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RESEARCH ARTICLE

ASSOCIATION BETWEEN LATENT AUTOIMMUNE DIABETES OF ADULTS (LADA) IN TYPE II DM IN IRAQ WITH *Helicobacter pylori* INFECTION

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ABSTRACT

Background: Latent autoimmune diabetes in adults (LADA) accounts for 2%-12% of all cases of diabetes. Patients are typically diagnosed after 35 years of age and are often misdiagnosed as type Ⅱ Diabetes Mellitus (DM). Glycemic control is initially achieved with sulfonylureas but patients eventually become insulin dependent more rapidly than with type Ⅱ DM patients. Although they have a type Ⅱ DM phenotype, patients have circulating beta (β) cell autoantibodies, a hallmark of type Ⅰ DM. Alternative terms that have been used to describe this condition include type 1.5 diabetes, latent type Ⅰ diabetes, slowly progressive Insulin Dependent Diabetes Mellitus, or youth onset diabetes of maturity. With regards to its autoimmune basis and rapid requirement for insulin, it has been suggested that LADA is a slowly progressive form of type Ⅰ DM. **Objective:** The objective of the present study was to evaluate the ratio of Latent Autoimmune Diabetes of Adults (LADA) patients among Iraqi type II DM, To quantify the association between H. pylori infection and (LADA) patients and To detection of H. Pylori IgG and detection of its virulence factors (anti-CagA and anti-VacA) and which of them is more prevalence with H. pylori positive samples. **Methods:** A total of 350 patients with type II DM admitted to National Diabetes center of AL-Mustansiriyah University/ Al-Yarmouk/ Baghdad. Blood sample where collected from 350 patients and serum sample collected from these patients and 40 healthy persons. Radioimmunoassay technique using to identify the C-peptide serum levels of these patients, ELISA technique for detection of anti-GAD65, anti-H.pylori IgG, anti-CagA and anti-VacA, also the Blood HbA1c concentration were measured. **Results:** The diagnosis of LADA patients was based on clinical findings, immunological and chemical analysis of blood. Fifty-seven of patients were have low C-peptide serum level, from which, 40 patients were have autoantibody to Glutamic Acid Decarboxylase (Anti-GAD65 Ab) diagnosed as LADA patients and the other seventeen patients as classical type II (positive control).The mean C-peptide level in patients with LADA was 0.30 ng/ml, 1.94 ng/ml in control and 3.15 ng/ml in classical type II. It is clear from the range of serum C-peptide level that there are no overlapping values seen for serum C-peptide in groups. **Conclusion:** Serum C-peptide levels can be used in the early diagnosis of LADA patients; Also Anti-GAD65 is more valuable marker than the other predictive marker. Similarly, they may be may be useful in differential diagnosis among types of diabetes to assess treatment efficacy.

Keywords: LADA, C-peptide, GADA, H. pylori IgG, anti-CagA, anti-VacA.

INTRODUCTION

Diabetes mellitus (DM) includes a group of carbohydrate metabolism disorders which is characterized by hyperglycemia and leads to long-term macrovascular and microvascular complications. The prevalence of DM is increasing considerably in most of the developed and many developing countries, and it is of great concern [1].

American Diabetes Association categorized DM mainly as types I and II diabetes and the others [2].

LADA is generally classified as a sub-type of diabetes mellitus type 1 although recent research suggests that is not exactly correct because LADA has some characteristics of diabetes mellitus type II (thus “diabetes 1.5”). The onset of LADA is slower than diabetes mellitus type I (thus “slow diabetes”) [3].

About 20% of the patients diagnosed with type II diabetes may have LADA. This accounts for 5–10% of the total diabetes population, the same number as type I diabetes [4].

LADA is defined by three features including: (1) adult age at diagnosis, (2) presence of diabetes-related autoantibodies and (3) delay from diagnosis in the need for insulin therapy to manage hyperglycemia. Because insulinoma-associated antigen-2 (IA-2) autoantibodies are usually found with GAD autoantibodies but rarely in LADA, this condition is widely defined by the presence of Glutamic Acid Decarboxylase (GAD) autoantibodies [5].

Helicobacter pylori is a micro-aerophilic spiral-shaped Gram-negative bacterium that colonizes the stomach for almost the entire lifetime of the host, and it infects more than half of the world's human population, and produces gastro-duodenal diseases, like peptic ulcer disease in about 10% and gastric adenocarcinoma in 1–2% of those that it infects. It is a common infection in diabetic patients who have inadequate metabolic control as these individuals are colonized by the *H. pylori* infection in gastric antrum, probably due to chemotactic factors such as tumor necrotic factors (TNF), interleukines-IL-1,IL-2, and IL-8 are present in the gastric epithelium [6].

