



Research article

Adherence to dietary treatment and clinical factors associated with anti-transglutaminase antibodies in celiac disease during the follow-up

Marta Miró^{a,1}, Manuel Alonso-Garrido^{a,1}, Manuel Lozano^{a,b,*}, Juanjo Peiró^c, Lara Manyes^a^a Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain^b Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain^c Department of Statistics and Operations Research, University of Valencia, Valencia, Spain

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ABSTRACT

Introduction: In clinical practice, celiac disease (CD) is monitored through anti-transglutaminase (TGA-IgA) antibody levels. The normalization of serum levels in successive periodic measurements indicates good response and adherence to dietary treatment.**Objectives:** To evaluate the factors associated with the evolution of TGA-IgA antibodies and their association with dietary non-compliance and diseases related to CD.**Methods:** This prospective observational study was carried out in 254 participants, who were recruited from patients from a hospital in southern Spain. Information about sex, age, serological test results, HLA DQ2/DQ8 haplotypes, mucosal atrophy, gastrointestinal and extra-intestinal symptoms, as well as diagnosis of diseases related to CD, was collected.**Results:** Clinical manifestations, such as diarrhoea, abdominal pain and weight loss, showed differences according to sex and age. Children under 18 years of age presented a degree of total or severe atrophy of the intestinal villi. TGA-IgA antibodies concentrations were directly associated with the number of digestive disorders manifested by the patient and the record of dietary non-compliance and inversely related to the number of extra-digestive disorders.**Conclusions:** Adolescents between 12 and 18 years old were the least monitored as well as the group with more extra-intestinal symptoms reported. Therefore, it is necessary to develop strategies in clinical practice aimed at this population group and continuous monitoring should be implemented to improve life quality and reduce complications that may arise in the long term.

1. Introduction

The only effective treatment for celiac disease (CD) is a strict gluten free diet (GFD). However, the reported celiac adult compliance varies between 17–45% [1]. Among other factors, it depends on the age of the patient or the age at the time of diagnosis, especially during the puberty and adolescence, where there is a greater autonomy in dietary decisions, making it hard to comply with the GFD [2]. The occasional ingestion of gluten does not usually produce any symptoms, although it does affect the intestinal mucosa causing inflammation and increasing the risk of long-term complications [3]. In clinical practice, CD is monitored through TGA-IgA antibody levels, since the normalization of serum levels in successive periodic measurements indicates good response and

adherence to treatment. A dietary interview, along with each antibody measurement, is recommended in order to detect non-compliance with the GFD [4]. Once gluten is removed from the diet, levels of TGA-IgA antibodies should stabilize in the next 6–12 months and damage to the digestive mucosa should be reversed in around 6–24 months [5].

CD is an inflammatory, chronic, and multisystem disorder mediated by the immune system, with a prevalence of 1–3%; It is triggered by the ingestion of water-insoluble proteins (gluten) contained in cereals such as wheat, barley and rye, in genetically predisposed individuals [6]. A delay in the diagnosis of CD increases the risk of developing other immune disorders due to the duration of exposure to gluten, so there is evidence of a gluten-related autoimmunity in genetically predisposed people, which may develop autoimmune disorders involving other

* Corresponding author.

E-mail address: manuel.lozano@uv.es (M. Lozano).¹ Both authors contributed equally to the manuscript.

organs than the intestine; Thus, CD has been associated with endocrine manifestations (autoimmune thyroiditis, Addison, type I diabetes mellitus), liver (autoimmune hepatitis, primary biliary cirrhosis), dermal (herpetiform dermatitis) and connective tissue (Sjögren's syndrome), among other immune diseases [7, 8]. Furthermore, previous studies reported that prevalence of concomitant autoimmune disease increases with age at the diagnosis of celiac disease [9].

Delay in diagnosis CD could also be associated with the atypical clinical presentation profile [10]. Currently, the atypical CD form is the most common with non-digestive symptoms. The increase in severity in these symptoms not related to CD has been associated with a heterogeneous immune response which is able to interfere with the diagnosis [11]. Therefore, early detection of CD is necessary in order to reduce the risk due to continuous gluten exposure, especially in patients with extraintestinal manifestations which can lead to a late diagnosis of the disease, unlike patients with classical CD whose typical gastrointestinal symptoms allow an early diagnosis [12].

