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Celiac Disease Prevalence and its HLA-genotypic Profile in Egyptian Patients with Type 1 Diabetes Mellitus

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ABSTRACT

The correlation between celiac disease (CD) and type 1 diabetes (T1D) mellitus has been known for decades. However, the data assessing celiac disease prevalence in type 1 diabetic patients among Arab population especially in Egypt were scarce. This study assessed celiac disease prevalence and its genotypic profile in Egyptian type 1 diabetic patients and their non diabetic relatives and determined effects of gluten-free diet on diabetes control and anthropometry. A total of 500 outpatients {15-49 years; with type 1 DM (300; F/M: 168/132) and their non diabetic relatives (200; F/M: 91/109)} and another 300 age and sex matched healthy control were randomly enrolled and screened for CD by tissue transglutaminase (TTG) antibodies, HLA genotyping and distal duodenal biopsy. The demographic, clinical, anthropometric data and effects of gluten free diet were assessed. CD prevalence was 10.3, 2 and 0.3% in T1D, relative and control groups, respectively. The majority of CD patients (77.42%) carried the HLA-DQ2 in linkage with HLA-DRB1*03 alone (41.9%) or with other alleles (DRB1*01,*04,*07,*08,*09,*13). Eleven patients (35.48%) carried HLA-DQ8 either alone in three patients or with other alleles (DRB1*03,*07 in five and three patients respectively. Only one patient with positive TTG-IgG and normal histopathology had negative DQ2 and DQ8 but positive DRB1*7 and *11 haplotypes. CD is a frequent but commonly under-diagnosed among Egyptian T1D patients. HLA-DQ2 and DQ8 genotypes (in linkage with HLA-DRB1*3 and HLA-DRB1*4 alleles) responsible for CD development are highly prevalent in Egyptian CD patients with T1D.

Key words: Celiac disease, type 1 diabetic patients, tissue transglutaminase antibodies

INTRODUCTION

Celiac Disease (CD), also known as celiac sprue or gluten-sensitive enteropathy, is a chronic systemic multi-factorial life-long immune-mediated disorder elicited by gluten and related prolamines consumption (component of wheat, barley and rye) in genetically susceptible individuals. Celiac disease is a common disorder affecting the general population worldwide with recent studies suggesting an increase in its prevalence (0.5-1%) compared with 4.4-11.1% in patients with T1D (Bhadada *et al*., 2011).

The increased prevalence of CD in recent decades strongly indicates that environmental factors other than gluten-exposure may have a significant impact on CD development (Popp *et al.*, 2013). Celiac disease is one of the most frequent autoimmune disorders associated with type 1 diabetes mellitus (T1D). The classical CD is commonly diagnosed at age of 2-3 years, while T1D is commonly diagnosed around 7-8 years. T1D occurs at younger age in patients with the double disease (CD and T1D) than in those with only T1D. However, CD diagnosis precedes diabetes onset only in 10-25%. In genetically susceptible subjects, one immunological disease could predispose to another (Kaspers *et al.*, 2004).

Susceptible subjects to T1D and CD are due to multiple genetic (HLA and non-HLA genes) and environmental factors which complexly interact with each other. The principal determinants of genetic susceptibility for CD are the major histocompatibility class II HLA -DQA and DQB genes coded by the major histocompatibility region in the short arm of chromosome 6 (Klapp *et al.*, 2013).

Carriers of HLA-DQ2 and DQ8 haplotypes are genetically predisposed to CD. DR3-DQ2 shows a strong association with CD; homozygosity for DR3-DQ2 in a population with T1D carries a 33% risk for the presence of TTG autoantibodies. The heterodimer DQA1*0501 and DQB1*0201 is detected in up to 95% of individuals with CD, with the remaining 5% expressing HLA-DQ8 allele; heterodimer DQA1*0301, DQB1*0302. Absence of these alleles (DQ2 or DQ8) is extremely unlikely to develop CD (Bao *et al.*, 1999; Saukkonen *et al.*, 1996).

The co-existence of both diseases may be explained by the similar genetic background as well as the similar trigger mechanisms for autoimmune processes. Wheat consumption triggers inflammation and injury of the small-intestinal mucosa and myriad of gastrointestinal and systemic manifestations (Friis, 1996).

CD is characterized by the presence of a variable combination of gluten-dependent clinical manifestations, organ-specific autoantibodies (tTG-IgA and EMA), HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy. The most frequent and severe CD-related symptoms are generally related to gastrointestinal malabsorption which include malnutrition, failure to thrive, abdominal pain, anorexia, nausea, vomiting, diarrhea, constipation and distension (Husby *et al.*, 2012).

With expanding knowledge about the natural history of CD, the spectrum of gluten sensitivity has become broader than previously thought and includes latent and potential CD. Patients with CD and T1D are either asymptomatic (silent CD) or present only mild gastrointestinal symptoms (less than 10%). Potential celiac disease (pot-CD) is defined as positive celiac-related antibodies without diagnostic small-bowel mucosal villous atrophy (Ludvigsson *et al.*, 2013).

The prevalence of pot-CD patients in T1D subjects is 12.2%, while the prevalence of pot-CD in the CD control population is 8.4% and only few of them present CD-related symptoms. Concerning to the natural history of patients with pot-CD, a recent study shows that 30% of these patients develops overt CD in a three years follow-up and underlines the necessity of re-testing. However, no data are available about the follow-up of patients with T1D and pot-CD (Tosco *et al.*, 2011).

