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RESEARCH ARTICLE

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Frequency of auto-antibodies of type 1 diabetes in adult patients with celiac disease

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Abstract

Both celiac disease (CD) and type 1 diabetes (T1D) are autoimmune diseases resulting from a complex interplay between genetic susceptibility and environmental factors.

Aim: In this retrospective study, we determined the frequency of auto-antibodies of T1D in adult patients with active CD.

Materials and methods: Eighty adult patients with active CD were included in our study. Ninety healthy blood donors (HBD) served as control group. Anti-glutamic acid decarboxylase IgG antibodies (GAD-Ab), anti-tyrosine phosphatase IgG antibodies (IA2-Ab), and anti-zinc transporter IgG antibodies (Zn-T8-Ab) were determined by enzyme-linked immunosorbent assay (ELISA) for patients and control group. For statistical analysis, we used Chi-square or Fisher's exact test.

Results: Out of 80 patients, 10 (12.50%) had auto-antibodies of T1D vs. only one in control group (1.11%) ($p = 0.003$). Simultaneous presence of GAD-Ab, IA2-Ab, and Zn-T8-Ab was found in one patient (1.25%). Nine patients had only GAD-Ab. IA2-Ab and Zn-T8-Ab were absent in all HBD. The frequency of GAD-Ab was significantly higher in CD patients than in HBD (12.5% vs 1.11%, $p = 0.003$).

Conclusion: The present study has shown that CD is associated with a high frequency of auto-antibodies of T1D. Screening for T1D in this population, at risk for other autoimmune diseases, may be useful.

KEYWORDS
adults, celiac disease, type 1 diabetes, type 1 diabetes auto-antibodies

1 | INTRODUCTION

Celiac disease (CD) is a multisystem autoimmune disease occurring in genetically predisposed people, in response to environmental factors and characterized by intestinal mucosal lesions and nutrient malabsorption.[1] The CD frequency in general population is 1% but the majority of cases remain undiagnosed.[2] This is mainly due to the high prevalence of paucisymptomatic or silent forms of CD.[3] Adult and pediatric gastroenterology societal guidelines recommend screening for CD in individuals at increased risk due to family history or the diagnosis of conditions associated with CD such as selective IgA deficiency, Turner's syndrome, autoimmune thyroid disease, and type 1 diabetes (T1D).[4] The coexistence of T1D and CD was attributed specially to a common genetic predisposition.

However, recent studies suggested the intervention of environmental factors in the co-occurrence of these two diseases. The aim of the current study was to investigate the frequency of T1D antibodies (IA2-Ab, GAD-Ab, and Zn-T8-Ab) in adult patients with active CD.

2 | STUDY PARTICIPANTS AND METHODS

2.1 | Study participants

In our retrospective study, sera of 80 adult patients (age ≥18 years) with active CD (newly diagnosed or known having CD but did not follow gluten-free diet [GFD]) were included from the database of our immunology laboratory. Patients with known preexisting T1D were not included in our study. Sera were collected over a 24-month period from four hospitals in the center of Tunisia. All patients had anti-endomysial and anti-transglutaminase 2 antibodies. Control sera were obtained from 90 healthy blood donors (HBD).

All sera were stored at −80°C until use. Ethical committee of our hospital gave approval for the study.

2.2 | Methods

2.2.1 | Type 1 diabetes auto-antibodies

Anti-tyrosine phosphatase IgG antibodies (IA2-Ab), anti-glutamic acid decarboxylase IgG antibodies (GAD-Ab), and anti-zinc transporter 8 IgG antibodies (Zn-T8-Ab) were determined using commercial ELISA kits (Euroimmun®). The assay was performed on microplate wells coated with human recombinant IA2, GAD, or Zn-T8 according to the manufacturer's recommendations. In the first reaction step, patient samples are incubated in the wells. If samples are positive, specific antibodies bind to the antigens. Bound antibodies are able to react divalently and form a bridge between the antigens on reagent wells and biotin-labeled IA2 or GAD or Zn-T8 added in a second incubation step. To detect the bound biotin, enzyme-labeled avidin (GAD-Ab or IA2-Ab) or enzyme-labeled streptavidin (Zn-T8-Ab) is added. The enzyme conjugate catalyzes a color reaction, and the intensity of the color is proportional to the concentration of antibodies. The photometric measurements are made at a wavelength of 450 nm then 405 nm within 15 min of adding the stop solution. The results were expressed in international units (IU/ml). The cutoff limit recommended by Euroimmun® is 10 IU/ml for IA2-Ab and GAD-Ab and 15 IU/ml for Zn-T8-Ab.

2.2.2 | Celiac disease auto-antibodies

Anti-endomysial IgA antibodies were performed by indirect immunofluorescence using cryostat sections (4 μm thick, done in our laboratory) of human umbilical cord as a substrate and fluorescein-labeled anti-human IgA antibodies (Bio-Rad®). A positive result was recorded if a connective tissue surrounding the muscle cells fluoresced brightly in a honey-comb pattern.

Anti-transglutaminase 2 IgA antibodies were determined by indirect ELISA (Orgentec®).

