HELICOBACTER PYLORI AND DIABETES MELLITUS TYPE 2 FREQUENCY IN EGYPTIANS: IS THERE AN ASSOCIATION?

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Abstract: *Helicobacter pylori* (*H. pylori*) is one of the most common chronic infections in patients with gastrointestinal disorders. Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is characterized by high levels of blood glucose resulting from insulin resistance and relative insulin deficiency. Many reports discussed that *H. pylori* might have high prevalence among patients with diabetes. Seventy nine diabetic patients and eighty non-diabetic as controls were included in the study. The aim of the present study was to determine the frequency of *Helicobacter pylori* infection in diabetic and non-diabetic patients and to compare the frequency of infection in both groups and evaluate a possible relationship between *H. pylori* and T2DM. Stool samples were obtained to measure *Helicobacter pylori* antigen (HpSA) and serum samples for biochemical investigations. A group with 49 (62%) of

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diabetics and other with 38 (47.5%) of controls were positive for *Helicobacter pylori* with no significant relation (p value = 0.066). Diabetics with *H. pylori* infection had a higher levels of inflammatory mediators, C reactive protein (CRP), Tumor necrosis factor-α (TNF-α) and IL 6 are significantly increased in diabetic group with *H. pylori* than non-diabetic group.

Key words: Diabetes mellitus type 2, *Helicobacter pylori*, Inflammatory mediators, non-diabetic.

1 Introduction and Background

Type 2 diabetes mellitus (T2DM) is an emerging pandemic, responsible for an estimated 3.8 million adult deaths worldwide [12]. The pathogenesis of DM type 2 is complex, with risk factors associated with lifestyle (e.g., diet, obesity, physical activity), genetic background, and socioeconomic factors [39] [27].

Diabetes mellitus is one of the important causes of dyspepsia [28]. The incidence of *Helicobacter pylori* (*H. pylori*) is increased in diabetes mellitus [30].

H. pylori is a gram-negative, spiral, flagellate bacillus and is the single most common cause of peptic ulcer. The high prevalence of this organism worldwide presents clinicians and other health care workers a formidable challenge with regard to its control in a large population. *H. pylori* is S-shaped, curved rod (0.5-0.9 μm wide by 2-4 μm long) when observed in vivo. Because of it spiral shape, bacterium burrows through the delicate stomach lining and sustain its life in stomach. These bacterial cells are actively motile in nature. The bacterium is microaerophilic, growing best at atmosphere of 5% oxygen, with 5-10% CO_2 on blood-containing media in vitro [19].

The role of *H. pylori* infection in diabetic dyspepsia is mainly related to blood glucose concentration [35]. Hyperglycemia may induce the infection by *H. pylori* or the silent infection may get reactivated and produce symptoms of dyspepsia in diabetes [33]. So, the presence of *H. pylori* and diabetes mellitus (DM) is one of the main causes of gastrointestinal diseases [8].

H. pylori infection may have an impact on cardiovascular conditions, insulin resistance, and metabolic syndrome potentially mediated by elevations

in inflammatory markers such as C-reactive protein (CRP) and Interlukin-6 (IL-6) [9].

The simplest test of *H. pylori* is serologic; including the assessment of specific IgG levels in serum but it cannot be used for early follow-up and has high rates of false positive results [33]. The urea breath test is non-invasive but the radioactive isotope ^{14}C exposes the patient to radiation. Another more specific, rapid and newly researched non-invasive test is *H. pylori* stool antigen (HpSA). It can be performed in 90 minutes with an overall specificity and sensitivity of 94% by doing HpSA [38].

Recent evidence implicates the pathological involvement of inflammation in T2DM, which is an important process induced by *H. pylori* infection [31].The aim of the present study was to determine the frequency of *H. pylori* infection in diabetic and non-diabetic patients and to compare the frequency of infection in both groups and evaluate a possible relationship between *H. pylori* and T2DM.

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2 Subjects and Methods

The study was an analytic observational study, performed through a case control method conducted from January 2016 to August 2016. One hundred and eight five patients who had attending the outpatient clinics in National Hepatology and Tropical Medicine Research Institute (NHTMRI), Egypt. They were divided into two groups Diabetic and Non-Diabetic group. Diabetic group were 79 patients (26 Females and 53 Males) and healthy Non-Diabetic group as control were 80 subjects (21 Females and 59 Males). Their ages were ranging from 20-54 years. all patients provided written informed consent to participate.

