Original Article

Association between Diabetes Mellitus type 1 and Celiac Disease: case-control study among Sudanese patients 2009-2011

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Abstract

Background: Gluten sensitive enteropathy (celiac disease (CD)) has a strong association with diabetes mellitus (type 1DM). Since, 2-3% of CD patients have selective IgA deficiency, the majority of the available tests may fail to show the auto-antibodies (the IgA endomysial antibody (EMA). To prevent such a false negativity, a new Enzyme Linked Immune Sorbent Assay (ELISA) test has been introduced to detect both IgG and IgA antibodies reactive with tissue transglutaminase (tTG), an autoantigen in CD patients.

Objectives: This study has been conducted to detect celiac disease among Sudanese patients with type 1 autoimmune diabetes using anti-tissue transglutinamase antibodies as a diagnostic tool.

Patients and Methods: Samples were collected from sixty nine randomly selected patients (38 males and 31 females) and their age ranged between 3-22 years with DM type 1 who were attending the outpatient clinics in Gabir Abu Eliz diabetic Center and Omdurman Pediatric Emergency Hospital. Blood samples were collected from 25 healthy individuals as controls. Levels of tTG specific IgA, tTG specific IgG and anti-endomysial antibodies of IgA class were measured in sera collected from both cases and from controls. All the results were analyzed using Statistical Packages of Social Sciences (SPSS) version 17 and MicroSoft office excel.

Results: Seven out of 69 patients with DM type 1 (10.1%) were identified as having CD using IgG anti-tTG and 5 (7.2%) of them were positive for IgA anti-tTG and IgA anti-endomysial antibodies. The mean of both anti-tTG IgA and IgG titers were higher in diabetic patients (M±SD = 12.30±41.0 and 7.2±13.1 respectively) when compared with the control group (M±SD =1.8±1.1 and 1.8±0.9 respectively), however, only anti-tTG IgG antibodies titer achieved statistical significance.

Discussion and conclusion: The present study revealed that patients with DM type I have an increased tendency to develop CD. The increased association of CD and selective IgA deficiency is a potential source of false-negative IgA, therefore testing for IgG class autoantibodies is recommended if celiac disease is suspected. Antibodies to tTG antigen fall once a gluten-free diet has begun, thus facilitating monitoring of dietary compliance. Thus, anti-tTG antibodies are highly sensitive marker for celiac disease with 95- 100 % sensitivty, and specificity of 90 to 97 %.

Keywords: Diabetes mellitus type I; Celiac disease and diabetes mellitus type I association; IgG anti-tissue transglutaminase antibodies; IgA anti-endomysial antibodies

Gluten-sensitive enteropathy or celiac disease (CD) is a common cause of chronic malabsorption in children and is characterized by mucosal damage of the possibly oats. An association between DM type 1 and CD has been previously reported[2]. 1.3 to 12% of children with DM type may suffer from CD all over the world and may contain a high proportion of clinically asymptomatic and atypical cases[3,4]. The reduced prevalence of type 1 DM after gluten deprivation in patients with celiac disease[5] and the reduced incidence of autoimmune diabetes in the non-obese diabetic mouse receiving a gluten-free diet[6] are some of the evidence supporting the association.

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Both CD and type 1DM are associated with the major antigen DQ2 encoded by the alleles *DQA1*501* and *DQB1*201*, thus providing a common genetic basis for expression of both diseases[7]. However in DM type 1 the genetic association has been found to be linked to DR3 and/or HLA DR4 loci. The high prevalence of CD in DM type 1 patients warrants routine screening of this group[2].

The diagnosis of CD was dependent on either detection of IgA anti-endomysial antibodies or endoscopic biopsy. Since 2-3% of patient with CD have selective IgA deficiency[8], it would be difficult to diagnose the CD among patients with DM type I. In Sudan diagnosis of celiac disease depends on biopsy from small bowel (invasive method) or detection of anti-endomysial antibodies of IgA class[9]. Recently new tests have been introduced. These tests have the capacity to detect IgG against the antigen tissue transglutaminase, an enzyme from intestine due to destruction by CD.

The current study is aiming at diagnosis the association of Celiac disease and diabetes mellitus in Sudanese children by using newly introduced immunological methods e.g., anti-tTG (IgA) & (IgG). As the sensitivity of anti-tTG is high, it is highly recommended for diagnosis of unrecognized celiac disease in patients with insulin-dependent diabetes mellitus as they may have an increased prevalence of celiac disease.

