Abstract. – Background and Objectives: We determined the prevalence and clinical features of celiac disease (CD) in family-members (FMs) of a population-based cohort of index cases.

Materials and Methods: We recruited 108 CD index cases: mean age at diagnosis, 23.0 years (range, 1.5-45.2 years); 81 (75%) female. Three-hundred twelve (mean age, 41.6 years; 219 [70%] female) of FMs were analyzed. 153 (49%) were parents, 24 (7.7%) were children, 69 (22.2%) were siblings, 66 (21.1%) were second degree FMs.

Results: CD was diagnosed in 63 subjects (20.1%, 21 males and 42 females, mean age 34.24 years, range 2-81 years). Classic, subclinical, and silent forms of CD were recognized in 18 [28.6% (6 siblings, 6 parents, 3 child, 3 second-degree FMs], in 27 [45.8% (9 siblings, 3 parent, 15 second-degree FMs], and in 18 [28.6% (6 siblings, 6 parents, 6 second-degree FMs) cases, respectively. Most of patients suffering from “classical” (18/63 patients, 28.7%) and “subclinical” (27/63 patients, 42.9%) form of CD were older than patients suffering from “silent “ CD (18/63 patients, 28.7%) (p=0.01). Most of patients suffering from subclinical disease showed autoimmune diseases (Hashimoto’s thyroiditis, and psoriasis), and other atypical symptoms, as gastroesophageal reflux disease (GERD), were also recorded.

Conclusions: We found an high-prevalence of CD between CD FMs, and most of them were olygo- or asymptomatic.

Key Words: Celiac disease, Family members, Prevalence, Symptoms.

Introduction

Celiac disease (CD) is a chronic inflammatory disease of the gut occurring in genetically susceptible individuals after ingestion of gluten. It is characterized by a flattened mucosa, villous atrophy and crypt hyperplasia in the small intestine and by the classic malabsorption syndrome (diarrhea, steatorrhea, weight loss) or by apparently minor symptoms such as iron-deficiency anemia, osteopenic bone disease, amenorrhea and infertility. The elimination of gluten from the diet generally leads to a return to normality of the morphological changes.

Familial aggregation is common; a large American study that recruited relatives during CD support group meetings identified 5% of first-degree relatives with CD. This is less than in Europe and might reflect different case selection, recruitment, and testing. The prevalence is higher in relatives of affected sibling pairs (17.2%) and monozygotic twins (75%), and HLA-identical siblings (40%).

The best strategy to detect CD in families is unknown, and most prior studies used referral populations, with variable selection and recruitment methodology of both probands and family members (FMs). Avoiding reliance on proband recruitment might improve risk estimates. The aim of this study was to determine clinical, histological, and serologic features of CD in parents, siblings, children, and second-degree family members of a population-based cohort of index cases.
from Rome and its Province in Lazio, Central Italy. Cases were identified from the CD patients followed by the different Gastroenterology Units involved in the study (Gastroenterology Service, ASL BAT, Andria (BAT); Division of Gastroenterology, ASL RMH, Albano Laziale (RM); Digestive Endoscopy & Nutrition Unit, “S. Eugenio” Hospital, Roma; Division of Gastroenterology, “Cristo Re” Hospital, Rome). Index cases were seen at Territorial Service of Gastroenterology of the ASL BAT, at the Division of Gastroenterology, ASL RMH, at the Division of Gastroenterology of the “Cristo Re” Hospital, Rome (Italy) and at the Digestive Endoscopy & Nutrition Unit of the “S. Eugenio” Hospital, Rome (Italy) which provide most of the outpatient gastroenterological healthcare in their provinces.

Institutional Review Boards approved this study.

We directly contacted all living first-degree relatives. Previously tested relatives were excluded. Sampling kits were evaluated at the time of diagnosis for processing. All enrolled families were caucasian.

**Laboratory Testing**

In all patients anti-tissue transglutaminase (anti-tTG) and anti-endomysium (EMA) were evaluated. HLA class II was also typed by low-resolution polymerase chain reaction-sequence specific primers and by high-resolution methods for DQA/B alleles.

**Endoscopic Procedures and Classification**

Esophagogastroduodenoscopy was performed in all patients who underwent to small-bowel biopsy.

