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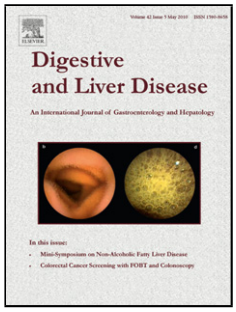

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BENEFIACIAL EFFECTS OF GLUTEN FREE DIET IN POTENTIAL CELIAC DISEASE OF THE ADULT

POTENTIAL CELIAC DISEASE OF THE ADULT

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AUTHORSHIP STATEMENT

ALL authors approved the final version of the article, including the authorship list.

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ABSTRACT

Background: To date, potential coeliac disease (PCD) occurring in adults remains an almost unexplored condition. **Aims:** to explore the prognostic role of Marsh grade in adult PCD patients, and to evaluate the effects of gluten-containing diet (GCD) in asymptomatic PCD patients. **Methods:** We retrospectively evaluated all consecutive adult PCD patients followed-up for at least 6 years. Patients were divided into: Group A (patients with Marsh 0 histology) and Group B (Marsh 1 patients). Symptomatic patients were started gluten-free diet (GFD), while asymptomatic subjects were kept on GCD and were followed-up. **Results**: 56 PCD patients were enrolled (21 in Group A and 35 in Group B). Forty-three patients were symptomatic and started GFD. Of these, none of 15 patients in Group A and 8 of 28 patients in Group B developed immune-mediated disorders (IMD) during follow-up (P=0.03; OR=4.2). The 13 asymptomatic PCD patients were kept on GCD. During the follow-up, 9 patients developed CD-related symptoms, 6 villous atrophy and 8 IMD. At the end, patients kept on GCD were at higher risk of developing IMD than those following a GFD (61% vs 18%, P=0.03, OR=3.3). **Conclusions:** Although PCD with normal mucosa seems to be a milder disease, the continuation of GCD places patients at a high risk of developing villous atrophy and IMD compared to commencement of GFD. Adult PCD patients should start GFD even if not symptomatic.

KEYWORDS: Potential coeliac disease, Marsh, villous atrophy, immuno-mediated disorders

INTRODUCTION

Coeliac disease (CD) is the most common immuno-mediated enteropathy in Western countries, triggered by exposure to gluten in genetically predisposed individuals (HLA DQ2/DQ8) [1,2].

At present, CD diagnosis in adults requires the serological assessment (anti-tissue transglutaminase [a-tTG] and anti-endomysial [EMA] antibodies [3]) of patients with suspected CD, and duodenal biopsy to evaluate the intestinal damage in patients with positive CD serology, although some patients are found and diagnosed based on histology only (only the minority of cases) [4].

In the majority of CD patients, small bowel damage consists of villous atrophy, intraepithelial lymphocytosis (IEL) and crypt hyperplasia [5]. These histological changes were classified by Marsh [6] into three different histological patterns: Marsh 1 (IEL>25/100 enterocytes), Marsh 2 (IEL>25/100 enterocytes and crypt hyperplasia), Marsh 3 (IEL>25/100 enterocytes, crypt hyperplasia and villous atrophy).

Although current CD diagnostic criteria require the presence of villous atrophy, this microscopic damage characterizes only the end stage of the disease; indeed many authors suggest that CD pathogenesis gradually leads from inflammatory damage to crypt hyperplasia and, ultimately, to villous atrophy [7].

Furthermore, a growing number of CD patients shows the absence of bowel damage or the sole increase of IELs at duodenal biopsy, findings which define the Marsh 0 and 1 types, respectively. At present, the term “potential CD” (PCD) is used to refer to those patients who have HLA-DQ2 and/or DQ8, a-tTG positivity but normal histology (Marsh 0) or low-grade enteropathy (Marsh 1) [8,9]. Moreover, since a-tTG antibodies display about 10% of false positive results, particularly when being positive at a very low titer, EMA positivity is also recommended before classifying a patient as affected by PCD.

