The Celiac Iceberg: Characterization of the Disease in Primary Schoolchildren


ABSTRACT

Objective: Celiac disease (CD) has a prevalence of 0.55% to 1% in Italy. Identifying CD in schoolchildren to characterize CD iceberg and evaluate the effect of diagnosis in screening-detected children.

Methods: A total of 7377 5- to 8-year-old children were invited to participate. A total of 5733 salivary samples were collected and tested for anti-transglutaminase antibodies (tTGAb), using a fluid-phase radioimmunoassay. Salivary tTGAb-positive children were analyzed for serum antibodies (anti-endomysium antibodies, radioimmunoassay, and enzyme-linked immunosorbent assay tTGAb). Positive children underwent endoscopy and then started gluten-free diet (GFD) and periodical follow-up.

Results: Forty-six subjects were found salivary tTGAb–positive and 16 border-line. Forty-five of 46 and 5 of 15 of them were also serum antibody–positive. Forty-two children showed duodenal villous atrophy and 1 had only type 1 lesions. Three children started GFD without performing endoscopy. CD prevalence (including 23 previously diagnosed children with CD) was 1.2%. Considering all 65 celiacs in our sample, a silent CD was found in 64%, typical in 28%, atypical in 7%, and potential in 1%. All patients showed strict adherence to GFD, weight and stature increase, and well-being improvement. Eighty-five percent and all but 2 screening-detected children had Italian parents.

Conclusions: Our sample size, representative of primary schoolchildren of our region, demonstrated that CD prevalence is growing in Italy, with a modified clinical spectrum and iceberg deepness.

Key Words: anti-transglutaminase antibodies, celiac iceberg, children, saliva, screening

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Celiac disease (CD) is a gluten-dependent autoimmune enteropathy, which occurs in genetically predisposed individuals. CD can manifest in a typical form (with gastrointestinal symptoms), with an atypical form (iron-deficiency anemia, headache, recurrent aphthous stomatitis), or be asymptomatic (silent form). The lifelong gluten exclusion from the diet leads to histological and clinical remission and prevents the development of long-term complications (1), such as autoimmune pathologies (2,3), osteoporosis (4), infertility (5), or malignancy (6).

Over the past decades, CD prevalence increased, mainly because of the improvement and diffusion of screening tools such as anti-gliadin, anti-endomysium (EMA), and anti-tissue transglutaminase antibodies (tTGAb), which play a crucial role in the selection of candidates for the intestinal biopsy, the criterion standard for CD diagnosis. We demonstrated that it is possible to perform a powerful, noninvasive, and sensitive CD screening using a radioimmunological assay (RIA) for tTGAb in human saliva, which proved to be particularly useful in children, bypassing the unpleasant blood sample collection (7–9). The availability of these tests made possible the emergence of silent forms, but the iceberg is still deep. In asymptomatic patients, the only chance of reaching a timely diagnosis is linked to screening programs, which need to be considered in terms of cost-effectiveness. In fact, the real challenge in the screened asymptomatic individuals is to achieve a long-lasting gluten-free diet (GFD) (10). Based on this assumption, the target age and periodic counseling should be strongly considered (11).

Historically, CD could be exemplified as an iceberg, in which clinically symptomatic patients are represented above sea level and below the water surface lie asymptomatic children with CD.

Our aim was to identify CD in an appropriate cohort of primary schoolchildren to characterize CD iceberg in Italy and evaluate the effect of CD diagnosis in asymptomatic screening-detected children.

