Original article

The relationship between tissue transglutaminase IgA antibodies and the clinical manifestations in a group of children, adolescent and adult patients with type -I diabetes mellitus

Background: *Type-1 diabetes mellitus (T1-DM) is the commonest endocrine-metabolic disease in childhood. The prevalence of CD in type-1 DM ranges from 0.6 to 16.4% compared with 0.01–0.03% in the general population. The mechanism of association between the two diseases involves a shared genetic background of HLA genotype. Serum tissue transglutaminase IgA antibodies (tTG IgA) are considered specific and sensitive markers for screening of Celiac disease in more than 95 % of patients.* ***Objective:*** *Screening for the presence of serum tissue transglutaminase IgA antibodies (tTG ab) as a specific and sensitive biochemical marker for Celiac disease in patients with type-1DM and its relation to the clinical manifestations of those patients.* ***Methods:*** *One hundred-forty-nine patients with type-1 DM attending the out-patient clinic of endocrine and metabolism, Minia University Hospital were screened for the presence of serum tissue transglutaminase IgA antibodies during the period from March 2014 to November 2015.* ***Results:*** *Out of 149 patients 8 patients (5.3%) were positive for IgA tTG antibodies. They who were predominantly of female gender (75% were females). According to each age group, there were four sero-positive cases in children (with age group between 9 and ≤ 12 years); two cases in adolescents (with age group between 12 and ≤ 16 years) and two cases in adults (with age group 16-21 years). Intestinal manifestations, chronic diarrhea, recurrent abdominal pain/ distension, recurrent aphtha's stomatitis, anemia and bleeding tendency were significantly more common in sero-positive cases (P=0.001, 0.001, 0.016, 0.00, 0.001and 0.04 respectively). All sero-positive cases (100%) had lower BMIs than normal. There were no correlations between the tTG antibodies levels and HbA1c levels.* ***Conclusions:*** *The presence of tTG IgA antibodies is associated with significant changes in the clinical status of patient with type-1 DM. Celiac disease related manifestations like weight loss; anemia and chronic diarrhea were more common in sero-positive diabetic patients. Serological screening for CD should be performed in all patients with type-1DM for early diagnosis and prevention of complications.*

Keywords: Type-1 DM, tissue transglutaminase, IgA antibodies.

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INTRODUCTION

Type 1 DM is the most common metabolic disease in childhood with incidence rate varies greatly between different countries, within countries, and between different ethnic populations[1, 2]. Incidence of T1DM in Egypt in children less than 15 years ranged from 5% to 9.9%[3]. Celiac disease (CD) is one of the most frequent autoimmune disorders occurring in type-1 DM. The prevalence of CD among patients with type-1 DM varies in different geographical populations and is observed to range between 0.6 - 16.4% compared with 0.01- 0.03% in the general population[4,5]. Type-1 DM and CD show the same genetic background and an abnormal small intestinal immune response with inflammation and variable grades of enteropathy[6]**.** The inflammation in Celiac disease occurs primarily in the mucosa of the small intestine, which leads to villous atrophy. The clinical presentation of CD in type-1 DM is symptomless in approximately half of patients, but a more accurate analysis often discloses a wide array of symptoms suggestive of CD[7].

A definitive diagnosis of CD requires a jejunal biopsy demonstrating villous atrophy. Given the

invasive nature and cost of the biopsy, serologic and genetic laboratory tests may be used to identify individuals with a high probability of having Celiac disease[8,9]. Subsequently, those individuals with positive laboratory results should be referred for small intestinal biopsy and/or exposed to gluten free diet, thereby decreasing the number of unnecessary invasive procedures.

The aim of this work was screening patients with type-1DM for the presence of serum tissue transglutaminase IgA antibodies (tTG ab) as a specific and sensitive biochemical marker for Celiac disease and its relation to the clinical manifestations of those patients.

METHODS

This study included one hundred-forty-nine (81 females & 68 males) patients with type-1 DM attended the out-patient clinic of endocrine and metabolism of both internal medicine and pediatric departments, Minia University Hospital. Their ages ranged from 9 to 21 years screened for the presence of serum tissue transglutaminase IgA antibodies during the period from March 2014 to November 2015. The diagnosis of type-1 DM was made according to the American Diabetes Association (ADA) using conventional criteria as classic clinical picture (polyuria, polydipsia and weight loss) and blood sugar > 200mg/dl and positive islet cell antibodies (ICA) and positive glutamic acid decarboxylase (GAD) antibodies [10].

We excluded from the study all patients with uncontrolled complications of DM (like diabetic keto-acidosis, diabetic coma, diabetic nephropathy, neuropathy or retinopathy), past history of lactose intolerance (for children), irritable bowel syndrome (for adults), treatment with systemic immune-modulatory agents in prior 30 days or treatment with systemic immune modifying biological agents e.g. infliximab in prior 6 months.

