Does *Helicobacter pylori* eradication therapy trigger or protect against Crohn's disease?

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Abstract

Helicobacter pylori (H. pylori) infection is involved in multiple gastrointestinal and extra-gastrointestinal disorders. This review focuses on possible link between H. pylori eradication and Crohn's disease (CD) which is a chronic inflammatory bowel disease (IBD). Fecal calprotectin and; to lesser extent; fecal lactoferrin are sensitive and specific markers for monitoring CD activity. Data about link between *H. pylori* eradication and CD are limited and inconclusive. The infection likely shifts equilibrium between T helper 1 (Th1) and Th2 immune responses to the Th2 pattern. In subjects genetically predisposed to CD (a Th1-related disease), H. pylori eradication increases Th1 proinflammatory cytokines causing development of CD. In contrast, clarithromycin and/or proton pump inhibitors that are used to eradicate H. pylori can suppress Th1 factors, and theoretically can protect against CD, but there are no data to support this supposition. This Th1/Th2 approach seems very simplistic. Another theory is that alterations in gut microbiota form "continuous antigenic stimulation" predisposing to IBD. H. pylori infection can inhibit such stimulation through activation of regulatory T cells, and thus eradication may predispose to CD. Probiotics weren't found useful in treatment of CD. The reported data about link between H. pylori eradication and CD are currently limited. Case reports, suggesting a positive association between both conditions, provide a very little evidence. On eradicating H. pylori in CD patients and/or patients with high risk for CD, patient counseling and follow-up in addition to measuring fecal calprotectin may help monitor CD activity. (Acta gastroenterol. belg., **2016**, 79, **349-354**).

Key words : eradication, Crohn's disease, Helicobacter pylori, therapy.

Introduction

Helicobacter pylori (H. pylori) infection is a widespread disease that is acquired in childhood and, if it is not eradicated, is carried through life. Although it may cause multiple gastrointestinal (GI) and extra-GI disorders, including cancer, it is mostly asymptomatic (1,2). It is diagnosed by positive IgG level in serum, H. pylori antigen in faeces, urea breath test (UBT), endoscopy, and biopsy. The IgG test has high sensitivity but low specificity; thus the UBT, a non-invasive test with high sensitivity and specificity, is considered the best diagnostic tool for H. pylori infection (3). H. pylori eradication regimens include the standard triple therapy regimen (a proton pump inhibitor (PPI), clarithromycin and amoxicillin; twice daily for ten days) (4) and the sequential therapy regimen (a PPI and amoxicillin; twice daily for five days and then a PPI, clarithromycin and tinidazole; twice daily for another five days). The salvage therapy for resistant infections is either bismuth-based quadruple therapy for 1-2 weeks or levofloxacin-based triple therapy for ten days (5).

Crohn's disease (CD) and ulcerative colitis (UC) are the two major types of the inflammatory bowel disease (IBD), a worldwide chronic disorder characterized by alternating exacerbations and remissions. Although both CD and UC have similar symptoms and predisposing factors, there are some variations between these diseases. CD occurs in the form of a patchy granulomatous lesion in any part of the GI tract, commonly the ileum and first part of the colon. It affects the entire bowel wall and is more difficult to treat than UC. Abdominal masses and perianal lesions are classic manifestations of CD. In contrast, UC is a superficial diffuse mucosal injury that affects only the colon, with the dominant sign being bloody diarrhea (6,7). IBD is thought to be caused by interactions of multiple environmental factors (such as infections, medicines, smoking, urban life, and diet) that provoke an autoimmune reaction in a genetically predisposed person (8-10). In addition, gut microbiota is assumed to be involved in pathogenesis of IBD through many mechanisms such as existence of opportunistic pathogens, dysbiosis, microbial dysfunction, and/or host genetic disorders. Whatever the theory is, the end result is that gut microbiota under these abnormal conditions form "continuous antigenic stimulation" that affects function of the different immune cells including T cells and finally leads to chronic intestinal inflammation (11). Loss of the inhibitory effect of regulatory T (Treg) cells, (a suppressive subtype of CD4+ T cells) on the immune response against gut bacteria, causes inappropriate immune reactions predisposing to IBD (12). IBD tends to run in families, with the highest risk occurring in first-degree relatives, and thus, "family members are at increased risk of both CD and UC irrespective of the type of IBD in the proband" (13).

