Rev. Toxicol (2021) 38: 64 - 71

Association between small-bowel mucosal damage and related diseases: Observational study in celiac patients

Miró M[1], Font G[1], Manyes L[1], Lozano M[1,2]

[1] *Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Avenue Vicent Andrés Estellés s/n, 46100, Burjassot, Spain*
[2] *Epidemiology and Environmental Health Joint Research Unit, FISABIO−Universitat Jaume I−Universitat de València, Valencia, Spain*

Abstract: Celiac disease is a systemic, chronic and autoimmune disorder that affects genetically susceptible individuals. Due to the increasing incidence of this pathology and the precision of new detection methods, celiac disease diagnosis has improved dramatically in recent years. Hereby, a study was performed to evaluate celiac disease's prevalence, attending to associated diseases as well as clinical determinants. A convenience sample of 254 patients diagnosed between 2007 and 2017 in the South of Spain was selected: 212 were confirmed for celiac disease, 18 remained with suspected celiac disease and 24 were considered silent patients. Multivariate logistic regression models were applied to patients' data. 95.3% of the subjects obtained a positive result in the genetic-molecular diagnosis, with prevalence of female patients' group (58.7%). Moreover, females were associated with diarrhea and abdominal pain to a greater extent (54.3% and 66.2%, respectively). Youngsters had accused villi atrophy and larger concentrations of anti-tTG antibodies compared to adults, but had more adhesion to treatment and recovered better than the older group. Deficit in Fe and multimorbidity were also factors associated with villi atrophy. The multivariate analysis adjusted for sex and age showed a direct association between intestinal lesion and Fe deficit, the presence of vomiting and the number of diseases associated with celiac disease. Novel results of the present study refer to the association between the level of intestinal injury and the multimorbidity associated with celiac disease.

Keywords: anti-tissue transglutaminase antibody, multivariate logistic regression, iron, extra-digestive disorders, multimorbidity..

Resumen: ***Asociación entre lesiones en la mucosa del intestino delgado y otras enfermedades: Estudio observacional en pacientes celiacos***

La enfermedad celíaca es un trastorno sistémico, crónico y autoinmune que afecta a individuos genéticamente susceptibles. Debido al incremento en la incidencia de esta patología y la precisión de los nuevos métodos de detección, el diagnóstico de la enfermedad celíaca ha mejorado drásticamente en los últimos años. De esta manera, se ha realizado un estudio para evaluar la prevalencia de la enfermedad celíaca, atendiendo tanto a las enfermedades asociadas como a los determinantes clínicos. Se seleccionó una muestra de conveniencia de 254 pacientes diagnosticados entre 2007 y 2017 en el sur de España: 212 fueron confirmados de enfermedad celíaca, 18 permanecieron con sospecha de enfermedad celíaca y 24 fueron considerados pacientes silentes. Se aplicaron modelos de regresión logística multivariante a los datos de los pacientes. El 95,3% de los sujetos obtuvo un resultado positivo en el diagnóstico genético-molecular, con predominio del grupo de pacientes del sexo femenino (58,7%). Además, las mujeres se asociaron en mayor medida con diarrea y dolor abdominal (54,3% y 66,2%, respectivamente). Los jóvenes se asociaron a una mayor atrofia de las vellosidades y concentraciones de anticuerpos-tTG superiors en comparación con los adultos, pero mostraron una mayor adherencia al tratamiento y se recuperaron mejor que el grupo de mayor edad. El déficit de Fe y la multimorbilidad también fueron factores asociados con la atrofia de las vellosidades. El análisis multivariante ajustado por sexo y edad mostró una asociación directa entre la lesión intestinal y el déficit de Fe, la presencia de vómitos y el número de enfermedades asociadas a la enfermedad celíaca. Los nuevos resultados del presente estudio se refieren a la asociación entre el nivel de lesión intestinal y la multimorbilidad asociada a la enfermedad celíaca.

Palabras clave: anticuerpo antitransglutaminasa tisular, regresión logística multivariante, hierro, trastornos extradigestivos, multimorbilidad.

Introduction

Celiac disease (CD) is a systemic, chronic and autoimmune disorder that affects genetically susceptible individuals (Tye-Din et al., 2018). CD is triggered by the combination of environmental (gluten), genetic (HLA DQ2 / DQ8) and immunological (innate and adaptive immunity) factors that lead to a loss of oral gluten tolerance. Incomplete digestion of gliadin and glutenin from gluten provokes large peptides accumulation that alter intestinal permeability (Perez-Gregorio et al., 2018; Reig-Otero et al., 2017) and are deaminated by anti-tissue transglutaminase-2 (tTG), released during inflammation. In the innate response, interleukin 15 is released by the enterocytes activating intraepithelial lymphocytes. The adaptive response is mediated by TCD4+ lymphocytes, HLA and proinflammatory cytokines (Arranz & Garrote, 2010; Moscoso & Quera, 2016).

