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Alimentary Tract

Faecal high mobility group box 1 in children with celiac disease: A pilot study

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ABSTRACT

Background: Celiac disease (CD) is a gluten-related immunological disorder resulting in inflammatory enteropathy.
Aims: We assessed a stool marker of intestinal inflammation, the HMGB1 protein, in children with CD on a gluten free diet (GFD) at baseline and at follow up (FU).
Methods: Thirty-nine children were investigated at diagnosis and at FU. Traditional serum markers of CD (anti-transglutaminase and anti-endomysial antibodies) and faecal HMGB1 (by enzyme-linked immunosorbent assay and immunoblotting) were tested.
Results: There was a marked increase at baseline in both serum anti-transglutaminase IgA (anti-tTGAs) and faecal HMGB1; the latter being undetectable in controls. A strong correlation occurred between the two markers. At 12-month FU in 24 patients on GFD, HMGB1 decreased in all subjects, yet still being detectable in six children: high anti-tTGAs where evident in three, while the three with normal anti-tTGAs were complaining of intestinal symptoms and reported a low GFD adherence.
Conclusions: Faecal HMGB1 is a valuable marker of intestinal inflammation and may have a role in complementing serology in the management of CD children. Future studies including larger patient cohorts and small bowel mucosa histology will be designed to assess the relationship between faecal HMGB1 levels and duodeno-jejunal histopathology.

1. Introduction

Celiac disease (CD) is an immune-mediated systemic disorder involving the small bowel (SB) mucosa, triggered by peptides from gluten and related prolamines in genetically susceptible subjects which leads to SB inflammation ranging from increased intraepithelial lymphocytes to villous atrophy [1,2].

The diagnostic approach for CD has recently undergone significant changes since serologic tests have been shown to be useful predictors of SB mucosal damage [3]. Thus, novel paediatric guidelines for CD have been proposed, defining cases in which a non-invasive approach is recommended or SB mucosa biopsy is needed [4].

Lifelong adherence to a gluten-free diet (GFD) is the key treatment of CD patients, both to promote mucosal healing and prevent complications; however, assessment of GFD compliances is the cornerstone in patient management. Periodic measurements of serum anti-transglutaminase IgA levels (anti-tTGAs) are widely thought to be useful in monitoring adherence to GFD [5]. However, anti-tTGAs may not always reflect the status of the SB mucosa since various degrees of mucosal damage may persist despite normal serum anti-tTGAs. The latter may not identify minor voluntary transgressions or unperceived dietary gluten contamination of GFD [6].

The high mobility group box 1 (HMGB1) protein has recently been described as a reliable faecal marker of intestinal inflammation in paediatric and adult inflammatory bowel disease (IBD), either in overt or subclinical status [7,8]. HMGB1 is a protein included in a group of endogenous molecules (also known as alarmins or damage-associated molecular patterns) with intestinal and systemic pro-inflammatory properties when secreted in the extracellular milieu [9]. HMGB1 serum levels have recently been

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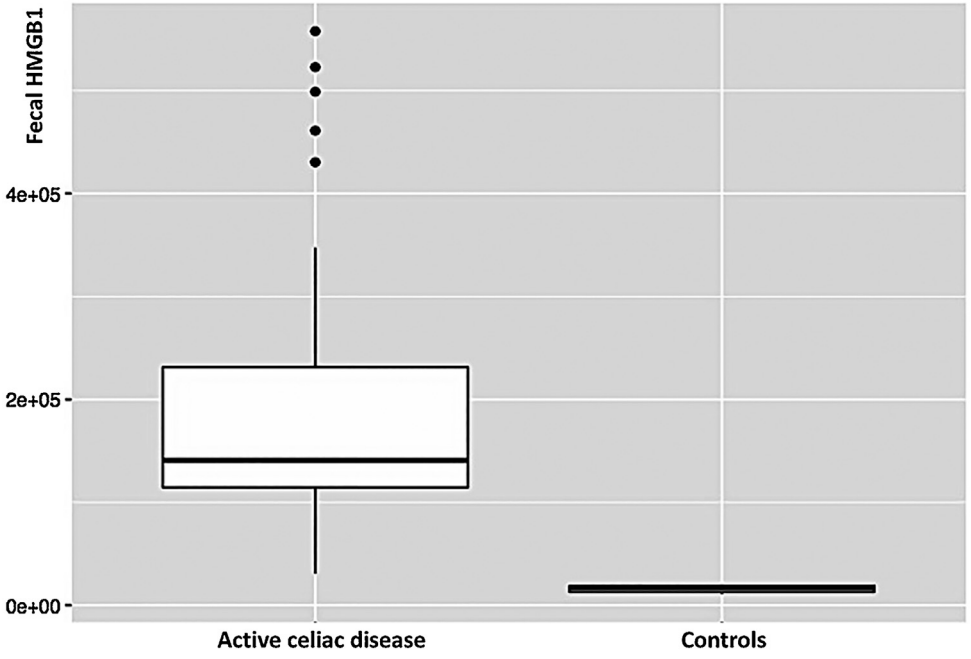