2.2.3 | Statistical analysis

Statistical analyses were performed by Epi Info version 3. The frequencies of T1D auto-antibodies in patients and in HBD were compared using Chi-Square or Fisher's exact test, and 95% confidence interval was calculated. All reported values were two tailed. A p-value <0.05 was considered significant.

3 | RESULTS

The present study involved 80 adult patients with active CD (60 females, 20 males). 20 patients had CD and did not follow strict GFD, and the others were newly diagnosed with CD. The mean age of our study population was 39.8 years (range 18–70). In the 90 control subjects, 56 were females. The mean age of our control group was 37.6 years (range: 20–64).

Out of 80 patients, 10 (12.50%) had auto-antibodies of T1D (7 females, 3 males) vs only one male of control group (1.11%) ($p = 0.003$). Simultaneous presence of GAD-Ab, IA2-Ab, and Zn-T8-Ab was found in one female patient (1.25%). Nine patients had only GAD-Ab. IA2-Ab and ZnT-8 were absent in all HBD.

The frequency of GAD-Ab was significantly higher in CD patients than in HBD (12.5% vs 1.11%, $p = 0.003$) (Table 1).

TABLE 1 Frequency of type 1 diabetes antibodies in patients and in control group

	Patients ($n = 80$)	Control group ($n = 90$)	p	95% CI
T1D	12.5% (10/80)	1.11% (1/90)	0.003	[4.202; 15.798]
GAD-Ab	12.5% (10/80)	1.11% (1/90)	0.003	[4.202; 15.798]
IA2-Ab	1.25% (1/80)	0.0% (0/90)	NS	__
Zn-T8-Ab	1.25% (1/80)	0.0% (0/90)	NS	__

Abbreviations: CI, confidence interval; GAD-Ab, anti-glutamic acid decarboxylase antibodies; IA2-Ab, anti-tyrosine phosphatase antibodies; NS, not significant; Zn-T8-Ab, anti-zinc transporter 8 antibodies.

Out of 80 patients with CD, 12.5% had GAD-Ab with a significantly higher frequency compared with IA2-Ab (1/10, $p = 0.005$) and Zn-T8-Ab (1/10, $p = 0.005$) (Figure 1).

Seven CD patients out of 10 with T1D antibodies were females (70%).

Levels of positive auto-antibodies and characteristics of patients are represented in Table 2.

4 | DISCUSSION

In the present study, we evaluated the frequency of different T1D-related antibodies in adult patients with active CD. We found that the frequency of T1D antibodies was significantly higher in active CD patients than in control group (12.5% vs 1.11%; $p = 0.003$). One patient had simultaneously GAD-Ab, IA2-Ab, and Zn-T8-Ab. Elfestrom et al.[5] suggested that almost 6% of patients with CD have T1D and that CD was associated with 2.4-fold increased risk of later T1D. The presence of one or more antibodies is usually required to have a positive immune diagnosis of T1D which distinguishes it from type 2 diabetes. In fact, 90% of T1D patients have IA2-Ab and/or GAD-Ab and/or Zn-T8-Ab. These antibodies are present months to years before symptom onset.[6,7] GAD-Ab was found to be the commonest[8] what is in agreement with what we found. The Zn-T8-Ab positivity decreases significantly from 57.38% at 1–2 years to 51.2% at 2–3 years of the duration of the disease.[9] GAD-Ab positivity is suggested to represent an evidence for general autoimmunity while IA2-Ab positivity may be a more specific marker of β cells destruction.[10]

Several studies have demonstrated elevated prevalence of CD among adults with T1D.[11-14] Only one study[15] was interested in determining auto-antibodies of T1D in adult patients with CD, and all patients were under gluten-containing diet. Findings of this study matched with our results. In fact, authors found that 10 patients with CD out of 92 (10.9%) had T1D-specific auto-antibodies. In their cohort, Kylökäs et al.[12] found that T1D was significantly overrepresented in CD. About one individual in six with both diseases was actually diagnosed with CD first.[5] In fact, untreated CD causes inflammation and nutritional deficiencies particularly vitamin D deficiency leading to the onset of T1D.[5]

The coexistence of CD and T1D could be explained by many similarities between both diseases. In fact, overlap in the susceptibility HLA haplotypes is the basis for the increased prevalence of T1D in CD. Thus, HLA regions DR3 or DR4 in association with DQ2 and DQ8 respectively are the strongest determinants of T1D and CD.[10,16-19] Over 90% of CD patients express HLA DR3 DQ2, as well as 55% of those with T1D, compared with <25% of the general population.[20] Genes other than HLA are associated with these two diseases such as interleukin 2 receptor, cytotoxic lymphocyte antigen 4, vitamin D receptor, and TNF alpha.[21,22]

