CASE REPORTS

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# Crypt hyperplastic enteropathy in distal duodenum in *Helicobacter pylori* infection – report of two cases without evidence of celiac disease

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## Abstract

*Objectives*: Patients with *Helicobacter pylori* (HP) infection have been reported to have in addition to duodenitis architectural changes of the duodenal bulb mucosa including villous atrophy and crypt hyperplasia. We here for the first time present two cases of HP-infected adult patients with a crypt hyperplastic enteropathy also in the distal duodenum mimicking celiac disease (CD). *Methods*: We evaluated separately the morphology of the anatomical bulb and distal duodenal mucosa using validated quantitative morphometric tools, *i.e.*, the villous height (VH) and crypt depth (CrD) and their ratios. The fresh frozen samples were evaluated for the presence of the CD-specific transglutaminase 2-targeted subepithelial IgA deposits. *Results*: Both patients had celiac-type crypt hyperplastic mucosal injury in the distal part of the duodenum in the absence of serum autoantibodies and subepithelial IgA deposits. After two years follow-up, having still a normal gluten-containing diet, none of the patients developed CD. Moreover, in the patient re-biopsied two years later, the CD-type enteropathy had healed after HP eradication. *Conclusions*: Prospective studies on HP-infected patients are needed in order to confirm our findings.

***Keywords***: celiac disease, *Helicobacter pylori*, enteropathy, crypt hyperplasia.

## Introduction

*Helicobacter pylori* (HP) is recognized as one of the most common bacterial infections worldwide and is associated with significant upper gastrointestinal tract pathology, mainly gastric-related but also in the duodenum [1]. Duodenal mucosal inflammation with increased densities of intraepithelial lymphocytes (IELs) is well described in HP infection [2]. In fact, the inflammation is similar to that of early developing celiac disease (CD), showing only infiltrative enteropathy in duodenal biopsies, which represents the so-called Marsh I lesion [3, 4].

The celiac-type histology is recognized as a diagnostic challenge nowadays, with a broad differential including gastrointestinal pathogens [5–7]. The histological features include both inflammatory (increased intraepithelial lymphocytes) and architectural changes (crypt hyperplasia and villous atrophy). When looking only at the inflammation from this spectrum of mucosal changes, previous data has shown that duodenal intraepithelial lymphocytosis with preserved villous architecture (Marsh I lesion) is a relative common finding in duodenal biopsies [2], and HP infection has been recognized as a possible etiology. Besides the lymphocytic duodenitis, HP has been also reported to induce morphological injury in the duodenal bulb: series of HP cases have been reported to have architectural changes of the bulb mucosa including villous atrophy and crypt hyperplasia in ulcer associated and in non-specific duodenitis [8]. Recently, it was shown that morphological injury is common also in children in the anatomical bulb without CD, increasing the risk of false positive diagnoses [9]. However, data on distal duodenum changes in HP-infected individuals are lacking.

We here present two cases of HP-infected adult patients having inflammation and crypt hyperplastic enteropathy in the distal duodenum mimicking CD.

## Methods

Serum anti-tissue transglutaminase 2 (TG2) antibodies were measured using the Quanta Lite® h-tTG IgA (Inova Diagnostics, San Diego, CA, USA) with a positive cut-off of 20 U. Serum anti-endomysial antibodies were measured with an indirect immunofluorescence Nova Lite® kit (Inova Diagnostics) and positive cut-off was set at serum dilution of 1:5.

Upper gastrointestinal endoscopy with multiple duodenal biopsies, both from the anatomical bulb and distal duodenum, was performed. Hematoxylin–Eosin-stained biopsy specimens were evaluated using validated quantitative morphometric tools using villous height (VH, μm), crypt depth (CrD, μm) and their ratio (VH:CrD) [10]. A ratio >2 was considered not CD. Duodenal mucosal inflammation was studied in freshly frozen samples by

counting the densities of intraepithelial CD3+ T-lymphocytes (IELs) (cut-off 37 cells/mm epithelium, corresponding to 25 cells/100 epithelial cells) as well as $\alpha\beta$+ and $\gamma\delta$+ IELs (cut-off 25 and 4.3 cells/mm epithelium, respectively) [10]. Freshly frozen duodenal biopsies were also double-stained for IgA targeting mucosal extracellular TG2 [11].

