SCREENING OF CELIAC DISEASE IN IRAQI PATIENTS WITH TYPE 1DIABETES MELLITUS [+]

Hanaa Naji Abdullah [□] **Hanaa Jabbar Saber** [□□] **Median Ali Hussein** [□□]

Abstract :

Type 1 diabetes mellitus is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic β-cells. The prevalence of celiac disease in patients with T1DM is approximately 20 fold that in the general population, ranging between 2%-6%. One hundred patients with type 1 diabetes mellitus and fifty apparently healthy volunteers were screened for celiac disease using the IgA, IgG anti-gliadin, anti-tissue transglutaminase and total serum IgA. The mean level of C-peptide among diabetic patients in comparison with healthy control which was (0. 901±0.088 ng/ ml) *vs.* (0.691±0.120ng/ml) (P< 0.0001). Anti-gliadin positivity in the current study showed (30%) for diabetic cases with non significant difference. Frequency of anti- tissue tranasglutaminase antibody positivity for diabetic patient's sera was (35%) while the control groups was 0.0% for healthy cases. It is clear that the concentration of IL-6 was highly elevated in the sera of diabetic patients with positive anti-tissue transglutaminase in comparison with diabetic patients without anti-tissue transglutaminase with significant differences (P<0.001). Additionally, there is non significant differences in the concentration of IgA in the sera of diabetic patients in comparison with healthy group. On the other hand, there was a highly significant finding between (AGA and Anti-tTG),(AGA and IgA) that were detected among diabetes patients (P<0.001).

Key word: Type 1 diabetes mellitus, Celiac disease, anti-gliadin, anti- tissue tranasglutaminase,

التحري عن داء حساسية الحنطة في المرضى العراقيين المصابين بداء السكري من النمط الاول

هناء ناجي عبدالله هناء جبار صبر مدين علي حسين

[+]**Received on 6/2/2012 , Accepted on 28/11/2012 .**

[*]**Lech . /College of Health and Medical Technology**

[**]**Ministry of Health, Teaching of Al-yarmouk hospital.**

المستخلص:

داء السكري النمط الأول هو مرض مناعي ذاتي مزمن مرتبط بتحطيم انتقائي ضد خلايا بيتا المنتجة لهرمون الأنسولين. أن نسبة تواجد حساسية الحنطة في مرضى داء السكري تقريبا عشرون ضعفا عن المجاميع عموما, تتراوح بين 2%- 6%. شملت الدراسة (100) مريضا مصابا بداء السكري من النمط الأول و 50 متطوعا من الأشخاص الأصحاء ظاهريا حيث تم التحري عن داء حساسية الحنطة باستخدام IgA,IgG anti-gliadin, anti-tissue transglutaminase, total serum IgA. وقد أظهرت النتائج ان متوسط (C-peptide) لمرضى السكري (0.901±0.088 ng/ml) مقارنة بمجموعة لأصحاء (0.691±0.120 ng/ml) . وبفارق معنوي لقيمة اقل من (P<0.0001). تم أجراء فحص التحري لأضداد الكلايدين للمجاميع الدراسة . حيث أظهرت (30%) من حالات السكري نتائج الايجابية للأضداد من نوع anti-gliadin مع عدم وجود فروق معنوية. في حين بينت النتائج ان نسبة ايجابية أضداد tissue transglutaminase في مرضى السكري كانت (35%) مقارنة بمجموعة السيطرة (0.0%). حيث أظهرت نسبة الايجابية anti-tissue transglutaminase زيادة معنوية في المرضى مقارنة بمجموعة الأصحاء (P<0.0001). يبدوا واضحا ان هنالك زيادة مرتفعة في تركيز IL-6 في مصول مرضى السكري ذوي الاجسام المضادةanti-tissue transglutaminase الموجبة مقارنة مع مرضى السكري من النمط الاول ذوي العينات الخالية من تلك الاضداد زبفرق معنوي ملحوظ (P<0.0001). بالإضافة الى ذلك, لم يكن هنالك فروق معنوية في زيادة تركيز ال IgA في مصول مرضى السكري مقارنة بمجموعة السيطرة. ومن جانب أخر, هنالك علاقة معنوية بين AGA (AGA and IgA ,and An ti-tTG) , عند التحري عن تلك المؤشرات في مرضى السكري (P<0.001).