Selection of Diabetic Subjects Persons were classified as diabetic according to the American Diabetic Association (ADA) criteria [3], or if they were on diabetic medication. Selection of Non-Diabetic Subjects. Non-diabetic (control) subjects were matched the same age of patients, they were identified

from the community if their venous blood glucose values were < 6.1 mmol/L and if they had never taken any diabetic medication. Control were tested by oral glucose tolerance test (OGTT) according to WHO criteria [1].

The Inclusion criteria of study were the following:

1. Either gender;
2. Diabetic (T2DM) who were newly diagnosed or known cases with positive or negative *Helicobacter pylori* infection; and
3. Non Diabetic individuals with positive or negative *Helicobacter pylori* infection.

The exclusion criteria of study were the followings:

1. Patients of type-1 diabetes;
2. Patients who refuse to give consent to participate in the study;
3. Patients with a history of *H. pylori* treatment; and
4. Patients with vascular and inflammatory diseases.

A full detailed history was taken from all patient and controls, demographics (age, gender, and Body Mass Index) of the patients were documented. All cases were subjected to the following investigations.

2.1 Stool Antigen Test

The amplified IDEIA Hp StAR^{TM} (Oxoid, Cambridge, UK) test, a sandwich-type Enzyme Immune Assay (EIA), was used to detect *H. pylori* antigens in stools, according to the manufacturer's instructions. Briefly, approximately 0.1 g of stool sample was mixed with the sample diluent. Fifty microliters of stool supernatant and 50 μL of conjugate were added to microwells and incubated for 60 min at room temperature. Following five wash cycles, 100 μL of substrate solution was added to each microwell and incubated for 10 min at room temperature. The reactions were stopped by adding 100 μL of stop solution.

The results were read by an EIA plate reader at 450 nm. OD values of $\geq$0.20 were considered positive; ODs between 0.2 and 0.5 weakly positive, and those > 0.5 strongly positive. Values < 0.20 were considered negative for other tests, Blood samples were obtained following an overnight fasting, samples were withdrawn from a cubital vein into blood tubes, then the serum was separated from the cells by centrifugation at 3000 r/min for 10 min and store samples at and immediately stored on refrigerator at 4C for 7 days or at -20°C for months. Avoid repeated freeze-thaw cycles.

2.2 Biochemical investigation

For each participant, a one-time sample of about 3-5 ml venous blood was collected and the following laboratory parameters were detected: Hemoglobin level (Hb), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), albumin (Alb), Glucose (Glu), serum total bilirubin (T-Bil), C-reactive protein (CRP) and serum creatinine (Creat) were determined on a Roche/Hitachi cobas c 311 analyzer (Basel, Switzerland), Tumor necrosis factor (TNF-α) were done by (Quantikine R&D systems, Inc, Minneapolis,USA), Interleukin 6 (IL-6) were done by Quantikine ELISA kits (R&D Systems, Wiesbaden, Germany) and alpha feto protein (AFP) (Roche Diagnostics, Basil) automated immuno-analyser.

2.3 Ethical Statement

All patients received written information concerning the background and procedures of the study, and the patients or their relatives gave written informed consent prior to entering the study.

2.4 Statistical Analysis

Continuous variables were expressed as mean plus standard deviation (SD). Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences - version 22). The significance of difference of different means (quantitative data) were tested using Students-t-test. The significance of difference of different percentages (qualitative data) were tested using Pearson chi-square test, Statistical significance was considered whenever the p value for the test of significance was equal or less than 0.05.

3 Results

In the present study, there were two groups. Seventy nine patients, twenty-six females (32.9%) and fifty three (67.1%) males in Diabetic group, and healthy Non-Diabetic group as controls were eighty subjects, twenty one females (26.3%) and fifty nine males (73.8%). Their ages were ranging from 40.23 ±7.97 and 39.35 ±8.18 respectively. The relations between patients and controls and some demographic variables are shown in Table 1. With regard body mass index (BMI), scores were higher among diabetics than Non-Diabetics as p value was < 0.001.