## Case presentations

### Case No. 1

A 50-year-old female, former smoker, presented for dyspepsia progressively worsening over the past few weeks. Her medical history was positive for autoimmune thyroiditis and mild cervical and lumbar discopathy. Daily substitution of the thyroid function with Levothyroxine 100 µg was declared. Chronic or recent use of non-steroidal anti-inflammatory drugs (NSAIDs) was denied. Physical examination was unremarkable and routine blood work-up reported normal values, except for a high titer of anti-HP IgG antibodies. Stool samples were negative for parasites, but positive for HP antigen. Abdominal ultrasound reported no pathological patterns. Upper gastrointestinal endoscopy revealed mild gastritis; several biopsies from the gastric and duodenal mucosa were taken. Gastric biopsy samples showed erosions, inflammatory infiltrates in the lamina propria, while the duodenal mucosa histological examination reported not only increased IELs but also morphological changes with crypt hyperplasia (VH:CrD <2) both in the anatomical duodenal bulb and distal duodenal samples (Table 1). In both sites, altogether six well-oriented villus-crypt units were measured.

**Table 1 –** ***Morphometric measurements in the bulb and distal duodenum of the two cases***

| Morphometric measurements | | Case No. 1 | | Case No. 2 | | Case No. 2 Follow-up after eradication therapy |
|---|---|---|---|---|---|---|
| | | *Bulb* | *Distal duodenum* | *Bulb* | *Distal duodenum* | *Distal duodenum* |
| Inflammation (IELs/mm epithelium) | CD3+ | 50 | 118 | 12 | 34 | 51 |
| | $\alpha\beta$+ | 43 | 103 | 9 | 29 | 42 |
| | $\gamma\delta$+ | 13.2 | 18.6 | 0 | 4.7 | 6.1 |
| Morphology (µm, means) | VH | 343 | 395 | 330 | 308 | 386 |
| | CrD | 245 | 243 | 268 | 288 | 166 |
| | VH:CrD | 1.4 | 1.6 | 1.2 | 1.1 | 2.3 |

IELs: Intraepithelial lymphocytes; VH: Villous height; CrD: Crypt depth.

The patient had ingested normal amounts of gluten and did not have detectable serum TG2 or endomysial antibodies (EMAs). No IgA deposits targeting extracellular TG2 were found in the duodenal samples. Her human leukocyte antigen (HLA) type was DQ2.

Stool HP antigen test was negative at 30 days after HP eradication. She continued her normal gluten-containing diet and no signs of CD have appeared upon two years on follow-up. Control endoscopy was not performed.

### Case No. 2

A 55-years-old male, non-smoker was remitted for anorexia and reflux symptoms. The patient's medical history was positive for autoimmune thyroiditis, diet treated mild hypercholesterolemia and chronic use of tricyclic antidepressants. There were no pathological findings on physical examination. Routine blood work-up and thyroid function reported normal values, with the exception of positive anti-HP IgG antibodies. On endoscopy, gastroesophageal reflux disease with grade A esophagitis (*Los Angeles Classification*) was reported, along with a mild hiatal hernia, gastritis and mild edema and hyperemia of the duodenal mucosa. Several biopsies were taken from the gastric and duodenal mucosa. HP was found on Giemsa-stained gastric biopsy specimens and consequently triple therapy with Esomeprazole, Amoxicillin and Clarithromycin was initiated.

The duodenal mucosal biopsy histological examinations did not show any marked inflammation but surprisingly reported a crypt hyperplastic lesion mimicking CD (Table 1).

In fact, the mean VH:CrD ratio in the distal duodenum was 1.1, measured from well-oriented villus–crypt units (Figure 1) work-up to exclude other diseases which might cause non-celiac villous atrophy was also negative. His HLA was negative for both DQ2 and DQ8 alleles.