Patients and Methods:

Ethical considerations: The current study had received an ethical approval from the ethical committee of Al Neelain University. Aims of the study was clearly explained to all volunteers and an informed consent was obtained from each volunteer. The confidentiality of patients was established by coding of samples. All investigations were carried out free of charge and patients were not claimed for analysis expenses.

Study design: case control: The study was conducted in 69 diabetic patients and 25 apparently healthy subjects. A comprehensive history was taken guided by the data collection sheet for each individual. Weight and height were measured to the nearest decimals and recorded. Body mass index (BMI) was calculated using the formula: BMI (Kg/m^2) = weight (Kg) / $(height)^2$ (m^2).

Study area and population: Sixty nine diabetic patients attending the outpatient clinic at Gabir Abu Eliz diabetic center, which is located in Khartoum state, and from Omdurman pediatric emergency Hospital were enrolled.

Sample processing: Five ml of whole blood were collected from each patient using acceptable medical techniques to avoid hemolysis and were left to clot for 2-3 hours at room temperature and then were centrifuged at 3000 rpm for 10 minutes and sera were stored at -20°c until used. Grouping of patients was made according to the WHO criteria[10].

Measurement of fasting blood glucose level: Glucose level was measured immediately before blood clotting by using Trinder's glucose oxidase method. In brief, glucose was essentially oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide produced was reacted in the presence of peroxidase with 4-aminoantipyrine and p.hydroxybenzene sulphonate to from aquinonimine dye. The intensity of the color produced was at 505 nm. Glucose concentration was calculated according to the following formula:

Glucose concentration of samples in mg/dl =

$$\frac{\text{A sample} - \text{A blank}}{\text{A sample} - \text{A blank}} \times \text{concentration of standard in mg/dl}$$

Diagnosis of Celiac disease using immunological methods: Screening for CD was done using three methods: anti-tissue-transglutaminase IgA, anti-tissue-transglutaminase IgG and anti-endomysial IgA. Non competitive solid phase Enzyme Linked Immuno-Sorbent Assay (ELISA) was used to detect tissue transglutaminase specific antibodies.

Anti-tissue-transglutaminase IgA or IgG. Using serological testing, many patients with Celiac disease can be identified quickly once the diagnosis is suspected. In this study, anti

tTG testing was undertaken with a commercially obtained ELISA kit (Orgentec, Germany) and performed essentially as described in details previously[11]. Briefly, stored samples were thawed and diluted 1:100 with sample buffer and tested in duplicate at room temperature along with appropriate negative and positive controls. is bound in 96 high binding microtitre well plates. Antibodies against this antigen, if present in diluted serum or plasma, bind to the human recombinant tissue transglutaminase immobilized to the bottom of wells. Following, plates were washed in triplicate to remove unspecific antibodies or plasma components. Then 100 µl of Horseradish peroxidase (HRP) conjugate anti human IgA or IgG immunologically were added to each well and incubate for 15 minutes at room temperature to detect the tTG specific antibodies in patients sera. Then plates were washed by wash solution in 3X to remove unbound conjugate. Reaction was catalyzed by adding 100 µl of enzyme substrate to each well and the plates were incubated for 15 minutes at room temperature. The reaction was stopped by adding 100 µl of stop solution and incubated 5 minutes. Results were read by ELISA reader at wave length 450 nm. An ELISA cut-off of for anti-tissue-transglutaminase IgA or IgG is **10 U/ml**.

Measurement of endomysial specific antibodies: This was performed by indirect immunofluorescence method, using a commercially available kit (Orgentec, Germany). In brief, samples were thawed and diluted 1:5 with sample buffer. Following that 50 µL from each sample were added in duplicate to slides coated with anti-endomysial antibodies (AEA) bind the corresponding antigens present in a section of the lower one-third of the monkey esophagus on cryostat sections of monkey esophagus and incubated at room temperature along with appropriate negative and positive controls for 15 minutes. Washing in triplicate gently was undertaken to avoid cross contamination of the sera. Then slides were rinsed gently with Phosphate Buffer Saline (PBS) by immersing in a washing tray filled with PBS for 5 minutes and slides were allowed to get dry by means of the blotting paper provided. Then 50 µL of conjugate on each well were added and slides were incubated for 30 minutes at room temperature then washed 3X and dried. Finally the resulting antigen-antibody complexes were detected by means of a fluorescein labeled anti-human immunoglobulin A, and visualized with the aid of a fluorescence.

