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Extension of the celiac intestinal antibody (CIA) pattern through eight antibody assessments in fecal supernatants from patients with celiac disease

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ABSTRACT

Background: Detection of anti-transglutaminase, anti-endomysium and anti-gliadin antibodies is commonly used to screen celiac disease patients. Besides that in serum, these antibodies are detectable in culture supernatants of oral, duodenal and colonic biopsy samples, saliva, gut lavage fluid samples, and fecal supernatants. Our aim was to extend the intestinal antibody pattern in fecal supernatants from patients with celiac disease.

Methods: The fecal supernatants obtained from 25 celiac disease patients and 12 healthy volunteers were used to determine IgA and IgG1 anti-endomysium by immunofluorescence analysis, IgA and IgG anti-transglutaminase, IgA and IgG anti-deamidated gliadin peptides, IgA/IgG anti-transglutaminase/deamidated gliadin peptides and IgA anti-actin by enzyme-linked immunosorbent assay.

Results: IgA anti-endomysium were found in 11 of 25 (44.0%) celiac disease patients and in none of healthy volunteers ($p = 0.0066$). The levels of IgA anti-transglutaminase, IgA anti-deamidated gliadin peptides, IgA/IgG anti-transglutaminase/deamidated gliadin peptides and IgA anti-actin determined in celiac disease patients were significantly higher ($p = 0.0005$, $p = 0.0018$, $p = 0.0061$ and $p = 0.0477$, respectively) than those measured in healthy volunteers. The ROC curve analysis showed a diagnostic significance in IgA anti-transglutaminase (AUC = 0.862, $p < 0.0001$), IgA anti-deamidated gliadin peptides (AUC = 0.822, $p < 0.0001$) and IgA/IgG anti-transglutaminase/deamidated gliadin peptides (AUC = 0.783, $p = 0.0003$) fecal tests.

Conclusions: Our data extend the intestinal antibody pattern detectable in fecal supernatants, thus increasing the knowledge in the humoral immunity of celiac disease. Further studies are needed to better evaluate the role of fecal antibody tests in identifying celiac disease patients.

1. Introduction

Celiac disease (CD) is a systemic autoimmune disorder triggered by the ingestion of wheat gluten (gliadins and glutenins), and related proteins of rye and barley, in human leukocyte antigen (HLA) DQ2 and/or DQ8 positive individuals (Guandalini and Assiri, 2014). In this condition, an incomplete digestion of gluten peptides, changes in intestinal permeability and the activation of innate immunity mechanisms seem to precede the development of an adaptive immune response (Abadie et al., 2011; Kupfer and Jabri, 2012). The tissue transglutaminase (tTG), known as the main autoantigen of CD, plays a key role in enhancing the immunostimulatory effect of gluten peptides (Di Sabatino et al., 2012).

Abbreviations: A_{450nm}, absorbance$_{450nm}$; AAA, anti-actin antibodies; AGA, anti-gliadin antibodies; anti-DGP, anti-deamidated gliadin peptides; AU, arbitrary units; AUC, area under curve; CD, celiac disease; CI, confidence interval; CIA, celiac intestinal antibody; ELISA, enzyme-linked immunosorbent assay; EMA, endomysium antibodies; FITC, fluorescein isothiocyanate; HLA, human leukocyte antigen; iIFA, indirect immunofluorescence analysis; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; tTG, tissue transglutaminase.

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The diagnosis of CD is currently based on clinical case identification, detection of specific circulating antibodies and histological finding of total, subtotal or partial intestine villous atrophy corresponding to type III of Marsh-Oberhuber classification (Husby et al., 2012; Oberhuber et al., 1999). Although gliadin is the culprit antigen of CD, serum anti-gliadin antibody (AGA) detection is not always an efficient tool to screen patients suffering from this condition. Conversely, serum anti-tTG and endomysium antibodies (EMA), showing a higher sensitivity and specificity, are commonly used in the screening and monitoring of CD patients (Giersiepen et al., 2012; Sandström et al., 2013).

Our previous study has demonstrated that EMA can be detected in culture supernatants of duodenal biopsy samples from CD patients (Picarelli et al., 1996). After some years, it has been shown that also anti-tTG antibodies can be measured in culture supernatants of duodenal biopsy samples (Carroccio et al., 2006), highlighting the ability of this organ culture system to identify CD (Picarelli et al., 2013a). The presence of EMA and anti-tTG antibodies has been subsequently demonstrated in culture supernatants of both oral and colonic biopsy samples, proving that also these mucosal sites are involved in CD (Picarelli et al., 2013b; Vetrano et al., 2007). The in vivo proof that CD-specific antibodies come from the intestinal compartment has been provided by studies detecting EMA and anti-tTG in fecal supernatants from CD patients. Consistently, high concentrations of AGA have been also found in these biological fluids (Halblaub et al., 2004; Picarelli et al., 2002). The hypothesis that the intestinal mucosa is the principal site of CD-specific antibody production has been therefore confirmed by molecular studies using phage display libraries (Sblattero et al., 2004). The literature also reports that the so-called celiac intestinal antibody (CIA) pattern (AGA, EMA, and anti-tTG antibodies) can be highlighted in gut lavage fluid samples from CD patients (Dahele et al., 2002; O'Mahony et al., 1990). Moreover, the anti-tTG detection in saliva from CD patients has opened the possibility to use this biological fluid for diagnostic purposes (Bonamico et al., 2004). Although a recent study has demonstrated that the anti-tTG salivary test is also effective in monitoring CD patients on a gluten-free diet (Bonamico et al., 2008), some adverse data have stopped its entry in the diagnostic protocol of CD (Baldas et al., 2004).