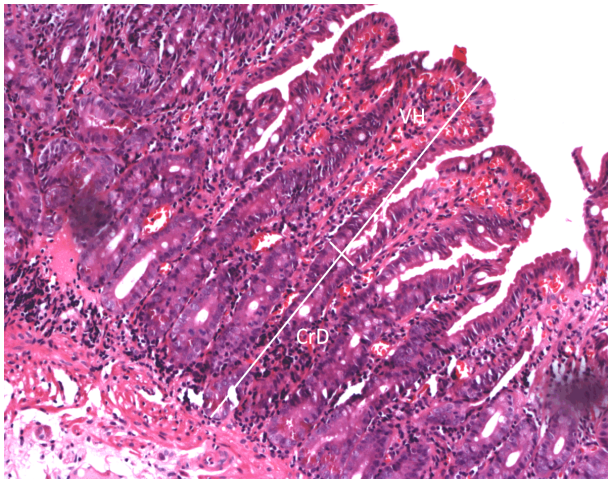


**Figure 1 –** ***Hematoxylin–Eosin-stained distal duodenal mucosa from a H. pylori-infected patient (Case No. 2). One villus–crypt unit is depicted in this biopsy cutting (white bar) showing crypt hyperplastic enteropathy (villus height 308 µm, crypt depth 280 µm, ratio 1.1). CrD: Crypt depth.***

The patient was on normal gluten-containing diet and all CD-specific serum autoantibodies were absent and the duodenal biopsies did not show any IgA targeting extracellular TG2.

The patient was symptom-free after successful eradication of the infection (negative HP stool antigen) at 30-day follow-up. Clinically, upon two-year follow-up when still eating normal amounts of gluten, he had not developed

any symptoms or signs suggesting CD. We performed a control endoscopy at the two-year follow-up visit and he was found to be HP negative (biopsy and stool test) and his distal duodenal biopsy specimen showed clear morphological healing, VH:CrD ratio being 2.3. TG2 autoantibodies and EMAs were still negative and no TG2 targeted IgA deposits in the duodenum were found.

## Discussion

Duodenal intraepithelial lymphocytosis with normal villous architecture is commonly seen in HP infection and the inflammation resolves after eradication therapy [12, 13]. However, it has not been shown that HP infection could induce distal duodenal architectural changes. This was recently hypothesized in a pediatric patient series where a crypt hyperplastic enteropathy mimicking CD was found in the anatomical bulb in non-celiac disease controls [9]. In the present report, we show for the first time HP infection to induce structural alterations also in the distal duodenum, *i.e.*, a crypt hyperplastic enteropathy similar to that seen in untreated CD, with resulting low VH:CrD ratios. Quantitative evaluation of the duodenal mucosal morphology requires oriented cutting of the biopsy specimens where crypts are cut longitudinally and not in cross-sections [10].

Our Case No. 1 had a marked duodenitis, villi present but crypts were hyperplastic corresponding with a Marsh IIIa CD lesion. Because the patient had autoimmune thyroid disease and her HLA type was typical for CD, she was suspected to have a gluten-induced enteropathy. However, we do not think the patient had an autoantibody-negative CD. In such patients, it has been shown that TG2-targeted autoantibodies were deposited in the distal duodenum even when absent in serum [14]. In fact, our case was negative for the IgA deposits. After two years follow-up, she had not developed any symptoms or signs of CD. We think her mucosal injury was caused by HP, similarly to that seen in other infectious disease [15]. Also, duodenitis in otherwise morphologically-normal mucosa is a relative common finding [2]. This so-called Marsh I lesion is common in peptic injury, small-intestinal bacterial overgrowth, NSAID-induced lesion, *Giardia lamblia* infections, inflammatory bowel disease, eosinophilic gastroenteritis, autoimmune enteropathy, common variable immunodeficiency and also in HP infections [13, 16]. Furthermore, a special attention should be also focused on patients with chronic kidney disease, known that this condition could be associated with CD including early development of small intestinal mucosal lesions [17–20]. In fact, it was shown in a prospective study that a Marsh I lesion in the duodenum shows a sensitivity and specificity for forthcoming CD in less than 60% [21]. On the other hand, it must be remembered that an increased IEL density is an early histological change in developing CD [21]. Although there have been some studies looking at the difference in the distribution pattern of IELs in CD (even distribution, villous-tip predominant) *versus* HP infection (patchy distribution, villous-base predominant), these differences lack sufficient specificity for CD [8, 22, 23].

Case No. 2, having a Marsh IIIb lesion but morphometrically even more damaged small-intestinal mucosa, could have been considered as seronegative CD, if not being genotyped. He was negative for both DQ2 and DQ8. But also in this case the duodenal mucosal IgA deposits were negative speaking against a gluten-induced CD [8, 11, 14, 21]. However, the strongest argument against CD is that the duodenal mucosa healed after HP eradication and continuation of a normal gluten-containing food consumption for two years.

