pylori between diabetic and non diabetic patients. H pylori infection was significantly associated with poor glycemic control and high prevalence of microalbuminuria in diabetic patients.

Introduction:

Helicobactor pylori (HP) is a gram-negative, spiral-shaped pathogenic bacterium that specifically colonizes in the gastric epithelium and causes chronic gastritis, peptic ulcer disease, and/or gastric malignancies. [1, 2]

The infection induces an acute polymorph nuclear infiltration in the gastric mucosa. If the infection is not effectively cleared, this acute cellular infiltrate is gradually replaced by an immunologically mediated, chronic, predominantly mononuclear cellular infiltrate. [3] The latter is characterized by the local production and systemic diffusion of proinflammatory cytokines,[4] which may exert their effects in remote tissues and organic systems and result in extragastric manifestations. [5]

The prevalence of HP infection varies between countries; generally, the prevalence is about 30% in developed and up to 80% in developing countries. [6]

Diagnosis of HP can be achieved by taking biopsies by endoscopy. However, this procedure is invasive and might not give accurate results if colonization is patchy. [7] For population screening, serodiagnosis remains one of the methods of choice for detecting the prevalence of infection. [8–10] The technique of choice is currently enzyme-linked immunosorbent

assay because it is a simple, quick, and low-cost technique that permits immunoglobulin class-specific determinations.[11–18]

Diabetes mellitus (DM), a chronic disease marked by high levels of sugar in the blood, is common and increasing around the world.[19]

The relationship between DM and HP infection is controversial. According to some studies, there is a high prevalence of HP infection in patients with either Type 1 or Type 2 DM which is correlated with the duration of DM, the presence of dyspeptic symptoms, cardiovascular autonomic neuropathy, age, gender, BMI, blood pressure, fasting glucose, and the glycated hemoglobin levels (HbA1c).[20–22] In contrast, other studies showed that HP infection is not associated with DM. [20–22]

According to some data, there is no relationship between HP infection and diabetic complications, such as nephropathy, retinopathy, and/or microangiopathy while other data showed that virulent strains of HP, such as cytotoxin-associated gene CagA+, are associated with macroangiopathy, neuropathy, and microalbuminuria in Type 2 diabetic patients. [23, 26]

The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria.

Patients and methods:

This study included two groups of patients:

Group I: Included 30 patients, non diabetic with dyspeptic symptoms.

Group II: Included 30 patients, with type 2 diabetes with dyspeptic symptoms.

Exclusion criteria included patients diagnosed previously to have H. pylori infection, patients receiving proton pump inhibitors or H_2 blockers before the endoscopy by 2 weeks, smokers, and patients with hypertension or any other cause of microalbuminuria or history of alcoholism or abuse of NSAI. All selected patients provided written informed consent before enrollment in the study.

All patients were subjected to the following: History taking and thorough clinical examination included neurological examination, laboratory investigation included; Routine laboratory investigation as CBC, fasting and postprandial plasma glucose, renal functions and liver function tests,[27&28] HbAlc,[28] Microalbuminuria in 24h collected urine [27] and upper gastrointestinal endoscopy with biopsy specimens obtained from gastric antrum and corpus. H. pylori status was evaluated in each patient by both the rapid urease test (CLO test) [29] and histopathological examination of endoscopic biopsies.[30] Data were collected, revised and transferred into statistical package for social science (SPSS/ version 10). Results were expressed as means and standard deviation. Statistical tests used in this study were (t.test, Fisher Exact test, Mann Whitney test, Monte Carlo test & Ci.square). A level of 5% was considered as the cutoff level of significance.

Result:

Our result shows that there were no statistical significant difference in the prevalence of H. pylori between the two groups by both histopathology and by CLO test (p=0.284 &0.118 respectively).Table (1) Also, the prevalence of H. pylori in both groups was statistically insignificantly higher by histopathology than by CLO test (p=0.093). Table (2)

Among the thirty diabetic patients, 17 patients were infected by H. pylori and 13 patients were not infected as shown by histopathological study. The mean age and the prevalence of obese were significantly higher in diabetic with H.pylori infection (58.88 ± 6.70 years & 100% resepectivly) than without H.pylori infection (44.23 ± 7.44 years & 0% resepectively) (P= 0.001&0.024 respectivly). There were no statistical significant difference

between diabetic patients with and without H.pylori infection as regard the prevalence of female sex and durations of DM. (p=1.000 & 0.136 respectivly). Table (3)

As regard the mean duration of dyspepsia, it is statistically significantly higher in diabetic patient with H.pylori (6.47 ± 1.94weeks) than those without H.pylori infection (4.62 ± 1.98 weeks). (P= 0.022). As regard the prevalence of dyspeptic symptoms, the prevalence of diabetic neuropathy and the mean systolic and diastolic blood pressure, there were no statistical significant difference between diabetic patients with and without H.pylori infection (P>0.05).Table (4)