Screening protocols for the diagnosis of CD are widely recommended and performed. The CD diagnosis is commonly performed by means of tTG-IgA (confirmed by EMA) or tTG-IgG if IgA-deficiency is present. Screening has to performed at followed times: (1) At the time of diabetes onset, (2) Yearly in the first 4 years of follow up, (3) Each 2 years in the successive 6 years of follow up. In the presence of CD-related antibodies positivity it is mandatory to perform bowel biopsy to confirm diagnosis of CD, even if in very recent guide-lines of ESPGHAN society it is proposed that in evident CD-cases it is possible to avoid biopsy (4 main criteria) (Camarca *et al.*, 2012).

Identifying and treating patients with overt CD by gluten free diet (GFD) surely is beneficial in reducing and resolving malabsorption, osteoporosis, malnutrition, retarded growth and mortality rate. Similarly, symptomatic CD patients with T1D benefit from GFD and also metabolic control of diabetes could be ameliorated. GFD has been suggested to have a benefit in treating pot-CD patients but there is no definite consensus exists among experts about to treat pot-CD patients by GFD (Hansen *et al.*, 2006). Surprisingly, intestinal inflammation has been described also in T1D patients without CD-related antibodies and structurally normal intestinal mucosa (Westerholm-Ormio *et al.*, 2003). Also, a gluten-related inflammation either in rectal or in small bowel mucosa of children with T1D has been observed (Maglio *et al.*, 2009).

Clinical data on patients with CD and T1D concerning the possible influence of CD on anthropometry or metabolic control are inconclusive and sparse. Moreover, the data assessing Celiac disease prevalence in type 1 diabetic patients among Arab population especially in Egypt were scarce. This study assessed celiac disease prevalence and its genotypic profile in Egyptian type 1 diabetic patients and their non diabetic relatives and determined effects of gluten-free diet on diabetes control and anthropometry.

MATERIALS AND METHODS

Subjects: A total of 510 adult consecutive outpatients, with age ranging from 12-60 years, with type 1 diabetes mellitus and their non diabetic relatives (sibling and parents) who attended the Diabetes Departments (Pediatric and Internal Medicine Departments) of Mansoura University Hospital were initially and randomly enrolled in this study from January 2012 through 2013 as patient group. Another 300 age and sex matched healthy subjects with no known disease (volunteers, medical students, paramedical and health workers) were also randomly included in this study as control group.

Inclusion criteria for the patient group were as follows: age between 12 and 60 years, non diabetic relatives; sibling and parents, diabetes mellitus onset before the age of 30 year, history of diabetic ketoacidosis and unremitting record of insulin treatment from the initial diagnosis of diabetes. Age, sex, anthropometric parameters (height, weight, body fat percentage and body mass index) were used as demographic features.

The study was approved by the Ethical Commission and Institutional Review Board of Mansoura University Hospital in Egypt. A written informed conscious consent was obtained from all subjects before their participation.

Exclusion criteria included advanced chronic or psychiatric illness, extremes of age <12 year; age >60 year; pregnancy; liver disease; coagulopathy; renal impairment; endocrinal and cardiopulmonary diseases; previous gastrointestinal surgery; smokers; drug or alcohol abuse; any special type of dieting for the previous 6 months and any family history of other autoimmune disease for control group.

Methods: Initially, all patients completed a detailed questionnaire regarding diet and habits, submitted to thorough history taking and detailed physical examinations performed at fasting in the morning. Ten patients from diabetic group were excluded; three on immunosuppressive drugs, two with pregnancy and five were non compliant. All patients were assigned into:

- Diabetic group: comprised 300 patients with type 1 diabetes mellitus
- Relative group comprised 200 non diabetic relatives; sibling and parents
- Control group: comprised 300 age and sex matched healthy subjects with no known disease (volunteers, medical, paramedical and health workers)

Three millilitres of venous blood were obtained from all patients and control subjects and serum samples were centrifuged at 3000 rpm then aliquoted and stored at -70°C until assayed. All the subjects were tested for TTG-IgA antibody. The total IgA level was tested in all sera to exclude selective IgA deficiency. It was determined by electro-immunodiffusion using routine nephelometric assay. TTG-IgG was analyzed in patients with selective IgA deficiency (if IgA level was below 0.05 mg dL^{-1}). Anti-tissue transglutaminase IgA and IgG (anti-TTG IgA and anti-TTG IgG) antibody detection based on antigen purified from guinea pig liver used a commercial anti-TTG ELISA kit (Catalog#MBS725995 ELISA kit, MyBioSource, Inc, SanDiego, CA29195-3308, USA).

Principle of the assay: TTG-IgA ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-TTG-IgA antibody and a TTG-IgA-HRP conjugate. The assay sample and buffer are incubated together with TTG-IgA-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the TTG-IgA concentration since TTG-IgA from samples and TTG-IgA-HRP conjugate compete for the anti-TTG-IgA antibody binding site.

Since the number of sites is limited, as more sites are occupied by TTG-IgA from the sample, fewer sites are left to bind TTG-IgA-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The TTG-IgA concentration in each sample is interpolated from this standard curve.

Standardized optical density values of more than 20 RU mL^{-1} were considered positive for IgA anti-TTG antibodies as recommended by manufacturer. In the present study, values more than 100 U (5 times UNL) were considered strong positive. This assay has excellent specificity and high sensitivity for detection of TTG-IgA. No significant cross-reactivity or interference between TTG-IgA and analogues was observed.

Genetic analysis (Human leukocyte antigen genotypes): All CD patients (positive TTG-IgA antibody) and 82 patients with T1D and negative TTG-IgA antibody (without CD) underwent genotyping for HLA class II antigens. Genomic DNA was prepared from EDTA-collected blood, either by salting out or using Qiagen protocol (Wizard genomic DNA purification kit, Promega Corporation, Madison, WI). HLA- DRB1, HLA-DQA1 and HLA-DQB1 genotyping was performed using a commercial PCR sequence-specific primer (SSP) (Olerup SSP; One Lambda Inc., Canoga Park, California, USA). The amplified products were visualized on 2% agarose gel, stained with 0.5 mg/mL of ethidium bromide, using the E-Gel precast agarose electrophoresis system (Invitrogen Life Technologies, PA4 9RF Paisley, UK).