Similar morphological changes in the small-intestinal duodenal mucosa to those seen in CD can be caused by a vast spectrum of conditions, for which we carefully checked our patients: stool was negative for parasites, NSAID use was denied, serum protein electrophoresis was normal, eosinophilic infiltrates were absent on multiple biopsy samples, enterocyte autoantibodies were absent, and previous ileocolonoscopy excluded inflammatory bowel disease (IBD).

Our two cases highlight a rather frequent scenario in clinical practice, that of seronegative celiac type-enteropathy, which requires extensive work-up in search for a cause (Table 2).

**Table 2 –** ***Differential diagnosis of seronegative villous atrophy [5–7]***

| |
|---|
| ▪ Seronegative CD; |
| ▪ Giardiasis; |
| ▪ Autoimmune enteropathy; |
| ▪ Small intestine bacterial overgrowth; |
| ▪ Common variable immune deficiency; |
| ▪ Eosinophilic gastroenteritis; |
| ▪ Drug-induced enteropathy (Olmesartan); |
| ▪ Intestinal lymphoma; |
| ▪ Crohn's disease; |
| ▪ Tropical sprue; |
| ▪ Collagenous sprue; |
| ▪ HIV-associated enteropathy; |
| ▪ Whipple disease. |

CD: Celiac disease; HIV: Human immunodeficiency virus.

If work-up is negative for a specific etiology, seronegative CD should be kept in mind. To exclude a case of seronegative CD, evaluation of duodenal biopsy samples for TG2-IgA subepithelial deposits is a powerful tool. Also, in the situation of equivocal small-bowel histological findings (Marsh I or II) in seronegative patients, *American College of Gastroenterology* (ACG) *Guideline* recommends HLA-DQ2/DQ8 genotyping testing [24] to rule out CD. Our patients were both genotyped and checked for TG2-IgA deposits, which allowed us to exclude CD as a cause for the crypt hyperplastic enteropathy seen in the duodenal biopsy samples.

The distal duodenal mucosal changes seen in our patients (crypt hyperplastic enteropathy with increased IELs) mimic those seen in CD, but they actually seem to be responses to an HP infection. We infer the CD pathology not to be operative in these two cases.

## Conclusions

Besides the duodenitis, HP can also induce architectural changes in the small-intestinal distal duodenal mucosa, leading to a celiac-type crypt hyperplastic enteropathy. Being a great mimicker of CD both clinically and at the duodenal biopsy level, HP infection should be considered as a differential diagnosis in front of a patient showing at least milder forms of celiac-type histopathology. The reported findings indicate that larger prospective studies on HP-infected patients are needed in order to confirm

our findings and establish a firm association with distal duodenal architectural changes.

**Conflict of interests**

None of the authors has any conflict of interests to declare.

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**Ethics**

The study was approved by the Ethics Committees of the Pirkanmaa Hospital Region, Finland, "Carol Davila" University of Medicine and Pharmacy, Bucharest, and "Alessandrescu–Rusescu" National Institute for Mother and Child Health, Bucharest, Romania. Patients gave written informed consent.

## References

[1] Chey WD, Wong BC; Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol, 2007, 102(8):1808–1825.

[2] Hammer ST, Greenson JK. The clinical significance of duodenal lymphocytosis with normal villus architecture. Arch Pathol Lab Med, 2013, 137(9):1216–1219.

[3] Memeo L, Jhang J, Hibshoosh H, Green PH, Rotterdam H, Bhagat G. Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in *H. pylori* gastritis. Mod Pathol, 2005, 18(8):1134–1144.

[4] Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, Laurila K, Huhtala H, Paasikivi K, Mäki M, Kaukinen K. Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. Gastroenterology, 2009, 136(3):816–823.

[5] DeGaetani M, Tennyson CA, Lebwohl B, Lewis SK, Abu Daya H, Arguelles-Grande C, Bhagat G, Green PH. Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. Am J Gastroenterol, 2013, 108(5):647–653.

[6] Volta U, Caio G, Boschetti E, Giancola F, Rhoden KJ, Ruggeri E, Paterini P, De Giorgio R. Seronegative celiac disease: shedding light on an obscure clinical entity. Dig Liver Dis, 2016, 48(9):1018–1022.