Statistical analysis: Statistical package for social sciences program (SPSS) and microsoft office excel were used for data. Screening studied variables for significant differences in the means between the groups was performed using the Student two-tailed, unpaired T-test. Cross-tabulation of diabetes mellitus against presence of celiac disease was performed to calculate the relative risk ratio for occurrence of celiac disease in diabetic patients. Association between diabetes mellitus and celiac disease was assessed using Chi square test. In all of these statistical tests, only $P < 0.05$ was considered significant.

Results:

The study involved two groups: a test group of 69 diabetic patients (38 (55.1%) males and 31 (44.9%) females) and control group of 25 apparently healthy subjects (9 (36.0%) males and 14 (64.0%) females). Table-1 shows a comparison between the diabetic patients and the control group as regarding age, weight, height, body mass index (BMI), fasting Blood Sugar. Table 2 shows fasting blood sugar, anti-tissue transglutaminase (anti-tTG) IgA and IgG titers in diabetic and non diabetic. 47.8% of the diabetic patients have history of recurrent diarrhea, abdominal pain and/or weight loss. In addition, 13% have history of other illnesses e.g. bronchial asthma, anemia and pneumonia. The mean BMI of diabetic patients (mean (M) ± standard deviation (SD) = 18.5±3.4) was significantly higher when compared with the non-diabetic subjects (M±SD = 17.1±1.9). The mean of both anti-tTG IgA and IgG titers were higher in diabetic patients (M±SD = 12.30±41.0 and 7.2±13.1 respectively) when compared with

the control group (M±SD =1.8±1.1 and 1.8±0.9 respectively), However, only anti-TTG IgG antibodies titer achieved statistical significance.

Figure (1) shows results of ELISA tests when used for detection of celiac disease. The anti-endomysal IgA, Anti-tTG IgA and Anti-tTG IgG antibodies titers were all negative for the control group. However, 10.1% (7 subjects) of the diabetic patients were diagnosed as having celiac disease. Two celiac disease patients out of the seven (28.6%) had negative anti-tTG and endomysal IgA antibodies titers.

As shown in table 3 the risk of developing celiac disease is 3.48 times in diabetic children compared with the control group. However, cross-tabulation of diabetes mellitus against presence of celiac disease markers failed to demonstrate statistical significance (Pearson Chi square = 2.740, P = 0.098).

Discussion:

The main objective of this study was to detect the association of celiac disease and diabetes mellitus type 1 in Sudanese children. To our knowledge, studies in this field are extremely rare in Sudan. Results were interesting and showed that more than 10% of the studied diabetic patients were suffering from celiac disease. The frequency of celiac disease in the population studied was approximately compared with studies conducted in Kuwait[12]

Table 1: Comparison between the diabetic patients and the control group

	Non Diabetic Subjects (N = 25) M±SD	Diabetic Patients (N = 69) M±SD	P Value	95% Confidence Interval of the Difference	
				Lower	Upper
Age (year)	9.8±2.9	11.7±4.0	0.014	-3.46	-0.41
Weight (Kg)	32.9±5.7	36.9±11.3	0.101	-8.64	0.78
Height (Cm)	138.2±5.7	139.9±19.8	0.677	-9.70	6.32
BMI (Kg/M2)	17.1±1.9	18.5±3.4	0.048	-2.83	-0.01

Table 2: Comparison between the diabetic patients and the control group: fasting blood sugar, anti-tTG IgA and anti-tTG IgG titre

	Non Diabetic Subjects (N = 25) M±SD	Diabetic Patients (N = 69) M±SD	P Value	95% Confidence Interval of the Difference	
				Lower	Upper
Fasting Blood Sugar (mg/dl)	88.9±9.3	244.6±103.7	0.000	-197.07	-114.27
Anti-tTG IgA Titer	1.8±1.1	12.30±41.0	0.205	-26.82	5.83
Anti-tTG IgG Titer	1.8±0.9	7.2±13.1	0.043	-10.59	-0.16

Table 3 Diabetes mellitus versus celiac disease cross-tabulation

		Diabetes Mellitus		Total
		Positive	Negative	
Celiac Disease	Positive	7	0	7
	Negative	62	25	87
Total		69	25	94