The aim of this study is to evaluate the factors association with the evolution of TGA-IgA antibodies and with dietary non-compliance and diseases related with CD.

2. Materials and methods

2.1. Study population

From 2007 to 2017, a prospective observational study was carried out in 254 participants, who were recruited from patients of the Hospital San Agustín (Linares, Jaén), which is responsible for a population area of 129,233 inhabitants (2018) in southern Spain. Only patients with IgA negative tissue transglutaminase antibody serology (<10 U/mL) were excluded from the study. From the total sample (mean age [95% CI] = $24.3 \pm [23.1-25.5]$ years), 212 were registered with a confirmatory diagnosis of CD, 18 were kept with a diagnosis of suspected CD (with symptomatology and positive serology but pending CD confirmation), and 24 were considered silent patients (positive serology but without symptomatology) [13]. The protocol of this study was approved by the Ethics Committee of the San Agustín Hospital. Study participants were informed of the objectives and the scope of the study and signed an informed consent for their participation.

2.2. Data collection

Criteria for patient's inclusion in the study followed the below protocol: positive serological test result (IgA tissue anti-transglutaminase antibodies ≥ 10 U/mL), determination of HLA DQ2/DQ8 haplotypes and confirmation by duodenal biopsy (evaluated by Marsh-Oberhuber classification [14]). TGA-IgA antibodies were determined by Fluorescent Enzyme Immunoassay (EliA Celikey® IgA Well), in the ImmunoCAP 250 autoanalyzer from Phadia Thermofisher®. The HLA DQ2/DQ8 haplotypes were determined using the GENVINSET HLA CELIAC® kit [15]. Briefly, preparation of the duodenal biopsy is performed using a formalin fixation (10% buffered formalin), paraffin inclusion and hematoxylin-eosin staining (it was used immunohistochemical staining to evaluate Marsh I and II stages) [16]. Serial biopsies prepared according to suggested were reviewed by a pathologist. Also, antibodies TGA-IgA, total IgA immunoglobulin and antibodies TGA-IgG in patients with selective deficit of IgA, were determined by an external laboratory. According to hospital protocol, first measurement of antibodies is recommended at 3–6 months after the introduction of dietary treatment, with successive measurements every 6 months until the patient's antibody level is normalized [16]. For every patient included in the study, at least 4 measurements were ensured in order to determine the existence of eating transgressions. The determination of non-compliance to GFD, based on this periodic evaluation of antibodies, was developed as follows: 1) 'inconclusive', when there were less than 3 TGA-IgA antibody values per patient (excluded from the study); 2) 'without transgression',

when the progression was clearly descending and evolving towards normal values; 3) 'with transgression' when the positivity of the abnormal level of antibodies persisted, or when the medical specialist, based on the interview with the patient, stated in the medical history that there had been transgressions. Antibodies concentrations depend on gluten, so a persistently positive serology or a lack of decrease in serum levels after one year of GFD indicates poor dietary compliance or unintentional exposure to gluten [17].

The covariates introduced in this study were: age (years), sex (man, woman), year of CD diagnosis, presence (yes/no) of digestive disturbances: 1) classic pattern (diarrhea, steatorrhea, weight loss); 2) non-classic pattern with unspecific digestive symptoms, actually more frequent (abdominal pain, vomiting, epigastralgia, constipation, bloating, dyspepsia, loss of appetite), presence (yes/no) of extra-digestive disorders derived from in much of micronutrient malabsorption (muscle, respiratory, skin, liver, neurology, anemia, allergies, osteopenia/osteoporosis, oral thrush), determination of the level of TGA-IgA antibodies, presence (yes/no) of food transgressions, presence (yes/no) of diseases associated with CD, detected in the patients included in this study (diabetes mellitus, thyroid disorder, neurological and psychiatric disorders such as attention deficit disorder and hyperactivity, dermatitis herpetiformis, irritable colon, Crohn's disease, ulcerative colitis, erosive duodenitis, alopecia areata, infertility, cancer, Down's syndromes, Asperger's, Sjögren and poliendocrine, obesity, Raynaud's disease, and results of biochemical tests associated with CD (iron ($\mu\text{g/dL}$) and ferritin (ng/mL)).

2.3. Statistical analysis

Mean (M) and standard deviation (SD) were calculated for antibodies TGA-IgA concentrations according to sex and the age-range of the study population. The Pearson Chi-square test and the ANOVA F-test were applied to the TGA-IgA concentrations to compare the mean of sex and age range across categories.