All patients were on regular insulin therapy. They were submitted to full history taking and thorough clinical examination. Egyptian growth charts for children were used for assessment of weight, length and BMIs for children less than 18 years. Urine and stool analysis were done in addition to the following laboratory investigations: CBC, serum creatinine, liver enzymes, HbA1c and tTG IgA antibodies.

They were classified about to the presence or absence of IgA tissue transglutaminase antibodies (tTG ab) into sero-positive and sero-negative groups. IgA tTG antibodies were considered positive if their levels were more than 10 U/ml. Correlations between tTG ab and HbA1c as a marker for control of diabetes were studied.

Written consents were taken from all adult patients and parents or care-givers of our children included in this study before conducting any steps and the study was approved by Faculty of Medicine Ethical Committee, Minia University.

Laboratory studies

Blood Sampling and Processing: About 7 mls of venous blood were taken from each patient by sterile venipuncture and divided as follow: One ml in EDTA containing tube for determination of CBC and HbA1c. Three mls were collected in dry tube, after clotting, the serum samples were obtained by centrifugation for 10 minutes at 3000 rpm and were stored frozen at (-20"C) until used. Three mls were collected in dry tube to be used in blood glucose, liver enzymes and renal function tests.

Principle of tissue-transglutaminase IgA antibodies: Human recombinant tissue transglutaminase was bound to microwells antibodies against this antigen. Horseradish peroxidase (HRP) conjugated anti-human IgA immunologically detected the bound patient antibodies forming a conjugate/antibodies/antigen complex. We washed the microwells to remove unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzed and formed a blue color. We added an acid to stop the reaction forming a yellow product. The intensity of this yellow color is measured photo metrically at 450 nm. The amount of color was directly proportional to the concentration of tTG IgA antibodies present in the original sample[11,12].

Statistical analysis

Data entry and analysis were done using software (SPSS version 19). Graphics were done using Excel. Quantitative data were presented by mean and standard deviation, while qualitative data were presented by frequency distribution. Student t-test and Chi-square tests were used to compare qualitative variables between groups. The probability of less than 0.05 was used as a cut off for significant tests.

RESULTS

This study included 149 patients with type-1 DM, their ages ranged from 9 to 21 years with mean age of 12.9±6.4 years. Eighty-one patients (54.3%) were females and 68 (45.6%) were males. One hundred and thirty-five (90.6%) were delivered by vaginal delivery and 14 (9.4%) were delivered by cesarean section. One hundred and twenty-nine

(86.5%) were breast fed with mean duration 20.6 ± 4.9 months and 20 (12.7%) were artificially fed. The mean time of gluten introduction was 6.9 ± 2.4 months with earlier introduction in sero-positive than the sero-negative patients (4.5±1 versus 7.1 ±2.4, p=0.023).

Out of one hundred and forty-nine diabetic patients only eight patients (5.4%) were positive for tTG IgA antibodies while 141 patients (94.6%) were negative. The antibodies titers were significantly higher in sero-positive than sero-negative patients (15.5 ± 1.8 versus 0.79 ± 0.44 respectively (p=0.001). (Table 1)

Sero-positive patients had a significant predominance of female gender as from 8 patients with sero-positive, 6 patients (75%) were females (figure 1) with lower body mass indices (BMIs) than sero-negative patients (p- 0.047).

The mean hemoglobin and corpuscular volumes were significantly lower in sero-positive patients (p= 0.032 and 0.017 respectively), while the mean values of liver enzymes were higher in sero-positive patients (AST = 86.2±9.5 IU/L versus 24.4±12.6 IU/L and ALT= 84±12.9 IU/L versus 28±18.5 IU/L, P=0.005). (Table 1)

Intestinal manifestations in the form of chronic diarrhea, recurrent abdominal pain and distension were significantly higher in sero-positive than sero-negative patients (p- value= 0.001and 0.016 respectively) (Table 2).

Extra-intestinal manifestations, weight loss, anemia, bleeding tendency, bone aches and abnormalities in dental enamel were significantly increased in sero-positive compared to sero-negative group (p 0.001, 0.001, 0.004, 0.004 respectively) (Table 3).

According to each age group, there were four sero-positive cases in children (with age group between 9 and ≤ 12 years); two cases in adolescents (with age group between 12 and ≤ 16 years) and two cases in adults (with age group 16-21 years). Also, intestinal and extra-intestinal manifestations were more frequent in children than in adolescents and adults (19,7 and 4 cases; p-value = 0.003 for intestinal and 27,10 and 11 cases; p-value=0.002 for extra-intestinal manifestations respectively)

There were no correlations between the tTG antibodies levels HbA1c levels (Table 4).

