The Relationship Between The Type 1 Diabetes And Celiac Disease: A Study Based On Anti Tissue Transglutaminase Antibodies And Anti-Glidine Antibodies Screening

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(Received 26 /12 /2013 , Accepted 9 / 2 / 2014)

الخلاصة

الخلفية :تهدف الدراسة الحالية إلى دراسة معدل انتشار والخصائص السريرية للمرض الجوف البطني بين الأطفال العراقيين يعانون من مرض السكري من النوع الأول باستخدام مزيج من الفحوص المصلية الأكثر حساسية ومحددة ومسح للأجسام المضادة للترانسكلوتامنيزIgA,IgG والأجسام المضادة للكليادين IgA,IgG وتحديد أدنى قيمة قطع المضادة الترانسكلوتامنيز ومستوى الاضداد الكليادين للأفضل تنبا لمرض الجوف البطني في المرضى المصابين بداء السكري من النوع الأول **المواد** : حققت اثنين من مجموعات المرضى (كل 30) في هذه الدراسة واحدة مع مرض الجوف البطني واخرى مع داء السكري من النوع الاول مقارنة مع المجموعة الثالثة من المجموعة اصحاء من (20)فردا المرضى مع إيجابية للأضداد الترانسكلوتامنيز واضداد الكليادين(مل / U 18 >)، والمرضى مع معتدل الأضداد الترانسكلوتامنيز واضداد الكليادين(مل / U 18-12)، والتخفيف السلبية(مل / U 18<) **النتائج** : وكانت حساب متوسط المضادة للغليادين IgA (U \ مل)؛ 1.6، 6.15 و 27.4 بين مجموعة صحية، ومرض السكري من النوع الاول ، ومرض الجوف البطني على التوالي: والقيم ROC مثيرة للاهتمام (AUC 0،949-0،000-1) لهذا أضداد ذاتية كاختبار مثالي في هذا الجانب. في نفس السياق ، فئة IgG من هذه الاجسام المضادة وقعت في تركيز متوسط 3.05، 5.7، و21.85 بين المجموعات الثلاث، على التوالي. فإنه يختلف اختلافا كبيرا بين هذه المجموعة وبالمقارنة مع كل الأخرى. الاضداد الترانسكلوتامنيز المقاس، وقدمت كلIgA وIgG مع أدوات تمايز كبير بالمقارنة بين المجموعات المدروسة الثلاثة كان كل من قدم من الصنوف لهROC عالية (1،000-0،947) **الاستنتاج**: من نتائج هذه الدراسة، وخلصنا تداخل أضداد ذاتية لمرض الجوف البطني ومرض السكري من النوع الاول

Abstract:

Background: The present investigation aims to study the prevalence rate and clinical characteristics of celiac disease(CD)among Iraqi children with type1 diabetes mellitus using a combination of the most sensitive and specific screening serologic tests anti-tissue transglutaminase antibodies IgA,IgG(tTG)and anti-gliaden antibodies IgA,IgG(AGA) and to determine the lower cut-off value of anti-tTG and anti-AGA level that best predicts celiac disease in patient with type1 diabetes. **Materials:** Two patient groups have investigated (each to NO =30) in this study one with celiac disease and the other with type1 diabetes mellitus compared to third group of apparently healthy control group of 20 individuals. patient with positive anti-tTG titer and AGA titer (>18 U/ml) and patient with equivocal of anti-tTG and AGA titers(12–18 U/ml) and negative titers (<18 U/ml) **Results:** The median calculation of Anti-gliadin IgA (U\ml) were ;1.6 ,6.15 and 27.4 among healthy group ,type1 diabetes mellitus, and celiac disease patient respectively. An interesting ROC values (AUC 0.949-1.000) for this auto-antibodies as a perfect test in this aspect. In the same context, the IgG ,class of this auto-antibody occurred in median concentration of 3.05, 5.7 , and 21.85 among the three groups ,respectively. It significantly differed among this group and as compared to each other's. For anti-tissue transglutamenase assays ,Both IgA and IgG were provided with significant differentiation tools when compared among the three studied groups both classes provided had high ROC (0.947- 1.000). **Conclusion:** From the result of this study, we concluded the overlapping auto-antibodies profile celiac disease and type1 diabetes mellitus

Keywords: Celiac disease, Type1 diabetes, Anti-tissue transglutaminase, Anti-gliadin antibody, Receiver operated characteristics .