Table 1: Relationship between studied groups and some demographic data

Paramenter		**Diabetic**	**Non-Diabetic**	**p value**
Age (years)		40.23 ±7.97	39.35 ±8.18	0.494 (NS)
Gender	**Female [un]**	26 (32.9%)	21 (26.3%)	0.357 (NS)
	Male [un]	53 (67.1%)	59 (73.8%)	
BMI [wt/(ht)^2]		32.54 ±7.29	25.99 ±8.76	< 0.001 (HS)
Range		15-42	18-45	

NS: non-significant, HS: highly significant

Figure 1 shows that, out of the 79 patients of type 2 diabetes, *H. pylori* infection was found in 49 (62%) while it was present in only 38 (47.5%) of 80 controls, which was found to be non-significant (p value = 0.066).

The mean Hb level among diabetics was highly significant than non-diabetics (258.97 ±49.36, 93.14 ±14.23 respectively and t value = 28.860, p value $<$ 0.001) (Table 2).

The diabetic patients had significantly high AST, ALT, serum creatinine (Creat) and Hemoglobin levels (Hb) than those with non-diabetics, moreover it was significant in serum Albumin (Alb) and non-significant in total bilirubin (T-Bil) and direct bilirubin (D-Bil) serum levels as depicted in Table 2.

The levels of CRP were significantly increased in diabetic patients in comparison with non-diabetics as the mean ±SD was 21.21 ±13.01 and 4.97 ±3.24 respectively, (t value = 10.826 and $p < 0.001$). The serum levels of TNF-α

Figure 1: Frequency of *H. pylori* in the studied groups (Source: Author)

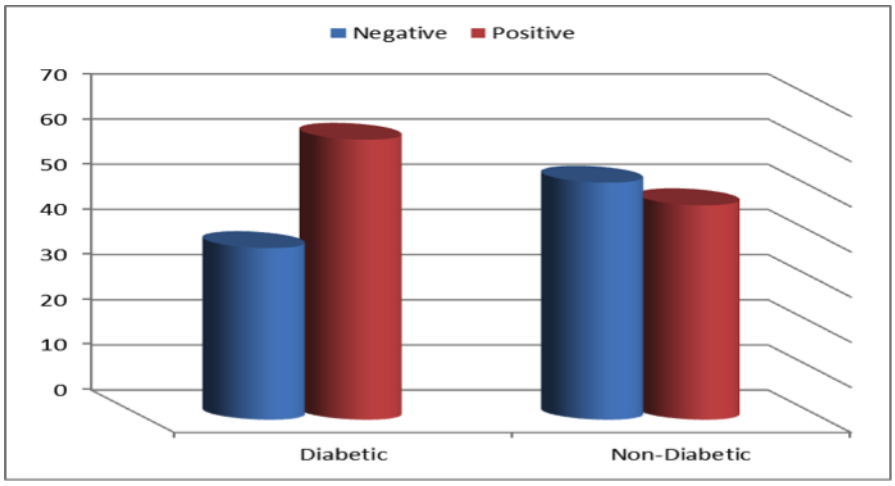

Table 2: Diabetic patients and controls in relation to some laboratory data

Laboratory Data	**Diabetic (mean ±SD)**	**Non-Diabetic (mean ±SD)**	**p value**
AST(u/L)	25.18 ±7.14	30.46 ±6.19	< 0.001 (HS)
ALT(u/L)	28.22 ±7.84	32.99 ±8.65	< 0.001 (HS)
T-Bil (mg/dL)	0.97 ±0.55	1.10 ±0.44	(NS)
D-Bil (mg/dL)	0.22 ±0.20	0.26 ±0.17	(NS)
Alb (g/dl)	3.73 ±0.43	3.85 ±0.35	0.048 (S)
Glu (mg/dl)	258.97 ±49.36	93.14 ±14.23	< 0.001 (HS)
Creat (mg/dl)	1.07 ±0.28	0.96 ±0.16	< 0.001 (HS)
Hb (g/dl)	10.23 ±1.28	12.18 ±1.80	< 0.001 (HS)