Small-bowel biopsies were obtained at the second duodenal portion during esophagogastroduodenoscopy. At least six duodenal endoscopic specimens were obtained from this portion using a disposable biopsy forceps with spike and evaluated by hematoxylin/eosin staining. Four additional biopsies were obtained from the duodenal bulb in case of endoscopic aspect of micronodular bulb.

Celiac disease was defined as a permanent gluten-sensitive enteropathy, primarily expressed by the presence of characteristic small intestinal lesions.

Classical CD was defined by the presence of a gluten sensitive enteropathy with extraintestinal symptoms (iron-deficiency anemia, alopecia, recurrent abortion, etc.) and without gastrointestinal symptoms. Silent CD was defined by the presence of a gluten-sensitive enteropathy not accompanied by any symptoms, but identified during the course of screening of high-risk groups (first-degree relatives of celiac patients, patients with insulin-dependent diabetes, Down’s syndrome, IgA deficiency and thyroid disorders).

**Histological Classification**

Histopathology was expressed according to the Marsh’s modified classification. As for the cut-off of intraepithelial lymphocytes, we used the cut-off of 25/100 according to Hayat et al. and Dickson et al. Other possible causes of villous atrophy or duodenal damage, such as Giardia lamblia infestation, tropical sprue, collagenous sprue, food protein hypersensitivity (cow’s milks, eggs, fish, rice, chicken) were excluded, as well as any other causes of inflammatory infiltration of duodenum, such as peptic duodenitis, were excluded before making diagnosis of celiac disease.

**Statistical Methods**

Data analysis was carried out by using Fisher’s exact test with Yate’s correction for small numbers, Student’s t-test for unpaired data, Mann-Whitney two samples U-test, as appropriate. Values of \( p < 0.05 \) were considered as statistical differences.

**Results**

**Cases**

We recruited 108 CD index cases: mean age at diagnosis, 23.0 years (range, 1.5-45.2 years); 81 (75%) female.

HLA was available in 54 (50%): 36 DQ2 (70.59%), and 12 DQ2/DQ8 (22.22%). Six CD patients were DQ2/DQ8 negative (11.11%). All index cases recruited permitted us to contact their relatives.

**Participating Relatives**

Three hundred and twelve (mean age, 41.6 years; 219 [70%] female) of FMs were analyzed. HLA was available in 54 (50%): 36 DQ2 (70.59%), and 12 DQ2/DQ8 (22.22%). Six CD patients were DQ2/DQ8 negative (11.11%). All index cases recruited permitted us to contact their relatives.
Prevalence of celiac disease and symptoms in relatives of patients with celiac disease

Overall Results

IgA-class tTGA was positive in 42 (13.5%) of 312: 9 (21.4%) parents, 3 (7.1%) children, 12 (28.6%) siblings, 18 (42.9%) second-degree FMs (cousins, uncles, grandparents). EMA was positive in 33 (8.6%): 6 (18.2%) parents, 0 (0%) children, 9 (27.3%) siblings, 18 (54.5%) second-degree FMs. All EMA-positive individuals were also tTGA-positive, but 9 tTGA-positive individuals were EMA-negative.

Intestinal biopsy was performed in all 42 individual tTGA-positive (9 EMA-negative). All tTGA-positive and EMA-positive individuals had biopsies according to CD diagnosis. Biopsies were done also in 135 FMs (43.2%) symptomatic or suspected for CD: 93 of them (29.8%) were seropositive DQ2 or DQ8 (or HLA was not assessed), 42 (13.5%) were seronegative DQ2 or DQ8. 21 EMA-negative and tTGA-negative individuals (but suspected for CD) had small bowel lesions compatible with CD: 3 individual, seronegative DQ2 or DQ8, had subtotal villous atrophy (stage 3b Marsh modified classification); 15 individuals (3 seropositive DQ2 or DQ8, 2 seronegative DQ2 or DQ8) had partial villous atrophy (stage 3b Marsh modified classification); 3 individual, seropositive DQ2, had a stage 2 lesion accordingly to Marsh modified classification.

Histologic Spectrum

Among 135 biopsied FMs, the Marsh/Oberhuber stage was 0 in 63 (46.7%), 1 in 9 (6.7%), 2 in 3 (2.2%), 3a in 21 (15.5%), 3b in 21 (15.5%), and 3c in 18 (13.4%).