METHODS

Study Design

A total of 7377 children (3838 boys, ages 5.3–9.0 years, median age 6.9 years), attending the first and the second classes of the primary school of 11 municipalities of Rome, were invited to participate in the study. Children, fasting for 3 hours, were asked to collect a salivary sample, spitting into a plastic tube, and underwent a physical examination. Salivary (TG IgA-positive or borderline subjects were tested for serum EMA, RIA, and enzyme-linked immunosorbent assay (ELISA) tTG IgA. Children confirmed antibody-positive to serum assays were addressed to the upper endoscopy (UE). After CD diagnosis, the patients were invited to start GFD and periodical follow-up.
3 months during the first year of GFD and then once per year. Adherence to GFD was evaluated by combining an interview with the antibody (Ab) reduction. After 6 months of follow-up, a multiple choice questionnaire asking information about school rendering and the number of episodes of upper respiratory infection was administered to parents. In addition, a validated iconographic questionnaire asking their feeling about “having CD” was submitted to children (12). Symptoms at time of diagnosis were investigated by telephone in previously diagnosed children.

Methods

Salivary tTG IgA were detected in the first 4048 salivary samples with a previously reported salivary fluid-phase RIA that has been demonstrated to have good sensitivity and specificity, 94.5% and 98.2%, respectively (7–9). The remaining 1685 saliva samples were analyzed with a similar method, modified by replacing single polystyrene tubes with 96-well filter plates. Salivary autoantibody levels were expressed as an Ab-index calculated as follows: 100 × (sample cpm – negative standard control sample cpm)/(positive standard control sample cpm – negative standard control sample cpm) (cpm is counts per minute). The same positive and negative salivary standards for Ab-index detection were utilized in the 2 assay variants. The positive autoantibody index of the 2 salivary assay variants was investigated separately and defined as the value above the 99th percentile of the first 500 salivary samples collected in the study. All the subjects having an antibody index between 97.5th and 99th percentile were considered borderline. By comparing the tTG indexes of 300 salivary samples collected from healthy subjects and celiac patients, a significant correlation (P < 0.001) was found between the antibody titers detected with the 2 assay variants. All the salivary samples resulting tTGAb-positive with 1 variant of the assay were confirmed by analyzing the same saliva sample also with the other assay variant. The presence of serum tTGAb was detected by a previously published RIA method (13,14).

Serum ELISA tTG IgA were evaluated using a commercial kit (Eurospital, Trieste, Italy). EMA IgA were detected by an indirect immunofluorescence method using as substrate sections of the distal portion of monkey esophagus (Eurospital).

All patients, fasting overnight, underwent UE after narcosis, using Olympus GIF E, PQ20 and GIF P140 gastroscopes (Olympus Italy s.r.l., Milan). During endoscopy, 3 samples from distal duodenum and 2 samples from bulb mucosa were taken, oriented on filter paper, fixed in 10% formalin, and separately embedded in paraffin blocks. Sections were serially cut, stained with hematoxylin and eosin, and assessed under light microscopy.

CD diagnosis was made according to the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition criteria (15). Our study was conducted in accordance with the ethical principles of the Helsinki Declaration. Approval from the local ethical committee was obtained (No. 1832).

Statistical Analysis

Sample size was computed as:

\[
N = \left(\frac{Z_{1-\alpha/2}}{E}\right)^2 \times \left(1 - P\right)
\]

where \( Z_{1-\alpha/2} = 1.96 \) is the value of the normal deviate associated with a 95% confidence interval; \( E \) = desired approximation, that is, maximum distance from prevalence to be considered; \( P \) = disease prevalence. For \( E = 0.00275 \) and \( P = 1\% \) \( N = 5029 \). The sample size of 5000 children permitted precise estimates (0.725% < \( P < 1.275 \)).

RESULTS

A total of 42 of 5733 (0.7%) children (13 boys, median age 6.9, range 5.9–8.7 years), who were positive to the salivary screening (38 of them highly positive and 4 with borderline Ab titers), were confirmed by the serology and showed CD-related duodenal lesions at UE (Fig. 1). In particular, 31 patients (74%) had total villous atrophy (type 3c) according to the Marsh classification as modified by Oberhuber et al (17), 10 (24%) showed different grading of villous atrophy (type 3a, 3b and 3c), and 1 had type 3b lesions localized only in the duodenal bulb. Moreover, 3 children, who had high levels of tTGAb (>100 IU/mL) twice, started a GFD; they showed Ab titer normalization after 6 months of follow-up. In 4 children, who were Ab-positive twice, the permission to be submitted to the UE was refused. In the cohort evaluated, 23 celiac children had already been diagnosed (Fig. 1).

