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Title: Prevalence of celiac disease in patients with severe food allergy.

Short title: Celiac disease and severe food-allergy

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Abstract

The association between food-allergy and celiac disease (CD) is still to be clarified. We screened for CD 319 patients with severe food allergy (IgE > 85 kU/l against food proteins and a history of severe allergic reactions) who underwent specific food oral immunotherapy (OIT), together with 128 children with mild allergy who recovered without OIT, and compared the prevalence data with our historical data regarding healthy schoolchildren. Sixteen patients (5%) with severe allergy and one (0.8%) with mild allergy tested positive for both genetic and serological CD-markers, while the prevalence among the schoolchildren was 1%. Intestinal biopsies were obtained in 13/16 patients with severe allergy and in the one with mild allergy, confirming the diagnosis of CD. Sufferers from severe food allergy seem to be at a five-fold increased risk of CD. Our findings suggest that routine screening for CD should be recommended in patients with severe food allergy.

Introduction

Celiac disease (CD) is a systemic gluten-dependent autoimmune disease often occurring in association with other autoimmune disorders (1). The relationship between CD and allergy is still unclear, but some reports suggest that patients with celiac disease have an increased frequency of allergic manifestations compared to general population (2-4), although two recent studies found no such association (5,6). One factor which might trigger allergies in celiac patients is abnormal

intestinal permeability which increases the flow of dietary antigenic protein, predisposing the mucosal immune system towards an atopic condition (7). Moreover, CD and allergy might share a similar predisposing genetic background (4). The aim of this study was to evaluate the prevalence of celiac disease in children with very severe food allergy, undergoing specific food oral immunotherapy (OIT). In addition, information regarding clinical symptoms, laboratory data and OIT outcome were collected about the newly diagnosed celiac patients both before and after diagnosis, so as to investigate whether the gluten-free diet (GFD) had any effect on the allergic manifestations.

Patients and methods

Three hundred-nineteen children (201 M, 118 F, mean age 9 ± 4 years) with very severe food allergy (245 with cow's milk allergy, 98 with egg allergy, 25 with wheat allergy) were included in our study according to the following criteria: elevated IgE levels against food proteins (IgE values > 85 kU/l) and a positive history of at least one severe allergic reaction (i.e., a reaction defined as class 4 and 5 according to Clark's classification) (8) after accidental or voluntary (oral challenge) exposure to the causal food. All subjects underwent specific oral tolerance induction at our hospital as previously described (9) between October 2011 and July 2014. The present study group represents the 40% of our allergic patients which were treated with OIT since 2003 at our hospital.

Serum samples were analysed for IgA antiendomysium antibodies (AEA) by indirect immunofluorescence on cryostat sections of human umbilical cord as previously described (10), and serum IgA-IgG anti-TG2 antibodies were measured using an ELISA assay (Eurospital, Italy) following the manufacturer's instructions (normal values <7 U/I). The susceptibility alleles for CD were determined by PCR with allele specific primers identifying HLA DQ2 and DQ8, using Eu-Gene-Risk kit (Eurospital, Italy). Intestinal biopsy was recommended to subjects testing positive to both the serologic and the genetic tests, so as to obtain definitive diagnosis of CD. Clinical and laboratory data were collected regarding the newly diagnosed cases of CD, and eventual changes before and after GFD were correlated.

CD prevalence in the study group was compared with that of 128 age-matched children (68M, 60F, 8±5 years) who had made a spontaneous recovery from food allergy without OIT and also with a control group made up of 3188 healthy schoolchildren screened for CD in Trieste (10). The immune assays and biopsy analysis were performed by operators blinded to the subjects' clinical and laboratory data. The study was approved by the independent Ethical Committee of the Institute of Child Health IRCCS Burlo Garofolo (IEC 311/2011).

Statistical analysis.

A two-tailed Fischer exact test and Kruskal-Wallis test were carried out to determine the significance of the differences in the categories which were defined according to the absence or presence of findings. A value of $p < 0.05$ was considered significant.

Results

Sixteen subjects (16/319, 5%, 11 M, 5 F) with severe food allergy tested positive for both the serological CD markers (AEA and anti-tTG), and HLA DQ2/8 haplotypes. All of them were retested for CD-related antibodies before gastro-intestinal endoscopy and three (2M, 1F) presented alternate negative-positive results. Endoscopy was postponed in these three patients, while the other 13 underwent small bowel biopsies that showed mucosal intestinal atrophy, and were placed on GFD. One patient was suffering from anaemia (haemoglobin 9.8 g/dl) and failure to growth (weight $< 3^{\circ}$ percentile) but showed improvement after 12 months of GFD (haemoglobin 12 g/dl, weight $> 10^{\circ}$). No difference was found between patients with or without CD as regards the clinical and laboratory data regarding their food allergy (mean of food specific IgE concentrations and severity of reaction). More specifically, with regard to children allergic to cow's milk, in 8 untreated CD-patients (before diagnosis), 4 treated CD-patients (on GFD for more than 12 months) and 40 non-celiac allergic patients, we measured the minimum trigger dose to induce

severe symptoms before oral immunotherapy and the maximum tolerated milk dose 10 days after oral immunotherapy. No statistical differences were found among these three groups regarding the cow's milk tolerated doses, with no differences among untreated and treated CD patients ($p=0.8$) (Fig.1).