9 seronegative (DQ2 or DQ8) FMs with significant symptoms had villous atrophy, and 26 had normal biopsies. The intestinal biopsy was abnormal in all (no.11) double-positive (tTGA and EMA). Biopsy was abnormal in 12 with positive tTGA but negative EMA (stages 1 in 6, 3b in 6, and 3c in 3). Biopsy was also abnormal in 18 of 39 (46.1%) patients negative for tTGA and EMA.

Clinical Features of New Cases

CD was diagnosed in 63 subjects (20.1%, 21 males and 42 females, mean age 34.24 years, range 2-81 years). Classic, subclinical, and silent forms of CD were recognized in 18 [28.6% (6 siblings, 6 parents, 3 child, 3 second-degree FMs)], in 27 [45.8% (9 siblings, 3 parents, 15 second-degree FMs)], and in 6 [28.6% (6 siblings, 6 parents, 6 second-degree FMs)] cases, respectively.

The signs and symptoms recorded in relatives affected by CD are described in Table I. Two interesting findings can be found:

1. Most of patients suffering from “classical” (18/63 patients, 28.7%) and “subclinical” (27/63 patients, 42.9%) form of CD were older than patients suffering from “silent ” CD (18/63 patients, 28.7%) (p=0.01);
2. Most of patients suffering from subclinical disease showed autoimmune diseases (Hashimoto’s thyroiditis, and psoriasis), and other atypical symptoms, as GERD, were also recorded.

Another important symptom recorded was iron-deficiency anemia (IDA), which was also often associated to abdominal symptoms.

All patients showed silent form of CD were enrolled by screening program, without any particular symptoms. But it is interesting to note that two girls with silent disease experienced recurrent headache, and one boy with silent disease experienced recurrent attack of severe fatigue with non-specific musculoskeletal pain.

Table I. Symptoms and classification of celiac disease in family members.

<table>
<thead>
<tr>
<th></th>
<th>Classic CD (15F, 3M, mean age 38.3 yrs)</th>
<th>Subclinical CD (18F, 9M, mean age 38.4 yrs)</th>
<th>Silent CD (12F, 6M, mean age 20 yrs)</th>
</tr>
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<tbody>
<tr>
<td>Symptoms</td>
<td>3 abdominal pain</td>
<td>6 Hashimoto’s thyroiditis</td>
<td>18 screening</td>
</tr>
<tr>
<td></td>
<td>1 diarrhea</td>
<td>3 psoriasis</td>
<td></td>
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<tr>
<td></td>
<td>3 abdominal pain, diarrhea, IDA</td>
<td>3 pathological fractures</td>
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<td></td>
<td>9 abdominal pain, IDA</td>
<td>9 IDA</td>
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<tr>
<td></td>
<td></td>
<td>6 GERD, IDA</td>
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Abbreviations: CD: celiac disease; IDA: Iron-deficiency anemia; GERD: Gastro-Esophageal Reflux Disease.
Finally, it is important to note that none of FMs suffering from type 1 diabetes mellitus (9 subjects) was found affected by CD.

Discussion

The prevalence of CD in relatives is increasing. Almeida et al. found recently a CD prevalence in Brazil of 4.8%, Rubio-Tapia et al. found a CD prevalence in USA of 11% in 2008, most of them (54%) affected by silent CD, higher than previously reported in US. In Europe the prevalence of the disease seems to be higher. A pooled prevalence among first-degree relatives of 16% was found in 2005 in Western Europe. This study found that CD prevalence in our FMs population was about 20%, higher that reported in US but not far from that reported by Dubé et al. The estimate of prevalence in family studies depends on accurate screening methodology and how index cases are selected and relatives recruited. This study uses several ways to obtain better results, in particular using a geographic basis for index case selection might avoid referral bias, direct recruitment of FMs rather than using the index case as the recruiter, and detection strategy not solely dependent on serology but augmented by HLA typing (if available) and symptoms.