Introduction:

Type 1 diabetes Mellitus (T1DM) is an autoimmune disease that leads to the destruction of pancreatic B cells and insulin deficiency. It is common in childhood and adolescence but can occur at any age [1]. It can be associated with other autoimmune disorder such as celiac disease and autoimmune thyroid disease. The associated autoimmune disease may influence the control of diabetes by impairing function of the respective organ [2]. The association between celiac disease (CD) and type 1 diabetes mellitus (T1DM) has been studied for almost 50 years. Both are autoimmune conditions resulting from a complex interaction between genetic, immunological and environmental factors [3]. The concurrence of two diseases may be explained by a similar genetic background associated with HLA-DQ2, HLA-DQ8 and similar trigger mechanism for autoimmune process [4,5]. The Prevalence of CD in patients with T1DM ranges between 1.3-12% through the world and may contain a high proportion of asymptomatic and atypical cases [6]. Several serological tests are used as a screening method for CD and included anti-gliadin, antireticulin, anti-tissue transglutaminase and anti-endomysial antibodies [7]. The main role of these tests in clinical practice is in screening patients with diseases associated with CD and who may have atypical or silent forms of CD, (e,g type 1 diabetes), in order to avoid an unnecessary intestinal biopsy [8]. Till now there are no available data on the incidence of celiac disease in Iraqi diabetes patients. The aim of this study was to determine the seroprevalence of celiac disease in T1DM by using (anti-gliadin antibody IgA, IgG, tissue transgultaminase antibody

IgA, IgG and total IgA level) for screening and association between celiac disease and T1DM .

Subjects and methods.

Patients and control subjects:

A total of 100 patient with T1DM (61 male, 39 female) with T1 DM who were attending National Center for Diabetes Management and Research. All patients were selected according to the revised criteria for diagnosis of DM as defined by the American Diabetes Association (ADA) (1997). The age range between (1-55y), with a mean of 14.37± 8.46y.

Fifty healthy control group (25 male, 25 female) whose ethnic back ground, age, and sex matched the patients group, and consisted of unrelated non-diabetic individuals according to the laboratory finding of fasting blood glucose value <6mml/L where considered as a control group. The age range between (6-45y), with a mean of 13.56± 4.63y.

Laboratory Methods:

- **Measurement of gliadin, tissue transglutaminase antibodies.**

IgA and IgG – AGA and IgA and IgG – tTG antibodies were measured by ELISA technique [9,10].

- **Measurement of C-peptide.**

Endogenous C- peptide was measured by ELISA Technique.

- **Measurement of Serum Immunoglobulin (IgA) by Using Single Radial Immunodiffusion (SRID).**

The concentrations of IgA were measured by a single radial immunodiffusion SRID method in which equal volumes of reference sera and test samples were added to wells in agarose containing monospecific antisera. The sample diffuses radically through this gel and the substance being assayed from a precipitin ring with the monospecific antisera. Ring diameters were measured and a reference curve is constructed on graph paper.

- **Statistical Analysis**

SPSS (Statistical Package for Social Sciences v. 10 was used for analysis. Descriptive statistics were used to describe the number and percentage of age groups and gender for patients and control. Mean and standard deviation was used to describe age, duration, Fasting blood sugar, C-peptide, IgA, AGA, AtTG among patients and control. P-value of less than 0.05 was considered statistically significant.

Results :

The level of both fasting plasma glucose and C-peptide were highly significantly altered among the patients' group in comparison with control group as shown in Table 1. It's clear from this table that there was a significant difference in fasting plasma glucose level among diabetic patients (266.38±11.08 mg/dL) in comparison with apparently healthy control group (92.980±1.943 mg/dL) (P< 0.0001). While the level of C-peptide among diabetic patients (0.

901±0.088 ng/ ml) appeared to be less than that for healthy control group (0.691±0.120ng/ml) with highly significant difference between the two groups (P< 0.0001).

Table 1: Levels of FPG and C-peptide among diabetic and control groups.

Groups	(FPG)mg/dL		(C- Peptide)Ng/ ml	
	Study	Control	Study	Control
N	100	50	100	50
Mean	266.38	92.980	0.901	0.691
Std. Error	11.082	1.943	0.0881	0.120
Std. Deviation	110.82	13.741	0.8814	0.854
	t=11.003 : P<0.0001 HS		t=-5.223: P<0.0001 HS	

The frequency of AGA positivity for TIDM patient's sera (30%) rather than the control groups 0.0% respectively (table 2). Anti-tTG positivity (35%) in the patients compared to the controls (P<0.0001), as summarized in (table 3).