Due to the high sensitivity and specificity of diagnostic tests, the incidence of CD has increased in recent years depending on age and geographic area; rates in adults have increased from 3.08 in 1995 to 11.13 / 100,000 inhabitants and in children from 2.08 (years 1981-1985) to 6.89 / 100,000 inhabitants in 2001-2005 (Hurley et al., 2012). The diagnosis of CD is characterized by the joint presence of clinical manifestations, tTG, endomysium and deaminated gliadin peptides; HLA and enteropathy (Husby et al., 2012). The degree of intestinal injury is described in the Marsh-Oberhuber classification (N Marsh et al., 2015; Oberhuber et al., 1999).

Nowadays, there is a void in evaluating the involvement of associated diseases causing enteropathy, which are capable of inducing atypical symptomatology. In addition, prevalence and descriptive studies in the Mediterranean arch are limited, as well as the analysis of clinical characteristics on the susceptible population (Navalón-Ramon et al., 2016). Therefore, the goal of this work is to study the level of intestinal damage in celiac patients, considering the CD associated comorbidity and identifying the clinical determinants.

Methods

Population description

A prospective observational study was conducted in a convenience sample of 254 participants diagnosed of CD between 2007 and 2017. Participants were recruited among patients of the San Agustín Hospital (Linares, Jaén), which serves a population area of 129,233 inhabitants (2018) from the north of Jaén. Patients with negative IgA tissue anti-transglutaminase antibody serology (<10 U/ml) were excluded from the study. Of the total sample (age mean [95% CI] =

*e-mail: lara.manyes@uv.es

24.3 ± [23.1-25.5] years), 212 were registered with a confirmatory diagnosis of CD, 18 remained with a diagnosis of suspected CD and 24 were considered silent patients. The study was approved by the Research Ethics Committee on May 31st, 2018.

Data collection

The required patient parameters included a positive serological test result (IgA tissue anti-transglutaminase antibodies ≥10 U/ml), determination of HLA DQ2 / DQ8 haplotypes, anti-endomysial antibodies and confirmation by duodenal biopsy, which is the gold standard technique of definitive diagnosis (Güngör et al., 2019). The preparation of the duodenal biopsy was carried out by means of a fixation (10% buffered formalin), inclusion in paraffin and hematoxylin-eosin staining (Grupo de trabajo del Protocolo para el diagnóstico precoz de la enfermedad celíaca, 2018). The HLA DQ2 / DQ8 haplotypes were determined using the GENVINSET HLA CELIAC® kit for the detection of DQB1 * 02, DQB1 * 03: 02 and DQA1 * 05 alleles of the HLA system (human leukocyte antigen). In the case DQB1 * 02, it is able to determine its presence in homo or heterozygosis based on real-time PCR technology using TaqMan® probes (Fasano et al., 2015). Anti-tTG IgA antibody determinations were performed by Fluorescent Enzyme Immunoassay (EliA Celikey® IgA Well), in the Phadia Thermofisher® InmunoCAP 250 autoanalyzer. These tests allow the determination of IgA antibodies directed against the tissue transglutaminase antigen (Sulkanen et al., 1998). According to the hospital protocol, after the implantation of the dietary treatment, a first measurement of antibodies is recommended at 3-6 months and successive every 6 months-examination until the patient's antibody level is normalized (Grupo de trabajo del Protocolo para el diagnóstico precoz de la enfermedad celíaca, 2018). In all patients included in the study, at least 4 measures were performed in order to determine the existence of food transgressions. The determination of food transgressions was performed based on the periodic evaluation of antibodies as follows: 1) 'inconclusive', when there were less than 3 values of anti-tTG antibodies per patient (excluded from the study); 2) 'without transgression', when the progression is clearly descending, evolving towards normal values; 3) 'with transgression' when the positivity of the abnormal level of antibodies persists, or when the medical specialist states in the clinical history that there have been transgressions based on the interview with the patient.

Several potentially predictive covariates of intestinal injury were collected at the beginning of the study through the history and biochemistry of the patients reflected in the clinical history. Thus, information on: age (years), sex (male, female), year of diagnosis of CD, presence (yes / no) of digestive disorders (diarrhea, abdominal pain, weight loss, vomiting, epigastralgia, constipation, bloating abdominal, dyspepsia, loss of appetite, steatorrhea), presence (yes / no) of extra-digestive disorders (muscular, respiratory, skin, liver, neurological, anemia, allergies, selective IgA deficit, osteopenia / osteoporosis, oral thrush), presence (yes / no) of diseases associated with CD (diabetes mellitus, thyroid disorder, neurological and psychiatric disorders such as attention deficit hyperactivity disorder, dermatitis herpetiformis, irritable colon, Crohn's disease, ulcerative colitis, erosive duodenitis, alopecia areata, sterility , cancer, Down, Asperger, Sjögren and polyendocrine syndromes, obesity, Raynaud's disease) (Dominguez Castro et al., 2017; Glissen Brown & Singh, 2019), and results of biochemical tests associated with CD (iron (µg/dl) and ferritin (ng/ml)) (Burger et al., 2018; Cilleruelo et al., 2014).