Although several pediatric studies have been published on PCD [10, 11], adult PCD remains an almost unexplored condition [12–15].

One of the most interesting (but unresolved) issues in the context of PCD regards the extent to which patients actually need to start a gluten-free-diet (GFD) and the long-term risk for those remaining on a gluten-containing diet (GCD) [11–15].

The aim of the present study was to explore the long-term prognostic role of Marsh grade in adult PCD patients, comparing clinical and serologic features at the time of diagnosis in subjects with Marsh 0 and 1 histology and monitoring the development of autoimmune disorders and neoplastic complications in the same subjects after PCD diagnosis. In addition, our study also examined the effects of a GCD in asymptomatic PCD patients to determine how many would develop gluten-related symptoms, villous atrophy, and/or autoimmune diseases, and whether the initial Marsh grade may be contributing to the outcome.

MATERIALS AND METHODS

Study population and study design

We carried out a retrospective study in adult PCD patients referred to our Gastrointestinal Unit (Tertiary Centre for Food Intolerance and CD) at the "Federico II" School of Medicine, Naples, Italy, during the years 2008 – 2010, for clinical suspicion of CD (diarrhea, steatorrhea, weight loss, growth failure, abdominal pain, bloating, anemia, osteopenia/osteoporosis, recurrent miscarriages, hepatic steatosis, dental enamel hypoplasia, alopecia, hypertransaminasemia, recurrent aphthous stomatitis) or risk of CD (Hashimoto's thyroiditis, Down Syndrome, type-1 Diabetes Mellitus, positive family history of CD), and who completed a follow-up period of at least 6 years. All study participants were tested for EMA (absent/present) and anti-tTG levels (U/ml) at our laboratory. Patients with positive serology underwent upper endoscopy with duodenal biopsies/histology. Diagnosis of potential CD was made in accordance with current Guidelines [8, 9], that is in the

presence of positive serology (for both a-tTG and EMA), normal histology (Marsh 0) or low-grade enteropathy (Marsh 1), and genetic susceptibility (HLA DQ2/DQ8).

On the basis of the Marsh grade at the time of PCD diagnosis, participating patients were divided into two groups: Group A comprised individuals with normal histology (Marsh 0) and Group B those with Marsh 1 type histology. All patients with symptoms of CD or with other conditions related to CD started GFD, in accordance with suggestions of current literature [12], while asymptomatic PCD individuals were left on GCD. We compared the two groups in terms of anthropometric and serological variables (body weight, haemoglobin, ferritin, blood glucose, albumin, cholesterol, triglycerides, anti-tTG IgA) and clinical features (gastrointestinal or extra-intestinal symptoms) at the time of diagnosis. We then periodically followed up patients on GFD to assess their clinical and serological response to the diet, and compared the development of autoimmune disorders and neoplastic complications despite GFD in the Marsh 0 and Marsh 1 sub-groups.

Asymptomatic PCD patients on GCD underwent clinical and serological follow-up every six months, and upper endoscopy with duodenal biopsies/histology every two years. In this group too we compared Marsh 0 and 1 patients with regard to the development of gluten-related symptoms, villous atrophy, immuno-mediated disorders (IMD) and neoplastic complications.

Patients who were diagnosed with PCD before the age of 18 were excluded from the study, as were patients who received the diagnosis of PCD at other institutes. Also, we excluded from the study all patients with discordant or negative antibodies, unclear histology or already on gluten-free diet (GFD).

Serology and HLA Typing

Anti-tissue transglutaminase (anti-tTG) IgA antibodies were measured by ELISA (Enzyme-Linked Immunosorbent Assay, automated system; Delta Biologicals SRL) using human recombinant tTG

as antigen: serum samples with antibody titer greater than 7 U/mL were considered positive. Tests for anti-endomysial (EMA) IgA antibodies were carried out by expert operators; the antibodies were identified using immunofluorescence on a section of monkey esophagus (Delta Biologicals SRL). Results were expressed qualitatively as either a positive or negative finding (sometimes mild positivity). All serological investigations were performed at our Centre's laboratory at the time of CD diagnosis and during the follow-up.