Probable association between TGA-IgA repeated measures, as the response variable, and predictive covariates, was assessed by using mixed multivariate models. Predictors for the response variable were adjusted according to the following procedure: (1) A basal model was built following a backward elimination procedure considering the complete 254 participants: starting from model including all covariates related to the TGA-IgA concentrations at $p\text{-value} < 0.20$ in univariate analysis, we sequentially excluded in a backward procedure those variables with an adjusted $p\text{-value} > 0.10$. The model with lower AIC [18] and higher determination coefficient R^2 was selected. Sex, age and time of each measure (from first to fourth time point) were included for adjustment purposes in all models, regardless of their statistical significance [17]. The predictors effect was considered statistically significant if the $p\text{-value} < 0.05$, although marginally significant effects ($p\text{-value} < 0.1$) were also considered. Sensitivity analyses were carried out to evaluate the robustness of the multivariate model by excluding all non-confirmed cases using Marsh scale ($< \text{level } 3$). The validity of the regression models was tested by residual analyses. No influential data were identified by graphical representation. Collinearity diagnostics were conducted on the final models. Analyses were conducted with the statistical software R, version 3.5.1 [19].

3. Results

3.1. Clinical manifestations

Clinical manifestations and mucosa atrophy at the time of the diagnosis, antibody concentrations during the follow up, and demographic characteristics of the study, are shown in Table 1. The mean age of the patients included in the study was 24.2 years, with no significant differences between sexes. A total of 254 patients (60.2% women and 39.8% men) were studied. Among them, 212 (83.5%) had a confirmed diagnosis of CD (59.9% were women with a mean age of 25.3 years and 40.1% men with a mean age of 22.7 years). Women showed a greater tendency to

Table 1. Descriptive variables based on age and sex.