Table 1. Laboratory data of studied patients

Item		**Sero-positive n = 8**	**Sero-negative n = 141**	**P - value**
HB (gm/dl)	Mean ±SD Median	10.2 ± 0.4 10	11.7 ± 1.47 12	0.032*
MCH (pg)	Mean ±SD Median	26.2 ± 0.73 26.4	27.7 ± 2.7 28.2	0.056
MCV (fl)	Mean ±SD Median	77.6 ± 2.1 77.7	83.6 ± 6.5 83.1	0.017*
WBC (1000/cmm)	Mean ±SD Median	9 ± 1.9 8.6	6.7 ± 2.6 6.1	0.021*
HbA1c	Mean ±SD Median	7.6±2.3 8.1	7.1±2.4 7.8	0.81
ALT(IU/L**)**	Mean ±SD Median	84±12.9	28±18.5	0.005**
AST (IU/L**)**	Mean ±SD Median	86.2±9.5	24.4±12.6	0.001**
tTG IgA antibodies (U/ml)	Mean ±SD Median	15.5 ± 1.8 15.2	0.79 ± 0.44 0.66	0.001**

**Significant ** highly significant*

Hb: hemoglobin; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; TTG IgA: tissue transglutaminase IgA antibodies; WBC: white blood cells;

Table 2. Intestinal manifestations of studied groups

Item		Sero-positive n = 8	Sero-negative n = 141	P – value
Intestinal manifestations	Yes No	8 (100%) 0 (0%)	22 (15.6%) 119 (84.4%)	0.001**
Chronic diarrhea	Yes No	4 (50%) 4 (50%)	2 (1.4%) 139 (98.6%)	0.001**
Recurrent abdominal pain/ distension	Yes No	4 (50%) 4 (50%)	16 (11.3%) 125 (88.7%)	0.016*
Recurrent aphtha's stomatitis	Yes No	4 (50%) 4 (50%)	6 (4.2%) 135 (95.8%)	0.001**

**Significant ** highly significant*

Table 3. Extra-intestinal manifestations of studied groups

Item		Sero-positive n = 8	Sero-negative n = 141	P – value
Extra-intestinal manifestations	Yes No	8 (100%) 0 (0%)	40 (28.4%) 101(71.6%)	0.003**
Weight loss	Yes No	6 (75%) 2 (25%)	16 (11.3%) 125 (88.7%)	0.001**
Anemia	Yes No	6 (75%) 2 (25%)	16 (11.3%) 125 (88.7%)	0.001**
Bleeding tendency	Yes No	2 (25%) 6(75%)	2 (1.4%) 139 (98.6%)	0.002**
Bone aches	Yes No	2 (25%) 6(75%)	2 (1.4%) 139 (98.6%)	0.003**
Abnormalities in dental enamel	Yes No	2 (25%) 6(75%)	2 (1.4%) 139 (98.6%)	0.004**

**Significant ** highly significant*

Table 4. Correlations between tTG-IGA antibodies and some of demographic, clinical and laboratory data

Item	tTG IgA r (p)	
	Sero-positive	**Sero-negative**
Age (years)	0.12 (0.5)	0.05 (0.8)
Weight (Kg)	0.51(0.09)	0.44 (0.06)
BMI	-0.47 (0.02*)	-0.45 (0.03*)
Duration of DM (years)	0.62(0.04*)	0.51 (0.06)
HbA1c %	0.24 (0.2)	0.20 (0.3)
Urea (mg/dl	0.13 (0.5)	0.24 (0.2)
Creatinine (mg/dl)	0.23 (0.2)	0.09 (0.6)
ALT (u/l)	0.17(0.4)	0.24(0.2)
AST (u/l)	0.21(0.3)	0.02(0.9)

**Significant ** highly significant*

BMI: body mass index; FBG: fasting blood glucose; 2-hour PPG:2-hours Post prandial glucose; HbA1c: glycated hemoglobin; ALT: Alanine transaminase; AST: Aspartate transaminase; tTG-IgA ab: tissue transglutaminase IgA antibodies

DISCUSSION

Celiac disease and type-1 diabetes mellitus are autoimmune in origin and develop as a result of complex pathological mechanisms, involving several common genetic, environmental and immunological factors[13]. In terms of serology, Celiac disease is associated with a variety of autoantibodies, including endomysial, tissue transglutaminase (tTG) and deamidated gliadin antibodies. Among the most sensitive and specific auto-antibodies were tTG and deamidated gliadin antibodies. For individuals with moderately to strongly positive results, a diagnosis of Celiac disease is likely and the patient should undergo biopsy and /or exposed to gluten free diet as a therapeutic test to confirm the diagnosis[14].