All patients on GFD were tested for EMA and anti-tTG every 12 months from the time of PCD diagnosis; all patients left on GCD were tested for EMA and anti-tTG every six months.

Furthermore, patients were genotyped for human leukocyte antigen (HLA) class II DRB1 and DQB1 molecules (Euroimmun SRL).

Endoscopy and Histology

Multiple endoscopic duodenal biopsies were obtained by esophagogastroduodenoscopy (EGDS). In particular, 4 well-oriented biopsies were obtained in the distal duodenum, while 2 biopsies were taken in the duodenal bulb. All patients confirmed to be consuming an unrestricted diet at the time of histological evaluation. Histological evaluation was carried out by a pathologist blinded to the clinical history. Histological findings were classified according to Marsh classification [6]. More specifically, in case of complete absence of intestinal lesions, both on hematoxylin-eosin stained sections and at immunohistochemistry for CD3, the patient was classified as Marsh 0, while the microscopic damage was estimated as Marsh 1 in the presence of IEL > 25/100 enterocytes and positive CD3 immunostaining. The presence of any disease other than CD detected on the basis of the results of the upper endoscopy or histology was also recorded. Endoscopic and histological evaluation was repeated every two years in PCD patients kept on GCD.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS software v.15.0) for Windows. The descriptive statistics used included calculation of mean values and standard deviation (SD) of the continuous variables, and the percentages and proportions of the categorical variables. Age at diagnosis, prevalence of symptoms (gastrointestinal or extra-intestinal), immuno-mediated disorders (IMD), family history and laboratory data were compared by using Student's t-test, chi-square test, Fisher's exact test and ANOVA, as appropriate. The odd ratio (OR) for quantifying the statistical difference between the dichotomous variables was also calculated. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

Clinical features of the study population

During the study period, a total of 56 adult PCD patients, out of the 452 adult patients diagnosed with CD at our tertiary centre (12.4%), completed a follow-up period of at least 6 years and met diagnostic criteria for inclusion in the study.

Of these 56 PCD patients (females 73%, mean age 31.4 years), 28 (50%) had at least a first-degree relative suffering from CD, and 6 (10.7%) had a previous diagnosis of immuno-mediated disorder (IMD). HLA DQ2 alleles were present in 95% of the patients, while 5% expressed HLA DQ8 haplotype; in particular, 19% of subjects with PCD were found to have DQB1*02 homozygosity, while the remaining displayed heterozygosity. With regard to signs and symptoms, 43 (76.7%) patients were symptomatic; of these 18 (41.8%) referred to our ambulatory for diarrhoea, abdominal pain and weight loss and 25 (58.2%) presented anemia, recurrent miscarriages, hepatic steatosis, and hypertransaminasemia. Thirteen patients (23.3%) were asymptomatic.

Duodenal biopsies showed Marsh 0 histology in 21 (37.5%) and Marsh 1 in 35 (62.5%) participants. In line with these microscopic findings, we divided our study population into two groups: Group A, comprised the 21 patients with complete absence of intestinal damage (Marsh 0), and Group B the 35 patients with isolated intraepithelial lymphocytosis (Marsh 1).

No significant differences were found between Group A and B in terms of gender, age at the time of PCD diagnosis, HLA haplotype, CD family history, pre-existent immuno-mediated disorders, symptoms, and anthropometric and serological variables (Table 1). In particular, mean a-tTG levels were 18.9 + 9.3 in Group A and 19.7 + 13.4 in Group B (p=0.8), while all patients displayed EMA positivity (titers varying from 1:80 to 1:160).

In addition, when confronting all PCD patients with those with villous atrophy, we found no statistically significant differences in term of age at diagnosis (31.4 vs 31.8, p=NS), gender (females 73% vs 71%, p=NS), prevalence of symptoms (77% vs 79%, p=NS), associated diseases at CD diagnosis (11% vs 16%, p=NS), and family history for CD (50% vs 48%). Moreover, no differences were seen in terms of serological variables (haemoglobin, ferritin, cholesterol, triglycerides, serum albumin) between PCD and atrophic CD patients (p=NS). On the other hand, patients with PCD displayed lower a-tTG titer at diagnosis than atrophic CD patients (19.4 vs 78.2, $p<0.001$).