Overall, we found a CD prevalence of 1.19% (68/5733), including the 3 children who were antibody-positive twice on GFD follow-up. This prevalence could reach 1.24% (71/5733) by including also the 4 children who were saliva and serum positive but who had not performed the UE yet.

Evaluating clinical presentation in screening-detected children with CD, we found 1 child with a typical form (abdominal pain), 4 with atypical forms (headache, iron-deficiency anemia), and the remaining 40 with a silent form (asymptomatic). Investigating the symptoms at CD diagnosis in patients already diagnosed, we found 18 children with a typical form, 1 with an atypical form, and the remaining 4 with a silent form. Moreover, a potential CD form was found in a girl with borderline Ab titers, who showed only type 1 lesions in the duodenal mucosa, and who was not included in the prevalence estimation. According to these data, the clinical appearance of patients with CD in our series can be figured as an iceberg where below the water level there are 66% of patients (diagnosed during the screening), while the remaining 34% of children, who were diagnosed before the screening, are above the water level (Fig. 2).

At the time of salivary collection, the percentage of children showing pallor and abdominal distension was higher among screening-detected patients with CD with respect to negative tTGAb healthy subjects (6.6% vs 3.2%, P = 0.06 ns; 37.7% vs 24.5%, P = 0.04; respectively). The mean ± standard deviation of weight and stature were 24.4 ± 4.4 kg and 123 ± 8.2 cm and 25.5 ± 5.5 kg and 124.1 ± 8.4 cm for boys and girls, respectively. All children but 1 were in the normal percentile.

Only 5 children have dropped out of the follow-up. Children who performed the follow-up have been following the GFD with strict adherence, 36 of them for >1 year, obtaining a considerable weight and height increase, particularly evident for the females after the first 3 months (Tables 1 and 2). Moreover, an improvement of school performance by 22% and a reduction of episodes of upper respiratory infections were registered by 26% of children. The 22% of children with CD felt neutral thinking about “having CD,” 45% bad, 15% good, and 18% very good. A subclinical Hashimoto thyroiditis was occasionally found in a girl at CD diagnosis. Up to
now, no other CD complications have been diagnosed. After 12 months of follow-up, 68% of children were salivary-negative and 75% were ELISA tTGAb–negative (Fig. 3). Interestingly, the screening performed on first-degree relatives of the newly diagnosed children revealed that 3 sisters and 2 mothers were celiac.

A total of 10 children with salivary tTGAb borderline values and 1 with low titers were not salivary or serum confirmed. The positive predictive value of the test was 97.6%. One child did not perform the serological confirmation yet (Fig. 1).

The compliance of the parents to the screening was 83.4%, and 93.2% of the children were able to collect their own saliva (Fig. 1). Analyzing the cohort of children enrolled, a slight predominance of boys (52%) over girls was found, in contrast with the high predominance of girls over boys (33%) among the children with CD ($P = 0.0009$). Sixteen percent of children with CD had at least 1 first-degree relative with CD, whereas among CD-negative subjects, it was found in only 1.1% ($P < 0.0001$). Moreover, comparing CD screening-detected children with previously diagnosed patients with CD, we found a significantly lower percentage of CD relatives (2% vs 44%, $P < 0.0001$). Furthermore, 85% of children enrolled had both Italian parents and 5% had only 1 Italian parent. Other nationalities more frequently found were Romanian (27%) and Filipino (15.5%), but there were also children from Peru (6.5%), China (4.7%), Bangladesh (4.4%), Ecuador (4.2%), and so on. Among screening-detected children with CD, 2 foreign girls, 1 from Venezuela and 1 from Sri Lanka, were found.