Biochemical profile, serum ferritin level, complete blood picture and radiography of small bowel were also accomplished for these subjects. The five hour urinary excretion of D-xylose was determined by colorimetry, after oral administration of 25 g of D-xylose. The qualitative analysis of fat in stool was done by microscopical examination of faecal samples using Sudan III stain and steatorrhea defined as a finding of more than eight fat globules in each high power field.

Endoscopy: All the participants with positive TTG-IgA antibody results underwent upper gastrointestinal endoscopic evaluation (XQ40, Pentax Fibreoptic, Tokyo) performed after an overnight fast between 08:00 and 10:00 am. During endoscopy, the macroscopic picture of the mucosa was assessed in terms of color, mobility, vascular network and villous atrophy. The distal duodenum was biopsied from at least five different sites to evaluate the histopathological evidence of CD and confirm its diagnosis (at least 4 samples from the second/third portion and at least 1 biopsy should be taken from the duodenal bulb) (Weir *et al*., 2010; Barada *et al*., 2014). Celiac disease was diagnosed according to a scoring system which took into account 4 Items: symptoms, auto-antibodies, HLA and biopsy findings and each contributing once. To make the diagnosis, a sum of 4 points was required. A gluten free diet should not be started before performing blood tests and biopsies as it can interfere with diagnosis giving false negative results (Husby *et al*., 2012).

Manifest celiac disease was defined as TTG-IgA positive patients with histological evidence of Celiac Disease (CD) while those with negative intestinal histology but seropositive test result and appropriate HLA class II antigens were considered potential CD (Sjoberg *et al*., 1998).

Age of onset, complications, regulation and control of the diabetes were recorded in diabetic patients. Gluten free diet was introduced just after confirmed CD diagnosis (manifest or latent) and reviewed at 3 months interval. Also, Gluten free diet was given to thirty patients with T1D without CD (age and sex matched with those with double disease). The compliance and adherence of patients to the gluten free diet was based on their diaries. Good compliance was defined as no major dietary faults on questioning and negative serology. Evaluation of diabetes control was made by serum glycosylated haemoglobin (HbA1c) level measured on High Performance Liquid Chromatography (HPLC). HbA1c range 4.2-6.2% was considered normal. Hypoglycaemic episodes, gastrointestinal symptoms, height, weight, Body Mass Index (BMI) and Body Fat Percentage (BFP) were recorded at diagnosis and at each visit. Informed written consent was obtained from all patients.

Anthropometric measurements: Body weight was measured with the use of a calibrated balance to the nearest 0.1 kg. Standing height was measured by a wall-mounted standiometer to the nearest 0.1 cm. BMI kg m^{-2} calculated as body weight in kilograms divided by the square of their height in meters. The appetites of all subjects were estimated using a 100 mm Visual Analog Scale (VAS) of appetite. Adult Body Fat Percentage = (1.20×BMI)+(0.23×Age)-(10.8×sex)-5.4; where sex is 1 for males and 0 for females (Deurenberg *et al*., 1991).

Histopathology: The biopsy samples were treated in a standard manner. The sections were stained with haematoxylin and eosin for conventional histopathological examination. Paraffin-embedded tissue blocks were used for microscopic section preparation. The intensity of various features of enteropathy was evaluated in accordance with modified marsh classification (Table 1; Fig. 1) (Marsh, 1992; Oberhuber, 2000). Villous height and crypt depth were measured and the ratio of villous height to crypt depth was calculated. The neutrophil infiltration in the lamina propria and epithelium was graded as absent, mild, moderate, or severe according to the updated Sydney system (Dixon *et al*., 1996). Histological evaluations were performed by two well-trained histopathologists who were blind to the study data.

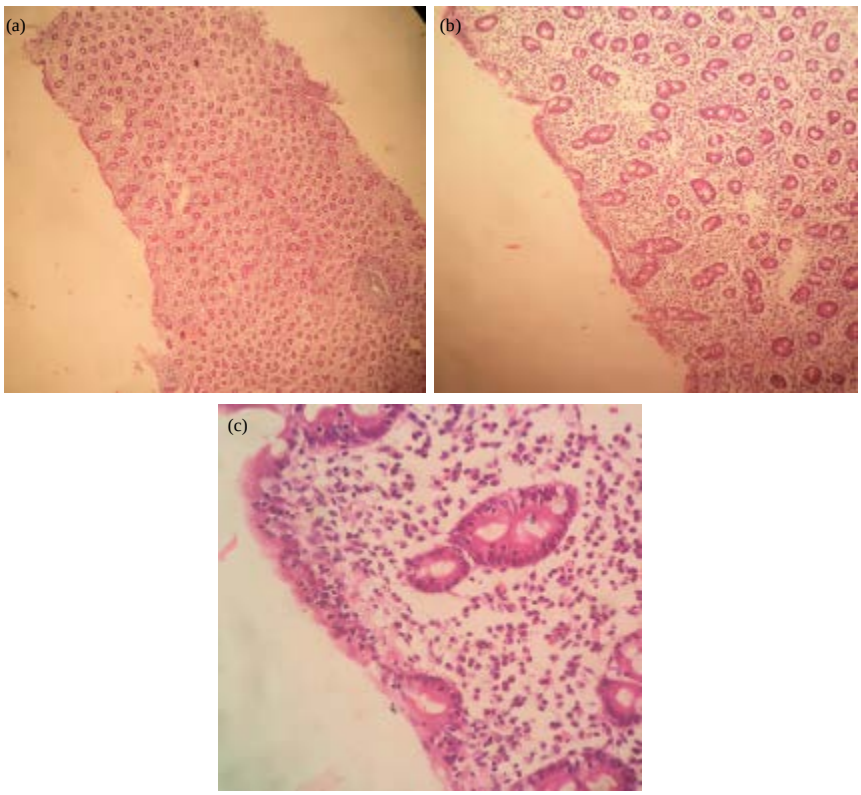

Fig. 1(a-c): Histopathological evidences of celiac disease (a) villous atrophy with (b) crypt hyperplasia and (c) increased intraepithelial lymphocytes

