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Study of Certain Immunological Aspects in Diabetes Mellitus Type 2 Patients Infected by Helicobacter Pylori

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Abstract

Type 2 diabetes mellitus (T2DM) is one of the metabolic diseases characterized by hyperglycemia and includes individuals who have relatively insulin deficiency and insulin resistance. Helicobacter pylori (H.pylori) is a spiral-shaped bacterium, infects 50% of the world's people, and inhabits in the human gastric epithelium, causing many diseases. It had been suggested that H. pylori infection is more frequent among T2DM patients. Aim of this study is to evaluate the levels of interferon gamma (IFN-γ) in Iraqi patients with type 2 diabetes mellitus infected by H.pylori bacteria. Anti-H.pylori IgG and IFN-γ concentrations were measured by enzyme-linked immunosorbent assay (ELISA). The study includes 140 patients, divided into 3 groups, which are a group of patients infected by T2DM and H.pylori (T2DMHp+ve), a group of patients infected by T2DM but not by H.pylori group (T2DMHp-ve), and the third group includes patients infected with H.pylori only (Hp), in addition to apparently healthy control group (AHC). Our study revealed significantly decreasing in serum IFN-γ in both T2DMHp+ve group ($P<0.05$) and T2DMHp-ve group($P<0.01$), compared with an apparently healthy control group(AHC). Also, this study shows decreased IFN-γ level in patients with Hp group compared with healthy control but without significant difference. Also, the concentration of IFN-γ in T2DMHp+ve is slightly more than that in T2DMHp-ve group.

Key words: *T2DM, Anti-H. Pylori IgG, IFN-γ*

Introduction

Diabetes mellitus (DM) is one of the metabolic diseases characterized by increased blood sugar (hyperglycemia) due to defects in insulin secretion, insulin action, or both (1). By the year 2030, DM will be the 7th principal cause of death according to the World Health Organization (2). Some pathogenic processes are associated with the development of diabetes. These processes include destroying the beta cells (β-cells) of the pancreas with subsequent insulin deficiency, and others that cause resistance to insulin activity (3).

Type 2 diabetes (T2DM), accounts for about 90–95% of all diabetes cases. This form includes individuals who have relatively insulin deficiency and peripheral insulin resistance (4). T2DM is the primary cause of death in only 13% of mortality cases, whereas the rest 87% is due to diabetes-associated complications–including infections (5).

Patients with diabetes are found to have a reduced cytokine response to acute infections, and the immune system induces inflammation in type 2 diabetes mellitus (T2DM) and triggers insulin resistance along with diabetic complications (6, 7).

Helicobacter pylori (H.pylori) is a microaerophilic, gram-negative and spiral-shaped bacillus. It infects 50% of the world's people, although most infected individuals have no clinical symptoms (8, 9).

H. pylori are capable to attack the stomach lining, and causes gastritis, peptic ulcer, gastric lymphoma, and gastric adenocarcinoma. It is adapted to survive in the acidic and harsh nature of the stomach which has an ability to change the stomach pH so they can survive (10, 11). It is indicated that the worsen glycemic and metabolic control rises the rates of H. pylori infections and in the same time, this pathogen is considered as one of the common problems of diabetic patients with

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conditions of gastrointestinal diseases (12).

Interferon-gamma is a key immunoregulatory protein which plays a major role in both innate and adaptive immune response. The biological effects of IFN- are widespread. IFN-γ although may be implicated in protection versus H.pylori infection but in a long-term infection, IFN-γ may play a vital role in gastric inflammation (13).

The aims of the current study is to evaluation of IFN-γ concentrations in diabetic patients infected by H.pylori, diabetic patients non- infected with H.pylori, non-diabetic patients infected by H.pylori, in comparison with apparently healthy control.

Materials and Method

Samples Collection

Ninety eight blood specimens were collected from suspected individual's revealed positive result by using H.pylori IgG rapid test. ELISA IgG test indicated that only (92) patients have H.pylori infection. 3 ml of venous blood were taken from patients and control group using 5 ml disposable syringes under sterile condition. The specimens were collected in gel tubes and left for 30 minutes until clotted at room temperature. The tubes were centrifuged at 3000 rpm for 5 minutes, and then the serum is divided into 3 equal parts in Eppendorf tubes and stored in (-400C) until it was used.