In early stages IBD may be asymptomatic, however it may later manifest with persistent diarrhea, rectal bleeding, abdominal pain, constipation, fever, loss of appetite, weakness, perianal abscesses, and anal fissures (14). IBD diagnosis is established by laboratory tests, radio-

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logy, endoscopy and biopsy. An elevated serum level of C-reactive protein (CRP) indicates active disease and is useful in predicting relapse. Calprotectin is a protein that is primarily released by neutrophils and monocytes into body fluids and tissues in cases of infection and inflammation. Fecal calprotectin (FC) is a valuable biomarker for the diagnosis and monitoring of GI inflammation, but it does not help in determining the site or cause of GI inflammation. The FC level is increased in IBD, colorectal cancer, and bacterial infections (15,16). Moreover, the first-degree relatives of UC patients have been shown to have higher FC levels, indicating that they "could have a subclinical intestinal inflammation" (17). FC is more sensitive than CRP for detecting CD activity and predicting relapse (18). In apparently healthy children, FC levels show non-significant variations in relation to H. pylori colonization (19). The level of fecal lactoferrin (FL; another neutrophil-derived protein) also increases in IBD, some bowel infections, irritable bowel syndrome, and cancer colon (20). It has been shown that H. pylori infection was not associated with elevated levels of FL (21).

The fecal markers (FC and FL) reflect the activity of neutrophils, and they are considered non-invasive and relatively inexpensive tests for detecting IBD. Although estimation of serum CRP and erythrocyte sedimentation rate (ESR) could predict the IBD activity, these systemic inflammatory markers are non-specific for IBD and poorly associate with endoscopic activity. FC and, to a lesser degree, FL are more sensitive and specific markers for monitoring CD activity compared with the CD activity index or systemic markers of inflammation (22-25). FC and, with lesser sensitivity and specificity, FL are clinically relevant markers for predicting postoperative recurrence for CD (26). Both FC and FL are increased in children with IBD, and then estimation of both provides a better initial diagnosis than each test alone (27). However, FC is more extensively studied and more ordered than FL (28).

Multiple antiinflammatory and/or immunomodulatory drugs are used for the treatment of IBD, including 5-aminosalicylic acid compounds (e.g., sulphasalazine andmesalazine), corticosteroids, methotrexate, and anti-tumor necrosis factor-α (anti-TNF-α) agents (e.g., infliximab). Antimicrobials, particularly those with antiinflammatory and immunosuppressive properties, can help alleviate symptoms of IBD by controlling infections and promoting the healing of abscesses and fistulas. Generally, antimicrobials are more useful in CD than in UC. Clinical trials have failed to prove a valuable role of antimicrobials in treating severe UC, except in cases of toxic megacolon. In contrast, metronidazole and sulfasalazine are equally effective in active CD, and ciprofloxacin is an alternative drug with fewer adverse effects (29,30).

Taken together, this review focuses on the possible link between H. pylori eradication and CD, including underlying explanations of this link and its clinical implications.

H. pylori infection and Crohn's disease

Data about the possible link between H. pylori infection and CD are controversial. In a retrospective study, files of IBD patients who had 'bidirectional endoscopy' (colonoscopy and upper GI endoscopy in the same day) with biopsies were studied. H. pylori infection showed opposing associations with IBD compared with non-IBD controls (31). In a retrospective study, H. pylori infection (diagnosed by UBT and culturing of a biopsy sample) was significantly lower in CD patients (diagnosed by endoscopy with biopsy) than in the control group (32). In a meta-analysis of 23 studies, a protective role of H. pylori infection against CD was reported. However, due to differences in the designs of those studies (e.g., use of IgG for H. pylori diagnosis, lack of data on pre-exposure to eradication therapy, and lack of distinct criteria for IBD diagnosis) and possibility of publication bias, the results are considered to have limited reliability (33). One explanation for this negative association is "the hygiene hypothesis". It proposes that subjects in urban areas are less exposed to enteric pathogens (e.g., H. pylori) in childhood and, therefore, the development of regulatory T cells is not promoted in such subjects. Consequently, they become more susceptible to the development of an improper immune response on exposure to GI infections later in life. Although the potential role of "the hygiene hypothesis" in development of IBD is still not proven, there are factors that support it such as the use of antibiotics and family size. In contrast, less hygienic environments that permit exposure to enteric infections are protective against the development of IBD later in life (34-36).