Statistical analysis

The association between MARSH score, as ordinal categorical response variable, and collected predictive covariates by using multivariate logistic regressions models with cumulative link models was analyzed.(Agresti & Kateri, 2017) Predictors were adjusted to the response variable in accordance with the following procedure: (1) A basal model was built following a backward elimination procedure considering all 254 participants: starting from a model including all covariates related to the response variable at $p < 0.20$ in univariate analysis, and then sequentially excluding those variables with an adjusted p-value >0.10. Sex and age were included in all models regardless of their statistical significance. (2) Stratified analysis by sex and age range (2-11, 12-18,> 18 years) was performed by building a multivariate model using only participants of each category. Analyzes were conducted with the statistical software R, version 3.4.3 (R Core Team, 2017).

Results

Results on the descriptive variables of the study are shown in Table 1. The average age of patients included in the study was 24.2 years, without differences between sexes. The mean of serum anti-tTG antibody decreased progressively between the first and fourth measurement, and significantly (p-value Tukey t-test <0.05) according to age during the first and second measurements. Patients aged 12-18 years registered a greater number of extra-digestive disorders (mean (SD) = 4.1 (1.3)). However, the lack of therapeutic follow-up was associated with older age (p-value Chi^2 test <0.001), which was also associated with greater weight loss and lower presence of vomiting (p-value Chi^2 test = 0.046 and <0.001, respectively). 95.3% of the subjects obtained a positive result in the genetic-molecular diagnosis (HLA), with prevalence of female patients' group (58.7%). Female sex was also associated with diarrhea and abdominal pain to a greater extent (54.3% and 66.2%, respectively). Most adults had a mild-moderate degree of villous atrophy, 66.7% type 3a and 61.8% type 3b. Regarding children under 18 years (age group 0-11 and 12-18 years), they presented mostly a degree of total or severe atrophy of the intestinal villi. According the criteria of ESPGHAN (Nevoral et al., 2013), biopsy was avoided in children <2 years (79.1% of non-biopsied participants).

The analyses of bivariate ordinal logistic regressions for each of the covariates included in the study and MARSH's diagnosis was also performed. The independent effect of each covariate at the level of intestinal injury (expressed as Odds Ratio (OR)), adjusted by sex and age are shown in Figure 1. An independent direct association in the first measure between the increase in intestinal lesion and the level of anti-tTG antibodies ($\beta = 1.002$; $p = 0.054$), the presence of vomiting (OR = 1,742; p-value = 0.056) and the number of diseases associated with celiac disease (OR = 1,845; p-value <0.001) were observed. Frequencies of associated diseases to CD and their known prevalence are listed in Supplemental Table S1.

The multivariate analysis showed a direct and significant association between intestinal lesion and Fe deficit (OR = 5,716; p-value = 0.028), the presence of vomiting (OR = 1.813; p-value = 0.042) and the number of diseases associated with celiac disease (OR = 1,920; p-value = <0.001). Figure 2 shows the significant associations for each covariate in the adjusted multivariate models stratified by sex and age. Level of intestinal injury is particularly affected by the presence of vomiting (OR = 2,717; p-value = 0,034) and the greater number of diseases associated with celiac disease (OR = 1,736; p-value = 0,030) in males, while in females the intestinal lesion was correlated with the set of digestive disorders (diarrhea (OR = 1,771; p-value = 0.098), abdominal pain (OR = 0.502; p-value = 0.058), weight loss (OR = 0.555; p-value = 0.082) and vomiting (OR = 1.877; p-value = 0.002). On the contrary, only an association between intestinal injury and diarrhea (OR = 0.251; p-value = 0.079) was found in children under 12 years of age. A direct association of injury with the level of anti-tTG antibodies was also observed in the first measure in patients over 18 years (OR = 1.003, p-value = 0.097). Intestinal lesion was also related to the last measure of anti-tTG antibodies (OR = 0.971, p-value = 0.085) and number of diseases was associated with CD (OR = 6.249, p-value = 0.002) among patients aged 12-18 years.

Table 1. *Descriptive variables based on age and sex.*

		Sex			Age			
Variable	**Total**	**Men**	**Women**		**0-11 years**	**12-18 years**	**> 18 years**	
	Media (SD)	**Media (SD)**	**Media (SD)**	**p[a]**	**Media (SD)**	**Media (SD)**	**Media (SD)**	**p[a]**
Ab (U/mL) – 1st 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) – 2nd 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) – 3rd 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) – 4th 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[b]**	**N (%)**	**N (%)**	**N (%)**	**p[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%)		64 (30,2%)	45 (21,2%)	103 (48,6%)	
Not confirmed	24 (9,4%)	6 (25%)	18 (75%)	0,131	7 (29,2%)	5 (20,8%)	12 (50%)	0,539
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	6 (2,4%)							
Negative		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%)	
Diarrhea								
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%)	
Transgression								
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%)	
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 biopsia‡	43 (16,9%)	17 (39,5%)	26 (60,5%)		34 (79,1%)	5 (11,6%)	4 (9,3%)	

