

# Celiac disease-associated antibodies in type 1 diabetes patients in Cuba

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RESEARCH

## ABSTRACT

Celiac Disease (CD) is present in 1 to 16.4% of patients with diabetes mellitus type 1 disease. The most important serological markers of CD are anti-endomysial, anti-tissue transglutaminase (tTGA) and anti-gliadin antibodies. In this work, the frequency of tTGA in the Cuban population of diabetic patients was determined, by analyzing 208 subjects (116 male and 92 female; 19.04-years-old in average, ranging 2-58 years) suffering from type 1 diabetes mellitus. The tTGA were determined in the sera of patients as whole tTGA by an immunochromatographic test (HeberFast Line<sup>®</sup> anti-transglutaminase), and also as IgA tTGA by ELISA. Fourteen subjects were identified as positive by both assays, two of them showing gastrointestinal symptoms, being submitted to duodenal biopsy. Six of them consented with this analysis, showing morphological changes consistent with celiac disease and accounting for 2.88% prevalence in the diabetic population. This study confirms the prevalence of the celiac disease in diabetic patients in Cuba, and highlights the relevance of screening for this disease among them even in the absence of celiac disease-associated symptoms.

**Keywords:** Celiac disease, type 1 diabetes, anti-tissue transglutaminase antibodies, immunochromatography, prevalence, biopsy

*Biotecnología Aplicada 2008;25:47-50*

## RESUMEN

**Anticuerpos asociados a enfermedad celíaca en pacientes cubanos de diabetes tipo 1.** La enfermedad celíaca (EC) está presente entre el 1 al 16.4% de los pacientes aquejados por la diabetes mellitus tipo 1. Los anticuerpos anti-endomisio, anti-transglutaminasa tisular (TGAt) y anti-gliadina son los marcadores serológicos más importantes. En este trabajo, se determinó la frecuencia de TGAt en la población cubana de pacientes diabéticos, siendo analizados 208 pacientes (116 hombres y 96 mujeres; de 19.04 años de edad como promedio, en un rango de 2-58 años). La TGAt se determinó como TGAt total mediante un ensayo inmunocromatográfico (HeberFast Line anti-transglutaminasa) y como TGAt IgA mediante un ensayo tipo ELISA. Se identificó a catorce sujetos como positivos en ambos ensayos (dos de ellos mostrando síntomas gastrointestinales), lo que fueron remitidos a biopsia duodenal. Seis de ellos consintieron con el análisis, determinándoseles cambios morfológicos consistentes con EC, los cuales significaron el 2.88% de prevalencia en la población diabética estudiada. Este estudio confirma la prevalencia de la EC en pacientes diabéticos en Cuba, y resalta la relevancia de la pesquisa de esta enfermedad incluso en ausencia de síntomas asociados a EC.

**Palabras clave:** Enfermedad celíaca, diabetes tipo 1, anticuerpos anti-transglutaminasa tisular, inmunocromatografía, prevalencia, biopsia

## Introduction

Type 1 diabetes mellitus disease has been described as associated to several autoimmune diseases, such as thyroiditis, vitiligo, chronic gastritis, pernicious anemia, alopecia, rheumatologic abnormalities and celiac disease (CD), among others [1-2].

CD, also known as gluten-sensitive enteropathy, is an autoimmune disorder characterized by permanent intolerance to gluten-containing cereals, wheat, barley and rye [3]. Malabsorption (particularly lactose, iron- or folic acid-deficient anemia), abnormal stools, chronic diarrhoea or steatorrhea, bloating flatulence, abdominal distention, weight loss and poor growth are among the classical clinical manifestations of celiac disease, which usually disappear on a gluten-free diet once clinically identified the condition. However, recent studies revealed that only 20-30% of patients show typical

symptoms, the rest of them showing atypical extra-intestinal manifestations, or asymptomatic (silent) or monosymptomatic CD. Other accompanying symptoms are recurrent aphthous stomatitis, dental enamel defects, arthritis, osteoporosis, epilepsy associated or not to intracranial calcification, ataxia, polyneuropathy, infertility, dermatitis herpetiformis and alopecia [3-5].

Therefore, other analyses are required for the appropriate diagnosis of the disease. From an anatomopathological point of view, a complete villous atrophy and crypt hyperplasia of the small bowel mucosa have been demonstrated [6].

Based on the immunological etiology of CD, through the humoral and cellular reactions found in CD patients against gluten, new diagnosis procedures have been developed to detect antibodies against gliadin, reticulin,

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intestinal matrix components and tissue transglutaminase. These methods have proved useful to pre-select individuals, further confirmed as CD positive by small-bowel biopsy [4, 6, 7].

Some reports described the prevalence of CD in 1.0-16.4% in persons with type 1 diabetes mellitus [7]. The coexistence of CD and this type of diabetes seems to be genetically predisposed by alleles HLA B8, DR3 and DQ B1\*02 [1, 8].