Variable	Sex		p-value ^a	Age			p-value ^a	
	Men	Women		0–11 years	12–18 years	>18 years		
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)		
Ab (U/mL) – 1 st measure	147.8 (118.4)	138.55 (119.0)	153.9 (117.9)	0.311	181.9 (125.1) [†]	136.2 (112.6) [†]	131.1 (112.8) [†]	0.009*
Ab (U/mL) – 2 nd measure	91.8 (92.3)	93.2 (99.8)	90.8 (87.3)	0.842	133.7 (106.3) [†]	92.7 (90.4) [†]	64.1 (71.5) [†]	<0.001*
Ab (U/mL) – 3 rd measure	44.1 (52.0)	42.6 (58.0)	45.1 (47.8)	0.712	47.5 (62.8)	42.8 (60.1)	42.5 (38.9)	0.789
Ab (U/mL) – 4 th measure	23.8 (26.7)	21.4 (22.5)	25.5 (29.1)	0.229	20.3 (21.2) [†]	17.8 (16.6) [†]	28.9 (32.3) [†]	0.013*
Age (years)	24.2 (19.3)	22.7 (18.4)	25.3 (19.9)	0.283	7.0 (2.8)	14.2 (2.1)	40.3 (17.0)	
Digestive alterations (N)	3.7 (1.2)	3.5 (1.2)	3.7 (1.2)	0.202	3.7 (1.0)	3.5 (1.3)	3.7 (1.3)	0.384
Extradigestive alterations (N)	3.7 (1.3)	3.7 (1.3)	3.8 (1.3)	0.433	3.9 (1.4)	4.1 (1.3)	3.5 (1.2)	0.014*
Associated pathologies (N)	2.6 (0.9)	2.5 (0.8)	2.7 (1.0)	0.200	2.7 (1.1)	2.6 (0.8)	2.6 (0.9)	0.709
	N (%)	N (%)	N (%)	p-value ^b	N (%)	N (%)	N (%)	p-value ^b
Sex	254 (100%)	101 (39.8%)	153 (60.2%)					
Men					30 (29.7%)	24 (23.8%)	47 (46.5%)	0.862
Women					48 (31.4%)	32 (20.9%)	73 (47.7%)	
CD diagnosis								
Confirmed	212 (83.5%)	85 (40.1%)	127 (59.9%)	0.131	64 (30.2%)	45 (21.2%)	103 (48.6%)	0.539
Not confirmed	24 (9.4%)	6 (25%)	18 (75%)		7 (29.2%)	5 (20.8%)	12 (50%)	
Suspicion	18 (7.1%)	10 (55.6%)	8 (44.4%)		7 (38.9%)	6 (33.3%)	5 (27.8%)	
Therapeutic follow-up								
No	62 (24.4%)	19 (30.6%)	43 (69.4%)	0.092	9 (14.5%)	12 (19.4%)	41 (66.1%)	<0.001*
Yes	192 (75.6%)	82 (42.7%)	110 (57.3%)		69 (35.9%)	44 (22.9%)	79 (41.1%)	
HLA diagnosis								
Negative	12 (4.7%)	1 (8.3%)	11 (91.7%)	0.023*	2 (16.7%)	1 (8.3%)	9 (75%)	0.140
Positive	242 (95.3%)	100 (41.3%)	142 (58.7%)		76 (31.4%)	55 (22.7%)	111 (45.9%)	
Diagnostic EMA								
Negative	6 (2.4%)	2 (33.3%)	4 (66.7%)	0.745	0 (0%)	0 (0%)	6 (100%)	0.032
Positive	248 (97.6%)	99 (39.9%)	149 (60.1%)		78 (31.5%)	56 (22.6%)	114 (46%)	
Ferritin								
Increased	2 (0.8%)	1 (50%)	1 (50)	0.766	0 (0%)	0 (0%)	2 (100%)	0.324
Deficit	252 (99.2%)	100 (39.7%)	152 (60.3%)		78 (31%)	56 (22.2%)	118 (46.8%)	
Iron								
Increased	5 (2%)	2 (40%)	3 (60%)	0.991	2 (40%)	1 (20%)	2 (40%)	0.900
Deficit	249 (98%)	99 (39.8%)	150 (60.2%)		76 (30.5%)	55 (22.1%)	118 (47.4%)	
Diarrhoea								
No	90 (35.4%)	26 (28.9%)	64 (71.1%)	0.009*	28 (31.1%)	21 (23.3%)	41 (45.6%)	0.907
Yes	164 (64.6%)	75 (45.7%)	89 (54.3%)		50 (30.5%)	35 (21.3%)	79 (48.2%)	
Abdominal pain								
No	100 (39.4%)	49 (49%)	51 (51%)	0.015*	43 (43%)	21 (21%)	36 (36%)	0.002*
Yes	154 (60.6%)	52 (33.8%)	102 (66.2%)		35 (22.7%)	35 (22.7%)	84 (54.5%)	
Weight loss								
No	189 (74.4%)	81 (42.9%)	108 (57.1%)	0.086	59 (31.2%)	48 (25.4%)	82 (43.4%)	0.046*
Yes	65 (25.6%)	20 (30.8%)	45 (69.2%)		19 (29.2%)	8 (12.3%)	38 (58.5%)	
Vomits								
No	155 (61%)	60 (38.7%)	95 (61.3%)	0.668	30 (19.4%)	24 (15.5%)	101 (65.2%)	<0.001*
Yes	99 (39%)	41 (41.4%)	58 (58.6%)		48 (48.5%)	32 (32.3%)	19 (19.2%)	
Dietary non-compliance								
No	138 (54.3%)	50 (36.2%)	88 (63.8%)	0.210	44 (31.9%)	34 (24.6%)	60 (43.5%)	0.375
Yes	116 (45.7%)	51 (44%)	65 (56%)		34 (29.3%)	22 (19%)	60 (51.7%)	

(continued on next page)

Table 1 (continued)

Variable	Total	Sex		p-value ^a	Age			p-value ^a
		Men	Women		0–11 years	12–18 years	>18 years	
		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	
Marsh diagnosis								
0	22 (8.7%)	12 (54.5%)	10 (45.5%)	0.673	2 (9.1%)	1 (4.5%)	19 (86.4%)	<0.001*
1	5 (2%)	1 (20%)	4 (80%)		1 (20%)	1 (20%)	3 (60%)	
2	1 (0.4%)	0 (0%)	1 (100%)		0 (0%)	1 (100%)	0 (0%)	
3a	54 (21.3%)	23 (42.6%)	31 (57.4%)		5 (9.3%)	13 (24.1%)	36 (66.7%)	
3b	68 (26.8%)	25 (36.8%)	43 (63.2%)		13 (19.1%)	13 (19.1%)	42 (61.8%)	
3c	61 (24%)	23 (37.7%)	38 (62.3%)		23 (37.7%)	22 (36.1%)	16 (26.2%)	
No biopsy [†]	43 (16.9%)	17 (39.5%)	26 (60.5%)		34 (79.1%)	5 (11.6%)	4 (9.3%)	

Ab: antibodies TGA-IgA.