Table 2:- The frequency of Anti-gliadin (IgA, IgG) antibodies among studied groups.

Studied groups		Anti-gliadin		Total	P-value
		Positive	Negative		
TIDM patients	N	30	70	100	(P> 0.05) NS
	%	30	70	100	
Healthy group	N	0.0	50	50	
	%	0.0	50	100	

Table 3: - The frequency of Anti-tTG Antibodies among studied groups.

Studied groups		Anti-tTG		Total	P-value
		Positive	Negative		
TIDM patients	N	35	65	100	0.0001<
	%	35	65	100	
Healthy group	N	0.0	50	50	
	%	0.0	50	100	

The concentration of immunoglobulin A (IgA) has been estimated in the sera of the studied groups. There is non significant differences in the concentration of IgA in the sera of diabetic in comparison with healthy group as shown in table (4).

Table 4: -The Mean of immunoglobulin (IgA) level among the sera of the studied groups (T1DM patients & apparently healthy control).

Immunoglobulin		No.	Mean	Std. Deviation	t-test P-value	Sig.
IgA	Healthy group	50	208.534	85.292		
	T1DM patients	100	221.924	135.731	0.525	NS

The concentration of some cytokines (IL-6) has been estimated in the sera of the studied groups. It is clear that the concentration of IL-6 was highly elevated in the sera of diabetic patients with positive anti-tTG in comparison with diabetic patients without anti-tTG with significant differences (P<0.001), as shown in table (5).

Table 5: -The Mean of interlukin-6 level among the sera of the studied groups (T1DM patients and apparently healthy control).

Studied groups	No.	Mean	Std. Deviation	t-test P-value	Sig.
T1DM patients with positive anti-tTG	50	11.33	2.22	.000	HS
T1DM patients without anti-tTG	100	134.40	164.25		

The result of this study proved that a non significant correlation was observed between (FBG and C-peptide, AGA, Anti-tTG, IgA) among type 1 diabetes patients and healthy control groups. On the other hand, there was a highly significant finding between (AGA and Anti-tTG),(AGA and IgA) which were detected among diabetes patients ,as shown in table (6).

Table 6: The association between anti-gliadin antibodies and anti-tissue transglutaminase, IgA.

		FBS	C-peptide	Ant-GA	Anti-tTG	IgA
FBS	Pearson correlation		0.025	0.030	0.090	0.169
	P value		0.861	0.836	0.533	0.242
	Significant		NS	NS	NS	NS
C-peptide	Pearson correlation	0.025		0.127	0.059	-0.109
	P value	0.861		0.378	0.683	0.451
	Significant	NS		NS	NS	NS
Anti-GA	Pearson correlation	0.030	0.127		0.313□□	0.439□□
	P value	0.836	0.378		0.002	0.001
	Significant	NS	NS		S	S
Anti-tTG	Pearson correlation	0.090	0.059	0.313□□		0.156
	P value	0.533	0.683	0.002		0.279
	Significant	NS	NS	S		NS
IgA	Pearson correlation	0.169	-0.109	0.439□□	0.156	
	P value	0.242	0.451	0.001	0.279	
	Significant	NS	NS	S	NS	

□□Correlation is significant at the 0.01 level (2-tailed).

Discussion:

Type one diabetes mellitus (TIDM) is an organ-specific disease characterized by cell destruction in glucose tolerance. The ADA recommended a FPG as a simplified diagnostic test for diabetes, it is a simple blood test which is done after eight hours (over-night) fasting, the value of 126 mg/dl (7.0 mmol/L) or higher is considered to be diabetes. In addition, the level of C-peptide is a measure of how much insulin is being produced in the body, most patients with T1DM have no detectable fasting C-peptide secreting remaining, So C-peptide were done to differentiate between T1DM and T2DM. A decrease of C-peptide concentration confirms the progression of insulitis and destruction of β- cells. The worsening of diabetes control in C-peptide negative patients may reflect the inability of organism to cope with abnormal metabolic situation, although a sufficient amount of exogenous insulin is being supplied [2]. The current study was nearly compatible with other studies which found the mean concentration of screening AGA-IgG, IgA was comparable in diabetes patient and healthy control ($P<0.01$), while AGA-IgA was significantly higher in the patients than in the control [2]. On other hand, these findings are higher than an Egyptian study who mentioned that AGA level was (4.3%) [11]. However, AGA is not disease specific, and some patients with active CD lack these antibodies. In contrast, patients with diseases other than CD and healthy individuals occasionally have elevated levels of IgA-AGA and IgG-AGA [18]. These antibodies disappear rapidly during a gluten-free diet [19]. It was noticed that the result of different studies were heterogeneous, this may be caused by the manufacturing companies with different serum dilution and different types of AGA (e.g. screening and AGA-IgA or AGA-IgG). Anti-tTG was a good serological marker for celiac disease and should be highly specific for the disease and be able to diagnose celiac disease in children and adult. These findings agreed with previous studies. It has been noted that there was significant difference of anti-tTG between Type 1 diabetes and healthy control ($P<0.03$) [2,20]. The prevalence of celiac disease in diabetes patients found in the present study was higher than rates observed in countries with socioeconomic conditions similar to ours, such as African studies using AGA or in India, with anti-tTG. In studies that used the human anti-tTG serological test for screening [3,21], it was shown that the result of different studies were heterogeneous, this may be caused by the manufacturing companies with different serum dilution and different methods of anti-tTG (e.g. screening and tTG-IgA or tTG-IgG). It's, however, known that patients with serology that is initially negative can become positive for CD over time. There is not yet consensus on the frequency with which serological tests should be repeated for diabetic patients. Some authors recommend serological screening at the time of diabetes type 1 diagnosis and annually or biennially thereafter [3]. Patients with T1DM showed a non significantly IgA level in sera compared with healthy control. This result seems to agree with previous studies which had shown that the concentration of IgA were non significant in diabetes mellitus patients compared with healthy control [3]. There is a strong evidence for the association between IgA deficiency and celiac disease, with frequency of approximately(3%). Individuals with IgA deficiency and CD do not form anti-IgA antibodies and the serological tests for anti-tTG commercially available are habitually limited to the IgA isotopes in antibodies. Serum IgA assay was included in the battery of tests in this study and it was observed that deficiency was confirmed in 3/35 (3%). Many studies reported that selective IgA deficiency is considered a risk factor for celiac disease and occurring with a high frequency of approximately 3% in both diabetes and CD [11,3].

Patients with type 1 diabetes showed a highly significant elevation of IL-6 in sera compared with healthy control. This result seems to agree with previous studies which had shown concentration of IL-6 that were significantly higher in diabetes patients with positive anti-tTG in comparison with diabetic patients without anti-tTG. These findings indicated that elevated serum IL-6 levels with positive anti-tTG compared with the patients without anti-tTG suggest that interleukin play a significant role in celiac disease development among T1DM might account not only for damage to kidney and eye but also for impairment of intestinal mucosa or might influence the incidence of celiac disease [21,22]. No relationships between high FBG and C-peptide, AGA, anti-tTG and IgA was observed in our study except that which was observed between (AGA and anti-tTG) , (AGA and IgA). A previous study found that single or combined autoantibody positivity had no influence on the control of diabetes if normal organ function was preserved. However, impaired organ function may be associated with worsened control of diabetes [2].

Conclusion:

Celiac disease is highly prevalent in patients with type 1 diabetes mellitus (35%) was diagnosed on screening. It is clear that the concentration of IL-6 was highly elevated in the sera of diabetic patients with positive anti-tTG in comparison with diabetic patients without anti-tTG.

References :

1. Redondo M., Yu L. ,Hawa M. ,Mackenzie T. , Pyke D. and Eisenbarth G. "Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States" *Diabetologia*,Vol. 44, No.3, pp.354–62, 2001.
2. Prazny M., Skrha J.,Limanova Z., Vanickova Z., Hilgerova J.; Prazana J. and Straiz I. "Screening for associated autoimmunity in type 1 diabetes mellitus with respect to diabetes mellitus with respect to diasbetes control" *Physiol* Vol. 54, pp.41-48, 2005.
3. Araujo J., Pontes G. and De Melo F. "Serum prevalence of celiac disease in children and adolescent with type 1 diabetes mellitus" *J Pediatr (Rio J)*, Vol.82, No.3, pp. 210-4, 2006.
4. Eisenbarth, G. (2008). Diabetes and related autoimmune disease . In Clinical Immunology Principle & Practice . Edited by rich, R.; Schroeder, H.; Fleisher, Th. ;Frew, A.;Shearer, W.& weyand, C. British Library publishers.767-787.
5. Schober E., Rami B., Granditsch G. and Crone, J. " Celiac disease in children and adolescents with type 1 diabetes mellitus to screen or not, to treat or not" *Horm Res*, Vol.57, pp.97-100, 2002.
6. Gronin C and Shanahon F. " Insulin dependent diabetes mellitus and celiac disease". *Diab Med , Vol.* 18, pp.169, 2001.
7. Kordonouri O., Dieterich W., Schuppn D., Webert G., Muller C. and Sarioglu A. "Autoantibodies to tissue transglutaminase are sensitive serological parameters for detecting silent celiac disease in patient with type 1 diabetes mellitus" *Diab Med*, Vol. 17, pp.441-4,2000.
8. Grodzinsky, E.; Hed, J. and Skogh, T. "IgA anti-endomysium antibodies have a high positive predictive value for celiac disease in asymptomatic patients" *Allergy*, Vol. 49, pp. 596, 1994.