The 43 (76.7%) patients who were symptomatic at the time of diagnosis (15 in Group A and 28 in Group B) were placed on GFD followed-up once a year for at least 6 years. After gluten withdrawal all these patients became negative for both anti-tTG and EMA antibodies and showed significant clinical improvement within 2 years. No specific difference was seen between the two groups in terms of response to GFD.

Despite these good responses and strict adherence to GFD, 8 patients in Group B (28.5%) – but none in Group A (P=0.03; OR=4.2 [95% IC 0.5-37.5]) - developed an immuno-mediated disorder during follow-up. No neoplastic complications were observed. Table 2 summarizes these results.

Clinical features of PCD patients kept on gluten-containing diet

Thirteen asymptomatic adult PCD patients were kept on a gluten-containing diet (GCD) and clinically monitored every six months; endoscopic evaluation was offered every 2 years. Of these, 9 were women and 38.5% had a first-degree relative suffering from CD. Nobody had a pre-existent CD-related IMD. Six patients were in Group A (Marsh 0) and 7 in Group B (Marsh 1).

During the first two years since PCD diagnosis, all subjects remained asymptomatic and showed no changes in Marsh grade at biopsy. However, 4 years after diagnosis 6 patients had developed CD-related symptoms; 5 patients developed IMD and 3 of them developed villous atrophy. Patients with villous atrophy showed higher anti-tTG value compared to non-atrophic patients (mean 54.3 U/mL vs 11.4 U/mL, respectively, $p<0.01$). The 6 patients developing CD-related conditions were placed on GFD, with substantial improvement of clinical symptoms and normalization of serum antibodies within 12 months.

The remaining 7 asymptomatic patients without villous atrophy were followed up every six months. Six years after PCD diagnosis, 3 of these 7 patients – 1 from Group A and 2 from Group B - had developed CD-related symptoms. All 3 patients were found to have atrophic changes on duodenal histology and were started on GFD. The two patients from Group B developed an IMD. Only 3 participants remained asymptomatic and showed persistent Marsh 0 histological features on GCD, without developing villous atrophy and/or IMD. In summary, over a 6-year follow-up period, 9 out of 13 patients (69%) developed CD-related symptoms, 6 (46%) developed villous atrophy and 8 (61%) an IMD, without statistically significant differences between groups A and B (Figure 1).

When we compared patients kept on GCD with those following a GFD, we found that the former had a statistically significant higher risk of developing CD-related IMD than the latter (61% vs 18%, $p=0.03$, OR=3.3 [95% IC 1.04-10.5]).

DISCUSSION

Potential coeliac disease of the adult is an almost unexplored condition [12–14]. Whilst numerous pediatric studies have been published [10,11], data on the condition in the adult population are still scarce. In view of this, more research is needed to understand the characteristics of this CD population and to establish the need for GFD and assess the long-term risk for patients remaining on gluten-containing diet (GCD) [11–15].

In this paper, we aimed to explore the long-term prognostic role of Marsh grade in adult PCD patients on GFD by comparing patients with Marsh 0 and Marsh 1, and the occurrence of autoimmune disorders and neoplastic complications after PCD diagnosis.

Within a study population of 56 adult PCD patients, we found a high rate (50%) of CD family history, and a large majority (76.7%) of symptomatic patients. Microscopic evaluation showed Marsh 0 histology in 37.5% of patients and Marsh 1 histology in 62.5%. These findings were in line with previous studies [12, 14-17]: the high rate of first-degree relatives with CD and the high predominance of symptomatic patients could actually explain an increase in serological investigations for CD suspicion and, as consequence, an increase in the prevalence of PCD over time. More specifically, Volta et al [17], Biagi et al [14] and Zanini et al [13] have reported a PCD prevalence of 10.5%, 18.3% and 11%, respectively. In line with these authors' results, the prevalence of adult PCD in our study was 12.4%. Moreover, only 19% of subjects with PCD were found to have DQB1*02 homozygosity, in accordance with Biagi et al [18]. Furthermore, we found no differences in terms of clinical presentation between PCD patients and age- and sex-matched atrophic CD subjects, except in lower a-tTG titer in PCD, as suggested by previous studies [14].