Many putative virulence factors have been identified in *H. pylori* that involved in its pathogenesis, most important among them is Cytotoxin Associated Gene A (CagA), it is 120-kilo Dalton (kDa) protein [7]. Another important virulence factor of *H. pylori* is a secreted toxin known as vacuolating cytotoxin (VacA) that induces the formation of intracellular vacuoles in epithelial cells [8].

MATERIALS AND METHODS

A total of 350 patients aged from 23 days to 70 years were enrolled in the study without any treatment with insulin, 57 patients have low C-peptide, from which, 40 patients have Anti-GAD65 positive and 17 patients have Anti-GAD65 negative. The remaining numbers of samples (293) were excluded.

This study was carried out at a consultation clinic for Diabetes Mellitus/ National Diabetes center /Baghdad. The control group was revealed by 40 healthy individual whose age range was between 25 to 65 years.

Patient divided into groups 40 LADA patients, 17 classical types II (positive control) and 40 controls (negative control) by using and Radioimmunoassay technique, biochemical tests, and ELISA technique.

Blood serum samples were taken from all cases, Sign & Symptoms of patients and findings at the time of diagnosis, demographical data also were reported.

C-peptide levels were measured by using Radioimmunoassay technique. Its detection when the value less than 0.75 ng/mL in LADA , more than 2.5 ng/mL in type II and the level in healthy individual as determined within normal range 0.75-2.5 ng/mL.

Anti-GAD65 Ab, Anti-*H.pylori* IgG, Anti-CagA Ab and Anti-VacA Ab were done by using ELISA technique.

Statistical analysis of data was carried out in a windows setting using the SPSS version 21.0 and excel program. Differences between groups in continuous variables were tested for significance with the t-test. A p-value of ≤0.05 was regarded as statistically significant.

RESULT

Serum level of C-peptide among studied groups

The mean value of serum C-peptide concentration in patients was 0.30 ng/ml± 0.24, 1.94 ng/ml ± 0.41 in control and 3.15 ng/ml ± 1.71 in classical type II and the P-value was 0.048 (≤ 0.05) as shown in table 1, this means that there is a significant decrease in serum C-peptide concentration in patient compared to control and classical type II patients.

Table 1: C-peptide serum concentration among LADA patients compared to controls and classical type II patients.

Subjects	Serum C-peptide (0.75-2.4ng/ml)
LADA Patients (Mean ± SD)	0.30 ± 0.24
Control (Mean ± SD)	1.94 ± 0.41
Type II patients	3.15 ± 1.71
P-value	*0.0481

*** P ≤ 0.05 (Significant)**

Anti-Glutamic Acid Decarboxylase (Anti-GAD65)

From 350 type II diabetic patients, there were 40(11.4%) GADA positive and 310(88.5%) GADA negative, as in table 2.

Table 2: Frequency of islet cell autoantibody Anti-GAD65 (LADA patients) among all type II diabetic patients:

Parameter	Positive		Negative		Total
	No	%	No	%	
Anti-GAD65 Ab	40	11.4	310	88.5	350(100)

Age group and GADA discover

The distribution of GADA according to the age group was illustrated in table (3).

It was shown that those who have DM before age of 50 years (the mean±SD to them was 38.67±6.68 years) significantly had higher prevalence of autoantibody (GADA) than those who developed the DM later after the age of 50 years (the mean±SD to them was 48.58±11.93 years), the 97.5% of GADA positive was in type II diabetic patients who developed the disease before age of 50 years in comparison to 2.5% of GADA positive in type II diabetic patients who developed the disease after this age.

Table 3: Distribution of anti- GAD Ab in studied groups according to the age group

Age groups	Anti-GAD65 Ab		Total
	Positive >5 U/ml	Negative <5 U/ml	
25-50 Count	39	42	81
% within age	48.1%	51.9%	100.0%
% within Anti-GAD	97.5%	73.6%	
>50 Count	1	15	16
% within age	6.2%	93.7%	100.0%
% within Anti-GAD	2.5%	26.3%	
Total Count	40	57	97
% within age	41.2%	58.8%	100.0%
% within Anti-GAD	100.0%	100.0%	100.0%
P-value	*0.001		

*** P ≤ 0.05 (Significant)**

Gender and LADA patients

The number of males was n=22 (55%) and females was n=18 (45%) and the p-value was 0.904 as shown in table 4. This means that there is no significant difference between female and male in LADA patients.

Table 4: Relation between gender and LADA.