Table 1: Modified Marsh classification of histological findings in celiac disease (Oberhuber, 2000)

	IEL/100 enterocytes			
Marsh type	Jejunum	Duodenum	Crypt hyperplasia	Villi
0	<40	<30	Normal	Normal
1	>40	>30	Normal	Normal
2	>40	>30	Increased	Normal
3a	>40	>30	Increased	Mild atrophy
3b	>40	>30	Increased	Marked atrophy
3c	>40	>30	Increased	Complete atrophy

IEL/100 enterocytes: Intraepithelial lymphocytes per 100 enterocytes

Type 0: Normal; celiac disease highly unlikely
Type 1: Seen in patients on gluten free diet (suggesting minimal amounts of gluten or gliadin are being ingested); family members of celiac disease patients, not specific, may be seen in infections
Type 2: Very rare, seen occasionally in dermatitis herpetiformis
Type 3: Spectrum of changes seen in symptomatic celiac disease

Statistical analysis: Data were analyzed using SPSS software (Version 17.0). Quantitative data were expressed as (Mean±SD) while qualitative data were expressed as number and percentage.

Continuous data are expressed as median (range) and were evaluated by appropriate statistical tests; t test (for paired data). Proportions were compared by means of Fisher's exact test. Correlations were evaluated using the Spearman rank correlation coefficient test. Kruskal-Wallis one way analysis of variance (ANOVA) compares more than two groups. Subgroups (percentages of patients) were compared by using the McNemar test. A value of $p<0.05$ was considered statistically significant. The correspondence analysis which represents an explorative multivariate statistical technique, is used to show the relationships which exist between the clinical and genetic variants. Sensitivity, specificity and predictive values were calculated to study the overall predictability and accuracy of other techniques.

RESULTS

The intensity of various features of enteropathy was evaluated histopathologically in accordance with modified marsh classification (Table 1, Fig. 1). There was total or subtotal villous atrophy with crypt hyperplasia and increased intraepithelial lymphocytes (a ratio of villous height to crypt depth less than 2 was indicative of celiac disease). Endoscopic patterns suggestive of CD were scalloped folds, paucity or absence of folds and mosaic (cracked-mud appearance) pattern of the mucosa, prominence of submucosal blood vessels and nodular pattern of mucosa between the folds. However, their reliability was limited to patients with total or subtotal villous atrophy. The demographic data, anthropometric, laboratory and clinical characteristics in studied groups were shown in Table 2. There were no statistical significant differences between studied groups regarding age, sex, anthropometric measurements (height, weight, BMI, BFP), ferritin and hemoglobin. TTG-IgA was positive (values more than 10 times upper limit of normal) in 29/300 (9.67%) and selective IgA deficiency together with positive TTG-IgG in 2/300 (0.67%) of diabetic patients. Seropositivity (TTG-IgA and/or TTG-IgG) for CD in diabetic patients was 10.3% (31/300) all of them underwent histopathological examination of their distal duodenal biopsies. All, except one, of the thirty one seropositive patients showed histological evidence of CD, thus the diagnosis of CD was confirmed. Four of them (4/31; 6.3%) showed normal mucosa (Marsh type I or II; they were considered latent CD), twenty six patients showed various degrees of villous atrophy

Table 2: Demographic data, anthropometric laboratory and clinical characteristics in studied groups

Parameters	T1D (300) Mean±SD	Relatives of T1D (200) Mean±SD	Control group (300) Mean±SD	ANOVA S≤0.05
Age	27.25±7.4; 25(17-48)	25.46±8.4; 24(18-49)	26.7±7.7; 23(16-48)	NS
Sex (♀/♂: 413/387)	168/132	91/109	154/146	NS
Height (m)	1.72±0.03	1.77±0.49	1.73±0.39	NS
Weight (kg)	64.4±10.2	66.4±19.2	63.4±15.2	NS
BMI	20.8±2.94	22.8±3.91	21.8±3.91	NS
BFP	17.4±8.78	18.4±8.4	18.3±8.9	NS
Ferritin	207.9±21.7	223±22.74	256±19.7	NS
Hemoglobin	10.9±1.5	11.7±1.4	12.5±1.1	NS
HbA1c at first visit	8.3±0.9	6.1±0.66	5.2±0.66	S
tTG-IgA (+ve/-ve)	9.67% (29/300)	4/200 (2%)	1/300 (0.33%)	S
Selective IgA def.(total IgA)	2 (0.67%)	0	0	S
TTG-IgG	2 (0.67%)	0	0	S
Marsh type (0,1,2,3a,3b,3c)	31(1,2,2,8,9,9)	4(0,0,0,4,0,0)	1(0,0,0,1,0,0)	S
Frequency and severity of CD	1 P, 4 L, 8 M, 9 Mod, 6 severe, 3 complicated	4 mild	1 mild	S

S: Significant, NS: Not significant, P: Potential, L: Latent, Mod: moderate

(Marsh type III; IIIa in 8 patients, IIIb in 9 patients and IIIc in 9 patients). The remaining one patient with marsh type 0 was considered potential CD (TTG-IgG positive and normal duodenal histology; highly unlikely to develop manifest CD). From patients with manifest CD, only nine patients had severe CD and microvascular complications of T1D. Severe CD was complicated by severe enteropathy in only three patients.

