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Alimentary Tract

Sampling of proximal and distal duodenal biopsies in the diagnosis and monitoring of celiac disease

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ABSTRACT

Background: Since celiac disease-associated mucosal lesions are patchy, the diagnosis of the disease requires histological evaluation of multiple duodenal biopsies.
Aim: To examine whether adequate biopsy sampling in either the bulb or distal duodenum is sufficient to diagnose celiac disease.
Methods: Twenty-five patients with positive celiac disease-specific serology and 17 patients with negative serology, who were on a gluten-containing diet, and 13 celiac disease patients on a gluten-free diet were consecutively and prospectively enrolled. Mucosal damage, anti-transglutaminase-2 IgA deposits, interferon-γ, interleukin-17A and interleukin-15 transcripts were evaluated in bulb and distal duodenal biopsies.
Results: All patients with positive celiac disease-specific serology exhibited villous atrophy in both duodenal sites. In this group, mucosal anti-transglutaminase-2 IgA deposits were found in 24/25 (96%) bulb samples and 22/25 (88%) distal duodenal samples. No villous atrophy was documented in patients with negative serology. Interferon-γ and interleukin-17A were over-expressed in both duodenal sites of patients with villous atrophy, unlike patients with normal duodenal morphology ($p < 0.001$). Among treated celiac disease patients, 2 (15.4%) had villous atrophy exclusively in the bulb and 6 (46.2%) had minimal histological abnormalities at both sites.
Conclusion: Sampling in the bulb and distal duodenum could be sufficient to diagnose/exclude celiac disease.

1. Introduction

Celiac disease (CD) is a gluten-driven enteropathy affecting approximately 1% of the general population [1,2]. In genetically-susceptible individuals, ingestion of gluten triggers a dual innate and adaptive immune response leading to mucosal damage and various grades of intestinal malabsorption [3]. Patients manifesting symptoms/signs suggestive of CD, asymptomatic individuals with a first-degree relative who has CD, and patients with other immune-mediated diseases, which frequently associate with CD (e.g. type I diabetes mellitus and psoriasis), should be tested for serum anti-tissue transglutaminase-2 (TG-2) antibody [4], whose sensitivity and specificity for untreated CD are greater than 95% in referral centres [5,6]. There are, however, differences in the performance of the commercial kits used to assess serum anti-TG2 antibody. This could explain why 5–16% of adult patients with a confirmed diagnosis of CD may have a negative test [5,7]. Similarly, negativity for serum anti-endomysium IgA antibody (EMA), which is also directed against TG2, has been documented in untreated CD patients with normal IgA levels [8]. Anti-TG2 antibodies are produced by plasma cells infiltrating the intestinal mucosa, and their presence in the duodenum of untreated CD patients can be observed even when they are not detectable in the serum [9,10]. Indeed, mucosal anti-TG2 antibody deposits are considered as a useful gluten-dependent marker in the diagnosis and dietary monitoring of CD [11].

Histological confirmation of the morphological features of the disease (i.e. villous atrophy, crypt hyperplasia and enhanced infiltration of the epithelial and lamina propria compartments with lymphocytes) is currently considered the gold standard to establish a diagnosis of CD in subjects with positive antibody testing

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[4,12]. Traditionally, multiple endoscopic biopsies were collected from the more distal segments of the duodenum, because the presence of Brunner glands in the proximal duodenum can interfere with the evaluation of the villous:crypt ratio [12–14]. It was also demonstrated that sampling of 4 or more specimens increases the diagnostic yield [15]. More recently it has been suggested that adding biopsies of the duodenal bulb increases the sensitivity of the test because nearly 10% of untreated CD patients can have villous atrophy exclusively in the bulb [16–20]. The reason why some patients should have villous atrophy exclusively in either the bulb or distal duodenum remains unknown. It is plausible that this may reflect the patchy distribution of the CD-associated lesions [20–22]. Another possibility is that ingestion of gluten can initially cause a localized tissue damage in the bulb as this portion of the intestine is rich in lymphatic structures [23]. The size, number, quality, and orientation of biopsies may also all affect the histological analysis and negatively influence the identification of CD-associated histological abnormalities [16,17].

Since evaluation of multiple biopsies taken at both duodenal sites is time-consuming and increases the costs for diagnosing CD [17], we aimed at examining whether adequate sampling of specimens at a single duodenal site is sufficient to identify CD-associated villous atrophy. To address this issue, we performed a prospective study in a single academic centre, where experienced gastrointestinal endoscopists and pathologists were involved in sampling and analysis of duodenal biopsies.

2. Materials and methods

2.1. Patients and samples

Fifty-five patients (aged 16–75 years) undergoing upper endoscopy at the Gastrointestinal Unit of Tor Vergata University Hospital (Rome, Italy) for CD suspicion and/or follow-up were prospectively studied from October 2011 to February 2013 and consecutively enrolled in the present study.