From an immunological perspective, different mechanisms were suggested to mediate the possible negative link between H. pylori infection and IBD. It was reported that H. pylori DNA attenuated the dextran sulphate sodium-induced colitis through inhibiting the pro-inflammatory responses from human plasmacytoid dendritic cells (37). Moreover, H. pylori infection protected against S.typhimurium-induced inflammation through suppressing the inflammatory T helper 1/17 (Th1/17) response in the mouse's lower GIT and increasing the production of the anti-inflammatory interleukin-10 (IL-10) in the mesenteric lymph nodes, suggesting an additional extragastric mechanism (38,39).

The gut microbiota also plays a role in the possible negative association between H. pylori infection and IBD. The gene polymorphisms associated with IBD indicate that microbiota 'the other genome' is involved in pathogenesis of IBD possibly through modification of the immune response, mucosal permeability, or metabolic microbial products. Thus, use of treatments directed at the microbes, such as antibiotics and probiotics, is progressing. The H. pylori infection of the gastric mucosa affects the intestinal microflora and markedly decreases gut inflammation (40). Persistent H. pylori infection in gerbils was found to change the distribution

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and numbers of microbiota in stomach and duodenum (41), and in large intestine (42). A vital concern in this respect in the activation of Treg cells which inhibit the inflammatory and immune responses against gut bacteria possibly through secretion of the anti-inflammatory and immunosuppressive cytokines such as IL-10. This explains why the immune system is found tolerant to many gut bacteria, although theoretically they are 'non-self' because they exist on the outer mucosal surface. Loss of this mechanism leads to abnormal immune reactions predisposing to IBD (12). It was found that H. pylori infection increases the number of Treg cells in the gastric mucosa and peripheral blood and also affects their functions possibly through modification of expression of certain receptors. H. pylori increases the gastric mucosal expression of Foxp3 (a Treg marker), which shifts the host immune response far from the inflammatory Th1/17 pattern. Treg cells maintain a constant H. pylori colonization state through suppression of the protective immunity (43,44). Moreover, H. pylori may stimulate production of "antibacterial peptide" which destroys other bacteria involved in pathogenesis of IBD (45). In addition, "it was hypothesized that H. pylori may inhibit GI infections by other yet unknown bacteria that are directly linked to the development of IBD" (46). Further, the lower prevalence of H. pylori infection in IBD may be due to mucosal alterations that prevent gastric colonization by H. pylori and/or its spontaneous eradication in response to IBD treatment, particularly treatment with 5-aminosalicylic acid (47,48).

Use of probiotics to restore the normality of the gut microbiota and/or reset the altered immune mechanisms, aiming to relieve the bowel inflammation, may be valuable (49). Through their anti-inflammatory and immune-modulatory effects, probiotics inhibit production of the pro-inflammatory cytokines while induce production of the anti-inflammatory ones. Results of the clinical trials support use of probiotics in treatment of UC, but not in CD (50).

The results about use of probiotics in eradication therapy of H. pylori are conflicting. Certain studies reported that addition of adjuvant medications (such as lactoferrin and probiotics) to triple therapy of H. pylori, especially in case of eradication failure, minimized side effects and probably improved the eradication rate (51,52). On the other hand, other studies reported that addition of lactoferrin or probiotics to the triple therapy failed to improve rate of H. pylori eradication, but decreased epigastric pain, vomiting, and diarrhea (53,54). This controversy may be attributed to differences in treatment schedules and antibiotic resistance in different populations (55).