Four patients in the relative group (4/200; 2%, two siblings and two parents of diabetic patients) were positive for TTG-IgA. None of them had selective IgA deficiency; their serum levels of total IgA were within normal limits). Histopathology showed sub-total villous atrophy in these four subjects (Marsh type IIIa). The frequency of biopsy-proven CD was found as 2% (4/200) in relatives of diabetic patients compared with 10% (30/300) in diabetic subjects. Only one of control group was positive for TTG-IgA and his duodenal biopsy showed subtotal villous atrophy (Marsh IIIa). Also, serum TTG-IgG and total IgA level were normal in all patients of control group. The prevalence of TTG (IgA or IgG) seropositivity for CD was significantly higher in patients with T1D than their non-diabetic relatives and healthy controls ($p \leq 0.05$). The prevalence of biopsy proven CD was also higher in diabetics than relatives and controls ($p \leq 0.05$). The prevalence of seropositivity and biopsy-proven CD in non-diabetic relatives was not different from healthy controls ($p > 0.05$).

The demographic data, anthropometric, laboratory and clinical characteristics in diabetic group with or without CD were shown in Table 3. Thirty one of 300 of diabetics were CD (10.3%). Twelve of them were adolescents (≤19 years) and nineteen were adults with no statistically significant difference between adolescents and adults regarding tTG-IgA titre, anemia, ferritin, enteropathy, diabetes complications and HbA1c ($p > 0.05$). On the other hand, cases with T1D and CD had significant differences in these parameters compared with patients with T1D only ($p \leq 0.05$). In patients with double disease, CD was diagnosed at age of 23.8±8.5; 24 (13-44)

Table 3: Demographic data, anthropometric laboratory and clinical characteristics in diabetic group with or without CD

Parameters	TID with CD (31) Mean±SD	TID without CD (269) Mean±SD	p value
Age	25.58±8.5, 25(15-45)	26.44±7.4, 26(17-49)	NS
Age at DM diagnosis.	19.2±4.3, 18 (14-27)	22.9±5.8, 21(12-26)	S
Age at CD diagnosis.	23.8±8.5, 24 (13-44)	-	S
Duration of diabetes	6.4±5.8	3.4±2.6	S
Insulin dosage	62.75±18.3	52.66±17.3	S
Diabetes complications	8,10 and 11(non, uncontrolled and microvascular)	66, 103 and 100	S
Sex (F/M = 15/14)	19/12	149/120	NS
FH of T1D or CD	27/31 (87.1%)	152/380 (40%)	NS
Height	1.73±0.04	1.72±0.03	NS
Weight	68±9.2	64.4±10.2	S
BMI	22.7±2.6	20.8±2.94	S
BFP	19±7.4	17.4±8.78	S
Ferritin	199.1±12.9	233±15.7	S
Hemoglobin (g dL^{-1})	10.4±1.3	12.3±1.8	S
HbA1c at diagnosis.	7.96±0.51	8.4±0.66	S
tTG-IgA titre	319.2±46.97	-ve	-
Marsh type (0,1,2,3a,3b,3c)	31(1,2,2,8,9,9)	-	-
Enteropathy	8 asymptomatic, 20 moderate and 3 severe	No	-

S: Significant and NS: Not significant

years, while T1D was diagnosed around 19.2±4.3; 18 (14-27) years. There was a significant relationship between CD and age of onset, complications and control of diabetes ($p>0.05$). T1D was firstly diagnosed at a statistically significantly younger age in patients with the double disease (T1D and CD) than in those with T1D only (19.2±4.3; 18(14-27) vs. 22.9±5.8; 21(12-26); $p\leq0.05$). However, CD diagnosis preceded T1D onset in only one patient of all studied CD patients (1/31; 3.23%). Also, CD was found to be frequent among patients with long standing T1D. Regarding sex, there was no statistical significant difference in sex between patients with T1D only (F/M; 149/120) and those with T1D and CD (F/M; 19/12). Also, female and male patients with T1D and CD (F/M; 19/12) had no statistical significant differences in anthropometric, serologic and metabolic parameters ($p>0.05$). CD Patients with positive family history (27/31) had a longer duration of diabetes and more severity of CD but not significant ($p>0.05$).

The Compliance and response to Gluten Free Diet (GFD) were shown in Table 4. Gluten Free Diet (GFD) was prescribed to all CD patients, but three of them did not tolerate it well and TTG-IgA and duodenal histology were still positive in these patients following three months of this dietary regimen while patients who did tolerate GFD showed a significantly good response. After GFD there were significant improvement in anthropometric, clinical, serologic, histological and metabolic parameters in patients with T1D and CD. Significant increase in body weight, BMI and BFP were evident three months after GFD ($p\leq0.05$). Also, TTG-IgA turned negative, iron deficiency anemia (IDA), mucosal inflammations, enteropathy and GIT manifestations markedly improved, diabetic state strictly controlled with striking improvement in T1D complications. Moreover, T1D patients without CD when given GFD the diabetic state, hypoglycemic and DKA attacks decreased significantly.