SD: standard deviation.

HLA: Human leukocyte antigen.

EMA: anti-endomysial antibody.

^a p-value from ANOVA F- test.

^b p-value from Chi-square test.

* significant ANOVA/Chi-square test (p value < 0.05).

[†] significant Tukey test (p value < 0.05).

[‡] Patients under 2 years had no biopsy following the ESPGHAN criteria [43].

suffer diarrhoea and abdominal pain (p-value Chi-square test = 0.009 and 0.015, respectively). 75.6% of the sample registered a correct therapeutic follow-up, which was stronger for the older group, which registered a higher frequency of abdominal pain and loss of weight (p-value from ANOVA F-test = 0.002 and 0.046, respectively). The youngest group reported the highest frequency of vomiting (p-value from ANOVA F- test <0.001). Participants aged between 12-18 years had the lowest degree of therapeutic follow-up (22.9%), being also the group with the highest number of extra-digestive disorders (mean (SD) = 4.1 (1.3)).

3.2. Mucosal atrophy

From the total of participants, 45.7% registered dietary transgressions at some point of the treatment, without observing significant

differences regarding sex or age. At the time of diagnosis, most of the adults presented a mild-moderate villous atrophy degree (66.7% type 3a and 61.8% type 3b). In contrast, children under 12 years of age showed a tendency to present a severe atrophy of the intestinal villi (see Table 1).

3.3. Diseases associated with CD

The mean of diseases associated with CD was 2.6 (0.9), with no differences between sexes or age ranges, highlighting: 1) thyroid disorders (36.6%), which included: autoimmune thyroiditis, hypo and hyperthyroidism (both confirmed and suspected by altered levels of Thyroid-Stimulating Hormone (TSH) or free T3 and T4 thyroxins); 2) diabetes mellitus (11.0%), including type I and II; 3) irritable colon (9.4%); 4)

Table 2. Frequencies of potentially related diseases to CD and known associated prevalence.

Diseases	Study frequencies N (%)	Associated prevalence according to bibliography
Thyroid disorder	93 (36.6%)	30.3% in adults [44] 26.2% in children [45]
Diabetes mellitus	28 (11%)	1–12% [46, 47]
Irritable colon	24 (9.4%)	0–11.4% [5, 6]
Obesity	21 (8.3%)	0–6% [48]
Attention deficit hyperactivity disorder	16 (6.3%)	6–11% [49]
Sterility	7 (2.8%)	2.1–4.1% [50]
Colon polyps	7 (2.8%)	20% [51]
Cancer	7 (2.8%)	Associated to all cancers with high prevalence variability [52]
Uterine fibroid	7 (2.8%)	–
Dermatitis herpetiformis	6 (2.4%)	13% [53]
Down syndrome	4 (1.6%)	3–12% [50, 54]
Alopecia areata	4 (1.6%)	1–2% [55]
Endometriosis	4 (1.6%)	2.5% [56]
Erosive duodenitis	3 (1.2%)	2–42% [57]
Raynaud's disease	3 (1.2%)	0.33–1.34% [58, 59]
Ulcerative colitis	3 (1.2%)	1.3% [49]
Crohn's disease	3 (1.2%)	1.3% [49]
Polyendocrine syndromes	3 (1.2%)	10–30% [60]
Sjögren syndrome	2 (0.8%)	2–15% [54, 61]
Asperger	2 (0.8%)	0.95–2.62% [62, 63]
Polycystic ovaries	2 (0.8%)	Associated in only 1 study [64]
Perthes	1 (0.4%)	–

