Prevalence of celiac disease in apparently healthy blood donors in the Autonomous Community of Madrid


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RESUMEN

Objetivo: conocer la prevalencia de enfermedad celiaca en la población adulta de la Comunidad de Madrid utilizando como método de despistaje los anticuerpos frente a la transglutaminasa tisular.

Población y métodos: han participado de modo voluntario 2.215 donantes de sangre. Todos ellos llenaron una encuesta. Se determinó la IgA sérica total y los anticuerpos antitransglutaminasa tisular. A los donantes con anticuerpos positivos, se les ofreció la realización de biopsia intestinal por endoscopia. La histología de la mucosa intestinal se graduó según los criterios de Marsh.

Resultados: mediante la encuesta se identificaron tres celiacos diagnosticados previamente. Once donantes presentaban anticuerpos positivos, todos ellos asintomáticos. Cuatro rechazaron la biopsia intestinal. De los siete en los que se realizó, tres tenían atrofia vellositaria y cuatro infiltrado linfocitario con vellosidades normales. En nuestro estudio el número total de donantes con enfermedad celiaca confirmada por biopsia fue de seis, lo que supone una prevalencia del 1/370. Considerando el grado I de Marsh, la prevalencia de la enteropatía por gluten sería de 1/222. La respuesta inmunológica anómala al gluten medida por la positividad de los anticuerpos fue de 1/201, que alcanza valores de 1/158 si consideramos los tres celiacos diagnosticados previamente.

Conclusiones: los datos de prevalencia hallados en este estudio confirman que la enfermedad celiaca constituye un problema sanitario de primer orden, que podría justificar la instauración de un programa de despistaje universal. Se ha detectado un alto número de casos de enteritis linfocitaria lo que obliga a plantearse la actitud a seguir.


ABSTRACT

Objective: the aim of this study was to determine the prevalence of celiac disease among the adult population of Madrid by measuring antibodies against tissue transglutaminase as serologic screening method.

Population and methods: 2.215 subjects participated voluntarily in this study. All of them completed a clinical questionnaire. We determined the levels of total IgA and antibodies to tissue transglutaminase (tTG). An intestinal biopsy by endoscopy was proposed to all subjects who were tTG-positive. The histologic lesion was classified in accordance to Marsh.

Results: three known CD cases were identified by the questionnaire. Eleven donors with tTG positivity were detected, all of them asymptomatic. Four subjects rejected the intestinal biopsy. Seven out of 11 positive subjects consented to undergo a duodenal biopsy –3 had villous atrophy and 4 had increased intraepithelial lymphocyte counts with normal villi. In our study the number of donors with biopsy-proven CD was 6, and the prevalence was 1/370. If we include the subcategories of gluten sensitive enteropathy (Marsh I), the prevalence would be 1/222. When we considered antibody positivity the prevalence of gluten sensitivity was 1 in 201, and it reached 1 in 158 when the three known CD cases were included.

Conclusions: data on CD prevalence in this study confirm that CD is a first-line healthcare problem that may warrant universal screening. We detected a high number of lymphocytic enteritis cases, and thus some sort of action is mandatory.

Key words: Celiac disease. Anti-transglutaminase antibodies. Prevalence. Lymphocytic enteritis.

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INTRODUCTION

Celiac disease (CD) is a gluten-sensitive enteropathy mediated by T lymphocytes in genetically susceptible individuals. It induces a characteristic, although non-specific, lesion of the small-intestine mucosa, and promptly improves upon compliance with a strict gluten-free diet (1).

The classical disease includes gastrointestinal symptoms, diarrhea, weight loss, abdominal distention, and anorexia, among others. Other patients may present mild or no gastrointestinal symptoms: dermatitis herpetiformis, dental enamel hypoplasia, short stature. Sometimes the subjects are asymptomatic, as is the case with those detected via the screening of first-degree relatives of patients with celiac disease, who can have villous atrophy without symptoms. These putative subphenotypes can be termed silent celiac disease.

It is difficult to establish the real prevalence of this disease, as the prevalence of CD varies when screening studies are made in different populations: general population, first-degree relatives, symptomatic subjects, increased-risk groups (2).

The studies performed in the general population of Europe, the United States, and Latin-America have showed a prevalence of CD between 1:100 and 1:300 (3-6). These studies have revealed an estimated ratio of known to undiagnosed CD of about 1 in 5-10, since CD may present with mild symptoms, atypical symptoms, or as silent CD. This finding shows that gluten-sensitive enteropathy is highly variable, but the frequency of CD diagnosis with different clinical forms depends on variables such as index of suspicion on the part of clinicians working in different specialties, or their possibilities to carry out screening tests.

An apparently healthy population for studies on the prevalence of silent celiac disease is blood donors.

