Original Article

Evaluation of Some Immunological Parameters in Type II Diabetic Patients Infected With *Helicobacter pylori*

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Abstract

Background: *Helicobacter pylori* is a pathogen of human stomach mucosa that leads to severe inflammation due to its virulence factor and stimulation of many cytokines. **Objectives:** To find the relationship between *H. pylori* and Diabetes mellitus type two (DMII) through the evaluation of some immunological markers. **Materials and Methods:** This study was conducted on 146 participants, divided into three groups: group A included 80 subjects suffering from DMII with a positive *H. pylori* stool antigen result; group B included 40 patients with DMII and a negative *H. pylori* test; and 26 were in the control group.Two types of samples were taken from all participants: (1) A stool sample for rapid detection of *H. pylori* antigen in stool by using immunochromatographic cassette technique. (2) 5 mL of blood samples for HbA1c (immunodetection), fast serum glucose (hexokinase/G-6-PDH) analysis, and immunological markers. By using the ELISA technique for quantitatively estimating serum *H. pylori* IgG and cytotoxin-associated gene A (CagA) IgG , interleukins (1β and 10). **Results:** *Helicobacter pylori* stool Ag test was positive in 80 individuals and negative in 66. There was a high significant increase ($P < 0.01$) in the concentrations of (FSG, HbA1c) in groups A and B. Also a high significant increase ($P < 0.01$) in the concentrations of IgG for *H. pylori* and CagA in addition to a high significant increase ($P < 0.01$) in the levels of interleukin-1β and interleukin-10 in group A compared to group B and control. **Conclusion:** Infection with *H. pylori* has a notable effect on the development of DMII, especially when the bacteria possess a virulence factor CagA which has severe influences on some inflammatory cytokines, which causes damage to extra-gastric organs.

Keywords: CagA, *Helicobacter pylori*, IL-10, interleukin-1β, type II diabetes mellitus

Introduction

Helicobacter pylori is a gram-negative, motile, microaerophilic bacteria that prefers an acidic environment, like the human gastric, due to urease activity. It is transmitted through many routes, but the most common is the fecal oral route. Through infection, which may be asymptomatic, can cause gastric inflammation and may progress to chronic gastritis, peptic ulcers, or duodenal ulcers, and in some cases, can lead to gastric cancer.[1,2] *Helicobacter pylori* has many virulence factors that have an impact on the severity of some inflammatory mediators, like the cytotoxin-associated gene A protein (CagA) and vacuolating cytotoxin A. Innate and adaptive immune responses of the host are essential for activating immune cell receptors, which leads to the production and release of a variety of proinflammatory cytokines.[3]

In some states of *H. pylori*, chronic infection causes systemic inflammation that affects other organs outside of the digestive system and leads to other diseases such as autoimmune thyroiditis, cardiovascular disease, asthma, iron deficiency anemia, and metabolic syndrome like diabetes mellitus.[4]

Diabetes mellitus is one of the most common diseases at present and results from prolonged elevation of blood glucose due to inadequate or absent insulin secretion.[5,6] DMII occurs due to genetic or environmental factors such

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as lifestyle, feeding pattern, aging, or microbial infection.[7] Chronic consequences of this metabolic disorder include microvascular and macrovascular complications, which, in addition, make individuals more susceptible to microbial infections.[8]

Interleukin-1β (IL-1β) is synthesized immediately by numerous immune and non-immune cell types and released in response to inflammatory signals, It is only cytokines that are processed by caspase-1 upon inflammasome-mediated activation.[9] It has been demonstrated to be involved in cell proliferation, differentiation, and apoptosis and is a key mediator of the inflammatory response.[10] Additionally, it is considered a vital pro-inflammatory cytokine during the pathogenesis of *H. pylori*-associated gastrointestinal disorders.[11]

Interleukin (IL)-10 has potent anti-inflammatory and immunosuppressive effects; it is a pleiotropic cytokine produced by a variety of immune cells such as T helper 2 and dendritic cells.[12] IL-10 inhibits excessive T cell activation and proliferation as well as the production of pro-inflammatory cytokines and antigen presentation.[13,14] Helicobacter pylori commonly utilizes IL-10's immunosuppressive function to escape the host's defenses, survive, and persistence for a long time in the gastric microenvironment.[24] So, we aimed to find the relationship between *H. pylori* in infected patients and the progression of DMII through the evaluation of the levels of *H. pylori* CagA IgG, IL-1β, and IL-10.