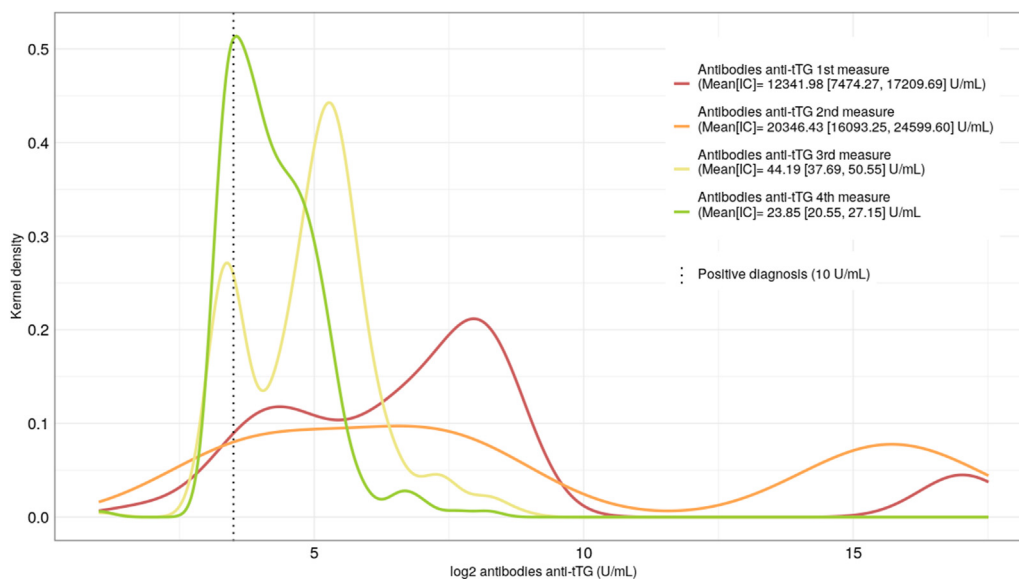


Figure 1. Kernel diagram with TGA-IgA antibodies concentrations at each measure. Reference concentration for positive diagnosis (>10 U/mL).

obesity (8.3%), considering a Body Mass Index (BMI) ≥ 30 kg/m²; and Attention Deficit Hyperactivity Disorder (ADHD) (6.3%) (Table 2).

3.4. GFD follow-up

Figure 1 shows the Kernel diagram with the TGA-IgA antibody concentrations in each of the four measurements versus the reference value proposed as a positive diagnosis of CD (10 IU/mL) [20]. Results also show that the mean of serum TGA-IgA antibody decreased progressively between the first and fourth measure (p-value from ANOVA F-test <0.001, p-value Tukey t-test <0.05 for every pair measured), being the steepest decrease among patients aged 0–18 years (see Table 1).

The multivariate mixed model (Table 3) showed a better fit (lower AIC score and higher determination coefficient R-square) for the direct association between TGA-IgA antibodies concentrations logarithm and the number of digestive disorders as well as the reported transgressions ($\beta = 0.146$, p-value = 0.115 and $\beta = -0.530$, p-value = 0.017, respectively). The model also showed an inverse relationship between

antibodies' concentrations and the number of extra-digestive disorders ($\beta = -0.164$, p-value = 0.056). Sensitivity analysis did not report significant changes (see Table 3).

4. Discussion

This study has observed differences in digestive and extra-digestive clinical manifestations according to sex and age. Also, TGA-IgA antibodies concentrations were directly associated with the number of digestive disorders manifested by the patient and the record of dietary non-compliance, and inversely related to the number of extra-digestive disorders.

Differences in digestive and extra-digestive symptoms were observed. According to sex and age, a higher level of villus atrophy in children under 18 years of age and a progressive decrease in the level of TGA-IgA antibodies, were directly associated with presence of digestive disorders, registry of dietary transgressions and inversely related to the presence of extra-digestive disorders.

Table 3. Output from the fitted mixed multivariate model before backward procedure and sensitivity analyses.

Covariates	Total sample (n = 254)		Excluding non-confirmed cases (n = 183)	
	β [CI 95%]	p-value	β [CI 95%]	p-value
N of digestive disorders	0.146 [−0.035, 0.327]	0.115	0.175 [−0.020, 0.370]	0.081
N of extra-digestive disorders	−0.164 [−0.331, 0.004]	0.056	−0.274 [−0.478, −0.070]	0.009*
Transgressions (reference 'no')	0.530 [0.095, 0.965]	0.017*	0.670 [0.162, 1.178]	0.010*
Adjustment variables				
Age (years)	0.006 [−0.005, 0.017]	0.299	0.008 [−0.005, 0.021]	0.236
Sex (reference 'male')	0.319 [−0.124, 0.762]	0.159	0.173 [−0.353, 0.700]	0.519
2nd measure time (reference 1st)	1.661 [1.146, 2.176]	<0.001*	1.960 [1.351, 2.569]	<0.001*
3rd measure time (reference 1st)	−2.402 [−2.916, −1.887]	<0.001*	−2.250 [−2.859, −1.641]	<0.001*
4th measure time (reference 1st)	−3.010 [−3.615, −2.585]	<0.001*	−2.908 [−3.518, −2.299]	<0.001*

N: number.