OBJECTIVE

The primary aim of this study was to determine the overall prevalence of CD in apparently healthy blood donors in the Autonomous Community of Madrid (CAM). Silent celiac disease was detected using antitransglutaminase antibodies (atTG) as screening test; the prevalence of diagnosed CD cases was obtained using a clinical questionnaire from each participant.

The secondary aims of this study included the identification of signs, symptoms, and other diseases associated with silent celiac disease.

POPULATION AND METHODS

Population

A sample size of 2100 donors was estimated for a hypothetical prevalence of 1/300, with an alpha error of 0.05 and beta error of 0.1.

The study period was from February 27, 2001 to June 25, 2001, and during June and July 2002; 2215 blood donors participated voluntarily after they were informed in writing of the study’s goals.

In all, 44.12% of donors were university students, 26.47% were donors at mobile units, 23.36% were employees of private companies, and 6% were public employees. All of them lived in Madrid. Sex: 1285 (58%) males and 930 (42%) females. Mean age, 32.2 ± 12.4 years (range 18-65 years).

All subjects completed a questionnaire about their family and personal history of CD, presence of autoimmune diseases associated with CD, cutaneous or mucous alterations, and thought-provoking gastrointestinal symptoms and signs (Table I).

All donors enrolled were volunteers, and signed a written informed consent form.

Table I. Questionnaire

<table>
<thead>
<tr>
<th>Family antecedents</th>
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<tbody>
<tr>
<td>Juvenile diabetes</td>
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<tr>
<td>Skin disease</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Celiac disease</td>
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<td></td>
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</tbody>
</table>

| Personal antecedents    |          |          |          |
| Thyroid disease         |          |          |          |
| Juvenile diabetes       |          |          |          |
| IgA deficiency          |          |          |          |
| Dermatitis herpetiformis|          |          |          |
| Infertility             |          |          |          |
| Recurrent fetal loss    |          |          |          |
| Celiac disease          |          |          |          |

| Symptoms and signs      |          |          |          |
| Constipation            |          |          |          |
| Chronic diarrhea        |          |          |          |
| Appetite loss           |          |          |          |
| Bleeding diathesis      |          |          |          |
| Recurrent aphthous stomatitis |      |          |          |
| Weight loss             |          |          |          |
| Abdominal distention    |          |          |          |
| Dental enamel hypoplasia|          |          |          |
| Drowsiness and cramps in limbs |      |          |          |

METHODS

Blood samples –5 cc– were obtained for serological studies. Serum samples were stored at –20 ºC until required.

In all subjects total serum IgA and transglutaminase antibodies (atTG) were measured.

Serum IgA levels were assayed with nephelometry (Dade-Behring®). A serum IgA concentration below 6.67 mg/dl was considered a selective IgA deficiency, and IgG antientomysium antibodies (EMA) were determined by indirect immunofluorescence using monkey esophagus as substrate.
Anti-transglutaminase antibodies were analyzed using enzyme-linked immunosorbent assay (ELISA) (Celikey, Pharmacia®) with recombinant human tTG as antigen. According to the laboratory experience a cut-off value of 8 U was considered positive; values of 5 to 8 U were considered doubtful.

Subjects with tTG positivity or increased EMA-IgG were asked to undergo an intestinal biopsy by upper gastrointestinal endoscopy. Almost three biopsies were obtained for routine histological analyses. Histological lesions were classified as described by Marsh (7).

— Preinfiltrative (stage 0).
— Infiltrative (stage I).
— Hyperplastic (stage II).
— Destructive (stage III).
— Hypoplastic (stage IV).

The diagnosis of active celiac disease was made in those subjects who had villous atrophy.

Statistical analysis

The statistical analysis was carried out with the statistical package SPSS version 11.

RESULTS

Two subjects were parental relatives of celiac disease patients, and 37 had family antecedents of diabetes mellitus (19 maternal and 18 paternal); none was atTG-positive.

The questionnaire identified 3 donors with known CD, one of them had presented with dermatitis herpetiformis that was diagnosed by a skin biopsy, and biopsy-confirmed villous atrophy. All 3 patients followed a gluten-free diet, and all of them were atTG-negative.

Therefore, in our sample, the prevalence of known CD was 1:738.

Other data detected by the questionnaire were: 2 insulin-dependent diabetes mellitus, 29 thyroid disease, 1 known IgA deficiency, 5 women with infertility, 7 women had had recurrent spontaneous abortions, and 24 had anorexia. None of them was atTG-positive.

One case of multiple myeloma and 8 IgA deficiency cases were detected by IgA measurements. All of them were IgG EMA-negative.