Correlation between histological degree, TTG-IgA titre and clinical symptoms in CD cases were shown in Table 5. There were significant positive correlations between histopathological marsh

Table 4: Response to Gluten Free Diet (GFD)

Parameters	TID with CD (Mean±SD)	After GFD (Mean±SD)	p value S≤0.05
Height	1.73±0.04	1.75±0.05	NS
Weight	68±9.2	70±11.2	S
Body Mass Index (BMI)	22.7±2.6	24.1±3.4	S
Body Fat Percentage (BFP)	19±7.4	20.2±7.4	S
tTG-IgA titre	333.3±28.6	-ve	S
Ferritin	197.9±14.7	284±23.7	S
Insulin dosage	59.75±18.3	45.41±11.3	S
HbA1c after GFD	7.4±0.67	5.9±0.65	S
Biopsy	+ve	-ve	S
Other clinical signs and symptoms	+ve	-ve	S

S: Significant, NS: Not significant

Table 5: Spearman (r) correlation between histopathological marsh types, CD severity and TTG-IgA titre in CD patients

Parameters	Statistics	Marsh classifications	CD severity
Marsh classifications	r	1.000	0.841**
	p		0.000
CD severity	r	0.841**	1.000
	p	0.000	.
TTG-IgA titre	r	0.954**	0.853**
	p	0.000	0.000

**Significant if p = 0.01 level, 2-tailed

Table 6: HLA-DRB1 (HLA-DQA1* and HLA-DQB1*) typing and histological marsh types in patients with T1D and CD

HLA class II genotyping			Histopathological marsh types						
									Total
HLA-DRB1*	HLA-DQA1*	HLA-DQB1*	0	I	II	IIIa	IIIb	IIIc	% (N/31)
DRB1 *03	0501	0201				4	4	5	13 (41.94%)
DRB1 *04	0301	0302			1	1	1		3 (9.68%)
DRB1 *03 *04	0501/0301	0201/0302				1	2	2	5 (16.13%)
DRB1 *03 *07	0501/0201	0201/0202					1	1	2 (6.45%)
DRB1 *03 *01	0501/0101	0201/05						1	1 (3.23%)
DRB1 *03 *08	0501/0401	0201/04					1		1 (3.23%)
DRB1 *03 *09	0501/0303	0201/0202				1			1 (3.23%)
DRB1 *03 *13	0501/0102	0201/06		1					1 (3.23%)
DRB1 *04 *07	0301/0201	0302/0202		1	1	1 (G)			3 (9.68%)
DRB1 *07 *11	0201/0505	0202/0319	1 (G)						1 (3.23%)
Total			1	2	2	8	9	9	31
Disease severity			Pot	L	L	Mild	Mod	S	
HLA-DQ2 and HLA-DQ8 typing in T1D patients with CD									
HLA-DR3-DQ2 (DQA1*0501-DQB1*0201) positive									24 (77.42%)
HLA-DR4-DQ8 (DQA1*0301-DQB1*0302) positive									11 (35.48%)
HLA-DR3,4-DQ2,8 (DQA1*0501/0301-DQB1*0201/0302 positive)									3 (9.68%)
DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 negative									1 (3.23%)

Pot: Potential, L: Latent, Mod: Moderate, S: Severe, G: TTG-IgG

types, CD severity and TTG-IgA titre in CD patients (Increase the degree of pathology was associated with increased antibody titre and more severe symptoms especially in GIT).

HLA-DRB1 genotyping and histological MARSH types in patients with T1D and CD were shown in Table 6. HLA genotype frequencies among the 31 diabetic patients with CD are as follows: HLA-DQ2 (DQA1*0501, DQB1*0201) was the most frequent allele present in our patients (24/31; 77.4%): DQ2 homo/hetero; 13/11CD patients). One of them (n=24) was carrier of HLA-DQA1*0303, DQB1*0202 in linkage with DRB1*09. Eleven patients (11/31; 35.48%) were carriers of DQ8 (DQA1*0301–DQB1*03:02) either alone in three patients or with other alleles (DRB1*03,*07 in five and three patients, respectively. HLA-DQ2 homozygosity had a remarkable prevalence in the anti-TTG-positive patient group (41.94%). In addition, the majority of CD patients (24/31; 77.42%) carried the HLA-DQ2 linkage with HLA-DRB1*03 alone (13/24) or associated with other alleles (DRB1*01, *04, *07, *08, *09, *13). The patient with positive TTG-IgG and normal intestinal histology (Marsh type 0 = potential CD), had negative DQ2 and DQ8 (DQA1*0501-DQB1*0201 and DQA1 *0301-DQB1*0302 negative) but positive DRB1*7 and *11 (HLA-DQA1*0201/0505 and HLA-DQB1*0202/0319) haplotypes. Histopathological Marsh grade I and II with normal mucosa (but increased intraepithelial lymphocytes) were infrequent in these CD patients (4/31; 12.9%) while small bowel mucosal atrophy (Marsh III) were found in twenty six (26/31; 83.87%) of the present Egyptian CD patients with T1D (Marsh IIIa in 8/26, Marsh IIIb in 9/26 and Marsh IIIbc in 9/26).

DISCUSSION

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPAGN) working group (2012) defined CD as an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals, characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 and

HLA-DQ8 haplotypes and enteropathy. Several classifications of CD had been used; classical, atypical, asymptomatic, latent and potential CD. Silent CD is defined as the presence of positive CD-specific auto-antibodies, HLA and small-bowel biopsy findings that are compatible with CD but without sufficient symptoms and signs to warrant clinical suspicion of CD. Latent CD is defined by the presence of compatible HLA but without enteropathy in a patient who has had a gluten-dependent enteropathy at some point in his or her life. The patient may or may not have symptoms and may or may not have CD-specific antibodies. Potential CD is defined by the presence of CD-specific antibodies and compatible HLA but without histological abnormalities in duodenal biopsies. The patient may or may not have symptoms and signs and may or may not develop a gluten-dependent enteropathy later (Husby *et al*., 2012).

Celiac Disease (CD) can be presented with unsuspected wide range of clinical manifestations, including typical malabsorption syndrome (chronic diarrhoea, steatorrhea, weight loss and abdominal distension) and could affect any organ or body system (neuropathy, anemia, decreased bone density and increased risk of fractures). However, CD is often presented atypically or even clinically silent; the vast majorities of CD cases remain undiagnosed for many years and expose long term complications (Ferguson *et al*., 1993).