The mean fasting blood sugar (FBS), the prevalence of HBA1c > 7 , The prevalence of Microalbuminuria & the mean 24 hours protein were statistically significantly lower (171.62 ± 111.35 mg/dl, 38.5%, 30.8% & 65.54 ± 87.79mg/24h respectively) in H.pylori negative patients than in H pylori positive patients (240.59±101.38 mg/dl, 88.2% , 82.4% 191.35±112.06mg/24h respectively) (p=0.013,0.007, 0.004&0,002 respectively). Table (5)

There were no statistical significant difference between diabetic patients with and without H. pylori infection as regard the endoscopic findings. (P> 0.05).Table (6)

The prevalence of chronic active gastritis was statistically significantly higher in diabetic patients with than without H.pylori infection. (p=0.001). While the prevalence of chronic quiscent gastritis was statistically significantly lower in diabetic patients with than without H.pylori infection (p<0.001). There were no statistical significant difference between diabetic patients with and without H.pylori infection as regard the prevalence of gastritis with metaplasia. (P> 0.05).Table (6).

Table (1) The prevalence of H. pylori infection by CLO test and histopathological study in both groups

	Non Diabetic (n=30)		Diabetic (n=30)		χ^2p
	No.	%	No.	%	
CLO test					
Negative	10	33.3	16	53.3	0.118
Positive	20	66.7	14	46.7	
H. Pylori by histopathology					
Negative	9	30.0	13	43.3	0.284
Positive	21	70.0	17	56.7	

p: p value for comparing between the two studied groups

χ^2: Chi square test

*: Statistically significant at p ≤ 0.05

Table (2): H. Pylori positive cases by CLO test and histopathology in both groups

H. Pylori +ve cases	CLO test	H. Pylori by histopathology	χ^2 (p)
Non Diabetic	20	21	0.093 (0.761)
Diabetic	14	17	

p: p value for comparing between the two studied groups

χ^2: Chi square test *: Statistically significant at p ≤ 0.05

Table (3): Relation between H. pylori infection with demographic and clinical data in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Sex					
Male	2	15.4	3	17.6	$^{FE}p=1.000$
Female	11	84.6	14	82.4	
Age					
Min. – Max.	30.0 – 55.0		40.0 – 70.0		
Mean ± SD	44.23 ± 7.44		58.88 ± 6.70		$^{t}p <0.001^{*}$
Median	44.0		60.0		
BMI (kg/m^2)					
<30	13	54.2	11	45.8	FEp=0.024
≥30	0	0.0	6	100	
Duration of DM (years)					
Min. – Max	5.0 – 17.0		4.0 – 22.0		MWp=0.136
Mean ± SD.	10.23 ± 4.09		12.76 ± 4.75		
Median	8.0		13.0		

p: p value for comparing between H. pylori categories FE: Fisher Exact test
t: Student t-test MW: Mann Whitney test χ^2: Chi square test

Table (4): Relation between H. pylori with medical history in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Duration of dyspepsia (weeks)					
Min. – Max.	2.0 – 8.0		3.0 – 9.0		$^{MW}p=0.022^{*}$
Mean ± SD.	4.62 ± 1.98		6.47 ± 1.94		
Median	4.0		7.0		
Vomiting	5	38.5	6	53.3	$^{FE}p=1.000$
Heart burn	9	69.2	10	58.8	$^{FE}p=0.708$
Abdominal pain	11	84.6	14	82.4	$^{FE}p=1.000$
Peripheral Neuropathy	9	69.2	10	58.8	$^{FE}p=0.708$
Systolic blood pressure					
Min. – Max.	90.0 – 130.0		90.0 – 130.0		
Mean ± SD.	116.15 ± 14.46		107.06 ± 13.12		0.082
Median	120.0		100.0		
Diastolic blood pressure					
Min. – Max.	60.0 – 90.0		60.0 – 90.0		
Mean ± SD.	76.15 ± 9.61		71.18 ± 7.81		0.129
Median	80.0		70.0		

p: p value for comparing between H. pylori categories FE: Fisher Exact test
MW: Mann Whitney test *: Statistically significant at p ≤ 0.05

Table (5): Relation between H. pylori infection with FBS, HBAIc and Microalbuminuria in diabetic patients