[7] Aziz I, Peerally MF, Barnes JH, Kandasamy V, Whiteley JC, Partridge D, Vergani P, Cross SS, Green PH, Sanders DS. The clinical and phenotypical assessment of seronegative villous atrophy; a prospective UK centre experience evaluating 200 adult cases over a 15-year period (2000–2015). Gut, 2016, Sep 7, pii: gutjnl-2016-312271.

[8] Hasan M, Sircus W, Ferguson A. Duodenal mucosal architecture in non-specific and ulcer-associated duodenitis. Gut, 1981, 22(8):637–641.

[9] Taavela J, Popp A, Korponay-Szabo IR, Ene A, Vornanen M, Saavalainen P, Lähdeaho ML, Ruuska T, Laurila K, Parvan A, Anca I, Kurppa K, Mäki M. A prospective study on the usefulness of duodenal bulb biopsies in celiac disease diagnosis in children: urging caution. Am J Gastroenterol, 2016, 111(1):124–133.

[10] Taavela J, Koskinen O, Huhtala H, Lähdeaho ML, Popp A, Laurila K, Collin P, Kaukinen K, Kurppa K, Mäki M. Validation of morhometric analyses of small-intestinal biopsy readouts in celiac disease. PLoS One, 2013, 8(10):e76163.

[11] Korponay-Szabó IR, Halttunen T, Szalai Z, Laurila K, Király R, Kovács JB, Fésüs L, Mäki M. *In vivo* targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut, 2004, 53(5):641–648.

[12] Nahon S, Patey-Mariaud De Serre N, Lejeune O, Huchet FX, Lahmek P, Lesgourgues B, Traissac L, Bodiguel V, Adotti F, Tuszynski T, Delas N. Duodenal intraepithelial lymphocytosis during *Helicobacter pylori* infection is reduced by antibiotic treatment. Histopathology, 2006, 48(4):417–423.

[13] Chang F, Mahadeva U, Deere H. Pathological and clinical significance of increased intraepithelial lymphocytes (IELs) in small bowel mucosa. APMIS, 2005, 113(6):385–399.

[14] Salmi TT, Collin P, Korponay-Szabó IR, Laurila K, Partanen J, Huhtala H, Király R, Lorand L, Reunala T, Mäki M, Kaukinen K. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut, 2006, 55(12):1746–1753.

[15] Lindfors K, Mäki M, Kaukinen K. Transglutaminase 2-targeted autoantibodies in celiac disease: pathogenetic players in addition to diagnostic tools? Autoimmun Rev, 2010, 9(11): 744–749.

[16] Binder HJ, Jain D. Does increased duodenal intraepithelial lymphocytosis always equate with Marsh 1 lesion? J Clin Gastroenterol, 2016, 50(8):613–614.

[17] Welander A, Prütz KG, Fored M, Ludvigsson JF. Increased risk of end-stage renal disease in individuals with coeliac disease. Gut, 2012, 61(1):64–68.

[18] Checheriţă IA, Turcu F, Dragomirescu RF, Ciocâlteu A. Chronic complications in hemodialysis: correlations with primary renal disease. Rom J Morphol Embryol, 2010, 51(1):21–26.

[19] Checheriţă IA, David C, Ciocâlteu A, Lascăr I, Budală L. Oral treatment of metabolic acidosis in hemodialyzed patients and the implications on the hemodynamic status. Rom J Morphol Embryol, 2013, 54(3):539–543.

[20] Jinga M, Checheriţă IA, Becheanu G, Jinga V, Peride I, Niculae A. A rare case of watermelon stomach in woman with continuous ambulatory peritoneal dialysis and systemic lupus erythematosus. Rom J Morphol Embryol, 2013, 54(3 Suppl): 863–865.

[21] Salmi TT, Collin P, Järvinen O, Haimila K, Partanen J, Laurila K, Korponay-Szabo IR, Huhtala H, Reunala T, Mäki M, Kaukinen K. Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. Aliment Pharmacol Ther, 2006, 24(3):541–552.

[22] Mino M, Lauwers GY. Role of lymphocytic immunophenotyping in the diagnosis of gluten-sensitive enteropathy with preserved villous architecture. Am J Surg Pathol, 2003, 27(9): 1237–1242.

[23] Goldstein NS, Underhill J. Morphologic features suggestive of gluten sensitivity in architecturally normal duodenal biopsy specimens. Am J Clin Pathol, 2001, 116(1):63–71.

[24] Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA; American College of Gastroenterology. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol, 2013, 108(5):656–676; quiz 677.

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