Introduction:

Celiac disease (CD) is a chronic, inflammatory disease of the small intestine induced by dietary proteins in wheat, rye, and barley. Only about one third of the identified patients presents with diarrhea; another third is diagnosed upon targeted screening, and one fifth presents with nonspecific, recurrent abdominal pain [1]. Moreover, they may be at increased risk for a number of CD-associated autoimmune disorders, like type 1 diabetes. The anti-gliadin antibodies (AGA) are synthesized by the diseased mucosa in patients with untreated coeliac disease [2]. It has been suggested that they may participate in an antibody-dependent cell-mediated cytotoxic reaction directed against absorptive cells or may form immune complexes, with gliadin components, releasing mediators such as arachidonate metabolites, histamine and eosinophilia cationic protein from mucosal inflammatory cells[3]. Anti-tissue transglutaminase (tTG) is Immunoprecipitation of proteins extracted from metabolically labeled fibro sarcoma cells with IgA from celiac disease patients led to the identification of tissue transglutaminase (tTG) as the prominent, if not sole, endomysial auto antigen [4]. tTG is a calcium-dependent ubiquitous intracellular enzyme that belongs to a family with 3 epidermal and 2 extracellular transglutaminases (Prostate transglutaminase and factor XIII)[5]. Activated endothelia, fibroblasts, and mononuclear cells are particularly rich sources of tTG. Gliadins that are glutamine-and proline rich proteins are excellent glut amyl donor substrates for tTG [6] . giving rise to gliadin-gliadin cross-links and even the covalent incorporation of tTG itself into high-molecular weight complexes[7]. Autoimmune endocrinological diseases such as AIDDM associated with celiac disease. The coexistence of these diseases could be explained by molecular mimicry by which gliadin or tissue transglutaminase activates T cells that are cross-reactive with various self-antigens. Such inflammatory responses may have the capacity to persist in genetically susceptible hosts and lead to chronic organ-specific autoimmune disease via epitope spreading[8] . However, it is unclear whether any sequence similarities exist between gliadin or tissue transglutaminase and, for example, glutamate decarboxylase antibodies associated with diabetes (GAD), insulin, thyroid peroxidase antibodies, or 21-hydroxylase. It is also possible that, apart from gliadin, tissue transglutaminase can modify other external or self-antigens by cross-linking or deamination and thus generate different neoantigens.These antigens and antibody production can further induce various autoimmune phenomena outside the intestine. On the other hand, apart from antiendomysial antibodies, celiac patients have an increased frequency of other autoantibodies; it is not known whether they play any pathological role[9]. There is evidence that in the development of autoimmunity in AIDDM, the failure to achieve tolerance to autoantigens derives from the gut. In patients with newly diagnosed AIDDM, the islet cell antigen GAD-reactive lymphocytes express the gut-specific homing receptor α4β7 integrin[8]. Interestingly, the prevalence of anti-tissue transglutaminase antibodies has been reported to be as high as 32% in HLA DQ2 homozygous AIDDM patients, as compared with 2% in patients without HLA DQ2 or DQ8[9]. **Aim of the study:** Evolution of Anti-gliadin and Anti-tissue transglutamense both of IgA and IgG classes in celiac and T1DM patients using ELISA .

Material and methods

This study was performed during the period from. November 2012 to September 2013. Subjects that were enrolled in this study. they were categorized into three groups, group 1 (30 subject of Celiac disease), group 2(30 subject of Diabetes mellitus), group3 (20 control healthy). In the current study the age of T1DM patients range was (4.3-18) years with mean (12.6) years and group of patients with celiac disease in the present work was clinically heterogeneous in ages with range (1-45) years with mean age (14.9) years whereas the age range for healthy group were (6-18) years with mean age (13.0)years. Blood samples were collected by vein puncture using disposable syringes under aseptic technique. Three milliliters of each sample were transferred to 10 milliliters sterile plain tube, centrifuge at 2500 rpm for 10 minutes and the separated serum was divided into several aliquots and immediately frozen at –20 c° till further use to avoid repeated thawing and freezing ELISA assay were used for detection and measurement of auto-antibodies specific for (anti-Gliadin IgA, IgG and anti-tTG IgA,IgG,) (Aesku Diagnostics Microform ring 2.55234 Wendelsheim Germany) were used in this work.