Index		LADA	p-value
Gender	Male	22 (55%)	
	Female	18 (45%)	0.904 NS
Total		n= 40	

P > 0.05 NS (Not Significant)

Anti-*Helicobacter pylori* IgG

The higher frequency of *H. pylori* infection was in LADA patients when compared with patient's controls (PC) and healthy controls (NC) (59.6%, 21.3% and 19.1%, respectively) as showed in table 5. Also according to the total number of each group, the higher frequency of H. pylori infection was in diabetic patients when compared with the controls (70%, 58.8%, 22.5%)

Table 5:Prevalence of *H. pylori* IgG among studied groups

Parameter			Type Patient	Control	Pt Ctrl	Total	p-value
HP IgG Group	>12 U/ml	Count	28	9	10	47	
		% within HP IgG Gp	59.6%	19.1%	21.3%	100.0%	
		% within Type	70.0%	22.5%	58.8%	57.7%	
	<=12 U/ml	Count	12	31	7	50	
		% within HP IgG Gp	24%	62%	14%	100.0%	*0.001
		% within Type	30.0%	77.5%	41.2%	42.3%	
Total		Count	40	40	17	97	
		% within HP IgG Gp	41.2%	41.2%	17.6%	100.0%	
		% within Type	100.0%	100.0%	100.0%	100.0%	

Anti-Cytotoxic associated gene A (Anti-CagA)

The total frequency of *CagA*$^+$ strains was 36/47 (76.6%) in all study groups. According to the total number of *H. pylori* positive patients the frequency was 42.6% in LADA group, 17% in Positive Control group, and 17% in Negative Control group. The difference in frequency of *CagA*$^+$ between groups was significant in *H. Pylori* positive Patients and negative cases (*p<0.05*) as in table 6.

Table 6: The frequency of *H.pylori* CagA strain (Anti-CagA) in positive IgG Anti-*Hp* samples among studied groups.

Positive IgG Anti-*Hp*/groups FR(%)	Anti-CagA IgG		Total FR(%)	P-value
	Negative <18 U/ml FR(%)	Positive >18 U/ml FR(%)		
LADA no=28/40 (70%)	8/47 (17)	20/47 (42.6)	28 (59.6)	<0.05*
Patient control no=10/17 (58.8%)	2/47 (4.3)	8/47 (17)	10 (21.3)	<0.05*
Healthy control no=9/40 (22.5%)	1/47(2.1)	8/47 (17)	9 (19.1)	<0.05*
Total no=47/97 (48.5%)	11/47 (23.4)	36/47 (76.6)	47/47 (100)	

***=Significant p-value <0.05**

Anti- Vacuolating Cytotoxin A (Anti-VacA)

The total frequency of *VacA*$^+$ strains was 16/47 (34%) in all study groups. According to the total number of each the frequency was 32.1% in LADA group, 30% in Positive Control group, and 44.4% in Negative Control group. The difference in frequency of *VacA*$^+$ between groups was not significant in *H.Pylori* positive Patients and negative cases (*p>0.05*) as in table (7).

Table 7: The frequency of the *H.pylori* VacA strain (Anti-VacA) in positive IgG Anti-*Hp* samples among studied groups.

Positive IgG Anti-*Hp*/groups FR(%)	Anti-VacA IgG		Total	P-value
	Negative FR(%)	Positive FR(%)		
LADA	19 (67.9)	9 (32.1)	28 (100)	0.501
Patients control	7 (70)	3 (30)	10 (100)	NS
Healthy control	5 (55.6)	4 (44.4)	9 (100)	
Total	31 (66)	16 (34)	47 (100)	

NS (Not Significant P > 0.05)

DISSCUSION

Latent autoimmune diabetes in adults (LADA) is a complex autoimmune form of diabetes that is sometimes referred to as type 1.5 diabetes. LADA is often mistakenly diagnosed and treated as type II diabetes (University of California, 2011)[9]. The use of immunological markers, especially C-peptide and Anti-GAD65, has been proposed to facilitate the accuracy of the initial diagnosis of LADA.

This study shows that C-peptide was significantly decreased in latent autoimmune diabetes of adults (LADA) patients when compared with controls and classical type II DM as in table 1. This finding is agreement with The American Diabetic Association 2007 [10], which suggests that LADA (also known as Ab positive type 2 diabetes) has lower C-peptide levels than patients with Ab negative type 2 diabetes suggesting that insulin deficiency could progress with time and lower C-peptide values may be achieved at a later stage. This indicates that these patients are likely to be patients of LADA.