Eleven donors were atTG-positive with median titers of 50.9 U (range 9.1-100). All of them were asymptomatic, according to the questionnaire and confirmed by a telephone interview. An intestinal biopsy by upper gastrointestinal endoscopy was proposed to all subjects; 4 donors refused. Median atTG titers were 53.7 U (range 16.5-100) in this group.

Seven atTG-positive patients agreed to undergo endoscopy and duodenal biopsy. Two patients had total villous atrophy with hyperplastic crypts and increased intraepithelial lymphocytes (Marsh IIIc). In these, median atTG titers were 73.8 U (range 21.5-100). The remaining five donors had mild histological features but increased intraepithelial lymphocytes (60-90%) (Marsh I); median atTG titers in this group were 25.05 U (range 9.1-40.2).

The first group was diagnosed as silent CD, and a gluten-free diet was indicated. The second group was maintained with a free diet under clinical control. During follow-up one of them developed increased atTG titers and subclinic autoimmune hypothyroidism. At the time the patient agreed to undergo a new endoscopy with intestinal biopsy, 6 months after the first one, which revealed partial villous atrophy and hyperplastic crypts. Then a gluten-free diet was indicated.

Therefore, in this sample, the total number of donors with biopsy-proven CD were 6, 3 with known CD and 3 with silent CD diagnosed during the present study, that means a prevalence of 1/370. The ratio of known to undiagnosed CD cases was 1 to 1.

If we had included the subcategories of gluten-sensitive enteropathy (Marsh I), the prevalence of CD would be 1/222.

In 11 of 2215 donors, gluten sensitivity was identified by the positivity of atTG; prevalence was 1/201, and reached 1/158 when we considered 3 previously known CD cases.

DISCUSSION

Since S. Gee first described CD in 1887, our knowledge has changed about CD’s age at symptom onset and clinical manifestations. Initially, the classic presentation

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>atTG titres</th>
<th>Intestinal biopsy</th>
<th>HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Male</td>
<td>54</td>
<td>49</td>
<td>Total villous atrophy and crypt hyperplasia</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Female</td>
<td>23</td>
<td>&gt; 100</td>
<td>Subtotal villous atrophy</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Male</td>
<td>31</td>
<td>&gt; 200</td>
<td>Villous atrophy</td>
</tr>
</tbody>
</table>
associated with CD was characterized by diarrhea, malabsorption and failure to thrive. In contrast, nowadays the usual form of presentation is oligosymptomatic or asymptomatic (8,9). This has been possible because of the new serological studies and screening tests in populations. This wide spectrum of clinical manifestations of CD has justified the so-called iceberg model –symptomatic cases make up the visible part of the celiac iceberg, and asymptomatic cases the submerged part of the iceberg (2,10,11). It justify that the true prevalence of CD is difficult to establish, although population-based studies in the United States suggest that the prevalence of CD is in the range of 0.5 to 1.0 percent, similar to estimates in Europe. Therefore, CD could affect around 450,000 individuals in Spain (only around 45,000 are currently diagnosed) (12).

Nevertheless, the numbers obtained by several studies about the prevalence of CD are different, and depend on several factors: studied populations (general, pediatric), blood donors, at-risk groups, symptomatic individuals. The methodology used also changes results: antigliadin antibodies (AGA), antiepithelial antibodies (EMA), anti-transglutaminase antibodies (atTG).

Our study was carried out in a sample of blood donors, a healthy and asymptomatic population, to explore the submerged part of the iceberg. However, this population has a bias both regarding drop-out and discharge rates. Individuals that presented with hypertransaminasemia were excluded of this study, as it could be the only manifestation of CD in adults. This illness is responsible for 2.7 to 5% of hypertransaminasemias of unknown origin (13-15). Subjects with chronic anemia refractory to replacement therapy were another group that was excluded from this study. In this sense it is estimated that 5-6% of asymptomatic CD cases present refractory anemia as their only sign of CD (16). On the other hand, donors with digestive symptoms or other illnesses were more likely to consent to be included in the study.

All studies of CD screening begin with a non-invasive test: a determination of the various antibodies against gliadin, endomysium, and transglutaminase, which have shown increasing sensitivity and specificity over time.

The initial studies with population-based detection of CD used a two-step model. This way, in those carried out in 1996 in Italy by Catassi et al. (17), directed to the schoolchildren population, the screening test was a measurement of both IgG-AGA and IgA-AGA. Then, those with either or both classes of AGA higher than the cut-off value were recalled for the second step, a determination of IgA EMA, since EMAs are more specific than AGAs, and AGAs have a lower predictive value when compared to EMAs (18). Afterwards, subjects with a positive serology for EMA underwent a small-intestinal biopsy. In this study the overall prevalence of CD was 1/184-198.