In this study, out of 149 diabetic patients 8 patients (5.3%) were positive for tTG IgA antibodies which are considered sensitive and specific auto-antibodies for Celiac disease and 141 patients (94.7%) were negative with higher levels in sero-positive (15.5 ± 1.8) than sero-negative patients (0.79 ± 0.44). Sero-positive patients had a significant predominance of female gender as 6 out of 8 patients (75%) were females.

These results were in agreement with other studies which reported that the prevalence of CD among patients with type-1 DM is approximately 4% and this risk is highest with diabetes of early onset in childhood (age < 4 years), with the longer diabetes duration and the onset of DM before the age of 20 years[14].

These results were similar to the results of John et al.**[15]**, 2011 who reported an overall prevalence of (3.3%) for CD in type-1 DM. Also, higher percentage for tTG Ig A antibodies in type-1 DM patients (17%) was reported by Abdulrahman et al.[16], 2012 who screened one hundred and six children with type-1 DM over a two-years period (2008–2010).

The present study showed that the time of gluten introduction was significantly earlier in sero-positive compared to sero-negative group (p=0.023). These results were in agreement with other studies [17,18] that reported an increased risk of CD in children exposed to gluten before 6 months with an incidence 4.24/1000 in children exposed to gluten before or at 4 months, compared with 3.68/1000 in children with late gluten introduction after 6 months. Different results were reported by Aronsson et al. [19], 2015 who reported that gluten introduction either before 4 months or after 6 months of age appeared to have no effect on Celiac disease risk.

Common clinical manifestations in Celiac disease related to gastrointestinal inflammation include abdominal pain, malabsorption, diarrhea recurrent aphthous stomatitis and constipation. Clinical symptoms of Celiac disease are not restricted to the gastrointestinal tract. Other common manifestations of Celiac disease include failure to thrive (delayed puberty and short stature), iron deficiency, recurrent fetal loss, osteoporosis, chronic fatigue, dental enamel hypoplasia, and dermatitis herpetiformis[20-22].

The results of this study showed that CD symptoms (both intestinal and extra-intestinal) are much more frequent in children than in adolescents and adults and significantly higher in sero-positive than sero-negative patients. There were many studies reported similar results[20-23].

Similar results were reported by Ehsani-Ardakani, et al.[22], 2013 as 34.9% had at least one GI symptom as well as the majority of patients had diarrhea (13.6%) followed by dyspepsia and constipation (4.0%).

The results of this study showed that extra-intestinal manifestations like weight loss, anemia, bleeding tendency, bone aches abnormalities in dental enamel were significantly increased in sero-positive compared to sero-negative group.

Anemia demonstrated with low hemoglobin percentage and mean corpuscular volume was significantly more common in sero-positive patients. The results which are in agreement with other studies reported that anemia is the most presenting non-GI symptom in the majority of diabetic patients with Celiac disease[,21.22].

Elevated liver enzymes were more common in sero-positive compared to sero-negative patients reflecting the involvement of liver in patients with CD and type-1 DM. These results were in agreement with other studies[23,24].

The results showed that there was no relationship between the tTG antibodies and the state of blood glucose control through Hb-A1c. These results were in agreement with others[25].

CD observed in type-1DM is classified as silent in approximately half of patients owing to the lack of symptoms suggestive for gluten-sensitive enteropathy. However, an accurate assessment of the clinical history and careful clinical examination allows the identification of more than one factor raising the suspicion of its diagnosis[26].

From the results of this study, serological screening for CD using tTG IgA antibodies is recommended for all patients with type-1DM for early diagnosis and prevention of complications due to high prevalence in this at-risk group for CD diagnosis with potential consequences of delayed diagnosis.

CONCLUSION

The presence of tTG IgA antibodies is associated with significant changes in the clinical status of patient with type-1 DM. Weight loss, anemia, chronic diarrhea and other complications related to Celiac disease were more common in sero-positive diabetic patients. Serological screening for CD using tTG IgA antibodies should be performed in all patients with type-1DM for early diagnosis and prevention of complications.

ABBREVIATIONS

CD: Celiac disease, HLA: Human leucocytic antigen, T1DM: Type-1- diabetes mellitus, WHO: World Health Organization, CBC: Complete blood count, FBG: Fasting blood sugar, PPG: Post prandial glucose, HbA1c: glycated hemoglobin, TTG IgA ab.: tissue transglutaminase antibodies, IgA: Immunoglobulin A.

AUTHORS' CONTRIBUTIONS

ES and ES conceived the study, carried out its designing, coordinated the implementation, helped to perform the statistical analysis and drafted the manuscript. GA and AE participated in the design of the study, analysis and interpretation of data and revised the statistics and final draft of the manuscript. AS participated in interpretation of the laboratory data of patients. All authors read and approved the final manuscript.

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