Estimation of GAD autoantibodies can be used as an important diagnostic marker for diagnosis of LADA (Clare-Salzler et al., 1999) [11]. The results of this study showed that the prevalence of LADA was 11.4% among diagnosed type II DM patients aged 23-70 years as in table 2; this determination is in accord with other investigators (Pham et al., 2012) [12] who mentioned a prevalence of 10% among age group 40–75 years. In a series of 256 patients > 25 years, found that 26 (10.2%) were GADA positive (Assicot et al., 1998) [13].

When age of patients is taken in consideration, it was found that the prevalence of these autoantibodies was more common in younger patients (25-50 years) who develop diabetes before age of 50 years (97.5%) in comparison with those who developed diabetes after age of 50 years (2.5%) which is statistically significant (P<0.05),as in table 3. The explanation of this finding may be due to the facts that those patients who develop the disease before age of 50 years have HLA-DR3/DR4 and show significant higher frequencies of anti-islet cell antibodies while those who begin the disease after 50 years were HLADRB1/DRQB1 which show much less evidence of autoimmunity (Groop et al., 2000; Pozzilli et al., 2001; Hosszufalusi et al., 2003) [14 -16]. This result is in agreement with Elbein *et al*., 1997[17], Turner *et al*., 1997 [18]**,** and Nabhan *et al.,* 2005 [19] who estimated a significantly higher prevalence of these antibodies (GADA) in young patients with type 2 DM.

According to the global prevalence of diabetes studies, overall, diabetes prevalence is higher in men than in women (Wild et al., 2004) [20]. In the current study the number of males was 22/40(55%) and females was 18/40(45%), this mean there is no significant difference between males and females in LADA patients as shown in table 4. the findings of current study are going with (Priyanka et al., 2012) [13] who revealed that diabetes was found to be more prevalent in men (61%) than in women (39%)

Since the discovery of *Helicobacter pylori*, it has been shown to have a world-wide distribution. It has been estimated that up to half of the world's population harbor the infection in their stomachs (Perez-Perez et al., 2004) [21]. The results of this study showed significantly higher positive *H. pylori* infection 38/47 (80.8%) among diabetic patients compared to controls 9/47 (19.2%) as shown in table 5. An increased prevalence of *H. pylori* infection among diabetes mellitus patients was first suggested by many reports. One of them documented a *H. pylori* prevalence rate of 74.4% in Type 2 diabetes mellitus patients as against 40% in non-diabetic controls (Gentile et al., 1998) [22].

The total frequency of *CagA*$^+$ strains was 36/47 (76.6%) in all study groups. According to the total number of *H. pylori* positive patients the frequency was 42.6% in LADA group, 17% in Positive Control group, and 17% in Negative Control group. The difference in frequency of *CagA*$^+$ between groups was significant in *H. Pylori* positive Patients and negative cases (*p<0.05*) as in table 6. In the current study, the prevalence of *CagA*$^+$ strains was (76.6%) in all *H. pylori* Positive patients among studied groups (Table 6). This result was usual when it compared with many other studies in different places in the world as 78% to 80% in Turkey (Erzin et al., 2006) [23], 82% in Japan (Azuma et al., 2004) [24], 61.6% in Tunisia (Ben-Mansour and Fendri, 2010) [25], and 47.6% to 63.4% in Mexico (Torres et al., 2005) [26].

The total frequency of *VacA*$^+$ strains was 16/47 (34%) in all study groups. According to the total number of each group, the frequency was 32.1% in LADA group, 30% in Positive Control group, and 44.4% in Negative Control group as in table 7. The difference in frequency of *VacA*$^+$ between groups was not significant in *H. Pylori* positive Patients and negative cases (*p>0.05*). The finding of current study is agreement with (Ogiwara et al., 2008) [27] were unable to confirm the *vacA* as a virulence determinant in 314 strains isolated from East Asia and Southeast Asia where gastric cancer is highly prevalent.

And not going with other studies that found the *VacA* strain have a higher prevalence in European, African and American strains (Togrul et al., 2009) [28].

In conclusion, C-peptide and Anti-GAD65 were a good markers for differentiating LADA patients from T2DM, The prevalence of LADA patients among Iraqi type II diabetic patients was 11.4%, there was no significant difference between gender and LADA. The number of *H. pylori* infected patients was higher in LADA patients compared with type II and control, Cytotoxic associated gene A protein (Cag A) protein is more prevalence with H. pylori positive patients than Vacuolating cytotoxin A Protein (Vac A) which is less prevalence. Cag A protein can be used as marker to discern the risk of developing serious gastroduodenal disease in the host. Further studies on large scale are needed to support our result.

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