In 1996 and 1998 two studies were carried out in blood donors in Sweden (19) and in the USA (20) using AGA and AGA plus EMA measurements, respectively. The prevalence was 1/250, lower than that detected in our study if we only take as reference antibody positivity. In Norway, Hovdenak et al. (21) studied 2096 blood donors who had been screened for IgG AGA and IgA AGA; then all those with increased AGA levels were tested for EMA, and those with EMA positivity underwent intestinal biopsy. The prevalence of silent CD was 1/340, and that of symptomatic CD in this country was 1/675. These results are very similar to ours.

The second method for serologic screening has been the initial determination of total IgA, EMA, and IgG AGA levels in cases with IgA deficiency, which increased the sensitivity and diminished the number of intestinal biopsies. In our country, a study carried out in 2002 by Cilleruelo et al. (3) in schoolchildren, using this strategy together with intestinal biopsy, showed a global prevalence of CD of 1/220. Riestra et al. (22) studied the general population in an area of Asturias using AGA and/or EMA measurements plus intestinal biopsy, and they found a total prevalence of CD of 1/389, very similar to that described by our study (1/370).

In the last few years a new screening method has been spread, the determination of antibodies directed against human transglutaminase (atTG). That is the specific protein against which EMAs are directed (23,24). Both EMA and atTG have a high sensitivity and specificity, but atTG is a cheaper, more reproducible, and quicker technique. In 2003, Mäki et al. (25) performed a study in schoolchildren from Finland, and found a prevalence of biopsy-proven celiac disease of 1/99 using atTG. With the same methodology, Fassano et al. (26) found a prevalence of 1/105 in the US general population. Nevertheless, in this latter work an intestinal biopsy was only obtained from 30% of cases, and therefore this prevalence is primarily based on serologic methods. In 2004, Neri et al. (27) screened for anti-tissue transglutaminase antibodies a population of blood donors previously studied in 1998 with AGA and EMA measurements, and they estimated that the prevalence of CD among blood donors was 1/125.

Despite the high sensitivity and specificity of serologic markers, the diagnosis of celiac disease requires a small-intestinal biopsy, which is the current gold standard. The National Institutes of Health (NIH) convened a Consensus Development Conference on Celiac Disease on June 28-30, 2004. One of the key questions was: how is celiac disease diagnosed? It was concluded that some degree of villous atrophy is necessary to confirm a diagnosis of celiac disease. This lesion is not pathognomonic of CD, since mucosal changes can also be due to other causes (28). This requirement was completed in 3 biopsied blood donors, and therefore the prevalence of biopsy-proven celiac disease was 1/370.

The finding of intraepithelial lymphocytes with crypt hyperplasia without villous blunting is less definitive to establish a diagnosis of CD, as the architecture is nearly normal. Nevertheless, it is now accepted that gluten-dependent enteropathy is not restricted to patients with a
flat mucosa during a gluten-containing diet. There are patients who have a high count of intraepithelial lymphocytes in an otherwise normal-looking jejunal mucosa. Intraepithelial cells decrease during a gluten-free diet and increase after gluten reintroduction (29). If we consider Marsh stages I and II as early stages in the spectrum of CD, the prevalence in our study would increase to 1/222. The true prevalence of celiac disease could be even higher, since more than one third of antibody-positive subjects refused to undergo an intestinal biopsy. For diagnosis confirmation in uncertain cases of CD a single best approach cannot be prescribed. Choices include: a) testing for certain genetic markers (HLA haplotypes) and additional small-bowel biopsies when susceptibility alleles are present; b) periodic monitoring with celiac disease serology tests; and c) as some authors advocate, a trial of gluten-free diet (30,31). While to this day subjects with Marsh stage I enteropathy were considered asymptomatic, short series of patients exist in the literature that show that these patients may develop symptoms (32), and they also improve on a gluten-free diet. It is a crucial fact in the diagnosis of CD, especially if antibodies are high, since these will be negative on a gluten-free diet.

Patients detected in our study as having low-grade enteropathy were asymptomatic, and they were monitored and followed up clinically. One of them developed autoimmune hypothyroidism, and then he had villous atrophy. This case is an example of latent, asymptomatic, little histologically expressive CD that showed up clinically in the course of follow-up. Hence, these individuals should be subjected to regular follow-up with the purpose of detecting their illness in early stages, to remove gluten from their diet and, in this way, to improve their quality of life by preventing the development of osteoporosis (33-35), autoimmune illnesses (36), lymphomas, or other forms of cancer (37).

Given the high prevalence of this disease even in a seemingly healthy population, and the high sensitivity and specificity of serologic tests available at this time, a screening of the general population for celiac disease would be warranted. Low dietary compliance has been reported in asymptomatic patients. All cases diagnosed in our study are compliant with their gluten-free diet after 2 years.

REFERENCES