Materials and Methods

This study included 146 participants (male and female) attending Azadi Teaching Hospital and Kirkuk General Hospital in Kirkuk City from November 2022 to April 2023. All of them answered the questioner's information, which included age, sex, BMI, type of drug, period of diabetic history, other chronic diseases, etc.

Samples

Three types of samples were taken from participants: Stool sample for rapid detection of *H. pylori* antigen by using (Neo nostics cassette, China) immunochromatographic method. As a positive result, a special, pink-colored band is obvious in the test line regions, in addition to a pink line in the control line areas. While negative the test line region has no lines. 5 mL of venous blood samples were drawn, and 2 mL samples were put in EDTA tubes for the HbA1c test by using (HbA1c ichroma™, Boditech Med Incorporated, Korea). And 3 mL of blood placed in plain tubes for the separation of the serum samples by centrifuge (Hettich, Germany) for 10 min at 3000 rpm. The serum sample was divided into two parts: the first part for fast serum glucose using (GluC Kit, Abbott, Germany). The HbA1c result of more than 6.5% and 126 mg/dL for FSG is considered to be that of a diabetic patient. The second part was put in eppendorf tubes and stored at –20ºC to perform other tests, *H. pylori* IgG, *H. pylori* CagA IgG, interlukin-1β and interleukin-10 (Sunlong Kits, China) using the ELISA (Sandwich-ELISA) technique (BioTek, USA).

Study groups

The volunteers have been divided into three groups according to the results of the *H. pylori* stool antigen, FSG, and HbA1c tests:

- Group A: included 80 patients (34 males and 46 females) suffering from DMII with a positive *H. pylori* stool antigen result, ranging in age from 37 to 78 years.
- Group B: included 40 (19 males and 21 females) suffering from DMII with a negative *H. pylori* stool antigen result, aged 41–78 years.
- Control groups: included 26 (13 males and 13 females) healthy individuals, ranging in age from 21 to 69 years.

Exclusion criteria

- Patients with type I diabetes mellitus.
- History of *H. pylori* antibiotic treatment.
- Patients who already had an *H. pylori* infection and were receiving treatment for it prior to being chosen as participants were included in the exclusion criteria.
- In addition, those who were receiving long-term, persistent non-steroidal anti-inflammatory drug treatment.
- Diabetic patients who did not exceed 5 years of diabetes history were also excluded.

Statistical analysis

Statistical analysis was done by the statistical programs SPSS IBM version 26 (Chicago, IL, USA) to measure the mean and standard deviation (mean ± SD) among groups using the one-way ANOVA. Probability (P value) > 0.05 is considered non-statistically significant; P value < 0.05 is considered statistically significant; and P value < 0.01 is highly statistically significant.

Ethical approval

The study protocol, subject data, and consent forms were reviewed and approved by a local ethics committee in accordance with the document number (1-11-2022/48204).

Results

Table 1 illustrates a significant increase in the mean levels of blood glucose and glycated hemoglobin (P value 0.01) in group A male (253.00 ± 28.6) (9.218 ± 1.922), female (256.70 ± 22.2) (8.917 ± 1.849) and group B male (252.70 ± 29.9) (8.468 ± 1.651), female (243.80 ± 18.3) (8.005 ± 1.317), respectively, compared to the control male (96.08 ± 7.08) (5.254 ± 0.701), female (99.31 ± 9.24) (5.538 ± 0.477). Moreover, there was a non-significant difference between the A and B groups.

In Table 2, there is a highly significant increase in the level of IgG antibody in groups A male (90.99 ± 30.56) female (94.77 ± 60.26) compared with group B male (71.67 ± 17.49) female (70.96 ± 15.68) and control male (47.92 ± 20.13) female (62.26 ± 19.03). Furthermore, there is a significant difference between group B and the control.

The result in Table 3 shows highly significant differences in the mean value of the *H. pylori*-CagA IgG titer of group A male (343.6 ± 25.0) female (326.4 ± 23.8) when compared to the control group male (229.5 ± 25.4) female (219.3 ± 27.1) and group B male (235.7 ± 49.3) female (250.0 ± 30.7). There is no significant difference between groups B and control.