In the end, we identified 13 biopsy-proven celiac subjects among 319 patients with severe allergy (prevalence 4%), but if the three further patients who tested positive for CD-related antibodies and HLA DQ2/8 are considered as having CD, the overall prevalence is 5%. Whereas among the group of patients who made a spontaneous recovery from mild allergy, one was confirmed as having CD by intestinal biopsy, giving a prevalence of 0.8%, similar to the prevalence (1%) among schoolchildren in Trieste. (10) The CD prevalence result in subjects with severe allergy was significantly different from that of the children with mild allergy ($p=0.03$) and from the healthy schoolchildren ($p<0.0001$).

The CD-associated HLA DQ molecule was detected in 120/319 patients (37%) with severe allergy and in 43/128 (33.5%) patients with mild allergy, figures similar to those found among the general Italian population. (11)

None of the patients we screened had total IgA deficiency (mean value of IgA: 185 ± 65 mg/dl).

Discussion

The CD-prevalence among patients with severe allergy is 4 to 5 times higher than in both the general population and in subjects with mild allergy. Severe food allergy is strongly associated with CD as we have seen among patients with autoimmune diseases, such as type 1 diabetes and autoimmune thyroiditis. (1,12) The frequent association of CD with these autoimmune diseases has usually been explained by increased frequency of the common genetic determinant, the HLA DQ2/8 haplotypes (13), but this correlation was not strong in our study population. It has been hypothesized that the impaired intestinal mucosal permeability of untreated CD patients might result in an increased flow of dietary antigens through the intestinal mucosa, stimulating food-dependent hypersensitivity. Indeed, also the reverse could be true because the impaired intestinal permeability, which was observed in some allergic patients, could break the tolerance to gluten, promoting the CD among the genetically predisposed subjects.

Furthermore, the chronic up-regulation of IL-15 (14) in the intestinal mucosa of celiacs, might promote the dysregulation of multiple immune mechanisms, influencing both Th1 and Th2 related diseases. (15) The IL-15 would seem to play a pivotal role in the induction of allergen-specific Th2-differentiated T-cells and allergic inflammation in vivo, while in an animal model, the inhibition of endogenous IL-15 suppresses a Th2-type allergic inflammation. (16) Theoretically, in some

patients with CD with chronic gluten-dependent inflammation, the IL-15 might function as the initial primer in determining the type-2 effector T-cells. In such cases, the untreated CD might be the causative agent of the severe allergy and consequently, GFD might be a novel, but rational therapeutic tool for treating food-dependent allergic inflammation. However, among our patients, there was no improvement in either clinical or biological allergic complaints during the GFD, but this may be due to the short follow-up. In fact, in our clinical practice, the GFD was related to the elimination of gluten-dependent organ-specific auto-antibodies (such as thyreoperoxidase antibodies, islet cell antibodies, glutamic acid decarboxylase antibodies, and anti-insulin antibodies) in more than 12 months (17), and other authors have even found an improvement in organ-specific dysfunctions involving the brain and liver (18,19).

Among our CD patients, only one had symptoms (anaemia and failure to thrive) that improved during the GFD, while the other 15 were asymptomatic, confirming that in the course of CD-screening programmes, symptoms are present only in a minority of CD subjects (10). To sum up, this study is the first to identify a new clinical condition at risk of CD, which calls for screening in order to prevent possible complications of untreated CD in both asymptomatic and symptomatic patients (20). Our study also points to the need for prospective studies to evaluate the potential

benefits of GFD in the management of the complaints among patients with both CD and severe food allergy.

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Figure 1. The minimal trigger cow's milk dose inducing severe symptoms before food oral immunotherapy (left) and the maximal tolerated cow's milk dose 10 days after the specific oral immunotherapy (right) among 8 untreated and 4 treated CD-patients and 40 non celiac allergic patients. No statistical differences of the cow's milk quantities were found among the three study groups (Kruskal-Wallis test $p=0,8$).

Author contributions: Roberto Pillon, Fabiana Ziberna, Irene Berti, Laura Badina, Stefano Martellosi, Giuseppe Patano, Tarcisio Not, Alessandro Ventura were involved in study conception and design and were responsible for data acquisition and data interpretation. Irene Berti, Laura Badina, Stefano Martellosi, Giorgio Longo, Alessandro Ventura managed clinical aspects and follow-up. Sara Quaglia, Luigina De Leo, Serena Vatta, Fabiana Ziberna performed the immunological and genetic tests and the analysis of intestinal biopsies. Irene Berti, Tarcisio Not wrote the manuscript. All authors read and approved the manuscript.

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Key words: celiac disease; gluten free-diet; severe food-allergy

Abbreviations: CD, celiac disease; GFD, gluten-free diet; SOTI, specific oral tolerance

Induction.

Conflict of interest: The authors declare that they have no conflicts of interest.