Rapid Anti-H.pylori Test

Helicabacter pylori rapid test is a lateral flow chromatographic immunoassay based on the principle of the double-antigen sandwich technique.

Diagnosis of Anti-H.pylori IgG by ELISA

The human body produces IgG when infected by H.pylori. This test was performed according to Human H.pylori ELISA Kit (14).

Method principle

The surface of microplate wells is coated with a target protein and incubated with a primary antibody to the target protein, followed by a (secondary antibody) versus the primary antibody. After washing, the microplate well-bound enzyme activity is estimated with the microplate reader at 450 nm.

Interferon Gama (IFN-γ) Determination

Determination of Interferon Gama (IFN-γ) was performed according to the protocol of Human IFN-γ ELISA Kit (15).

Principle of the assay

This kit is based on the sandwich technique. Anti IFN-γ monoclonal antibodies were coated on microtiter well. Calibrators and samples react with the capture monoclonal antibody and with a monoclonal antibody labeled with HRP (sandwich formation). After incubation and washing, the bound enzyme-labelled antibody is measured through a chromogenic reaction by added TMB solution.

Results and Discussion

Distribution of the groups

This study includes 140 individuals divided into 3 groups including patients with diabetes mellitus type 2 infected by H.pylori (T2DMHp+ve) were 45 cases, patients with diabetes mellitus Type 2 not infected by H.pylori (T2DMHp-ve) were 48 cases and non-diabetic patients infected by H.pylori (Hp) were 47 cases. In addition to these groups, there is an apparently healthy control group (AHC) were 20 cases. According to the Specialized Center for Endocrinology and Diabetes-Baghdad/Al-Rusafa, diagnosis of T2DM was performed, while infections with H.pylori were determined initially by antibody rapid test as a screening test, then followed by ELISA as a confirmatory test.

Serum Anti H.pylori IgG levels

A. Rapid test

Ninety eight from 140 individuals gave positive results in rapid anti-H. pylori IgG as screening and qualitative test, and when confirmed by using ELISA method, only 92 of individuals gave positive result while all negative results in the rapid test were corresponding to ELISA results. So, the sensitivity of Anti- H.pylori rapid test were 100%,and the specificity was 87.5%, as shown in table 1. IgG Ab against H.pylori typically appears approximately 21 days after infection and can remain for a long period beyond eradication (16).

The low specificity of the immunoassays is may be due to cross-reaction between prepared crude antigen containing proteins with antibodies directed against

other bacteria (17).

Table 1: Comparison of Anti-H.pylori IgG results between the rapid test and ELISA.

Method		ELISA		
	Results	**Positive**	**Negative**	**Total Result**
H. Pylori Rapid test	Positive	92	6	98
	Negative	0	42	42
Total results		92	48	140

Relative sensitivity: 100%, Relative specificity: 87.5%

B. Using ELISA method

Table 2 revealed a highly significant difference (P<0.001**) of serum anti-H. pylori IgG in T2DMHp+ve group were 41.6980±4.85127and Hp group were 45.0590 ± 5.132 AU/mL in compared with a control group were (3.12 ± 0.211).Also, this study showed non-significant differences between T2DMHp-ve and healthy control group.

Table 2: Anti H.pylori IgG serum levels, detected by ELISA test in the study groups

Type of groups Anti-H.pylori IgG (M±SE)	**Type of groups Anti-H.pylori IgG (M±SE)**	**P value**
AHC (3.1264±0.211)	T2DMHp+ve (41.6980±4.851)	0.001**
	Hp (45.0590±5.13277)	0.001**
	T2DMHp-ve (3.0212±0.13873)	0.67 N.S

AHC: apparently healthy control; T2DMHp+ve: a group with type 2 diabetes mellitus infected by H.pylori; Hp: group infected with H.pylori; T2DMHp-ve: type 2 diabetes mellitus non infected by H.pylori; M±SE: Mean ± Standard error; ** highly significant P<0.01; N.S: Non-significant P>0.05.

The ELISA is an easy, cheap, effective and reliable immunological test, an alternative to UBT (Urea breath test) for detection of H. pylori infection (18, 19). Also, this study agreed with Mansour (2) who confirmed elevated the immunoglobulin (IgG) during infection with H.pylori. The immune system of the host produces antibodies (Abs) against H.pylori immediately after bacterial gastric colonization. The immunologic tests developed for detection of immunoglobulin g (IgG) antibodies that specific for H. pylori in the serum of patients.