	H. pylori by histopathology		Test of sig.
	Negative (n=13)	Positive (n=17)	
FBG(mg/dl)			
Min. – Max.	110.0 – 526.0	105.0 – 526.0	
Mean ± SD.	171.62 ± 111.35	240.59 ± 101.38	$^{MW}p= 0.013^{*}$
Median	140.0	225.0	
HBA1c			
6.5 – 7	8 (61.50%)	2 (11.8%)	$^{FE}p= 0.007^{*}$
>7	5 (38.5%)	15 (88.2%)	
Min. – Max.	6.10 – 12.50	8.20 – 11.40	
Mean ± SD.	7.91 ± 2.41	9.46 ± 0.91	$^{t}p= 0.044^{*}$
Median	6.40	9.30	
Microalbuminuria	4 (30.8%)	14 (82.4%)	$\chi^2p= 0.004^{*}$
Urine protein 24 hour (mg/24h)			
Min. – Max	13.0 – 275.0	14.0 – 300.0	
Mean ± SD.	65.54 ± 87.79	191.35±112.06	$^{MW}p=0.002^{*}$
Median	16.0	230.0	

p: p value for comparing between H. pylori categories FE: Fisher Exact test t: Student t-test
MW: Mann Whitney test χ^2: Chi square test *: Statistically significant at p ≤ 0.05

Table (6): Relation between H. pylori endoscopic finding in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Gastritis	13	100.0	17	100.0	-
Duodenitis	2	15.4	5	29.4	0.427
Hiatal hernia	0	0.0	1	5.9	1.000
GERD	0	0.0	3	17.6	0.238
Erosion	1	7.7	4	23.5	0.355
Mass	0	0.0	0	0.0	-

Table (6): Relation between H. Pylori with histopathological findings in diabetic

	H. Pylori				FEp
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Chronic active gastritis	4	30.8	16	94.1	0.001^{*}
Chronic quiscent gastritis	9	69.2	1	5.9	$<0.001^{*}$
Gastritis with metaplasia	1	7.7	1	5.9	1.000

FE: Fisher Exact test
*: Statistically significant at p ≤ 0.05

Discussion:

The link between diabetes and HP infection has been inconsistently reported. Researchers have hypothesized an association between infection with Helicobacter pylori and diabetes mellitus.[31&32] However, studies to date have failed to confirm this hypothesis as results have been discordant.[33-35] The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria. We studied two groups of patients, group I included 30 patients non diabetic with dyspepsia and group II included 30 patients diabetic with dyspeptic symptoms.

In our work, assessment of Helicobacter pylori (HP) Infection was done by both rapid urease test, also known as the Campylobacter-like organism (CLO) test and histological study of antral and corporal biopsies. When either the histopathology or the CLO tests were positive, the infection was confirmed.[36] The prevalence of HP infection was insignificantly higher in histopathological results than in CLO test .Since the diagnosis of HP has a sensitivity of 93–99% and specificity of 95-99%, the diagnosis established by the histopathological method accepted as the "gold standard".[36]

The relationship between DM and HP infection is controversial. In our study we found that the prevalence of H pylori infection by both CLO test and histopathological study was statistically insignificantly lower in diabetic than in non diabetic.

In agreement with our work, Demir et al[37] and other investigator,[38-40] showed that the prevalence of HP infection did not differ significantly between diabetic patients and nondiabetic controls.

The decreased chance of growth of HP in diabetics may due to presence of microangiopathy of gastric mucosa which may decrease the mucous layer which is essential for H pylori survival. [41]

On the contrary Bener A, et al [31] and other investigator [42] reported that Helicobacter pylori infection was significantly higher in diabetic patients than non-diabetic subjects. Several hypotheses were presented for confirmation of higher prevalence of HP infection in diabetic patients such as immune system impairment (cellular and humoral immunity) in patients with diabetes mellitus, the reduction of both gastrointestinal motility and acid secretion (autonomic neuropathy) and higher secretion of pro-inflammatory cytokines related to the HP gastric infection itself.[31&42]

In our work we found that significantly higher mean age was associated with HP infection in diabetic patients.

Pounder RE, Ng D.[43] stated that there is positive correlation between HP infection and increasing age of the subjects. Also MalatyHM et al[44] stated that the risk of HP infection increases with advancing age. These associatiation could be explained by repeated exposure to the infection in old subjects.

In our work we found that significantly higher BMI were associated with HP infection in diabetic patients.

Also Cho I, Blaser MJ, Francois F, et al.[45] concluded that, among older individuals and especially those with a higher BMI, glucose intolerance associated with HP could remain significant.

Glycated hemoglobin (HbA1c) results from the non enzymatic glycosylation of hemoglobin, reflecting integrated blood glucose levels during the preceding 3–4 months.[46-48] HbA1c levels are predictive of both prevalent and incident diabetes and are useful in diagnosing prediabetes, diabetes and long term control of diabetes.[46-48]

In our study we found that diabetic patients with HP infection were characterized by poor diabetic control as regard significantly higher mean fasting blood sugar and high prevalence of HAIC > 7 than in HP negative patients.