Table 4 shows the results of IL-1β. A highly significant difference was found (*P* value 0.01) in groups A male (89.04 ± 14.26) female (88.63 ± 15.13) compared with the control group male (45.83 ± 10.49) female (49.90 ± 10.26) and there was also a significant difference between group B male (67.07 ± 10.37) female (59.16 ± 13.38) compared with the control group.

Table 1: Concentration of blood glucose and HbA1c among study group

Groups	Gender	No.	FSG mg/dL (M ± SD)	HbA1c % (M ± SD)	*P*-value
Group A	Male	34	253.00 ± 28.6[a]	9.218 ± 1.922[a]	0.01**
	Female	46	256.70 ± 22.2[a]	8.917 ± 1.849[a]	
Group B	Male	19	252.70 ± 29.9[a]	8.468 ± 1.651[a]	
	Female	21	243.80 ± 18.3[a]	8.005 ± 1.317[a]	
Control	Male	13	96.08 ± 7.08[b]	5.254 ± 0.701[b]	
	Female	13	99.31 ± 9.24[b]	5.538 ± 0.477[b]	

[a, b] Small letters indicate presence significant differences between groups ($P < 0.01$)

**highly significant increased ($P < 0.01$)

Table 2: *H. pylori* IgG antibody concentration among study groups

Groups	Gender	No.	*H. pylori* IgG pg/mL (M ± SD)	*P*-value
Group A	Male	34	90.99 ± 30.56[a]	0.01**
	Female	46	94.77 ± 60.26[a]	
Group B	Male	19	71.67 ± 17.49[b]	
	Female	21	70.96 ± 15.68[b]	
Control	Male	13	47.92 ± 20.13[c]	
	Female	13	62.26 ± 19.03[b]	

[a, b, c] Small letters indicate presence significant differences between groups ($P < 0.01$)

**highly significant increased ($P < 0.01$)

Table 3: Estimation of *H. pylori*-CagA IgG concentration among study groups

Groups	Gender	No.	*H. pylori* CagA IgG pg/mL (M ± SD)	*P*-value
Group A	Male	34	343.6 ± 25.0[a]	0.01**
	Female	46	326.4 ± 23.8[a]	
Group B	Male	19	235.7 ± 49.3[b]	
	Female	21	250.0 ± 30.7[b]	
Control	Male	13	229.5 ± 25.4[b]	
	Female	13	219.3 ± 27.1[b]	

[a, b] Small letters indicate presence significant differences between groups ($P < 0.01$)

**highly significant increased ($P < 0.01$)

Table 4: Concentration of serum IL-1β among study groups

Groups	Gender	No.	IL-1β pg/mL (M ± SD)	*P*-value
Group A	Male	34	89.04 ± 14.26[a]	0.01**
	Female	46	88.63 ± 15.13[a]	
Group B	Male	19	67.07 ± 10.37[b]	
	Female	21	59.16 ± 13.38[b,c]	
Control	Male	13	45.83 ± 10.49[c]	
	Female	13	49.90 ± 10.26[c]	

[a, b, c] Small letters indicate presence significant differences between groups ($P < 0.01$)

**highly significant increased ($P < 0.01$)

Table 5: Level of serum IL-10 concentration among study population

Groups	Gender	No.	IL-10 pg/mL (M ± SD)	*P*-value
Group A	Male	34	67.53 ± 10.30[a]	0.00**
	Female	46	67.59 ± 11.37[a]	
Group B	Male	19	41.03 ± 8.270[b]	
	Female	21	37.73 ± 8.620[b]	
Control	Male	13	46.00 ± 8.060[b]	
	Female	13	47.97 ± 8.920[b]	

[a, b] Small letters indicate presence significant differences between groups ($P < 0.01$)

**highly significant increased ($P < 0.01$)

Table 5 shows the results of serum interleukin-10 level among the study groups, that indicate a significant increase (P value < 0.01) in the level of IL-10 in A male (67.53 ± 10.30) female (67.59 ± 11.37) compared to the control group male (46.00 ± 8.060) female (47.97 ± 8.920), whereas a non-significant decrease was observed in the level of interleukin-10 in group B male (41.03 ± 8.270) female (37.73 ± 8.620) compared to the control group.