Statistics analysis

Data were translated into a computerized database structure. The database was examined for errors using range and logical data cleaning methods and inconsistencies were remedied. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010. The ROC method was used to evaluate the performance of a quantitative test in differentiating between a disease status (or an outcome) and a second comparison group.

Result

The median titer of Anti-gliadin Antibody of IgA class has significantly (P<0.001) raised among celiac disease patient (27.4 U\ml) compared with (6.15 U\ml) among diabetes mellitus type1 patient and (1.6 U\ml) among healthy control table(1) shows the higher range for this antibody among different studied groups, in celiac disease patients, the level range was (17.5-472.9 U\ml) followed by (2.1-58.3 U\ml) and (1.2 - 3.5 U\ml) in diabetes mellitus type1 patient and healthy control, respectively. The different parameters of anti-gliadin of IgG class over the three studied groups is presented in table (1). It shows statically significant differences in the median level of this auto-antibody (P <0.001) in the studied groups. It has risen in celiac disease patients (21.85 U\ml) compared with T1DM patients (5.7 U\ml) and healthy control group (3.5 U\ml). Moreover, the level range of Anti-gliadin IgG was (2.7 - 216.9 U\ml) in celiac disease group and (2.7-16.3 U\ml) in diabetes mellitus group and (2.7-6 U\ml) among healthy control individuals.

Table(1):Different(AGA-IgA,IgG)level parameters among study groups.

Anti-GliadinAntibody-IgA (U/ml)	Study group			
	Healthy controls (20)	Diabetes Mellitus (30)	Celiac disease (30)	P
Range	(1.2 - 3.5)	(2.1 - 58.3)	(17.5-472.9)	<0.001
Median	1.6	6.15	27.4	<0.001
P (Mann-Whitney) for difference in median between:				

Celiac disease x Healthy controls	<0.001			
Celiac disease x DM (positive controls)	<0.001			
DM x Healthy controls	<0.001			
Anti-GliadinAntibody(IgG)(U/ml)				
Range	(2.7 - 6)	(2.7 - 16.3)	(2.7 - 216.9)	<0.001
Median	3.05	5.7	21.85	<0.001
P (Mann-Whitney) for difference in median between:				
Celiac disease x Healthy controls	<0.001			
Celiac disease x DM (positive controls)	<0.001			
DM x Healthy controls	0.013			

The median titer of IgA anti-tissue transglutaminase for three group evaluted in thes study are shown in table (2)**.**There was signafecant differances in the median,range of IgA of this auto-antibody between the three groups studied. The median range in celiac disease group was (37.65 U\ml) ranged in (18.6-301 U\ml), in diabetes mellitus group median was (8.2 U\ml) ranged in (2- 69.4 U\ml) , while the median healthy control group was (2.4 U\ml) ranged in (1.8-4.8 U\ml). The results of the present study revealed highly significant differences (P <0.001) comparing celiac disease group with healthy control group or with T1DM group (positive control). A similar result has been shown when comparing T1DM group with healthy control group. The median of anti-tTG IgG class level were (15.35 , 4.6 , 1.65 U\ml, respectively) with range (3 - 109 , 2.2-31.9, 1.1 - 2.8 U\ml, respectively). A significant difference observed in diabetic patient's was compared with control groups(p<0.001).In addition, the anti-tTG IgG median concentration in celiac disease patient was significantly higher than in diabetes mellitus patient and healthy control (p <0.001).

Table(2): Different Anti-tTG-IgA, IgG)level parameter among studied groups.