In agreement, Buell C et al [46] found a positive association between HP status and HbA1c levels. Also, Cho I and Blaser[45] report that HP seropositivity, and especially HP cagA positivity, was associated with higher mean

HbA1c levels, an association that persisted after excluding individuals with a history of diabetes mellitus and controlling for potential confounders.

The most plausible hypothesis is that H. pylori directly or indirectly increases levels of HbA1c H. pylori plays a role in the regulation of leptin and ghrelin, [49&50] which are central to energy homeostasis and metabolism.[51] These 2 hormones are involved in the regulation of appetite and energy expenditure. Ghrelin decreases energy expenditure and promotes weight gain,[52] whereas leptin, which is expressed mainly by adipocytes, reduces food intake and increases energy expenditure. [53]HP infection was associated with lower levels of circulating ghrelin through decreases in the ghrelin producing cells in the gastric mucosa and increases in gastric leptin levels.[54]

Gino et al[55] also found a poor glycemic control in type two DM patients who were infected by HP. The significant decrease in the glycemic control in patients infected with the virulent CagA-positive of HP could be explained by its ability to increase insulin resistance,[56] and decrease serum concentration of somatostatin,[57] which has an inhibiting effect on insulin release.[57] Also, CagA-positive strains are associated with increased production of cytokines such as tumor necrosis factor (TNF alpha), interleukin (IL)-1, -6, and -8,2 which may affect carbohydrate metabolism and stimulate the secretion of insulin counter-regulatory hormones leading to hyperglycemia in diabetic patients.[58]

In contrast, other studies did not find an association between infection by HP and glycemic control,[59] and explained their findings by the fact that gastritis increases glucose-and meal-stimulated insulin release by increasing gastrin secretion, which inhibit glucose absorption in the small intestine, and amplifies glucose stimulated insulin release.[59]

Obesity is an established risk factor for diabetes and it is known that high BMI is associated with elevated HbA1c. Separately, the presence of HP is also associated with elevated HbA1c. In the present study we hypothesized that having both high BMI and the presence ofHP would have a synergistic effect, increasing HbA1c even more than the sum of the individual effect of either risk factor alone.

In agreement, Yu Chen and Martin Blaser[60] showed a synergistic effect of H. pylori and body mass index (BMI) on increased levels of HbA1c in that higher levels were found in HP –infected subjects with BMI >25.

Microalbuminuria (Malb) is a confirmed marker of diabetic nephropathy.[61] The appearance of albumin in urine is thought to be the consequence of generalized endothelial damage along the vascular area including the glomerulus.[61] Various infectious diseases may be listed among the etiologic factors related with this vascular endothelial damage and consequently developing atherosclerosis. As shown in recent studies, HP are these microorganisms.[62-65]

In our study microalbuminuria was present in 4 out of 13(30.8%) of H pylori negative patients and present in 14 out of 17 (82.4%) of H pylori positive patients. This relation was found to be statistically significant. Also the mean 24 hours protinuria was statistically significantly higher in diabetic with than without HP.

In agreement, Pietroiusti A, et al[66] showed that virulent strains of HP are associated with microalbuminuria in type 2 diabetic patients. This may be due to an immune-mediated injury at the level of the endothelium caused by a systemic immune response to the infection, leading to albumin leakage.[67&68] Although a certain value has not been determined about the frequency, it has been demonstrated that persistent systemic inflammatory response related with HP increases the vascular injury in diabetics and predisposes them to pulmonary, cardiovascular and cerebral diseases.

HP infection has been hypothesized to contribute to a strong inflammatory response, atherogenesis and plaque instability.[69] It is thought that pro-inflammatory factors are produced at excessive amounts in this infection, and cross-reaction between the released mediators and host antigens causes

gastric injury and extra-digestive manifestations. Studies have demonstrated a significant relation between LPa, HDL-C, oxidant lipids, LDL-C, thrombotic activation-related anti-thrombin (AT)-III, von-Willebrand factor, interleukin-1, tumor necrosis factor, and interleukin-6 and HP infection.[67&68]

Oshima et al[65] reported that HP was associated with elevated C-reactive protein and soluble intercellular adhesion molecule-1. This indicates that chronic HP infection might be involved in the pathogenesis of atherosclerosis which is the corner stone of the development of microalbuminuria.

Conclusion: No statistical significant difference in the prevalence of H.pylori between diabetic and non diabetic control H.pylori infection was significantly associated with poor glycemic control and high prevalence of microalbuminuria in diabetic patients.

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