The types of ELISA are more suitable for diagnosis of H. pylori infection in epidemiological studies of peoples. Different antigen preparations have been prepared in coating ELISA wells, in these methods: crude antigens, such as (whole cell extracts, glycine extracts, sonicated cell extracts), heat-stable antigens and recombinant antigens. Antibody levels remain in the blood for a long time. The best performance of these tests is dependent on specificity for detection of (local) H. pylori strains in each country by choosing antigens for coating the (ELISA) wells. Serological tests are recommended in many studies for epidemiological studies, and they based on the many reasons: rapid, inexpensive, availability, and easy in use, but it is not recommended for the confirmation of H. pylori elimination after treatment (20).

Comparison of IFN-γ levels among the study groups

The level of IFN-γ in patients in the study groups (T2DMHp+ve, Hp, T2DMHp-ve, and healthy control group) were (2.83 ± 0.29, 3.85 ± 0.65, 2.65 ± 0.28, and 4.07 ± 0.58 IU/mL) respectively.

Data in the table 3, revealed decreased significantly in serum IFN-γ in both T2DMHp+ve group (P<0.05) and T2DMHp-ve group(P<0.01) in compared with an apparently healthy control group(AHC).

Also, this study shows decreased IFN-γ level in patients with Hp group compared with healthy control but without significant differences between them. Also, it has demonstrated that the concentration of IFN-γ in T2DMHp+ve is slightly more than that in T2DMHp-ve group.

Table 3: Serum interferon gamma levels in the study groups

Type of groups M±SE of IFN-γ(IU/ml)	M±SE of IFN-γ (IU/ml)	P value
AHC (4.0738±0.58197)	T2DMHp+ve (2.8319 ± 0.29659)	0.05*
	Hp (3.8584 ± 0.65944)	0.8 (N.S)
	T2DMHp-ve (2.6520 ± 0.28704)	0.01**

AHC: apparently healthy control; T2DMHp+ve: a group with type 2 diabetes mellitus infected by H.pylori; Hp: H.pylori infected group; T2DMHp-ve: type 2 diabetes mellitus non infected with H.pylori; M±SE: Mean ± standard error; *significant; ** highly significant P<0.01; N.S: Non-significant P>0.05.

Type 3 diabetic patients obviously had a decreased capacity of IFN-gamma production by a down-regulated production of cytokine molecules in the endoplasmic reticulum of both (CD4 and CD8) T-lymphocytes compared to healthy individuals (21). Many studies agreed with our result that decreased IFN-γ in T2DM patients (22-25). But disagree with Amin and his co-workers (26), who found elevated IFN-γ concentration in T2DM and that indicate activation of circulating T-cells against infection.

IFN-γ may be suppressed by H.pylori as a strategy to escape immune destruction through interference with IFN-γ signaling and that may cause permanent infection by H.pylori in gastric epithelial cells (27). The expression of cholesterol-α-glucosyl transferase (cgt) by H.pylori reduces cholesterol concentrations in infected gastric epithelial cells which result in blocks IFN-γ signaling, allowing H.pylori to escape the inflammatory response of the host (28).

In 2015, Yang and his coworkers (29) agreed with this study who found non-significant differences of IFN-γ in H.pylori infected persons compared with the control group. In contrast, different studies disagreed with this study (30, 31) who revealed that raised production of lipopolysaccharides, may trigger innate inflammatory processes which associate with increased inflammatory cytokines, also Abdollahi (13) and Bimczok(32) showed increased IFN-γ concentration in H.pylori infected patients and that indicates T-cells activation against infection ,and the IFN-γ maintains mucosal inflammation and may stimulate disease development to gastric ulcer.

Conclusions

1. Concentrations of serum interferon gamma (IFN-γ) decreased significantly in type 2 diabetic patients whether infected or non-infected by H.pylori. Also, slightly decreased IFN- γ level in H.pylori infected patients who are non-diabetic.

2. Anti-H.pylori IgG rapid test is important for the primary diagnosis of H.pylori infection, although ELISA IgG gives more sensitivity and specificity for confirmation of this infection.

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