**DISCUSSION**

In the present study, we provide a clinical updation of CD in Italian primary schoolchildren and we gain a new iceberg
Even the disease clinical spectrum seems to be modified over the time. We identified some children with mild symptoms, whereas some children with a silent CD form had already been diagnosed (Fig. 2). This may be because of the widely accepted use of screening projects in high-risk groups, such as first-degree relatives (24), patients with autoimmune diseases (25), or iron-deficiency anemia (26). Moreover, some pediatricians include CD-related autoantibodies detection at least once in routine analysis of their patients. Consequently, the overall ratio between symptomatic and silent forms of CD was 1:1.7, which was slightly lower than the 1:2 found in another Italian screening on 3188 schoolchildren (22). We identified some sign in screening-detected children with CD; none of them had been judged ill or sent for investigation by the pediatrician. The finding of abdominal distension (that was significantly more frequent in screening-identified celiac children than in negative subjects) emphasizes the need for CD-related antibodies detection, particularly in these children; however, in our series, among screening-detected children, only 1 patient had first-degree relatives with CD, and CD-related disorders were present in none except the girl with autoimmune thyroiditis that, nevertheless, was present but not recognized, at the time of CD diagnosis. Thereafter, the majority of our patients did not belong to high-risk groups, and therefore their chance of receiving a timely diagnosis was low.

The ratio between “visible” (previously diagnosed) and the overall size (overall prevalence) is 0.36; this is higher than in other studies performed in Italian (0.12) (19) and Hungarian (0.13) (27) children and in European adults (0.06) (21). Our data could be explained by the high level of CD awareness in Rome pediatricians, because of the knowledge of its health, nutritional, and economic implications.

In our study, screening-detected children with CD, whose growth percentiles were all but one in the normal range at diagnosis, showed good weight and stature growth, which improved in terms of percentiles during the follow-up (Table 1), an improvement of displaying the emergence of almost all typical forms and some atypical and silent children, but the submerged part is still deep. To obtain a correct CD prevalence evaluation, we had to analyze at least 7316 children who permit an approximation of 0.00228. The population investigated was highly representative of the primary school-age population of Rome. The male/female ratio was also extremely similar to the 5- to 8-year-old primary schoolchildren of our region, and no significant difference was found in terms of the foreign scholars’ percentage, as reported by the National Institute of Statistics data (18).

It was possible to diagnose CD in 45 children, and the disease prevalence in our series, as including 23 previously diagnosed patients, reached to 1.24%. In this study only positive children were biopsied because of obvious ethical reasons. There was no justification in biopsying negative subjects and, moreover, was not even our aim. In our study, salivary test showed a high positive predictive value (97.6%), but it has not been possible to determine the sensibility of our test because tTG IgA has not been tested in all participants; however, previous study demonstrated a high sensitivity and specificity of our test (8,9) and CD prevalence in our sample is even higher than expected (19,20).

In any case, for screening purposes, we chose to retest children with positive and borderline RIA tTG values not to lose any detectable patient with CD.

In 1996 a CD screening (using the less sensitive anti-gliadin antibody assay) was conducted on 17,201 primary and secondary schoolchildren, revealing a prevalence of 0.55% (20). During the last decades, growing evidence (7,21,22) supports the increase of CD prevalence in our region, and no significant difference was found in terms of the foreign scholars’ percentage, as reported by the National Institute of Statistics data (18). The recent years have seen a significant increase in CD diagnoses among children. This may be related to increased awareness of the disease among parents and healthcare professionals, improved diagnostic techniques, and changes in dietary habits.