Pearson Chi square = 2.740
P = 0.098
Relative Risk = 3.48

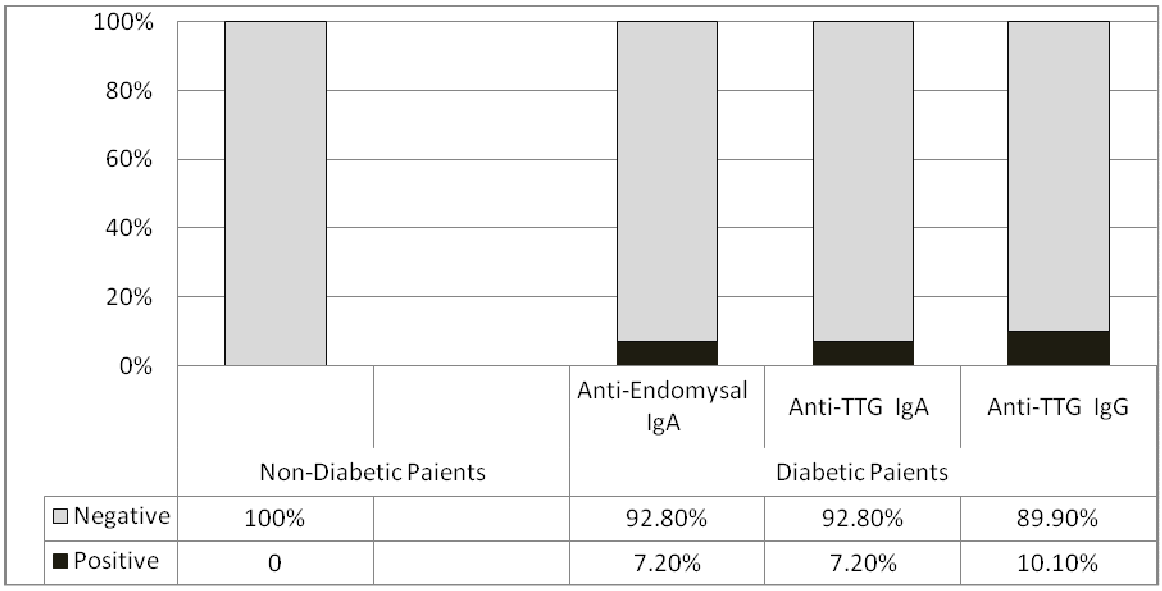

Figure (1): Celiac disease detection by anti-endomysal IgA, anti-tTG IgA and anti-tTG IgG antibodies in studied subjects

and Saudia Arabia[13]. The risk of developing celiac disease is 3.48 times in diabetic children compared with the control group. This increased association of CD and DM type 1 is most probably due to a common genetic predisposition as suggested by the increased occurrence of HLA-DR3, DQ2 encoded by the alleles *DQA1*501* and *DQB1*201*, thus providing a common genetic basis for expression of both diseases[7].

It is worth mentioning that most of the studied diabetic patients were poorly controlled (M±SD of fasting blood sugar = 244.6±103.7 mg/dl) in spite of insulin therapy. In addition, there was significant difference in the mean body mass index when diabetic patients with celiac disease (M±SD = 20.0±4.6 Kg) were compared with the control group (M±SD = 17.1±1.9 Kg) (P = 0.029). However, the mean body mass index of the diabetic patients with no celiac disease (M±SD = 18.4±3.2 Kg) was not significantly increased compared with non-diabetic control subjects (P = 0.083). These results are attention-grabbing because celiac disease is expected to reduce weight and consequently body mass index. An explanation for this high body mass index is co-existence of insulin resistance which is commonly associated with obesity. Nonetheless, early-onset non-insulin dependent diabetes mellitus i.e. insulin resistance is less common in pediatric patients. In the present study indices of insulin resistance were not measured and

remained to be explored by further studies in diabetic patients with celiac disease.

In contrast to anti-tTG IgA antibodies titer, anti-tTG IgG antibodies titer was significantly higher in diabetic patients when compared with the control group. This proved the competence of anti-tTG IgG antibodies in detection of celiac disease in diabetic patients. The study by Rittmeyer and others 1996[14] 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. This finding is further confirmed in the present study by the higher detection capability of anti-tTG IgG compared with both anti-tTG IgA and anti-endomysal IgA (table1).

Conclusion

The present study confirms that patients with insulin-dependent diabetes have an increased tendency to develop celiac disease and most cases are clinically unrecognized. Consideration should be given to screening all insulin-dependent diabetes mellitus patients for gluten intolerance. Screening for celiac disease should be part of the routine investigation to diagnose diabetes mellitus especially in children because of the high prevalence and the potential benefits of treatment with a gluten free diet. This also includes control of symptoms, stabilization of diabetes and prevention of complications associated with celiac disease. Thus, there may be considerable benefits derived from screening and early detection of CD.

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