NS: non-significant, S: significant, HS: highly significant

Table 3: The relationship between inflammatory mediators and the studied groups

Inflammatory Mediators	**Diabetics (mean ±SD)**	**Non-Diabetic (mean ±SD)**	**p value**
CRP	21.21 ±13.01	4.97 ±3.24	< 0.001(HS)
αFP	9.14 ±3.26	11.55 ±10.95	0.063 (NS)
TNF-α	254.49 ±79.44	177.08 ±84.85	< 0.001(HS)
IL-6	208.80 ±61.50	158.44 ±34.32	< 0.001(HS)

NS: non-significant, HS: highly significant

Table 4: relationship between *Helicobacter pylori* positivity and Age and Laboratory Data

Parameter	**Diabetic**				**Non-Diabetic**				**p value**
	H.p (+)		**H.p (-)**		**H.p (+)**		**H.p (-)**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age	41.39	8.58	38.33	6.54	39.95	8.85	38.81	7.59	> 0.05
AST	25.41	7.56	24.80	6.50	30.18	5.84	30.71	6.55	> 0.05
ALT	29.22	7.74	26.57	7.83	32.42	8.09	33.50	9.19	> 0.05
T-Bil	1.01	0.63	0.91	0.38	1.04	0.34	1.15	0.51	> 0.05
D-Bil	0.25	0.24	0.18	0.08	0.25	0.15	0.27	0.19	> 0.05
Alb	3.73	0.44	3.72	0.44	3.93	0.29	3.78	0.39	> 0.05
Glu	255.20	47.19	265.13	52.96	94.24	14.96	92.14	13.64	> 0.05
αFP	8.84	3.35	9.63	3.11	11.21	7.45	11.85	13.44	> 0.05
Creat	1.08	0.29	1.07	0.27	0.95	0.13	0.96	0.18	> 0.05
Hb	10.40	1.26	9.96	1.28	12.41	1.83	11.98	1.77	> 0.05
BMI	32.12	7.48	33.23	7.04	27.89	9.41	24.26	7.85	> 0.05
IL-6	208.18	56.86	209.80	69.43	156.42	32.24	160.26	36.40	> 0.05
TNF-α	252.35	92.03	258.00	54.15	180.58	71.43	173.90	96.17	> 0.05

were 254.49 ±79.44 in diabetic group which is also highly significant compared to control group, t value = 5.937 and p ≤0.001. In addition, the levels of interleukin-6 (IL-6) were increased in patients with diabetes and they were 208.80 ±61.50 and 158.44 ±34.32 in diabetic patients and non-ones respectively, which is highly significant (t value = 6.386 and p < 0.001). However, the mean levels of αFP were 9.14 ±3.26 in diabetics and 11.55 ±10.95 in non-diabetics which was non-significant as t test = -1.875 and p value = 0.063.

As regard the relationship between *H. pylori* and Ag and some laboratory investigations in diabetic and non-diabetic cases, it was non-significant, p value were > 0.05 in all.

4 Discussion

Helicobacter pylori is a gram-negative bacterium that colonizes the stomach and causes persistent infection [36] [29]. It has been shown to have a world-wide distribution [27].

DM patients are usually prone to chronic infections due to cellular and humoral immune deficiency [7].

There was no significant difference in the prevalence of *H. pylori* in diabetic and non-diabetic group as 49 (62%) were positive for H.p in Diabetic group and 38 (47.5%) in Non-Diabetic group (p < 0.05), Although diabetics have nearly 15% higher prevalence of *H. pylori* infection than control group. Our results showed that *H. pylori* infection is not significantly associated with type 2 diabetes mellitus in this studied groups. These findings were in agree with some authors [4] [11]. Moreover, in 2008, Ugwu *et al.* [37] reported the same in a study done in South East region of Nigeria between diabetic and diabetic groups (35% and 28% respectively, p = 0.432). The results are also in keeping with the others from various other regions of the world. Australia. [13], Italy. [40] and China [32], Turkey [11], Romania [24] and Egypt [31].

Although these findings were not in consistent with others [17] [22] [42] who found that the prevalence of *H. pylori* was significantly higher in the DM group compared to the control group.