The screening of CD is feasible and involves only slight discomfort to the screened population. So, many subclinical cases of CD would be detected by screening not only patients at-risk but also the general population.

There was a paucity of data assessing CD prevalence in T1D patients among the Arab population however; recently, CD is increasingly reported from North African countries including Egypt. Patients with T1D are considered a high risk group for CD. The frequency of CD diagnosis among T1D patients was investigated in this study. The present study found that CD prevalence in the Egyptian population is similar (0.3%) when compared with US and European studies (Maki *et al*., 2003; Ertekin *et al*., 2005). However, the prevalence was distinctly higher (10.3%) in the Egyptian type 1 diabetic population (at-risk patients) when compared with several previous studies.

The present study confirmed that CD prevalence in the Egyptian type 1 diabetic population is much higher (10.3%) when compared with US and European studies assuming that celiac disease could be under-diagnosed. Simple, non-invasive serologic tests detected celiac disease in this genetically predisposed population who had not previously been given a diagnosis of the disease. According to our findings, celiac disease diagnosed on clinical background represented the tip of the iceberg (0.3%, 1/300).

In this study, the serum IgA anti-TTG test was adopted as first-level screening of CD because it is an accurate, reliable, easy to perform and operator-independent test (Hill, 2005). However, CD was confirmed after positive distal duodenal biopsy. Actually, there is evidence in the literature suggesting that anti-TTG-IgA test has high positive predictive value for CD diagnosis. Although IgA anti-TTG does not lack specificity, we elected to be conservative in recommending a small intestinal biopsy for patients with positive results. Only few articles mentioned the prevalence of CD in Egypt either in general or at-risk populations (El-Shabrawi *et al*., 2011). This study indicates that CD is commonly under-diagnosed among Egyptian patients. The prevalence observed in T1D Egyptian patients was much higher than that previously reported by El Dayem *et al*. (2010) (10.3% versus 3%) but overlaps with previous studies from Europe and north African countries reporting a prevalence up to 16.4% (Bouguerra *et al*., 2005; Abu-Zekry *et al*., 2008).

A significantly higher prevalence of CD was found in this study in T1D patients (especially in long standing T1D) than non diabetic T1D relatives and healthy controls (10.3% vs. 2% and 0.3%, respectively). Most of the diagnosed Egyptian CD patients showed atypical, asymptomatic or mild to moderate forms of CD confirming that many screening-detected CD cases in Egyptians are subclinical. Thus at-risk individuals should always be screened serologically for CD. In T1D, Celiac disease should be screened not only at diabetes onset, but also in long-standing patients.

Patients with selective IgA deficiency (SIgAD) have low levels of IgA anti-TTG. So, CD diagnosis in patients with SIgAD will be missed by the IgA anti-tTG test (or any other IgA-based test such as EMA) but could be detected by a 2-step diagnostic approach (low total IgA levels; <0.5 U and positive IgG anti-tTG) (Villalta *et al*., 2007).

In this study, the use of this 2-step diagnostic approach determined a higher frequency of SIgAD in patients with CD (0.65%) than in the general population, a finding consistent with previous studies (Cataldo *et al*., 1998). It is of interest that the vast majority of Egyptian CD patients with T1D were found to have small bowel mucosal atrophy (Marsh IIIa,IIIb,IIIc), a finding that confirms a previous report from a study on Turkish CD patients which found that most patients had severe mucosal atrophy (Djuric *et al*., 2010). In contrast, patients who had minimal small bowel villi impairment were diagnosed by studies reported by Tanure *et al*. (2006) and Abu-Zekry *et al*. (2008).

By contrast, variable degrees of non specific damage of the intestinal mucosa were frequently found in patients without CD, probably related to what is called environmental enteropathy; intestinal infections and/or parasites (Gandolfi *et al*., 2001). All together highlight that CD of diagnosis in developing countries may be missed or delayed if it is based only on mucosal changes and support the important diagnostic role of serological testing for CD. The difference in histopathology between studies could be explained by the genetic diversity (HLA-DQ2 and DQ8 genotypes) of CD patients, the amount of gluten-containing food consumed and also by different stages in the course of CD when the diagnosis is made.

Celiac disease is a common multi-factorial disorder in which specific HLA-DQA1 and HLA-DQB1 alleles represent the major genetic predisposition. However, HLA typing does not have an absolute diagnostic value but only allows assessing the relative risk of CD. A positive test indicates genetic susceptibility but does not necessarily mean the disease development. Gluten intolerance rarely occurs in the absence of specific HLA predisposing alleles (good negative test). HLA genes are stable markers throughout life, so their typing can distinguish genetically CD-susceptible from non susceptible individuals before any serological or clinical signs. HLA test is increasingly considered as a solid support in the diagnostic algorithm of CD. New ESPGHAN guidelines for the diagnosis of CD have established that duodenal biopsy can be omitted in cases with elevated serum anti-TG2 antibodies (>10 times upper limit of normal), positive serology and at-risk HLA (Hill and Horvath, 2012; Klapp *et al*., 2013).

Both the genetic (HLA-DQ2 and DQ8 genotypes) and the environmental (gluten-containing food consumption) factors responsible for CD development are highly prevalent in Egypt. In the Egyptian population, the prevalence of HLA-DRB1*3 and HLA –DRB1*4 alleles (which are in strong linkage disequilibrium with the cis HLA-DQ2 (DQA1*0501, DQB1*0201) and DQ8 (DQA1*0301–DQB1*03:02) haplotypes, respectively) was recently reported to be 22.4 and 21.4%, a figure that is high in comparison with data from Europe and the United States (Bakr *et al*., 2007). The present study reported a significantly higher prevalence of these alleles (HLA-DR3 and HLA-DR4) than those reported by Bakr *et al*. (2007).