A positive link between Helicobacters and CD was suggested by some studies. The enterohepatic and gastric Helicobacters have been detected in stool of children having CD, suggesting a potential role in its development (56). In CD patients, H. pylori infection is most commonly caused by toxic strains (57). Other studies showed a higher prevalence of Helicobacteraceae in CD patients (58) and concluded that Helicobacteraceae may be involved in the pathogenesis of CD and that different strains of H. pylori may adapt to colonize extragastric areas. Further, the occurrence of H. pylori in the intestine was found to be associated with the UC-like phenotype of CD (59). It is likely that the H. pylori-induced stimulation of the immune system (which occurs more with the toxic stains) results in increased production of interleukin-12 (IL-12) and the Th1 immune response. Increased levels of Th1 cytokines, such as TNF-α and IL-2, participate in the development of CD, which is considered a Th1-related disease (60,61). Moreover, the inflamed gut epithelium due to H. pylori infection secretes IL-8, which recruits neutrophils involved in the pathogenesis of IBD (62). In addition, the marked increase in both IBD and H. pylori-related metabolic syndrome in developed countries over the past 50 years may suggest a role of this syndrome and insulin resistance in development of IBD (63). Consequently, the relation between H. pylori and IBD is still uncertain and needs further clarification (64).

H. pylori eradication and Crohn's disease

In a case report, a 28-year-old patient suffered from abdominal pain, fever, watery diarrhea, elevated CRP, and neutrophilia at six months following H. pylori eradication with omeprazole and amoxicillin. Additionally, CD in the stomach, jejunum, and upper part of the ileum was detected by endoscopy (65). Another two similar cases were reported in two successive years, a 34-year-old man and a 39-year-old woman, both of whom suffered from profuse diarrhea 3-4 months after H. pylori eradication by triple therapy (esomeprazole, amoxicillin, and clarithromycin). Laboratory investigations confirmed inflammation, stool culture excluded clostridium difficile infection, and colonoscopy indicated ileal and colonic CD in the male and female patient, respectively (66). A possible explanation for the development of CD after H. pylori eradication in these patients is that long-term H. pylori infection shifts the equilibrium between the Th1 and Th2 immune responses to the Th2 pattern and that in subjects who are genetically predisposed to CD (a typical Th1-related disease), H. pylori eradication therapy diminishes Th2 cytokines (especially IL-4, IL-5 and IL-6) and increases Th1 proinflammatory cytokines triggering the onset of CD (67) (Fig. 1: A and B). Moreover, the antimicrobials used to eradicate H. pylori may affect the gut microbiota in a manner that increases the risk for IBD (68). However, the immune interactions are much more complex and thus the Th1/Th2 pattern is considered very superficial explanation. Most studies that used helminthes or probiotics, to modify the immune response of CD patients, have shown unpromising results (69,70). It's clear that patients in the previously mentioned case reports might have developed CD apart from the H. pylori eradication. In addition, the data from the case reports are only of evidence level 5 and

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thus they just suggest a possible association between H. pylori eradication and CD (71). Unfortunately, solid large-scale epidemiological data are unavailable.

Clarithromycin and/or PPIs used to eradicate H. pylori may suppress Th1 factors and may thus theoretically protect against CD (Fig. 1: C). However there are no epidemiological data to support this supposition. It is well known that the antimicrobial clarithromycin has anti-inflammatory and immunomodulatory effects; it was shown to markedly inhibit the release and gene expression of Th1 factors and to lesser extent inhibit Th2 factors (72). In another study, clarithromycin (but not amoxicillin) reduced Th1, Th2, and Th17-associated immune factors. Interestingly, clarithromycin reduced Th2 factors in steroid-sensitive asthma and Th1/Th17 factors in "infection-induced", steroid-resistant asthma (73). In a placebo-controlled randomized clinical trial, treatment of patients with active CD with clarithromycin was effective at one month but not at three months, possibly due to bacterial resistance (74). In regards to PPIs, microbial growth in the upper GIT can significantly increase as a result of inhibition of gastric acid secretion (75). In contrast, PPIs have direct bacteriostatic and occasional bactericidal effects against certain gut microorganisms (e.g., H. pylori) by inhibiting their membrane H^+-ATPase (76), and they have antiinflammatory effects (77). Omeprazole was shown to induce the full remission of UC symptoms within five days (78). Lansoprazole was suggested to be beneficial for the treatment of IBD, including CD, through the inhibition of the production of relevant proinflammatory cytokines by macrophages (79). In vitro, lansoprazole reduced the production of inflammatory cytokines (TNF-α and IL-1β) from peripheral blood monocytes (80). Thus, based on the antiinflammatory and immunomodulatory effects of clarithromycin and PPIs, these drugs may theoretically protect against CD, but there are no epidemiological data to support this supposition.