In the present study, we determined the frequency of anti-tissue transglutaminase antibodies (tTGA) in a cohort of subjects with type 1 diabetes mellitus, also corroborating the usefulness of using the HeberFast Line® anti-transglutaminase immunochromatographic test to diagnose CD in diabetic patients.

## Materials and methods

### Subjects

A transversal study was carried out to determine the presence of tTGA in 208 type 1 diabetes mellitus patients. They were 116 males and 92 females patients, 19.04 ± 12.35 years-old in average (ranging 2-58 years), and showing a median diabetes duration of 3.78 ± 7.18 years, and a mean age at diagnosis of diabetes of 15.12 ± 11.52 years. Inclusion criteria comprised diabetic patients diagnosed as positive for antibodies against islet cells (ICA) and/or glutamic acid decarboxylase isoform 65 (antibodies against GAD65; GADA), also requiring insulin treatment at diagnosis. All the subjects included in the study agreed previously with the terms of the informed consent.

### Antitissue transglutaminase antibodies (tTGA)

The anti-tissue transglutaminase whole antibody responses were detected in all the patients by using a fast one-step immunochromatographic assay (HeberFast Line® anti-transglutaminase, CIGB, Havana, Cuba) [9]. Briefly, the HeberFast Line® anti-transglutaminase assay is an immunochromatographic (lateral flow technology) nitrocellulose-based strip as solid phase of the system, sensitized with ticular transglutaminase in the first line (positive line) and a control reagent capable of binding to tGT-colloidal gold conjugate in excess in the second line (control line). Plastic cassettes, containing transglutaminase-immobilized nitrocellulose strips and tGT-colloidal gold conjugate (mobile phase), were filled with direct blood serum or plasma samples and the test was let to run for up to 20 min. The assay was read in the reactive zone of the strips, with control colored lines evidenced for all the samples applied, and positive samples were identified by the presence of the respective colored line in the positivity area in the strips [9].

Anti-tissue transglutaminase IgA antibodies were specifically determined by ELISA (Celikey Pharmacia & Upjohn, Freiburg, Germany). Results were expressed as the ratio (R) between patient sample and cut-off optical density values at 492 nm ( $R = OD \text{ Patient Sample} / OD \text{ Cut-off}$ ). Values were considered positive for  $R > 1.4$  and negative for  $R < 1$ , with values considered as equivocal for  $1.0 < R < 1.4$ .

### Islet cell antibodies (ICA)

The antibody levels against ICA were determined by an indirect immunofluorescence technique with a prolonged incubation period as previously described [10]. All the subjects with ICA titers higher or equal to 10 JDF units (Juvenil Diabetes Foundation units) were considered positive. The assays were carried out in a laboratory from the National Institute of Endocrinology (lab 274, Havana, Cuba), previously certified as having 75% sensitivity and 75% specificity in the 8th ICA Proficiency Test [10].

### GAD 65 antibodies (GADA)

GADA response was detected by a quantitative radioimmuno-precipitation assay by using *in vitro* synthesized 35S-methionine-labeled human recombinant GAD65, followed by adding 50% protein Asepharose to separate free from antibody-bound labeled GAD65 [11]. Results were expressed as an index ( $\text{index} = \text{unknown sample cpm} - \text{negative standard control cpm} / \text{positive standard control cpm} - \text{negative standard control cpm}$ ). A GAD65 index of 0.035 was used as positivity threshold. This method has been previously certified as having 100% of sensitivity and specificity (First International Diabetes Workshop GAD Proficiency Program), and was carried out in the laboratory 155 (Rome, Italy), which was certified as having 97% specificity and 88% sensitivity (DASP 2002 IDS/CDC autoantibody program) [10].

### Duodenal biopsy

The patients positive for tTGA were respectively informed and submitted for gastro-intestinal endoscopy, with six of them consenting for it, from whom distal duodenum biopsies were taken at the endoscopy services in the "William Soler" Hospital. Standard morphological CD criteria were used for diagnosis [12].

### Statistical analysis

Data were given as mean ± SD. To assess differences between groups on the presence or absence of tTGA, statistical differences of the frequencies among groups were analyzed by using a two-tailed Aspin-Welch test for unequal variance. A p value below 0.05 was considered as statistically significant. Data was analyzed by using the Statical Package for the Social Sciences software (SPSS 11.0; SPSS, Chicago, IL).

## Results

In this study, we attempted to evaluate for the first time the prevalence of CD among diabetes patients, due to the negative impact of CD in the progression and/or control of diabetes. Only 14 subjects (9 female and 5 male) out of the 208 diabetic subjects included in the study were positive for tTGA, determined either by immunochromatographic assay or by ELISA, accounting for 6.73% seroprevalence. Of these 14 positive patients, two (14.28%) of them showing gastro-intestinal symptoms (abdominal pain, anorexia and diarrhoea) and four of the rest twelve symptoms-free patients (85.72%) were subjected to biopsy analyses. The morphological changes detected were consistent with CD in the six patients studied, accounting for a 2.88% (6/208) biopsy-proven CD prevalence. Five

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of them showed partial villous atrophy with elevated intraepithelial lymphocyte (IEL) counts, and the other one with subtotal villous atrophy. The mean age at the onset of diabetes for these six subjects was  $9.00 \pm 1.79$  years, statistically different ( $p < 0.0044$ ) to the same parameter for the group negative for tTGA, with a mean age at CD diagnosis of  $11.00 \pm 4.56$  years.