On the basis of the mentioned literature, our aim was to re-evaluate and extend the CIA pattern in fecal supernatants in order to increase the knowledge in the humoral immunity of CD and possibly enrich its diagnostic armamentarium.

2. Materials and methods

2.1. Patients

Twenty-five serum EMA/anti-tTG positive CD patients (4 male/21 female, mean age 36.3, range 18–59 years), diagnosed according to the British Society of Gastroenterology guidelines (Ludvigsson et al., 2014), and 12 serum EMA/anti-tTG negative healthy volunteers (4 male/8 female, mean age 31.5, range 18–65 years) were enrolled in the study (Table 1). A spot stool sample was collected from all participants at the time of study entry, when they were on a gluten-containing diet. All procedures followed were in accordance with the ethical standards of the institutional committee responsible for human experimentation. Furthermore, an informed consent was obtained from each participant being studied.

2.2. Fecal supernatants

Fecal supernatants were obtained from stool aliquots of 2 g diluted in 8 ml of normal saline solution (0.9% sodium chloride) and centrifuged for 40 min at 3000 rpm (1700 g). Thereafter, supernatants were removed and centrifuged three times in microfuge for 5 min at 6000 rpm (3000 g). Supernatants were then collected and stored at −80 °C until used to perform the following antibody assessments: IgA and IgG1 EMA, IgA and IgG anti-tTG, IgA and IgG anti-deamidated gliadin peptides (anti-DGP), IgA/IgG anti-tTG/DGP, and IgA anti-actin antibodies (AAA).

2.3. Immunofluorescence antibody detections

IgA and IgG1 EMA were searched in undiluted fecal supernatants by indirect immunofluorescence analysis (iIFA) on cryostat sections of monkey esophagus (Eurospital, Trieste, Italy). After supernatant incubation, the sections were stained with a fluorescein isothiocyanate (FITC)-conjugated anti-human IgA or IgG1. The results, expressed as positive/negative, were evaluated blindly by two trained observers, whose agreement rate was 99.6%. EMA positive results were identified by the typical honeycomb-like staining pattern along muscularis mucosae (endomysium), that marks the collagenous matrix of type 3 connective tissue surrounding the smooth muscle fibers of the primate esophagus.

2.4. Immunoenzymatic antibody determinations

IgA and IgG anti-tTG, IgA and IgG anti-DGP, and IgA/IgG anti-tTG/DGP antibodies were measured in undiluted fecal supernatants, while IgA AAA were measured in fecal supernatants diluted 1:2 by enzyme-linked immunosorbent assay (ELISA) on microtiter-plate wells coated with recombinant human tTG for anti-tTG, deamidated gliadin peptides for anti-DGP, and filamentous (F)-actin for AAA (IgA and IgG anti-tTG, IgA/IgG anti-tTG/DGP, and IgA AAA: INOVA Diagnostics, San Diego, CA – distributed by Instrumentation Laboratory, Milan, Italy; IgA and IgG anti-DGP: IVAX Diagnostics, Miami, FL – distributed by Delta Biologicals subsidiary, Rome, Italy). The results, quantified by an ELISA plate reader at 450 nm, were expressed in absorbance (A_{450nm}) values because, in the kits used, the calculation systems to obtain antibody concentrations were standardized only for serum samples.

2.5. Statistical analysis

Qualitative data obtained from different groups of study participants were expressed as frequencies (both absolute and relative) and compared by the Fisher exact test because the presence of some frequencies equal to zero. For each comparison, the relative risk and its 95% confidence interval (95% CI) were calculated.

Quantitative data achieved in this study were firstly analyzed by the D'Agostino–Pearson omnibus test to verify the normal distribution hypothesis within each statistical sample. Given that some resulting p values were significant ($p < 0.05$), it is reasonable to assume that not all data obtained from every group of participants fall into Gaussian distributions and therefore, were synthesized as mean values ± standard deviations and processed by means of parametric tests, the results of which were verified by the relative non-parametric tests. In detail, comparisons between the parameters determined in different groups of participants were performed by using the Student t test for unpaired data confirmed by the Mann–Whitney test. The relationship between different parameters was assessed by the Bravais–Pearson linear correlation that in turn, was verified by the Spearman rank correlation. The analysis of receiver operating characteristic (ROC) curve was carried out to determine the optimal threshold value of fecal antibody tests, as well as to evaluate their diagnostic performance through the assessment of both area under curve (AUC) and Youden index calculated as (sensitivity + specificity) − 1. In detail, each optimal threshold value was identified as the point of the ROC