A previous meta-analysis for the association between *H. pylori* infection and DM reported a significantly high odd ratio (OR) (1.33, 95% CI 1.08-1.64) [15], although the analysis mainly included case-control studies and only few studies in East Asian populations.in addition Fayed *et al.*,[21] stated that prevalence of *H. pylori* infection regarding total anti-*H. pylori* antibodies was significantly higher in the diabetic group (75.5%). Kayar *et al.*, [34] who had shown a significant relationship between *Helicobacter pylori* infections and metabolic syndrome, insulin resistance, inflammations, and diabetic complications. Moreover, Tamura *et al.*, [41] report findings that did not support an association between *H. pylori* infection and DM.

The variability of prevalence rates of *H. pylori* may be related to the epidemiological distribution of *H. pylori* or the kind of diagnostic method to detect infection. The presence of micro-angiopathy in patients with DM may be an inhibiting factor for colonization by *H. pylori* because micro-vascular changes in the gastric mucosa may create an unsuitable media for the growth or survival of *H. pylori* [14].

In this study we used *Helicobacter pylori* Antigen ELISA Kit For the in vitro Determination of *Helicobacter pylori* in stool because it is rapid and non-invasive method with high sensitivity (97.7%) and specificity (96.3%), it is potentially very helpful in diagnosing active and repeated *H. pylori* infection in primary care centers [5], as it is considered as a more simple sampling method (only one stool specimen is required) and relatively cheap [23]. There is no need for trained personnel at the testing site or expensive equipment as well as indicating the presence of a current active infection; the stool antigen test is very helpful in the diagnosis of *H. pylori* [18].

In the current study CRP, TNF-α and IL-6 are inflammatory mediators that showed highly significance relations between Diabetic group and no diabetic one as P value was < 0.001. *H. pylori* infections cause microvascular damage and trigger premature development of atherosclerosis in patients. [9]. This may be due to vessel wall invasion of the bacteria, an increase in maturation and activation of monocytes and an increase in the proliferation of smooth muscle or endothelial cells occur, and, as a result, thrombosis and ischemia develop. It is thought that the endotoxins produced by the bacteria play a role in the maturation of monocytes and atherosclerotic plaques due to *H. pylori* triggers high amounts of IL-6 and TNF-α [16]. Increased levels of inflammatory cytokines in our study were in disagreement with Al-Shukaili *et al.*, [2] who carried out a study for analysis of inflammatory mediators in

Type 2 diabetes patients. So still *H. pylori* may have a role in releasing of inflammatory mediators in diabetic group.

BMI was significantly higher in our diabetics group as p value was < 0.001.This can be explained that, diabetic patients usually had increase in insulin resistance and it lead to increase in BMI of diabetics, this supports that higher BMI is a well-established risk factor for diabetes mellitus Type 2 which was in agreement with Costanzo *et al.* [10].

Since the risk of DM is influenced by lifestyle factors, age and sex, our results found that no statistically significant difference in sex in between studied groups.

As regard age, in our study there were statistically non-significant relation in mean value of age in relation to *H. pylori* infection in diabetic and non-diabetic groups. These results were in agree with El-Eshmawy *et al.* [25].

The mean value of age was 41.39 ± 8.58 in diabetic patients have *H. pylori* infection. Although in other studies the mean age was 60 years [37] [20]. Also, a study by Sargýn showed that the mean age of diabetic patients with *H. pylori* infection is 56 years.

Our data reported that there was no relation between BMI and *Helicobacter pylori* infection. In an American study by loannou *et al.* [26] showed that *H. pylori* seropositive had no statistically significant relation to body mass index or fasting serum leptin level. Although Papamichael *et al.* [26] and Arslan *et al.* [6] considered that obesity can be a risk factor for *H. pylori* infection.

5 Conclusion

Our results indicate that *H. pylori* infection may not have an association with DM and don't consider as a risk factor for diabetes among the studied groups. Patients with established T2DM had different cytokine profile than controls. This indicates that, there is an alteration in the function of the immune system in T2DM patient. As Diabetes mellitus is a multifaceted and multistep disease, a larger set of samples and different methods to diagnose *Helicobacter pylori* in diabetics are needed for further studies in the future.

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