9. Ascher H. and Lanner A. "A new laboratory kit for anti-gliadin IgA at diagnosis and follow-up of childhood celiac disease" *J Pediatr Gastroenterol Nutr*, Vol. 10, pp.443,1990.
10. Dieterich W. , Ehnis T. and Bauer M. " Identification of the tissue transglutaminase as the autoantigen of celiac disease" *Nat Med*, Vol. 3, pp.797,1990.
11. Saadany S.; Farrag W.; Saleh M.A., Saleh, M. Esmail, S.A., Menessy A. and Hamouda H. "Prevalence of celiac disease in Egyptian children and adolescent with diabetes mellitus: a clinical, Biochemical and histopathological study "Pediatr,100,pp84-91, 2010.
12. Mahmud F., Murray J. El-youssef M., Van Dyke C. and Brogan D. "Celiac disease in type 1 diabetes mellitus in a north American Community: prevalence, Serological screening and clinical features "*Myo Clin Proc*, Vol. 80, No.11, pp. 1429-1434, 2005.
13. Hsieh A. and Twigg S. "Celiac disease with diabetes: More than through chance alone" *The Australian celiac*, p.13, 2011.
14. Weets I., Rooman R., Coeckelberghs M., Deblock C.; Van Gaal L . "The age at diagnosis of type 1 diabetes continues to decrease in Belgian boys but not in girls: a 15-year survey″ Diabetes Metab Res Rev,Vol. 23, No. 8, pp. 637-43, 2007.
15. Scott CR ., Smith JM., Cradock MM . et al. " Characteristics of youth-onset noninsulin-dependent diabetes mellitus & insulin –dependent diabetes mellitus at diagnosis" *Pediatr* , Vol. 100, pp.84-91, 1997 .
16. Karvonen M. , Viik-Kajander M. , Moltchanova E. et al. Incidence of childhood type 1 diabetes worldwide . Diabetes mondiale (DiaMond) project group " *Diabetes Care*, Vol. 23, pp.1516-26 , 2000.
17. William E., Neil H. and Desmond S. "Immunological Markers in the Diagnosis and Prediction of Autoimmune Type 1a Diabetes" *Clini diabet*, Vol. 20, No.4, pp.183-91, 2002.
18. Bürgin-Wolff A., Gaze H., Hadziselimovic F., Huber H., Lentze MJ, Nussle D., et al. " Antigliadin and antiendomysium antibody determination for celiac disease" *Arch Dis Child* , Vol. 66, pp. 941-7, 1991.
19. Chirdo FG., Rumbo M., Carabajal P., Castagnino N. ; Mavromatopulos E. Cirincione V. , et al. "Analysis of anti-gliadin antibodies by immunoblot analysis and enzymelinked immunosorbentassay using gliadin fractions as antigens". *J Pediatr Gastroenterol Nutr* , Vol. 29, pp171-177, 1999.
20. Mysliwiec M., Balcerska A., Zorena K., Mysliwska J. and Wisniewski P." Immunological and biochemical factors of coincident celiac disease and type 1 diabetes mellitus in children" *Diab Med*, Vol. 64, No. 6, 677-81, 2008.
21. Sari S. , Yesilkaya E., Egritas A., Bideci A., Cinaz P. and Dalgic B. "Prevalence of celiac disease in Turkish children with type 1 diabetes mellitus and their non-diabetic first-degree relative" *Tur J Gastroenterol*, Vol. 21, No. 1, pp.34-38, 2010.
22. Mysliwiec M., Balcerska A., Zorena K., Mysliwska J., Wisniewski P., et al. " The activity of tumor necrosis factor-alpha and interlukin-6 at early stage of celiac disease development in type 1 diabetes mellitus children" *Pediatr Res*, Vol. 2, No.19, pp.1-2, 2007.