Based on the limited data available and presence of multiple explanations, monitoring the exact link between H. pylori eradication and development of CD is difficult but important. H. pylori infection is becoming less prevalent, and then its impact on chronic inflammatory diseases including CD must be assessed (81). Certain precautions may be of value if taken prior to eradicating H. pylori in CD patients (21 in the original = 32). The present review suggests two precautions to be taken on eradicating H. pylori in CD patients and/or patients with a high risk for CD, e.g., those with a positive family history. These precautionary measures include:

1) Measuring the FC level: The FC level should be measured before starting H. pylori eradication, upon the development of any post-eradication GI complaints, and at 3-6 months after eradication. This time point was chosen because it was the time of CD development in the cases reported. Interestingly, the FC level didn't significantly increase in patients with chronic gastritis, with H. pylori infection, or with chronic use of PPIs (82). Thus, it is considered an ideal biomarker for early screening of CD in this situation.

2) Patient counseling and follow-up after H. pylori eradication: Patients should be given an information leaflet about CD symptoms and should be instructed to immediately revisit their doctors upon the development

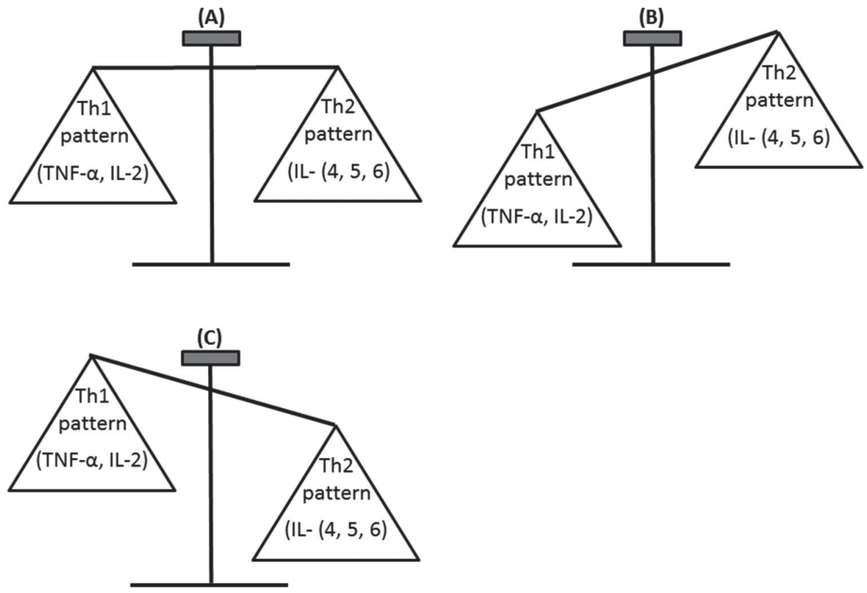

Fig. 1. — A postulated simplified immune link between *H. pylori* eradication and CD based on the Th1/Th2 immune pattern: (A) normal balance, (B) proposed effect of *H. pylori* eradication (triggering CD), and (C) effect of PPIs and/or clarithromycin (83).

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of any of them. Moreover, patients should be called regularly once/month for at least six months after H. pylori eradication.

Conclusion

The current data that suggest a positive association between H. pylori eradication and development of CD are limited and provide a very little evidence. Consequently, monitoring the exact link between the two conditions is difficult but important because H. pylori infection is becoming less prevalent, and then its impact on chronic inflammatory diseases including CD must be assessed. The present review suggests that measuring fecal calprotectin, and patient counseling and follow-up, on eradicating H. pylori in CD patients and/or patients with a high risk for CD, may help monitor CD. More clarifying studies, especially randomized controlled clinical trials, are needed.

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