CI: Confidence interval.

* Significant p-value < 0.05.

The female trend in the frequency of diarrhoea and abdominal pain had been previously observed in other studies [21]. However, in another study, also in Minnesota, it was observed that the prevalence of abdominal pain and diarrhoea was higher in men [22]. Possible causes of atypical manifestations, such as abdominal pain, could be based on the superposition of other functional disorders such as irritable bowel syndrome, where CD is 3–4 times more prevalent, or an undiagnosed intolerance to food components [23].

In our study, we observed that patients >18 years were associated with a higher frequency for abdominal pain and weight loss. A recent pilot study in adult patients with seronegative CD, showed weight loss and abdominal pain as a characteristic form of presentation of the illness [24]. There are other studies in celiac adults they also include weight loss and abdominal pain among its main symptoms [25]. Another study carried out in the Netherlands in 412 celiac adults, reported the following results in order of prevalence: diarrhea, fatigue, weight loss and abdominal pain [26], similar to those obtained in the present study. On the other hand, children under 18 years of age were more frequently associated with vomiting in our study, which is consistent with research carried out in 16 countries in the Mediterranean area on 749 celiac children, divided by age groups, which it related vomiting as the most prevalent symptom in the age group between 6–10 years [27]. However, in a multicenter study carried out in Spanish children, divided by age groups (0–2, > 2–6, > 6–15 years), it was associated with other symptoms such as bloating (<6 years) and loss of appetite and iron deficiency anemia (>6 years) [28]. Regarding non-classical or atypical extra-intestinal alterations, our study related them to the group of 12–18 years. This fact is also supported by other studies. In this sense, it is worth mentioning a study developed in the United States, which observed that the mean age at the time of diagnosis for patients with classic CD was 8.09 years with symptoms such as diarrhoea and developmental delay. However, the mean age for children with non-classical CD was 10.19 years and it was associated with extra-intestinal manifestations [29]. Another study carried out in 165 children (2–18 years old) from New York, showed a mean age of diagnosis for CD of 10.7 years, most of them associated with non-classic manifestations such as abdominal pain and constipation. The authors pointed out that this increase in the presentation of CD in its non-classical form could be due to changes in eating habits and a greater consumption of processed food products that contribute to a greater exposure to the gliadin antigen [30].

It is well known that the antibody titer decreases with age [31, 32]. A proposed mechanism for this phenomenon is based in the theory of the sequestration of TGA-IgA antibodies over time by the intestinal mucosa [32]. The results of the present study indicate a higher antibody titer at younger ages, although they reach similar values to the group of adults in the fourth serological measure. This result was also observed in a study conducted in northern Spain (97 children and 227 adults), in which the correlation of TGA-IgA antibodies and the degree of villous atrophy was evaluated to avoid duodenal biopsy. Adults showed less severe histopathology and lower antibody titers than children [20]. The results of the present study also highlight that those under 18 years were associated with the highest degree of intestinal villous atrophy (3c-severe lesion according to the Marsh-Oberhuber classification in duodenal biopsy), compared to those over 18 years of age, associated with a degree of injury 3a-mild and 3b-moderate. This association has also been observed in previous studies. In a multicenter study developed on 974 celiac Spanish children under the age of 15, 46.4% showed a level of atrophy 3b, followed by level 3c in 28.4% of cases, associated with a high prevalence of classic forms (digestive symptoms) [28]. Another Canadian study of 140 celiac children under 17 years of age found that 42.8% of patients had severe villous atrophy or Marsh 3c [33].