Ab: antibodies anti-TGt
SD: standard deviation
[a] p-value from ANOVA F- test
[b] p-value from Chi^2 test
* significant ANOVA/Chi^2 test (p value <0.05)
† significant Tukey test (p value <0.05)
HLA: Human leukocyte antigen
EMA: anti-endomysial antibody
‡ Patients under 2 years had no biopsia following the ESPGHAN criteria

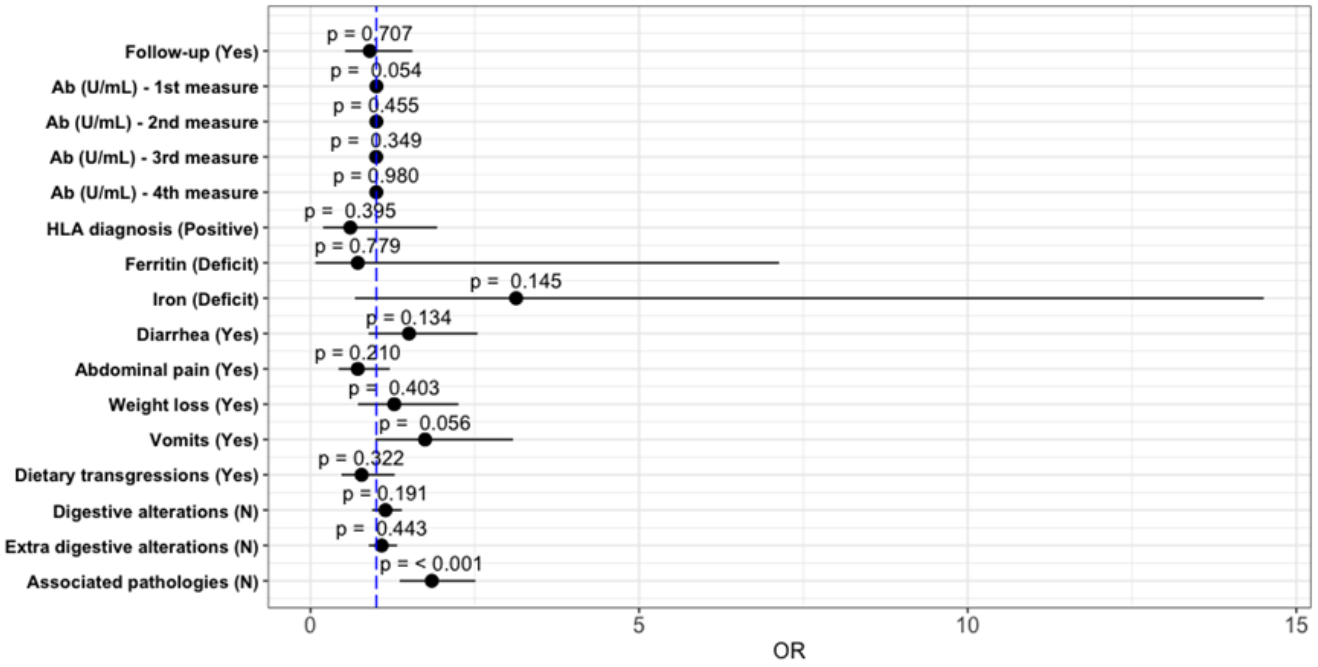

Figure 1. Bivariate ordinal logistic regression models, adjusted for sex and age.