Discussion

There are numerous hypotheses and concepts regarding the association between *H. pylori* and diabetes, including those that focus on the possibility of systemic infections triggered by bacterial infection, the impact of *H. pylori* on certain hormones, or the possibility that individuals with diabetes mellitus have weakened immune systems that facilitate bacterial infection. that is, why we noticed a high level of *H. pylori* IgG in people with diabetes. *Helicobacter pylori* infection is mostly associated with cytokine production,[15] but these cytokines may also be released into the bloodstream and influence other tissues and organs, which could increase the incidence of extra-gastrointestinal disorders. These inflammatory cytokines most likely result in insulin resistance, which, when hyperglycemia is present, increases the long-term effects of diabetes.[4] In our research, we have noticed a high level of virulence factor IgG in diabetes mellitus, According to some studies, *H. pylori* Cag+ strains prevent the pancreas from producing insulin by interacting with the somatostatin hormone. ADditionally, *H. pylori* increases ghrelin and leptin levels as well as the risk of diabetes and obesity in people.[16] Cytotoxin-associated gene A, one of *H. pylori's* major virulence factors, has been recognized. CagA-positive *H. pylori* strains are believed to induce more severe stomach mucosal inflammation than CagA-negative strains. The *H. pylori* strains that are cagA-positive are related to higher risks of peptic ulcer disease and gastric cancer.[16]

From a different angle on this association, we observe that diabetes is related to a weakened immune system. Uncontrolled diabetes mellitus, especially hyperglycemia, impairs diabetics' immune systems, making DM a known risk factor for various infectious diseases.[17] Numerous studies support our findings, such as the meta-analysis conducted by Mansori in 2020, which found a positive correlation between infection with *H. pylori* and DM.[16]

IL-1β is a regulator of the inflammatory which is produced in response to antigenic exposure, infection, and tissue injury.[18] Research has shown that long-term exposure to low concentrations of IL-1β results in epigenetic modifications that lead to the suppression of insulin production and loss of beta-cell identity, indication marker of type II diabetes.[19] Many researches indicate that DMII have low grade-inflammation due to elevation of some proinflammatory cytokines such as IL-1β.[20] We noticed that this result was in agreement with our results. Thus, we can assume that the elevation of IL-1β for long periods leads to systemic inflammation that may affect other organs, such as the stomach, inhibiting gastric acid secretion, which creates a perfect environment for *H. pylori* colonization. IL-1β plays a critical role in both initiating and increasing the inflammatory response to *H. pylori* infection, in addition to being a powerful inhibitor of gastric acid production. The activity of the gastritis and the pH of the gastric juice are related to the expression of the IL-1β mRNA. ADditionally, early on following *H. pylori* eradication, patients without stomach cancer displayed significant declines in IL-1β mRNA and protein levels.[21]

IL-10 is a multipurpose anti-inflammatory cytokine. The production of proinflammatory cytokines, antigen presentation, and T cell proliferation have all been demonstrated to be inhibited by IL-10.[22,23] The immunosuppressive property of IL-10 is frequently used by *H. pylori* to evade the host's defenses, live, and remain for a long period in the gastric milieu.[24]

According to a study, children with *H. pylori* infections had higher levels of IL-10 (134.3500 ± 8.18391) than healthy controls (96.2000 ± 7.20088).[25] Additionally, serum IL-10 levels were greater in individuals with mild to severe ulcerative colitis who had positive *H. pylori* 13C-urea breath test results.[26]

In diabetic patients, many studies have suggested that a high level of HbA1c is associated with a low level of IL-10.[27] Alkaisey showed that IL-10 was linked to the pathophysiology of T2DM and that a low level of IL-10 was related to an elevated level of HbA1c.[28] The result of

this study has supported our result in terms of a decrease in IL-10 in diabetic patients, where a low level of IL-10 can impact the immune system and make it susceptible to many infections.[29-30]

Conclusion

We concluded that there was an association between *H. pylori* infection and the progression of diabetes as a result of alterations in some immunologic parameters such as IL-1β and IL-10, especially when *H. pylori* have an increased level of the virulence factor cytotoxin associated gene A.

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Conflicts of interest

There are no conflicts of interest.

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