Anti-TissueTransglutaminase IgA(U/ml)	**Study group**			
	Healthy controls (20)	**Diabetes Mellitus (30)**	**Celiac disease (30)**	**P**
Range	(1.8 - 4.8)	(2 - 69.4)	(18.6 - 301)	<0.001
Median	2.4	8.2	37.65	<0.001
P (Mann-Whitney) for difference in median between:				
Celiac disease x Healthy controls	<0.001			
Celiac disease x DM (positive controls)	<0.001			
DM x Healthy controls	<0.001			
Anti-Tissue Transglutaminase IgG(U/ml)				
Range	(1.1 - 2.8)	(2.2 - 31.9)	(3 - 109)	<0.001
Median	1.65	4.6	15.35	<0.001
P (Mann-Whitney) for difference in median between:				
Celiac disease x Healthy controls	<0.001			
Celiac disease x DM (positive controls)	<0.001			
DM x Healthy controls	<0.001			

The diagnostic and differentiating value of Anti-gliadin Antibody of IgA has been also calculated using ROC curves . In the differentiation of Celiac disease from healthy control, this test has offered perfect test value as it gained a 1.000 ROC area (Figure 1, Table 3). ROC curves to evaluate the diagnostic value of this auto-antibody, revealed a high ROC value (0.938) for anti-gliadin Antibody of IgG when used as test to predict Celiac disease differentiating them from healthy control.The Receiver operated characteristics curve analysis of tTG IgA ,IgG autoantibody has offered area under of curve (AUC of 1.000) a perfect test in the prediction of Celiac disease differentiating them from healthy control .

Table (3): ROC area for selected antibodies when used as test to predict Celiac disease differentiating them from healthy controls.

	ROC area	P
Anti-Gliadin Antibody-IgA	1.000	<0.001
Anti-Gliadin Antibody-IgG	0.938	<0.001
Anti-Tissue Transglutaminase-IgA	1.000	<0.001
Anti-Tissue Transglutaminase-IgG	1.000	<0.001

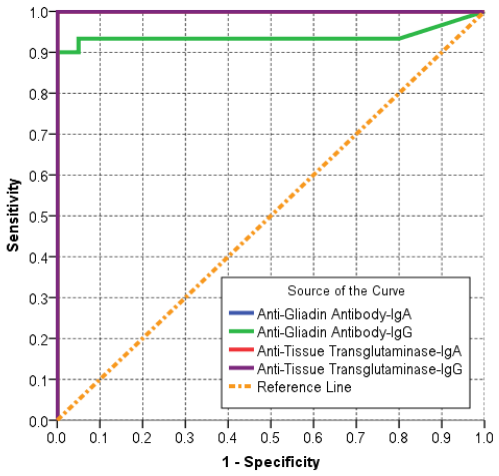

Figure (1): ROC curve showing the trade-off between sensitivity (rate of true positive) and 1- specificity (rate of false positive) for different auto-antibodies' serum levels (AGA IgA ,AGA IgG ,tTg IgA , tTg IgG) when used as tests to diagnose celiac disease cases differentiating them from healthy controls.

In the differentiation of this disease from T1 DM, the ROC area was 0.971, anti-gliadin antibody of IgA which mean a very g ood diagnostic value (Table 4, Figure 2). Whereas, The reciprocal Operative Curve value for Anti-gliadin Antibody of IgG (0.947) showing high specificity and sensitivity when this test used to predict Celiac disease differentiating them from DM (positive controls). In the differentiation of this disease from T1DM, the ROC area was(0.812) for anti-tissue Transglutaminase-IgG , which mean a good diagnostic value . In addition, a similar ROC area for Anti-Tissue Transglutaminase-IgA (0.863) has been

calculated using this test to predict celiac disease differentiating it from diabetes mellitus type1 (positive control).

Table(4): ROC area for selected antibodies when used as test to predict Celiac disease differentiating them from DM (positive controls).