Besides genetic susceptibility, several environmental factors are involved in the predisposition to CD and T1D. For CD, the main factor is the gluten. Ventura et al.[23] suggested, in a study carried out on 909 adolescents and young adults, that the prevalence of the onset of autoimmunity in CD is related to the duration of exposure to the gluten. In fact, exposure of an immature immune system to gliadin, in genetically predisposed individuals, alters the immune response early in life predisposing them to CD and other autoimmune diseases[23] including T1D.[24] Thus, a GFD helps prevent the occurrence of other autoimmune diseases in celiac patients. It was demonstrated that serum insulin-related antibodies disappeared in children with CD during GFD[25] and that the GFD allows a better control of diabetes and decreased frequency of insulin reactions.[26] On the other hand, other authors[27,28] reported that gluten withdrawal had no effect on the development of autoimmune diseases in adults. Both Ventura et al.[23] and sategna G[27] showed that the prevalence of autoimmune disease in children in whom CD was diagnosed before the age of two years was comparable with that of healthy control. The prevalence of autoimmune disorders is sevenfold higher in

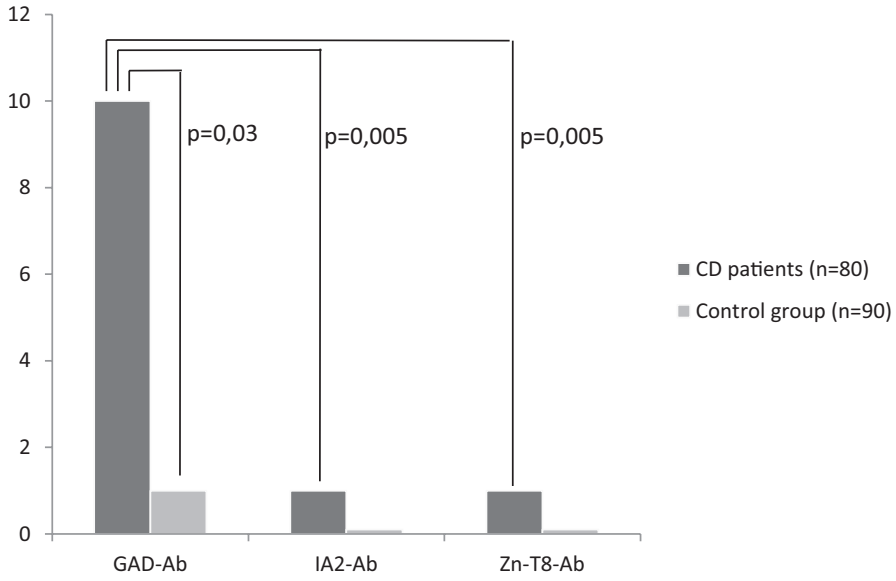

FIGURE 1 Frequency distribution of type 1 diabetes-specific auto-antibodies in patients with active celiac disease and in control group. CD, celiac disease; GAD-Ab, anti-glutamic acid decarboxylase antibodies; IA2-Ab, Anti-tyrosine phosphatase antibodies; Zn-T8-Ab, anti-zinc transporter 8 antibodies

TABLE 2 Levels of positive type 1 diabetes antibodies and characteristics of patients

Patient	Age	Sex	GAD-Ab (IU/ml)	IA2-Ab (IU/ml)	Zn-T8-Ab (IU/ml)	Medical records
1	25	F	>2000	-	-	Abdominal bloating-weight loss
2	30	F	24	-	-	Systemic lupus-rheumatoid arthritis-chronic diarrhea
3	27	M	>2000	-	-	Abdominal pain, asthenia
4	22	F	>2000	-	-	Diarrhea, abdominal pain
5	24	F	26	-	-	Chronic diarrhea
6	22	F	110	140	60	Persistent anemia
7	29	M	50	-	-	CD-not compliant with GFD
8	23	F	>2000	-	-	Anemia, constipation
9	19	M	140	-	-	Chronic diarrhea
10	23	F	1915	-	-	Chronic diarrhea

Abbreviations: CD, celiac disease; F, female; GAD-Ab, anti-glutamic acid decarboxylase antibodies; GFD, gluten-free diet; IA2-Ab, anti-tyrosine phosphatase antibodies; M, male; Zn-T8-Ab, anti-zinc transporter 8 antibodies.

patients with CD diagnosed after 10 years of age than in control group.[23] Having a CD for more than 15 years was associated with a 2.8-fold increased risk of death in individuals with T1D.[29] But the early detection of CD in the general population to prevent the co-occurrence of additional autoimmune diseases is a matter of continuous debate.[30]

Other mechanisms could explain this association. During active β cells destruction in T1D, TG2 expressed in pancreatic cells is released and might be presented in an immunogenic form. Such an inflammatory response could have the capacity to persist in genetically susceptible hosts and lead to chronic organ-specific autoimmune diseases.[10] In addition, a molecular mimicry by which gliadin or TG2 could activate T cells that are cross-reactive with autoantigens of T1D was suggested.[10]

On the other hand, the human microbiome might be a major player in autoimmunity, as the loss of immune tolerance can be caused by microbial composition changes.[31] Dysbiosis is involved in the pathogenesis of other autoimmune diseases.[32-34] A significant alteration in its composition was described in CD[35] and T1D patients.[32,34] Our study has shown that CD is associated with a high frequency of antibodies of T1D. Screening for T1D in this population, at risk for other autoimmune diseases, may be useful.

DATA AVAILABILITY STATEMENT

Data are not shared.

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