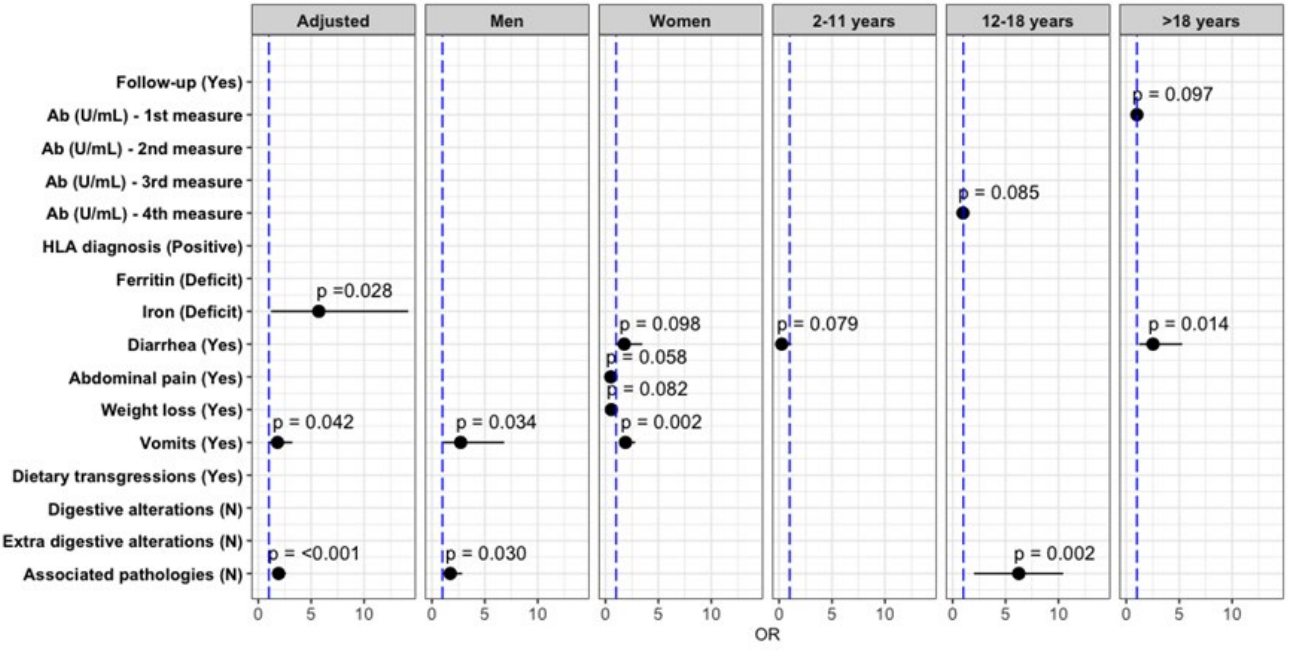

Figure 2. Multivariate ordinal logistic regressions models, adjusted and stratified by sex and age.

Discussion

Firstly, results pointed to higher female prevalence of CD, associated with positive genetic - molecular diagnosis (HLA) and digestive disorders such as diarrhea and abdominal pain. These results are consistent with other studies. A prospective study conducted in 178 Hungarian patients related female gender to a higher level of injury according to Marsh-Oberhuber classification (Kocsis et al., 2013), while a study conducted in a cohort of 385 American patients associated, in addition to sex, other digestive factors such as the presence of vomiting (Rubio-Tapia et al., 2016). Also, a study with a much larger number of subjects (n = 2264) carried out in Sweden, confirmed the correlation between female sex and the prevalence of CD during childhood, without finding significant causalities (Wingren et al., 2012). Only few studies found no significant differences between sexes (Brar et al., 2007).

Secondly, according to MARSH classification, children under 18 years of age presented a greater degree of atrophy on the intestinal villi than the other groups. Also, youngsters responded better to dietary treatment, showing a pronounced decrease in serum anti-tTG antibodies when analyzing differences between the first and fourth measure. This could be due to the larger degree of therapeutic follow-up and weight maintenance observed compared to the older group, but it seems there could be other variables involved. In this sense, in a Sudanese population study conducted in 60 celiac patients between 2-70 years old, where 41.6% of the participants were under 10 years old, authors also found an inverse association between Marsh's score for intestinal injury and age (Mokhtar et al., 2016). Another Spanish study conducted in 66 children and 54 adults showed greater villous atrophy and a higher level of anti-TGt antibodies in 86% of children versus a 52% of adults, although it did not determine the evolution of the antibodies during the study period (Vivas et al., 2008).

Additionally, the multivariate analysis adjusted for sex and age showed a direct association between intestinal lesion and Fe deficit, the presence of vomiting and the number of diseases associated with celiac disease. Association between the degree of villous atrophy and the deficit of Fe is also shown in the Sudanese study, in which 41.7% of the sample presented deficiency (Mokhtar et al., 2016). The relationship between Fe deficit and celiac disease is well established in the scientific literature, linking patients with anemia to causality based on CD, as indicated in a recent meta-analysis that included 2998 patients with anemia (Mahadev et al., 2018). The presence of vomiting was also observed in an American study as a common gastrointestinal symptom in CD (Rubio-Tapia et al., 2016). In a US cohort consisting of 215 patients, nausea was reported by 42% of participants before dietary treatment and vomiting occurred in a fifth of these patients. These symptoms usually show remission with the follow-up of a gluten-free diet (Murray et al., 2004).

Finally, novel results of the present study refer to the association between the level of intestinal injury and the multimorbidity associated with CD. There are few studies trying to relate villi damage, related with unique pathologies, to other diseases such as type I diabetes (Fröhlich-Reiterer et al., 2011). Some studies have linked CD with several associated diseases, but only to assess the prevalence of these diseases in patients previously diagnosed with CD. A study developed in India in 363 celiac patients which exploring other autoimmune diseases or a recent multinational study conducted in Italy and the US, which included 289 patients, associated CD and abdominal pain-associated functional gastrointestinal disorder (Nijhawan et al., 2013). However, these studies have some limitations. On one hand, the convenience of the sample, which could lead to a bias in data acquisition. On the other hand, data on the symptoms and diagnosis of associated diseases were collected from the clinical history and not through the use of validated questionnaires that contemplate all possible symptoms and related clinical pathologies, which could induce possible tendencies and vagueness.