	ROC area	P
Anti-Gliadin Antibody-IgA	0.971	<0.001
Anti-Gliadin Antibody-IgG	0.863	<0.001
Anti-Tissue Transglutaminase-IgA	0.947	<0.001
Anti-Tissue Transglutaminase-IgG	0.812	<0.001

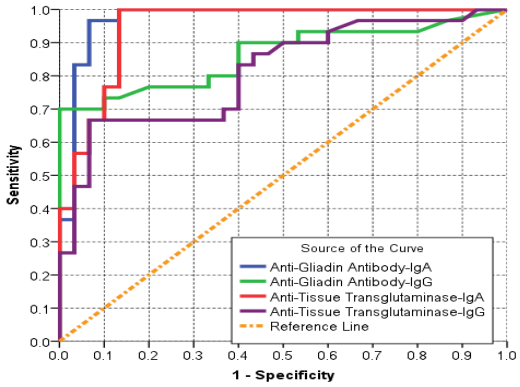

Figure (2): ROC curve showing the trade-off between sensitivity (rate of true positive) and 1-specificity (rate of false positive) for different auto-antibodies (AGA IAgA , AGA IgG , tTG IgA , tTG IgG) when used as tests to predict celiac disease differentiating them from T1DM (positive control).

A similar diagnostic value has shown when differentiating T1DM patients from healthy control using the Anti-Gliadin Antibody-IgG and Anti-Gliadin Antibody-IgA (Table 5, figure. 3). A lesser ROC (Area Under Curve =0.708 , 0.949 respectively). Anti-Tissue Transglutaminase-IgA and ,IgG has been observed using this test to differentiate diabetes mellitus type1 from the control group. and good serological marker (AUC= 0.911 0.991 respectively)

Table (5): ROC area for selected antibodies when used as test to predict DM differentiating them from healthy controls.

	ROC area	P
Anti-Gliadin Antibody-IgA	0.949	<0.001
Anti-Gliadin Antibody-IgG	0.708	0.013
Anti-Tissue Transglutaminase-IgA	0.911	<0.001
Anti-Tissue Transglutaminase-IgG	0.991	<0.001

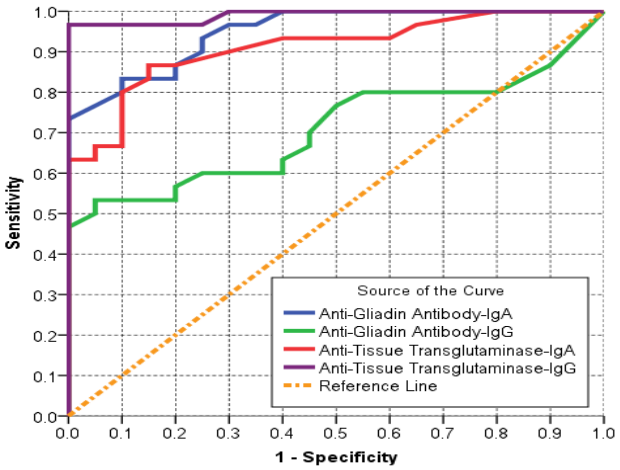

Figure (3): **ROC** curve showing the trade-off between sensitivity (rate of true positive) and 1- specificity (rate of
false positive) for different serum (AGA IgA ,AGA IgG ,tTg IgA,tTg IgG) when used as tests to diagnose diabetes mellitus type 1 cases differentiating them from healthy controls**.**

Discussion

The AGA test appears specific to detect gluten sensitivity rather than celiac disease, since positive AGA was also seen in other diseases and normal people .The test is of less value in confirming a diagnosis of celiac disease, if used as a single test, but it is good for monitoring diet therapy in established celiac cases this was consistent with Trier [11] study . Previous studies have reported the value of AGA assay in screening for a typical or silent celiac disease, example in patients with insulin dependent diabetes mellitus [12] and the first degree relative of celiac disease. Anti-tTG of IgG class was a good serological marker for celiac disease and should be highly specific for this disease and give us the ability to diagnose celiac disease in children and adults. These findings agreed with previous studies. The seroprevalence 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[13] ,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 class or tTG-IgG class). It is, 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 celiac disease. Some authors recommend serological screening at the time of diabetes type1 diagnosis and annually or biennially thereafter[14]. The confusing auto-antibody detecting in the celiac disease and type1DM that need further accurate quantitative assays for the purpose of appropriate management of the suspected cases.

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