**TABLE 2.** Percentiles of weight and stature growth during the follow-up of children with CD

<table>
<thead>
<tr>
<th>M/F</th>
<th>M increase percentile (m ± SD)</th>
<th>F increase percentile (m ± SD)</th>
<th>M increase percentile (m ± SD)</th>
<th>F increase percentile (m ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>6.1 ± 5.5</td>
<td>10.4 ± 8.8</td>
<td>6.7 ± 10.5</td>
<td>10.3 ± 7.9</td>
</tr>
<tr>
<td>6 mo</td>
<td>10.8 ± 5.5</td>
<td>7.9 ± 7.9</td>
<td>5.4 ± 11.4</td>
<td>7.1 ± 8.6</td>
</tr>
<tr>
<td>9 mo</td>
<td>17 ± 4.1</td>
<td>11.3 ± 10.2</td>
<td>3</td>
<td>11.7 ± 6.6</td>
</tr>
<tr>
<td>12 mo</td>
<td>16.7 ± 18.2</td>
<td>10.1 ± 15.8</td>
<td>2 ± 9.8</td>
<td>6.8 ± 13.1</td>
</tr>
<tr>
<td>18 mo</td>
<td>27.5 ± 20.5</td>
<td>1.8 ± 2.5</td>
<td>10.7 ± 8.9</td>
<td>4.4 ± 13</td>
</tr>
</tbody>
</table>

m ± SD = mean ± standard deviation.
school performance, and reduction of episodes of upper respiratory infections. Up to now no CD-related complications have been detected in any celiac children on a GFD. The relation between gluten assumption and the induction of endocrine autoantibodies and organ dysfunction is documented (28), but the existence of a close relation is debated. In our opinion, the age 5 to 8 years could be an advantage at which the CD duration, and consequently the disease activity, is long enough for CD onset but short enough for the development of complications and the puberty is still far. Although some children may develop CD later on, this age range allows to gain a good compliance to the GFD in these young children. It is also important to consider that children usually eat more frequently at home or at school and consequently they are more compliant to the diet, which is not the case for adolescents and adults.

Our study confirms that the salivary test is a powerful screening tool, well accepted by the general population, as shown by the high compliance (83.4%), that was even higher than the simple, rapid Biocard test kit (76%) (27). The salivary test was seen as a game by children and only 2% of them were not able to collect autonomously the sample. We decided not to use any instruments (swabs, syringes) for help in collecting because our priority was to make a completely noninvasive test. Five percent of children were absent at the time of collection, but the possibility of alternative appointments and the strong willingness that drove parents to take their children on the day agreed permitted us to recover some children, 2 of whom had CD.

We had a low dropout from the second-level test (1.6%). Among the 50 children who were positive twice, 7 dropped out of the endoscopic evaluation, but 3 of them performed the follow-up on a GFD. Only 4 schoolchildren’s parents (8%) refused further evaluation. Some studies on adult patients report higher rates of dropout from the screening (50%) (29). Even the study of Catassi et al (19) reported a dropout from second-level tests of 4.6% and from the endoscopic evaluation of 11.7%. An explanation of our results can be found in the clear and attractive illustrative forms, as in the timely communication of results by telephone for positive
children. In addition, it is well known that intensive counseling is associated with high compliance.

The foreign nationalities of our series cover the entire world and 2 screening-detected children with CD had parents who were foreign. Unfortunately, among children whose parents refused the study, there were many foreign subjects. In future projects, translations of the forms could help to overcome the possible mistrust because of the language barrier.

In the present study, we tested only RIA anti-transglutaminase IgA. IgA deficiency, which has a prevalence of 0.14% in Italy, is reported in 1.7% of patients with CD (30). These children could be missed in our study, but the decision to use only a test for IgA is derived from the need to minimize costs, which is the basis of a screening project.

Nevertheless, serological IgA deficiency does not fully reflect salivary IgA deficiency, as shown by borderline salivary tTG IgA levels detection in a patient with serum IgA deficiency. Moreover, the informative form included the investigation concerning repeated upper respiratory tract infections and recurrent diarrhea, aimed to detect indirect signs of IgA deficiency in children to be eventually submitted to IgG tTGAb afterwards.

CONCLUSIONS

To our knowledge, this is the first study reporting an updated clinical picture of CD in children. A massive screening program could permit the emergence of subclinical CD forms and reduce the clinical picture of CD in children. A massive screening program derived from the need to minimize costs, which is the basis of a screening project.

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REFERENCES