In conclusion, patients with CD who have associated diseases, as well as those who suffer from vomiting among their gastrointestinal symptoms, have almost double the risk of showing a greater degree of atrophy of the intestinal villi. Likewise, patients with Fe deficiency may present almost 6 times greater risk of worsening the level of atrophy. Additionally, female prevalence of CD, diarrhea and abdominal pain has been observed. Younger patients, who have better therapeutic follow-up and weight maintenance, are associated with a greater degree of villi atrophy, although they respond better to dietary treatment by showing a larger decrease in anti-tTG antibodies.

Supplementary material

Supplemental Table S1. *Frequencies and known prevalence of associated diseases with celiac disease.*

Diseases	Study frequencies N (%)	Associated prevalence according to bibliography
Thyroid disorder	93 (36,6%)	30.3% in adults (Sategna-Guidetti et al., 2001) 26.2% in children (Baharvand et al., 2020)
Diabetes mellitus	28 (11%)	1-12% (De Leo et al., 2016; Escribano-Serrano et al., 2016)
Irritable colon	24 (9,4%)	0-11.4% (Capriati et al., 2016; Güngör et al., 2019)
Obesity	21 (8,3%)	0-6% (Capriati et al., 2016)
Attention deficit hyperactivity disorder	16 (6,3%)	6-11% (Güngör et al., 2019)
Sterility	7 (2,8%)	2.1-4,1% (Rostom et al., 2006)
Colon polyps	7 (2,8%)	20% (Pereyra et al., 2013)
Cancer	7 (2,8%)	Associated to all cancers with high prevalence variability (Han et al., 2015)
Dermatitis herpetiformis	6 (2,4%)	13% (Salmi, 2019)
Down syndrome	4 (1,6%)	3-12% (Lindfors et al, 2019; Rostom et al., 2006)
Alopecia areata	4 (1,6%)	1-2% (Rios et al., 2013)
Endometriosis	4 (1,6%)	2.5% (Aguiar et al., 2009)
Erosive duodenitis	3 (1,2%)	2-42% (Perez-Cuadrado-Robles et al., 2018)
Raynaud's disease	3 (1,2%)	0.33-1.34% (Bartoloni et al., 2019; Gabrielli et al., 2003)
Ulcerative colitis	3 (1,2%)	1,3% (Güngör et al., 2019)
Crohn's disease	3 (1,2%)	1,3% (Güngör et al., 2019)
Polyendocrine syndromes	3 (1,2%)	10-30% (Kahaly et al., 2018)
Sjögren syndrome	2 (0,8%)	2-15% (Lindfors et al, 2019; Zylberberg et al., 2018)
Asperger	2 (0,8%)	0.95-2.62% (Batista et al., 2012; Calderoni et al., 2016)
Polycystic ovaries	2 (0,8%)	Associated in only 1 study (Grode et al 2018)
Perthes	1 (0,4%)	-

References

1. *Aguiar F, Melo S, Galvão L, Rosa-e-Silva J, dos Reis R, Ferriani R.* Serological testing for celiac disease in women with endometriosis. A pilot study. Clin Exp Obstet Gynecol. 2009;36(1):23–25.
2. *Agresti A, Kateri M.* Ordinal probability effect measures for group comparisons in multinomial cumulative link models. Biom. 2017; 73(1), 214–219.
3. *Arranz E, Garrote J.* Immunology of celiac disease. Gastroenterol Hepatol. 2010; 33(9), 643–651.
4. *Baharvand P, Hormozi M, Aaliehpour A.* Comparison of thyroid disease prevalence in patients with celiac disease and controls. Gastroenterol Hepatol Bed Bench. 2020;13(1):44–49.
5. *Bartoloni E, Bistoni O, Alunno A, Cavagna L, Nalotto L, Baldini C, et al.* Celiac Disease Prevalence is Increased in Primary Sjögren's Syndrome and Diffuse Systemic Sclerosis: Lessons from a Large Multi-Center Study. J Clin Med. 2019;8(4):540.
6. *Batista I, Gandolfi L, Nobrega Y, Almeida R, Almeida L, Campos Junior D, et al.* Autism spectrum disorder and celiac disease: no evidence for a link. Arq Neuropsiquiatr. 2012;70(1):28–33.

7. *Brar P, Kwon G, Egbuna I, Holleran S, Ramakrishnan R, Bhagat G, et al.* Lack of correlation of degree of villous atrophy with severity of clinical presentation of coeliac disease. Dig Liver Dis. 2007; 39(1), 26–29.

8. *Burger J, Van der Laan J, Jansen T, Drenth J, Roovers E, Wessels M, et al.* Low yield for routine laboratory checks in follow-up of coeliac disease. J Gastrointestin Liver Dis. 2018; 27(3), 233–239.

