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Helicobacter pylori and gastro-esophageal reflux disease (GERD)

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ABSTRACT

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Helicobacter pylori is the major cause of gastritis and duodenitis, and is responsible for the majority of gastric and duodenal ulcers. It is thought that *H.pylori* may have a benign influence in the prevention of reflux esophagitis and gastro-esophageal reflux disease (GERD). GERD is due to failure of the gastro-esophageal anti-reflux mechanism, thus allowing gastric acid to damage the esophageal mucosa, but the main abnormalities may also be found in the stomach and duodenum. The recently decreased prevalence of *H.pylori* has led to a lower incidence of duodenal ulcer and gastric carcinoma, but increasing acid secretion resulted in an increase in GERD. Eradication of *H.pylori* does not aggravate the symptoms of GERD and also does not induce the development of GERD in the normal population or in patients with duodenal ulcer. Pharmacological suppression of acid secretion is more effective in patients infected with *H.pylori* in comparison to non-infected individuals. The evidence on the efficacy of acid suppression therapy and its association with *H.pylori* infection is still contradictory. The sharply differing opinions regarding the role or association of *H.pylori* infection with GERD have not been reconciled and contradictory study results are still widespread.

Keywords: *Helicobacter pylori*, gastro-esophageal reflux, eradication

INTRODUCTION

The discovery of the bacterium *Helicobacter pylori* (*H.pylori*) in 1982 was the starting point of a revolution in the concept and management of gastroduodenal disorders. *H.pylori* was known to be asscociated with inflammation of the gastric mucosa, and it was agreed that the common gastric disease known as peptic ulcer was an infectious disease, and that the causal organism was *H.pylori*. The isolation of this bacterium opened a new era in gastric microbiology.[1,2] Ample evidence showed that once an infection of *H.pylori* has occurred, this bacterium persists in the patient's body for life and causes chronic active

gastritis.[2]

The National Institute of Health (NIH) Consensus Conference of 1994 concluded that *H.pylori* was the main cause of gastric ulcer and recommended a therapy of eradication of the causative organism.[3] Based on reports indicating an association between *H.pylori* infection and gastric carcinoma, the WHO also reviewed the existing data and stated that there was sufficient epidemiological and histological evidence demonstrating that *H.pylori* is carcinogenic for humans.[4] The most effective approach to reduce the pravalence of gatric carcinoma is to prevent childhood *H.pylori* infection.[5]

The principal abnormality in the esophagus, stomach and duodenum is associated with *H.pylori* infection or with Gastro-Esophageal Reflux Disease (GERD). *H.pylori* causes chronic infection and has a severe impact on the gastric mucosa, whereas GERD occurs as a result of abnormal exposure of the esophagus to gastric acid. In developed countries such as the United States and Europe, GERD is a controversial problem because it is associated with *H.pylori* infection.[6,7] GERD has since long considered to be independent of colonization or infection with *H.pylori*. GERD may occur with the same frequency and grade of severity both in *H.pylori* positive as well as in *H.pylori*-negative patients. However, this concept was revised in connection with the results of several studies indicating that *H.pylori* protects against the development of GERD.[6] Some additional aspects such as the reduction in the prevalence of *H.pylori* in GERD patients and the increase in GERD following measures for eradication of *H.pylori*, led to the widespread opinion that *H.pylori* has a protective effect for the esophagus: *H.pylori* can prevent the occurrence of GERD with its various complications. However, the existing data and their interpretation are still controversial such as to cause the relationship between GERD and *H.pylori* to be a problem without a definitive solution.

MICROBIOLOGICAL CHARACTERISTICS OF *H.PYLORI*

H.pylori is a helical gram-negative micro-aerophilic organism with rounded ends. The bacterium has 4-6 sheathed unipolar flagella with swollen ends. The flagella have a propulsive function, allowing the bacterium to move at great speed within viscous liquids such as the mucus on the surface of gastric epithelial cells.[1,2]

There is ample evidence linking *H.pylori* with chronic superficial gastritis. Eradication of *H.pylori* through antimicrobial therapy cures the patient of gastritis and the titer of specific antibody decreases after eradication; in contrast, nearly all patients infected with *H.pylori* show a persistent immunological response against this organism.[7]