The high prevalence of CD among FMs found by this study confirms the results recently published in Northern Italy by Biagi et al., who found an high prevalence of CD among first degree relatives (28/158, 17.7%). This relatively high prevalence is probably related to our approach in searching new cases of CD, which combines a laboratory and clinical approach to select patients undergoing to intestinal biopsy. Someone may argue that the patients anti-tTG, EMA, and DQ2/DQ8-negative may be affected by histological lesions suspected, but not definitely classified as CD lesions, and this may affect the real prevalence of CD in FMs. We think that these patients are affected by real CD. Several considerations may be done about these patients, confirming the real celiac nature of these histological lesions. The first, is that the concept of “seronegative” CD is now accepted, especially in patients with mild-moderate histological damage; the second, is that CD DQ2/DQ8-negative is now increasing in clinical practice; third, in all of patients other causes of small bowel damage (food’s allergy, parasitic infection, gastric H. pylori infection, Inflammatory Bowel Diseases) were excluded before making diagnosis of CD; finally, all these patients experienced symptoms suspected for CD, and intestinal biopsy is still the “gold standard” for the diagnosis of CD. Interestingly, we found an increasing number of CD cases in second-degree members. This is probably related to the higher sensibility to the “CD problem” in families in which one member is affected by CD, increasing the number of members undergoing to screening.

Only 18/63 (28.6%) of FMs with CD were symptomatic, suffering from “classical” CD (see Table I). This is an important point, that strengthens the importance of the screening programs in FMs. We found also that “silent” CD was more frequent in younger FMs, whilst “classical” and “subclinical” forms of the disease were more frequent in older FMs (see Table I). Duration of gluten exposure may be a key point to explain this different clinical appearance of the disease. When CD is diagnosed early it is probable that gluten had not yet caused serious damage, with consequent slight/mild histological damage associated with subclinical or silent disease at the time of the diagnosis. On the contrary, in older patients gluten would cause prolonged damage which would show itself as more severe histological damage with consequent classical, active disease. Therefore, according to these data, it is hypothesized that those patients suffering from classical CD may be at higher risk of complications over patients suffering from subclinical/silent CD. Our data seem to support this hypothesis.

We found an high prevalence of autoimmune diseases in FMs affected by CD, especially in patients affected by “subclinical/silent” form of the disease. This is probably related to the overall gluten exposition, as previously hypothesized. A long-term exposition to gluten may increase the susceptibility to develop autoimmune diseases, but we cannot forget that CD and the other diseases share a similar pathogenic autoimmune mechanism or a genetic defect in the same responsible genes. For example, Neuhausen et al. found a significantly increased number of cases, relative to the expected number, of insulin-dependent diabetes mellitus (IDDM) in CD patients and first-degree relatives, and hypothyroidism among subjects with CD. Juvenile rheumatoid arthritis/juvenile idiopathic arthritis was increased among first-degree relatives of
celiacs. These results indicate that the presence of type 1 diabetes, as well as any other autoimmune disease, within CD families may be due to shared genetic susceptibility predisposing to these diseases or autoimmune diseases in general.

How can we improve our strategy in diagnosing new CD cases among FMs? Chang and Green performed a robust cost analysis on 3 different follow-up strategies based on anti-tTG antibodies and HLA typing. They showed that a strategy based on testing anti-tTG antibodies in 2 different moments (“tTG at t0 and t1”) without HLA testing may offer the best ratio between false-negative FMs and costs. This may be a very reasonable strategy and the best timing for the second serologic test could be after 4 or 5 years, since the majority of first-degree relatives who subsequently developed CD described in the literature developed CD less than 4 years after the first testing. Again, most of them were younger than 40 years. The study of Chang and Green was performed on children and adolescents, and it suggests that incidence tends to decrease in older age groups. On the contrary, we cannot forget that the first-degree relatives found to be suffering from CD by Goldberg et al and Niveloni et al were adults.

Moreover, it is likely that a negative selection bias exists toward the older ages. In fact, it is a common experience that infant and younger FMs are far more likely to be tested for CD than elderly first-degree relatives. However, we found an high participation of older ages too to our screening program, and we found 18 FMs older than 50 years affected by CD.

In conclusion, we found an high-prevalence (20%) of CD between CD FMs, and most of them were olygo- or asymptomatic. We cannot exclude that improved screening program among FMs, as suggested by Chang and Green, and especially in asymptomatic subjects, may increase further this prevalence.

References