When comparing our two groups (Marsh 0 and Marsh 1) at the time of PCD diagnosis, we found no significant differences in terms of gender, age at diagnosis, HLA haplotype, CD family history, pre-existent immuno-mediated disorders, symptoms and anthropometric and serological variables.

Recently, Volta and colleagues [17] reported in their series of CD patients a population of adults with normal mucosa, finding no significant difference between this population and that with Marsh 1 in terms of clinical features. In line with these Authors' findings, we did not detect any differences between the two groups at the time of PCD diagnosis. However, during a 6-year follow-up period and despite participants' adherence to a strict GFD, we found that 28.5% of Marsh 1 patients– but none in Marsh 0 Group - developed an immuno-mediated disorder (p=0.03; OR=4.2). In our interpretation, this means that patients diagnosed with a completely normal mucosa showed a milder disease compared to subjects with intraepithelial lymphocytosis at microscopic evaluation and placed on GFD. Also, it suggests that the latter may be the subgroup of PCD patients which most benefit from early treatment in the long term.

In our study we also examined the effects of a GCD in asymptomatic PCD patients. Over a six-year follow-up period, 69% of patients on GCD developed CD-related symptoms, 46% developed villous atrophy and 61% IMD, without statistically significant differences between the two histological groups. As mentioned earlier, one of the most important questions with regard to adult PCD concerns the actual need for commencing GFD in PCD patients and the long-term risks of continuing GCD instead, especially in terms of the possible development of villous atrophy, IMD and neoplastic complications [11–15, 17].

In a pediatric population of 175 asymptomatic cases, Auricchio et al [11] found that the progression from PCD to overt CD in children was observed in 14%, 27%, and 33%, over a 3-year, 6-year, and 9-year follow-up period, respectively. A study by Lionetti, with a shorter follow-up, has reported progression from PCD to overt CD in 5% of the children studied [19]. Data for the adult population are limited. In a 3-year study including 16 asymptomatic patients on GCD, Volta et al [17] reported that only one of them (6%) developed mucosal flattening. The Authors concluded that GFD was not recommended in asymptomatic adults with PCD, since they did not tend to develop villous atrophy. Very recently, in a 12-month study conducted with 57 adult Indian PCD patients, Kondala et al [20]

observed the development of overt CD (Marsh 3) in only four patients (7%). The Authors did not justify starting GFD in all patients with PCD in India.

By contrast, in a study by Biagi et al [14] on 24 PCD cases followed-up for 20 months, approximately 21% of the subjects progressed from PCD to villous atrophy. These authors proposed that the cumulative probability of mucosal flattening was high, but the time of flattening was totally unpredictable. Moreover, in a randomized controlled clinical study, Kurppa et al [12] found that the small-bowel mucosal villous architecture deteriorated - and the symptoms and abnormal antibody titers persisted - in all patients in the GCD arm (Marsh 1–2), while in the GFD arm (Marsh 1–2) symptoms improved, antibody titers decreased, and mucosal inflammation diminished in the same way of CD patients with atrophy. The Authors concluded that all subjects with non-atrophic enteropathy benefited from GFD. Zanini et al [13] demonstrated that mild enteropathy did not mean mild disease, suggesting that CD patients with Marsh 1-2 deserved treatment with GFD on clinical grounds, independently of any consideration around the natural history of the disease. In accordance with findings from these Authors, our study documented an evolution from PCD to overt CD in 46% of cases during a six-year follow-up period. We therefore suggest that adult PCD patients deserve early treatment with GFD. We also suggest that, given the high prevalence of family history for this condition [13,21], the lower prevalence of villous atrophy found in some studies could be related to a reduced gluten intake in a family setting where another member is already on GFD.