The most outstanding result associating the level of TGA-IgA antibodies directly with the number of digestive disorders and dietary non-compliance, as well as inversely with extra-digestive disorders is a study of 445 celiac patients in Finland which correlated the highest TGA-IgA antibody titre with the greatest deterioration of the digestive mucosa,

and also with the highest presence of digestive symptoms [32]. In a prospective study in 170 Hungarian patients an increase in the level of TGA-IgA antibodies was shown in CD patients with more severe enteropathy (Marsh 3b and 3c) and a greater degree of gastrointestinal clinical presentation. Furthermore, the study associated a higher degree of recovery in the level of antibodies in children after the GFD [34]. On the other hand, a study conducted in the Netherlands in 116 celiac children showed that those with TGA-IgA antibodies ≥ 100 U/mL had a higher prevalence of extra-intestinal symptoms than children with levels of TGA-IgA antibodies <100 U/mL, which manifested more gastrointestinal symptoms. The authors suggested that those patients with high antibody titers, extra-digestive manifestations and more severe digestive injuries, were related to a more advanced or generalized disease [35]. However, other studies do not link the level of antibodies with age or the symptoms of CD. For example, a study carried out in 59 celiac children from Saudi Arabia studied the clinical presentation, serology and the degree of atrophy of the disease, noting that although the children had the highest degree of villous atrophy, it was not related to TGA-IgA antibody levels [36]. In another wider study conducted in the Netherlands which included 4,442 children for screening of CD TGA-IgA antibodies, there was also no relationship between gastrointestinal symptoms, the degree of enteropathy and the level of TGA-IgA antibodies [37]. On the other hand, celiac patients are more susceptible to develop other diseases associated with CD, possibly by sharing the same genetic component, which show extra-digestive symptoms. The most common are type 1 diabetes mellitus, autoimmune thyroiditis, and inflammatory bowel disease. These comorbidities present an heterogeneous immune response which can be confound the diagnosis of CD based on the antibody titer [38, 39].

Little is known about the dynamics of antibody reduction after GFD and the effect of non-compliance. After treatment, around 80% of patients offer negative serologies at 6–12 months and 90% at 5 years [17]. Non-compliance with GFD, has been significantly related to increased serum TGA-IgA antibody level [17] (45.7% of patients committed dietary non-compliance during the present study). The relationship between the level of TGA-IgA and dietary non-compliance has been well established in previous studies [40, 41]. An Italian study in 204 patients, which divided the sample into two age groups, reported eating transgressions in 26.5% of the sample, with the serum level of TGA-IgA antibodies increased in children (<13 years old) and adolescents (>13 years old) [40]. Another Italian study in 2,245 celiac patients, developed a follow-up strategy with annual serologies (TGA-IgA antibodies) and periodic clinical visits, observing that a third of the patients presented fluctuating serologies due to unstable adherence to GFD [41].

There are limitations in the present study: (1) a bias derived from the study design is the lack of knowledge about the presence of previous diseases associated with CD before the diagnosis; (2) not known is if the patient starts the gluten-free diet right after the CD confirmed diagnosis; a precise period of time has been studied including patients with a previous EC diagnosis and new diagnosed; (3) a relatively small sample size, although the follow-up period was long and the low prevalence of CD in southern Europe (260–1000 cases/100,000) should be considered [42]; (4) the lack of inclusion in the study of other serum antibodies such as antiendomysium or antigliadin, although tissue antitransglutaminase was chosen for its sensitivity and specificity in diagnosing and monitoring the disease. On the contrary, this study strengths are: (1) obtention of the data directly from the medical records and not by means of questionnaires, which avoids biases both from the interviewer and patient's suggestion; (2) the statistical analysis, which also includes the performance of sensitivity analysis and confounding assessment; and (3) the study's prospective design, which made it possible to obtain detailed information concerning biological and clinical characteristics that may affect antibodies concentrations outcomes.

In conclusion, clinical manifestations on CD showed differences in digestive and extra-digestive symptoms according to sex and age. Women showed a greater tendency to suffer from diarrhea and

abdominal pain, those over 18 years were associated with a higher frequency of abdominal pain and weight loss, and those under 18 years were associated with the presence of vomiting, as well as a higher level of villous atrophy. TGA-IgA antibodies levels were directly associated with the number of digestive disorders manifested by the patient and the record of dietary non-compliance, and inversely related to the number of extra-digestive disorders. The less monitored group and with more extradigestive disorders reported were those between 12 and 18 years old. Therefore, it is necessary to develop strategies in clinical practice aimed at this population group and continuous monitoring should be implemented to improve life quality and reduce complications that may arise in the long term.

Declarations

Author contribution statement

Marta Miró: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Manuel Alonso-Garrido: Analyzed and interpreted the data; Wrote the paper.

Manuel Lozano, Lara Manyes: Conceived and designed the experiments.

Juanjo Peiró: Analyzed and interpreted the data.

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The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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