Fig. 1. Faecal HMGB1 level in active coeliac disease at diagnosis and in controls. P < 0.001 between the two groups (Wilcoxon rank sum test).

shown to have increased in CD at diagnosis helping to characterize patients according to their phenotypical subtype [10].

In this preliminary report we investigated the usefulness of faecal HMGB1 as a non-invasive marker in the diagnosis and in the follow-up of children with CD.

2. Material and methods

We enrolled 39 patients (24 girls; median age: 8.2 years; range: 15 months-18 years) with a CD diagnosis and 20 sex- and age-matched controls (median age: 11.0 years; range: 5–18 years) referred to the Paediatric Gastroenterology and Liver Unit of "Sapienza University of Rome" in a 24-month period. All patients were positive for serum anti-tTGAs and anti-endomysial antibodies (EMA); 38 out of 39 underwent upper gastrointestinal (GI) endoscopy and SB mucosa biopsies, in agreement with the ESPGHAN criteria published in 1990 [11]. One child was diagnosed as stated by the novel guidelines released by ESPGHAN in 2012 [4].

At diagnosis, 24 children had a combination of classical gastrointestinal (GI) features such as diarrhoea, abdominal pain, abdominal distension, bloating and growth failure. Fifteen had no GI features and were investigated owing to a family history (in 5) or non-specific symptoms and signs (in 10) such as pallor, asthenia, poor school performance, mood changes, iron deficiency anaemia, thyroid disease and neurological manifestations.

Stool samples from all children were collected at diagnosis; 24 samples were obtained at 12-month follow-up on GFD. Histology was graded in line with Marsh-Oberhuber criteria [12]. Anti-tTGAs were assayed at diagnosis and at follow-up with enzyme linked immunosorbent assay (ELISA) commercially available kits from Eurospital (Trieste, Italy; cut-off value >9 UA/ml). In outpatient setting GFD compliance was assessed at a 12-month follow-up; a dietary interview by an expert paediatric gastroenterologist was performed, coupled with anti-tTGAs levels.

Protein extraction from stool samples was performed to assess HMGB1 concentration in accordance with a prior reported method [7]. Twenty micrograms of faecal extracts were fractionated by sodium dodecyl sulfate-(12%) polyacrylamide gel electrophoresis. Proteins were transferred in polyvinylidenefluoride membrane (Bio-Rad Laboratories) and blocked with TBS-Tween 20 0.1%, containing 5% non-fat dry milk. Anti-human HMGB1 antibody (1:1.000; R&D, Minneapolis, Minnesota) was diluted in TBS-Tween 20 0.1%, containing 3% non-fat dry milk and incubated overnight at 4 °C. Membranes were washed in TBS-Tween 20 0.1%, incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, California), washed in TBS-Tween 0.1%, and developed with LiteBlotEXTEND (Euroclone, Milan, Italy). Densitometric analysis of the western blot bands was performed using the Software ImageQuant Las500 (GE Healthcare Life Science, Uppsala, Sweden).

2.1. Statistical analysis

Shapiro–Wilk test of normality rejected the normal distribution hypothesis for HMGB1 values. Wilcoxon signed-rank test was therefore used to compare mean HMGB1 values between patients with CD at diagnosis and the control group (significance taken as P < 0.001). The same test was then used to compare mean HMGB1 values at CD diagnosis and during follow-up. The correlation between HMGB1 and anti-tTG value was evaluated by Spearman's rank correlation coefficient (rho) following non-parametric distribution. All statistical analyses were performed using R v 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Faecal HMGB1 was significantly increased in all 39 CD patients at diagnosis compared to controls, while it was undetectable in the latter (Wilcoxon rank sum test with continuity correction p < 0.001) (Fig. 1). A strong correlation was found between faecal HMGB1 and serum levels of anti-tTGAs (Spearman's rho = 0.79) (Fig. 2).

Patients underwent physical examination and nutritional counselling by an expert paediatric gastroenterologist to investigate probable dietary pitfalls at 6 and 12 months following GFD. Stools were tested at 12 months post-GFD. Twenty-four of 39 patients were available for faecal HMGB1 and serum anti-tTGAs measurements.

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