In our study, patients who continued on GCD had a statistically significant higher risk of developing CD-related IMD than patients who were placed on GFD (61% vs 18%, p=0.03, OR=3.3). Although the GFD does not prevent the occurrence of IMD [22], in this population the early onset of treatment could reduce the incidence, or at least delay the occurrence, of autoimmune diseases. In our opinion, our findings, along with those in the literature mentioned above, suggest that all PCD patients should start an early dietetic treatment, regardless of the presence or absence of symptoms.

In our study, we found no antibodies disappearance or fluctuation in patients on a GCD. This result was in contrast with previous studies [10,11,14,17], which found a high prevalence (up to 31.5%) of antibody markers disappearance or fluctuation in asymptomatic PCD patients on GCD. Probably, this observation is due to small size of our population.

Our study presents some limitations, which we briefly discuss. Firstly, our sample size is relatively small, although not dissimilar from that found in other studies [14,17]. This is partly due to the high percentage (76.7%) of symptomatic patients found in our study population and, more generally, in the adult PCD population [14,17]. Moreover, our study is based upon current clinical management practice that has been retrospectively analyzed. Further prospective and multicentric studies including larger population samples are needed to confirm our data and to inform official guidance on the question of dietary treatment. Secondly, we did not evaluate anti-tTG2 IgA intestinal deposits. Several previous studies have underlined the importance of tissue deposits in supporting CD diagnosis, especially in EMA-negative patients [10,11,17,23,24]. However, this investigation would have not added essential value to our study as, in order to avoid selection biases, we enrolled only PCD patients with EMA positivity, although all subjects in Group A expressed a slight positivity, as it is to be expected in the absence of mucosal damage [13,23,25]. Moreover, we were unable to evaluate the eventual reduction of gluten intake in our patients.

In summary, the present study demonstrated that all symptomatic adult PCD patients clinically improved after gluten withdrawal. After commencing GFD, patients with positive CD serology but normal mucosa (Marsh 0) showed a milder disease compared to patients with Marsh 1 histology; probably, these patients can be considered the subgroup of PCD patients which most benefits from early GFD. Remarkably, also asymptomatic PCD patients should start GFD, because they are at risk of developing villous atrophy and autoimmune diseases in the long term.

Specific author contributions:

Nicola Imperatore: planning the study, drafting the article, analysis and interpretation of data, follow-up coeliac patients

Raffaella Tortora: analysis and interpretation of data, follow-up coeliac patients

Giovanni Domenico De Palma: execution of endoscopy, revision of the paper

Pietro Capone: acquisition and interpretation of data

Nicolò Gerbino: analysis of data

Sara Donetto: editing English grammar and paper revision

Anna Testa: acquisition and interpretation of data

Nicola Caporaso: critical revision of the manuscript

Antonio Rispo: planning the study, drafting the article, revision of the paper, analysis and interpretation of data

Guarantor of article: Dr Antonio Rispo

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CONFLICT OF INTEREST: None declared

AUTHORS WITH NOTHING TO DECLARE SHOULD PROVIDE A STATEMENT TO THAT EFFECT

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FIGURE LEGENDS

Figure 1. Development of gluten-related symptoms (diarrhoea, abdominal pain, weight loss, anemia, recurrent miscarriages, hepatic steatosis, dental enamel hypoplasia, hypertransaminasemia), villous atrophy and immune-mediated diseases (Hashimoto's thyroiditis and psoriasis) during a 6-year follow-up in patients in Group A (individuals with normal histology - Marsh 0) and Group B (subjects with Marsh 1 type histology) kept on gluten containing diet (GCD).