H.pylori has a characteristic tissue specificity with an exclusive affinity to gastric mucosal cells but not to intestinal epithelial cells.[8,9] After ingestion by the host, the organism has to be able to protect itself against the bactericidal activity of the gastric luminal contents and subsequently enter the mucus layer (Figure 1). Within the gastric lumen, *H.pylori* is capable of spatial orientation in the mucus layer, adhere to the epitelial cell, colonize it and settle in the gastric lumen.[10] The pathogenic determinants of the microorganism may be divided into two main groups, i.e. (i) virulence factors that play a role in the pathogenic effect of the microorganism, and (ii) maintenance factors that allow the microorganism to colonize and settle in the body of the host.[9] The two important factors here are urease production and motility of the microorganism. Urease hydrolyzes urea into carbon dioxide and ammonia, thus enabling *H.pylori* to persist in an acid environment, whilst motility is essential for the microorganism in order to colonize the stomach. Urease is a strong stimulus for activation of mononuclear phagocytes and production of inflammatory cytokines. In vitro,

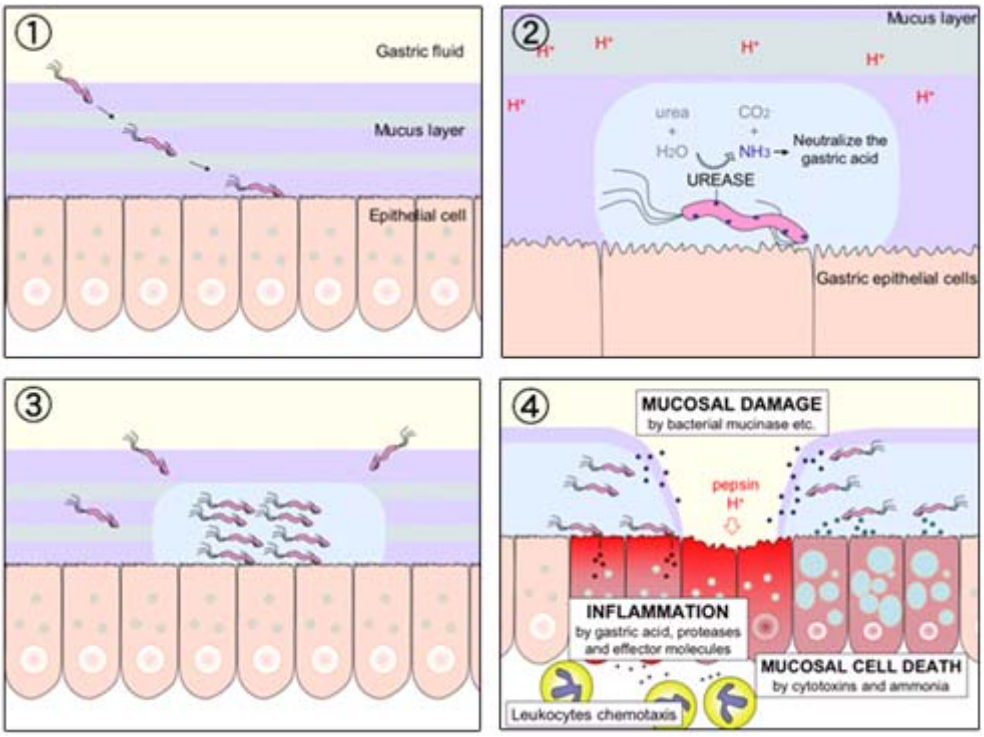

Figure 1. Mechanism of *H.pylori* colonization of the gastric mucosa[10]

urease activity is also toxic for human gastric cells. Therefore, urease probably functions simultaneously as virulence factor and maintenance factor.[1,9,10] Patients whose gastritis is situated predominantly in the antral region of the stomach (which is the most common form of gastritis) tend to suffer from duodenal ulcers, whereas patients whose gastritis is predominantly in the gastric corpus and is multifocal and atrophic, tend to have gastric ulcers, gastric atrophy, intestinal metaplasia, and ultimately gastric carcinoma.[4,7,11] Gastric or duodenal ulcers are called peptic ulcers, which are mucosal defects ≥0.5 cm in diameter, that penetrate the muscularis mucosae. Gastric ulcers commonly occur along the minor curvature at the transition between the mucosa of the body and the gastric antrum. Duodenal ulcers generally affect the duodenal bulb, which is the site most exposed to acid.