9. *Calderoni S, Santocchi E, Del Bianco T, Brunori E, Caponi L, Paolicchi A, et al.* Serological screening for Celiac Disease in 382 pre-schoolers with Autism Spectrum Disorder. Ital J Pediatr. 2016;42(1):98.

10. *Capriati T, Francavilla R, Ferretti F, Castellaneta S, Ancinelli M, Diamanti A*. The overweight: A rare presentation of celiac disease. Eur J Clin Nutr. 2016;70(2):282–284.

11. *Cilleruelo M, Roman-Riechmann E, Sanchez-Valverde F, Donat E, Manuel-Ramos J, Martín-Orte E, et al.* Spanish national registry of celiac disease: Incidence and clinical presentation. J Pediatr Gastroenterol Nutr. 2014; 59(4), 522–526.

12. *De Leo S, Lee S, Braverman L.* Hyperthyroidism. Lancet. 2016;388(10047):906–918.

13. *Dominguez Castro P, Harkin G, Hussey M, Christopher B, Kiat C, Liong Chin J, et al*. Changes in Presentation of Celiac Disease in Ireland From the 1960s to 2015. Clin Gastroenterol Hepatol. 2017; 15(6), 864-871.e3.

14. *Escribano-Serrano J, Mancera-Romero J, Santos-Sánchez V, Payá-Giner C, Méndez- Esteban M, García-Bonilla A, et al.* Prevalence of Hypothyroidism in Andalusia, Spain, Determined by Thyroid Hormone Comsumption. Rev Esp Salud Publica. 2016;90:e1–12.

15. *Fasano M, Dametto E, D'Alfonso S.* HLA Genotyping: Methods for the Identification of the HLA-DQ2,-DQ8 Heterodimers Implicated in Celiac Disease (CD) Susceptibility. Methods Mol Biol. 2015; 1326, 79–92.

16. *Fröhlich-Reiterer E, Kaspers S, Hofer S, Schober E, Kordonouri O, Pozza S, et al*. Anthropometry, metabolic control, and follow-up in children and adolescents with type 1 diabetes mellitus and biopsy-proven celiac disease. J Pediatr. 2011; 158(4), 589-593.e2.

17. *Gabrielli M, Candelli M, Santarelli L, Nista E, Santoliquido A, Gasbarrini G, et al.* Raynaud's phenomenon and celiac disease. Am J Gastroenterol. 2003;98(11):2578–2579.

18. *Glissen Brown J, Singh P*. Coeliac disease. Paediatr Int Child Health. 2019; 39(1), 23–31.

19. *Grode L, Agerholm I, Humaidan P, Parkner T, Bech, B, Ramlau-Hansen C, et al.* Unrecognised coeliac disease among men and women undergoing fertility treatment: A screening study. Un Eur Gastroenterol J. 2018;6(10):1477–1484.

20. *Grupo de trabajo del Protocolo para el diagnóstico precoz de la enfermedad celíaca*. Protocolo para el diagnóstico precoz de la enfermedad celíaca. Ministerio de Sanidad, Servicios Sociales e Igualdad. Servicio de Evaluación del Servicio Canario de la Salud (SESCS); 2018.

21. *Güngör D, Nadaud P, LaPergola C, Dreibelbis C, Wong Y, Terry N, et al.* Infant milk-feeding practices and cardiovascular disease outcomes in offspring: A systematic review. Am J Clin Nutr. 2019; 109(Suppl_7), 800S-816S.

22. *Han Y, Chen W, Li P, Ye J*. Association between coeliac disease and risk of any malignancy and gastrointestinal malignancy: A meta-analysis. Medicine. 2015;94(38):e1612.

23. *Hurley J, Lee B, Turner J, Beale A, Jenkins H, Swift G.* Incidence and presentation of reported coeliac disease in Cardiff and the Vale of Glamorgan: The next 10 years. Eur J Gastroenterol Hepatol. 2012;24(5), 482–486.

24. *Husby S, Koletzko S, Korponay-Szabó I, Mearin M, Phillips A, Shamir R, et al*. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012; 54(1), 136–160.

25. *Kahaly G, Frommer L, Schuppan D*. Celiac Disease and Glandular Autoimmunity. Nutrients 2018;10(7):814.

26. *Kocsis D, Miheller P, Lőrinczy K, Herszényi L, Tulassay Z, Rácz K, et al*. Coeliac disease in a 15-year period of observation (1997 and 2011) in a Hungarian referral centre. Eur J Intern Med. 2013; 24(5), 461–467.

27. *Lindfors K, Ciacci C, Kurppa K, Lundin K, Makharia G, Mearin M, et al.* Coeliac disease. Nat Rev Dis Primers. 2019;5(1):3.

28. *Mahadev S, Laszkowska M, Sundström J, Björkholm M, Lebwohl B, Green P, et al.* Prevalence of Celiac Disease in Patients With Iron Deficiency Anemia-A Systematic Review With Meta-analysis. Gastroenterology. 2018; 155(2), 374-382.e1.