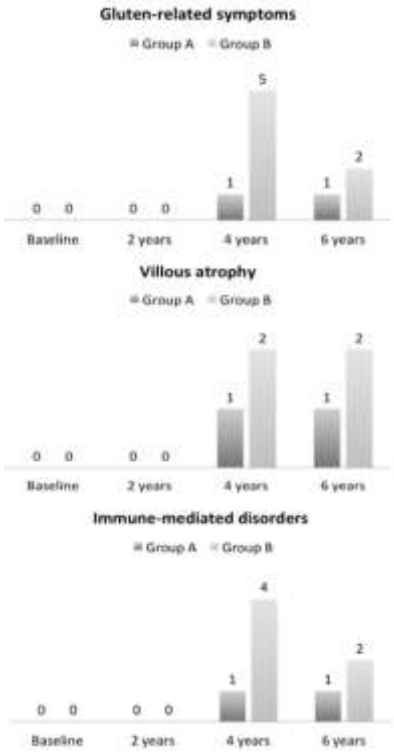

Table 1. Baseline demographic and serological features of potential CD patients at time of diagnosis. Group A (individuals with normal histology - Marsh 0); Group B (subjects with Marsh 1 type histology).

	Group A	**Group B**	***p***
Number of patients	21	35	0.1
Males n (%)	4 (19)	11 (31)	0.4
Age (years) (mean + SD)	31.3 + 15.7	32 + 19.5	0.9
CD family history n (%)	10 (47)	18 (51)	0.8
Pre-existent IMD n (%)	2 (10)	4 (11)	0.8
Symptoms n (%)	15 (71)	28 (80)	0.8
Weight (Kg) (mean + SD)	66.8 + 16.3	60 + 10	0.06
Anti-tTG (U/mL) (mean + SD)	18.9 + 9.3	19.7 + 13.4	0.8
EMA positivity n (%)	21 (100)*	35 (100)	1
HLA DQ2 n (%)	20 (95)	33 (94)	0.8
Hemoglobin (g/dL) (mean + SD)	12.7 + 1.1	13.2 + 1.6	0.3
Ferritin (ng/mL) (mean + SD)	64.6 + 110.3	58.5 + 58.7	0.7
Cholesterol (mg/dL) (mean + SD)	172.9 + 34.2	170.3 + 38.6	0.8
Triglycerides (mg/dL) (mean + SD)	101 + 57	98.6 + 49.4	0.8
Serum albumin (g/dL) (mean + SD)	4.5 + 0.3	4.3 + 0.5	0.1

*Although slight positivity in all subjects
n=number; SD=standard deviation; CD=coeliac disease; IMD=immuno-mediated diseases; anti-tTG=anti-tissue transglutaminase antibodies; EMA=anti-endomysial antibodies

Table 2. Clinical and serological features of potential CD patients after six years of gluten free diet regimen. Group A (individuals with normal histology - Marsh 0); Group B (subjects with Marsh 1 type histology).

	Group A	**Group B**	***p***
Number of patients	15	28	0.1
New IMD n (%)	0 (0)	8 (28)	**0.03**
Response to GFD n (%)	15 (100)	28 (100)	1
Weight (Kg) (mean + SD)	69.1 + 12.4	68.4 + 15.6	0.8
Anti-tTG (U/mL) (mean + SD)	1.3 + 0.9	1.2 + 0.9	0.9
EMA positivity n (%)	0 (0)	0 (0)	1
Hemoglobin (g/dL) (mean + SD)	13.2 + 1.3	13.7 + 1.7	0.3
Ferritin (ng/mL) (mean + SD)	76.6 + 98.7	74.5 + 68.9	0.9
Cholesterol (mg/dL) (mean + SD)	184.9 + 38.4	192.3 + 41.8	0.5
Triglycerides (mg/dL) (mean + SD)	138.4 + 64.2	135.3 + 76.8	0.8
Serum albumin (g/dL) (mean + SD)	4.6 + 0.2	4.5 + 0.3	0.3

n=number; SD=standard deviation; CD=coeliac disease; IMD=immuno-mediated diseases; anti-tTG=anti-tissue transglutaminase antibodies; EMA=anti-endomysial antibodies