Moreover, HLA-DR3-DQ2 (DQA1*0501-DQB1*0201) positive individuals manifested a severe form of CD compared with HLA-DR4-DQ8 (DQA1*0301-DQB1*0302) positive subjects who exposed latent, atypical or mild CD. Additionally, in the present study, there were significant differences in the frequency of the two alleles among CD with T1D patients with different grades of Marsh histopathological types assuming that HLA-DR3-DQ2 and possibly HLA-DR4-DQ8 alleles not only confer susceptibility to CD but also could determine the severity of the disease. The carriage of both HLA-DQ2 (DQA1*0501, DQB1*0201) and DQ8 (DQA1*0301-DQB1*03:02) haplotypes was associated with a higher susceptibility to CD than either alone which was greater than individuals not carrying either. The rarity of CD (one patient in this study) in the absence of these DQ alleles suggests that the lack of possession of these DQ alleles makes CD very unlikely although not impossible. These observations are similar to and affirm those obtained by Biagi et al. (2012) reporting that the more severe forms of CD are marked by the DQ2 allele, mainly in the homozygous condition. All together, indicates that HLA molecular typing for CD is a genetic test with a negative predictive value. Nevertheless, it is a very important tool that discriminate genetically susceptible individuals to CD in either general population or in at-risk groups.

To explain these observations, it could be hypothesized that HLA-DQ2 is more efficient than HLA-DQ8 in presenting gliadin to the immune system. The dissimilarity of DQ2 and DQ8 in the binding site topology, charge distribution, gliadin deamidation and the degree of T-lymphocytes activation support this hypothesis (Kim *et al*., 2004; Tollefsen *et al*., 2006). Lastly, DQ8-positive patients had a reduced risk of CD compared with DQ2-positive patients. This could be related to the protease-sensitive nature of the á2-gliadin peptides which activate DQ8 (Henderson *et al*., 2007). All together, these findings could suggest that proteins coded by HLA-DQ genes are responsible for the histopathological, immunological and clinical diversity of the disease. Such suggestion is likely to have an impact on how to manage CD. However, it is not possible to predict whether and when the disease will develop in these patients.

We should not forget environmental factors responsible for CD development such as gluten-containing food consumption (the amount and time exposure to gluten and the patient's compliance to a gluten-free diet) (Murray *et al*., 2007; Biagi and Corazza, 2010).

The correlation between either tTG antibody titers or severity of intestinal impairment, demonstrated in this study, suggests that these autoantibodies may have a role in immunologic injury, even though such injury is cell-mediated. This finding may be important in understanding the heterogeneity of inflammatory response. Evidence had been presented specifying a predominantly Th1 pattern of cytokine production by the CD associated HLA-DQ restricted T cell clones. Occasionally, the inflammatory destruction of the small intestinal integrity initiated by gluten peptides goes further developing a proper autoimmune process which necessitates immunosuppressive drugs in addition to a gluten-free diet (Pena *et al*., 1998).

From this study, we could suggest that CD diagnosis should be through early case-finding by knowledge about protean presentations of the disease, serological testing in patients with vague symptoms and screening of at-risk groups. Despite the limitations of intestinal biopsy (invasive procedure, patchy lesions, proper specimens orientation is not standardized and subjective interpretation), it will remain the cornerstone for definitive diagnosis of CD in patients with immunological reaction to gluten for the foreseeable future.

After GFD there were significant improvement in anthropometric, clinical, serologic, histological and metabolic parameters in patients with T1D and CD. A highly significant observation was that such improvement was compliance and age-dependent; better response in compliant children than

adults. Encouragement of diagnosed patients with CD and T1D to receive a gluten-free diet as early as possible is highly recommended to avoid serious complications attributed to this additional burden. This is consistent with Kurppa *et al.* (2009) who demonstrated using a randomized, controlled study that patients with mild enteropathy (Marsh type I-II) with positive autoantibodies benefited from GFD in terms of improving symptoms, antibody response and mucosal inflammation. We could suggest that seropositivity in the absence of enteropathy may be an early predictor for later development of villous atrophy.

Type 1 diabetes is an autoimmune disease caused by the destruction of pancreatic islet β-cells. In addition to the genetic determinants (HLA), environmental factors (enteroviral infections and cow's milk proteins) are important in T1D development, probably by initiating or modifying an autoimmune process (Atkinson and Eisenbarth, 2001). An interesting finding in this study was that, T1D patients without CD when given GFD the diabetic state, hypoglycemic and DKA attacks decreased significantly. Together with the genetic similarities (HLA) of CD and T1D, this finding could raise the supposition that gluten may be a further environmental triggering factor for T1D.

CONCLUSION

This study showed that CD is a frequent but commonly under-diagnosed disorder among Egyptian patients, both in the general population and at-risk patients (T1D and their non diabetic relatives). HLA-DQ2 and DQ8 genotypes (in linkage with HLA-DRB1*3 and HLA-DRB1*4 alleles) and the environmental (gluten consumption) factors responsible for CD development are highly prevalent in Egyptian CD patients with T1D.

RECOMMENDATIONS

Being aware of the expanding epidemic of CD over populations consuming wheat, we hope that simplified diagnostic criteria, possibly avoiding the invasive and expensive biopsy, could help to diagnose CD. Increased awareness of CD and case-finding policy are highly recommended in Egypt. Celiac disease is a global health issue necessitating a multidisciplinary and collaborative multinational research. Long-term and follow-up studies of a larger population of at-risk groups, especially T1D will be required to determine environmental triggers other than gluten and the need for gluten-free diet. Finally, in the future, further efforts are warranted to improve knowledge about the genetic background of Egyptian patients with coexisting type1 diabetes and CD and to develop novel alternative or complementary therapeutic approaches for CD management.

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