29. *Mokhtar M, Mekki S, Mudawi H, Sulaiman S, Tahir M, Tigani M, et al.* Histopathological features of coeliac disease in a sample of Sudanese patients. Malays J Pathol. 2016; 38(3), 267–272.

30. *Moscoso F, Quera R.* Update on celiac disease. Rev Med Chile. 2016; 144(2), 211–221.

31. *Murray J, Watson T, Clearman B, Mitros F*. Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. Am J Clin Nutr. 2004; 79(4), 669–673.

32. *Marsh M, Johnson M, Rostami K*. Mucosal histopathology in celiac disease: A rebuttal of Oberhuber's sub-division of Marsh III. Gastroenterol Hepatol Bed Bench. 2015; 8(2), 99–109.

33. *Navalón-Ramon E, Juan-García Y, Pinzón-Rivadeneira A.* Prevalence and features of coeliac disease in the Mediterranean area of Spain. Semergen. 2016; 42(8), 514–522.

34. *Nevoral J, Kotalova R, Hradsky O, Valtrova V, Zarubova K, Lastovicka J, et al.* Symptom positivity is essential for omitting biopsy in children with suspected celiac disease according to the new ESPGHAN guidelines. Eur J Pediatr. 2013.

35. *Nijhawan S, Katiyar P, Nagaich N, Saradava V, Nijhawan M, Gupta G, et al.* Prevalence of associated disorders in Indian patients with celiac disease. Indian J Gastroenterol. 2013; 32(5), 330–334.

36. *Oberhuber G, Granditsch G, Vogelsang H*. The histopathology of coeliac disease: Time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999; 11(10), 1185–1194.

37. *Pereyra L, Gonzalez R, Mohaidle A, Fischer C, Mella J, Panigadi G, et al.* Risk of colorectal neoplasia in patients with celiac disease: A multicenter study. J Crohns Colitis. 2013;7(12):e672–677.

38. *Perez-Cuadrado-Robles E, Lujan-Sanchis M, Elli L, Juanmartinena-Fernandez J, Garcıa-Lledo J, Ruano-Dıaz L, et al*. Role of capsule endoscopy in alarm features and non-responsive celiac disease: A European multicenter study. Dig Endosc. 2018;30(4):461–466.

39. *Perez-Gregorio M, Días R, Mateus N, de Freitas V*. Identification and characterization of proteolytically resistant gluten-derived peptides. Food Funct. 2018; 9(3), 1726–1735.

40. *R Core Team. R:* A language and environment for statistical

computing. Viena: R Foundation for Statistical Computing; 2017.

41. *Reig-Otero Y, Mañes J, Manyes L.* Non-Celiac Gluten Sensitivity: Nutritional management of the disease. Nutr clin y diet hosp. 2017; 37, 171–182.

42. *Rios L, Khan A, Sultan M, McAssey K, Fouda M, Armstrong D.* Approach to diagnosing celiac disease in patients with low bone mineral density or fragility fractures: multidisciplinary task force report. Can Fam Physician. 2013;59(10):1055–61, e441-448.

43. *Rostom A, Murray J, Kagnoff M.* American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006;131(6):1981–2002.

44. *Rubio-Tapia A, Jansson-Knodell C, Rahim M, See J, Murray J.* Influence of gender on the clinical presentation and associated diseases in adults with celiac disease. Gac Med Mex. 2016; 152(Suppl 2), 38–46.

45. *Salmi T.* Dermatitis herpetiformis. Clin Exp Dermatol. 2019;44:728–731.

46. *Sategna-Guidetti C, Volta U, Ciacci C, Usai P, Carlino A, De Franceschi L, et al.* Prevalence of thyroid disorders in untreated adult celiac disease patients and effect of gluten withdrawal: An Italian multicenter study. Am J Gastroenterol. 2001;96(3):751–757.

47. *Sulkanen S, Halttunen T, Laurila K, Kolho K, Korponay-Szabó I, Sarnesto A, et al.* Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology. 1998, 115(6), 1322–1328.

48. *Tye-Din J, Galipeau H, Agardh D.* Celiac Disease: A Review of Current Concepts in Pathogenesis, Prevention, and Novel Therapies. Front Pediatr. 2018; 6, 350.

49. *Vivas S, Ruiz de Morales J, Fernandez M, Hernando M, Herrero B, Casqueiro J, et al.* Age-related clinical, serological, and histopathological features of celiac disease. Am J Gastroenterol. 2008; 103(9), 2360–2365; quiz 2366.

50. *Wingren C, Agardh D, Merlo J.* Sex differences in coeliac disease risk: A Swedish sibling design study. Dig Liver Dis 2012; 44(11), 909–913.

51. *Zylberberg H, Lebwohl B, Green P.* Celiac Disease-Musculoskeletal Manifestations and Mechanisms in Children to Adults. Curr Osteoporos Rep. 2018;16(6):754–762.