The study population included 25 patients on a gluten-containing diet who were found to be both EMA and TG2 antibody-positive (group A), 17 individuals negative for both antibodies while receiving a gluten-containing diet (group B) and 13 patients with a previously confirmed diagnosis of CD, who were EMA and TG2 antibody-negative, and on a strict gluten-free diet (GFD) (group C). Nine out of the 13 treated CD patients (69.2%) had persistent malabsorption symptoms (i.e. abdominal bloating and/or pain, diarrhoea, chronic fatigue and iron-deficiency anaemia) despite adherence to a GFD (median age: 7 years; range: 2–14). No patient was IgA-deficient. Demographic data and indications for upper endoscopy are shown in Table 1.

Patients were excluded from the study if they were pregnant, had an active gastrointestinal bleeding, or a suspected neoplasia.

Upper endoscopy was performed by 3 experienced gastrointestinal endoscopists. An independent observer trained in gastrointestinal endoscopy supervised all procedures thus ensuring consistency in biopsy sampling from both the bulb and second part (distal) of the duodenum. Endoscopy was performed with an Olympus GIF-Q 165 Exera II system endoscope (Olympus Italia s.r.l, Milan, Italy) and biopsies were collected using standards forceps. All procedures were performed with the use of pharyngeal local anaesthesia and, when requested by the patient, with the addition of conscious sedation (midazolam). The presence of macroscopic features suggestive of CD (i.e. loss of duodenal folds, scalloping, mosaic pattern) was also recorded. In total, 8 duodenal biopsies were taken from all the patients: four biopsies were collected from the bulb and 4 from the distal duodenum. Biopsies were used for histological analysis and assessment of mucosal anti-TG2 deposits. To provide consistency among the endoscopists, biopsy samples were collected from the 3, 6, 9, and 12-o' clock positions within the bulb, with the patients in left lateral position. Following sampling, biopsies were oriented on tissue paper and immediately either fixed in formalin and then embedded in paraffin, or snap-frozen in optimal cutting temperature compound (OCT, NEG50 Thermo Scientific, Langeselbold, Germany) and stored in liquid nitrogen. Two additional biopsies (one from the bulb and one from the distal duodenum) were taken from 9 patients of group A and 9 patients of group B and used for total RNA extraction and evaluation of interferon-γ (IFN-γ) expression.

Specimens were analysed by experienced pathologists in a blinded manner using the Corazza–Villanacci criteria to identify CD-associated histological abnormalities [24–26].

Each patient who took part in the study gave written informed consent, and the study protocol was approved by the local Ethics Committee.

2.2. Double immunofluorescence and microscopic analysis

Mucosal deposits of anti-TG2 antibody were examined by double-immunofluorescence using previously described procedures [27]. Briefly, cryostat sections were cut, fixed in acetone for 10 min and pre-incubated with protein block (Dako, Milan, Italy) for 15 min. Sections were then incubated with a monoclonal mouse antibody against human TG2 (CUB 7402) (1:200, NeoMarkers, Fremont, CA, USA) for 1 h at room temperature in a humidified chamber, washed in PBS containing 1% bovine serum albumin (BSA, Sigma–Aldrich, Milan, Italy), and incubated with a mixture of a R-phycoerythrin (RPE)-labelled rabbit anti-mouse secondary antibody (1:60, Dako) to detect TG2 and a fluorescein isothiocyanate (FITC)-labelled rabbit antibody against human IgA (1:100, Dako) for 30 min in the dark. Finally, the sections were washed several times in PBS with 1% BSA, mounted with Mowiol (Millipore, Milan, Italy), and analysed by a fluorescence microscope (Olympus BX51, Byosystem82, Rome, Italy). Evaluation of all the sections was performed by 2 blinded investigators who were not aware of the clinical history and CD-associated serologic tests of the patients. The pattern and the intensity of the yellow-orange co-staining indicative of the presence of mucosal deposits of anti-TG2 IgA were evaluated and graded using the following scores: negative (1), very weak (2), weak with patchy distribution (3), strong with patchy distribution (4), and strong with a homogeneous distribution (5).

2.3. RNA extraction, cDNA preparation, and real-time PCR

Total RNA was extracted using the Pure Link mRNA mini kit according to the manufacturer's instructions (Life Technologies, Milan, Italy). A constant amount of RNA (1 μg/sample) was retro-transcribed into complementary DNA (cDNA) and then 1 μl of cDNA/sample was amplified using the following conditions: denaturation, 1 min at 95 °C; annealing, 30 s at 58 °C for IFN-γ, at 61 °C for interleukin (IL)-17A, and at 60 °C for β-actin, followed by 30 s of extension at 72 °C. The primers' sequence was as follows: IFN-γ forward 5′-TGGAGACCATCAAGGAAGAC-3′; reverse 5′-GCGTTGGACATTCAAGTCAG-3′; IL-17A forward 5′-ACTACAACCGATCCACCTCAC-3′; reverse 5′-ACTTTGCCTCCCAGATCACAG-3′; β-actin forward, 5′-AAGATGACCCAGATCATGTTTGAGACC-3′; reverse, 5′-AGCCAGTCCAGACGCAGGAT-3′. β-actin was used as internal control gene, and IL-15 was evaluated using commercially available TaqMan probes (Applied Biosystems, Foster City, CA, USA).

RNA expression was calculated relatively to the housekeeping β-actin gene on basis of the $\Delta\Delta$Ct algorithm.