The mean interval between diabetes and CD diagnoses was  $2.00 \pm 4.00$  years (see table 1), with four patients (including the two showing CD symptoms) concomitantly diagnosed as CD positive at the onset of diabetes. The other two patients were diagnosed as CD positive two and ten years after diabetes onset, respectively. No patient had a first degree relative with CD record.

## Discussion

The association between type 1 diabetes mellitus and CD has been documented for several decades [1, 4, 13]. In fact, these entities share susceptibility gene alleles such as HLA B8, DR3 and DQ B1\*02, [1, 8]. In a recent study, we have found in our celiac patients a proportion carrying the DQ2 allele (86.3%), similar to that reported in other populations [14].

The occurrence of CD can accelerate diabetes worsening. Features of CD specific to type 1 diabetes include poor glycaemic control, decreased bone mineral density with increased risk of developing osteoporosis and fractures in adults like in non-diabetic CD patients [15, 16]. Then, patients with type 1 diabetes and celiac disease will potentially benefit from improved control of diabetes and less hypoglycaemic episodes, and few complications associated to celiac disease when they are treated with a gluten free diet [15, 16]. Therefore, the use of massive antibody screening helps to detect CD among diabetic patients with a high accuracy level (manuscript in preparation).

Instead of this advantage, the histological examination of a biopsy specimen of distal duodenum or small intestine remains the gold standard for definitive diagnosis. Therefore, we established the diagnosis of CD by sequentially determining tTGA and positive biopsy [17, 18]. Among the 208 subjects studied, six were fully diagnosed as CD following these two procedures, showing a consistent lesion [19]. The other eight tTGA positive diabetic patients did not consent to be biopsy studied for CD confirmation. We have found a 2.88% CD prevalence among diabetic patients, similar to that reported in other countries where massive serologic screening detected 1.0-16.4% [7].

Asymptomatic CD frequently occurs in type 1 diabetes and detected by serological screening [20]. We found that the 85.72% (12/14) of the tTGA positive

**Table 1.** Demographic characteristic of patients with type 1 diabetes according to the presence of tTGA and biopsy-proven CD

	N	Sex (F/M)	Age at DM <sup>a</sup> onset Mean $\pm$ SD <sup>b</sup> (years)	Age at CD <sup>c</sup> diagnosis Mean $\pm$ SD (years)	Interval between diagnosis of DM and CD (years)
tTGA <sup>d</sup> negative	194	83/111	19.26 $\pm$ 12.26	-	-
tTGA positive	14	9/5	12.50 $\pm$ 9.72	16.21 $\pm$ 13.19	3.71 $\pm$ 6.49
tTGA positive Biopsy-proven CD	6	4/2	9.00 $\pm$ 1.79*	11.00 $\pm$ 4.56	2.00 $\pm$ 4.00

<sup>a</sup>Diabetes mellitus

<sup>b</sup>Standard deviation

<sup>c</sup>Celiac disease

<sup>d</sup>tTGA: anti-tissue transglutaminase antibodies.

\* $p < 0.0044$  (p values compared to tTGA negative).

patients were asymptomatic and only 14.28% (2/14) had CD-related symptoms. The risk of gastrointestinal malignancy increases in patients with symptomatic disease, a phenomenon remaining to be demonstrated in patients with silent or sub-clinical CD [4, 20].

The prevalence of CD among type 1 diabetic subjects is approximately 20 times higher than in the general population. Sixty percent of the cases already suffering from CD at the onset of diabetes remain undetected, and the rest 40% of patients develop CD a few years after diabetes onset [21]. We confirmed that most of the patients biopsy-diagnosed for CD were also recently diagnosed diabetic at a younger age (4/6; 66.66%).

Additionally, we found a complete agreement between tTGA results obtained either by the immunochromatographic assay or by ELISA. Nevertheless, false negative results can arise due to IgA deficiency, a concomitant condition among CD patients [22]. On the other hand, the HeberFast Line<sup>®</sup> anti-transglutaminase assay can detect both IgA and IgG antibodies in only 10-20 minutes by a very simple procedure and starting from the direct sample. In previous studies, it has proven highly sensitive for CD positive samples from non-treated patients [9, 23], also shown accurate to screen for CD among diabetic subjects.

In summary, we conclude that CD is common among type 1 diabetes mellitus Cuban patients. Further coverage of tTGA positive subjects by biopsy screening would bring a more complete picture of the prevalence of CD, emphasizing in the follow-up diagnosis among the diabetic population for the control of this disease. The HeberFast Line<sup>®</sup> anti-transglutaminase (CIGB, Havana, Cuba) has proven to be an accurate biotechnological tool for this purpose.

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*Received in September, 2007. Accepted for publication in March, 2008.*