Some experts are of the opinion that certain strains of *H.pylori* are more virulent than others. Preliminary studies indicated that the pathogenicity was associated with the activity of a protein called cagA (cytotoxigenic -associated gene A).[1,2] CagA is an extremely immunogenic protein encoded by the cagA gene, which is possessed by around 50-70% of *H.pylori* strains, where cagA is a marker of bacterial virulence. Patients infected with cagA-positive *H.pylori* experience a more severe inflammatory response. Infection with these cagA-positive strains increases the risk for the occurrence of duodenal ulcer and adenocarcinoma of the distal part of the stomach.[11,12] In the United States and Europe around 60% of *H.pylori* strains reportedly possess the cagA gene.

Another virulence factor also considered to play a role in *H.pylori* infections is vacuolating cytotoxin (VacA), that is a strongly immunogenic protein capable of inducing vacuolization of epithelial cells. VacA accumulates in the mitochondria and causes tissue necrosis.[7]

EPIDEMIOLOGY AND TRANSMISSION OF THE BACTERIUM

H.pylori infection may be found worldwide, but its prevalence varies considerably by country and by population group in the same country. The overall prevalence is intimately associated with the socio-economic conditions of the involved region, as the infection predominantly affects the lower socio-economic groups.[7] In developing countries 70-90% of the population is *H.pylori*-positive, approximately 80% are elderly people and nearly all acquired the infection under the age of 10 years. In contrast, in industrial countries the prevalence of the infection is only around 25-50%.[6] To date there is no evidence for a zoonotic transmission, while a reservoir other than humans has not been found for *H.pylori*.[1,2] Therefore it is thought that there are 3 routes of transmission from one individual to another, namely (i) by means of endoscopic apparatus (an extremely rare event); (ii) fecal-oral transmission; (iii) oral-oral transmission, e.g. in Africa where mothers masticate food before giving them to their infants. In general, infection is acquired by oral ingestion of *H.pylori* organisms and is transmitted among family members. Transmission may be effected through saliva, utensils, fecal matter, and water.

Unlike other regions,Western countries have an approximately fourfold frequency of duodenal ulcer compared with gastric ulcer. Duodenal ulcer primarily affects the age range of 20-50 years, while gastric ulcer is more frequent in patients older than 40 years.[7] *H.pylori* infection in adults is usually chronic and does not heal without specific treatment. On the other hand, spontaneous elimination of the bacteria may occur in children, particularly if these children receive antibiotic treatment for other infections. In developed countries the prevalence of *H.pylori* has substantially decreased in the last decades in connection with improved nutrition, living standards, and sanitation in these countries, but it is to be feared that without intervention *H.pylori* will still be endemic.[12]

DETECTION OF *H. PYLORI* INFECTION

Currently there are several tests available for the diagnosis of *H.pylori* infection, comprising noninvasive and invasive methods. The latter require endoscopic biopsy of the gastric mucosa. The endoscopic specimen may be subjected to histological examination, bacterial culture, rapid urease test, DNA probing and PCR analysis. Noninvasive methods are the urea breath test, serological tests, and fecal antigen assays. The choice of test depends mainly on the required clinical information, test availability and cost.

Testing endoscopic biopsy specimens

The best specimens for detection of *H.pylori* organisms are endoscopic biopsy specimens. The specimens are collected before adminstration to the patients of antibiotics or antisecretoric agents, particularly proton pump inhibitors (PPI), resulting in abnormal distribution of the organisms. Two weeks before endoscopic specimen collection the patients should not be given the abovementioned drugs.

The distribution of *H.pylori* and associated inflammation is patchy, such that it frequently leads to endoscopic sampling errors and thus yielding negative results. Therefore at least two biopsy specimens should be taken from the antrum at a distance of around 2-5 cm from the pylorus.

The biopsy specimens may be subjected to (i) culture and (ii) rapid urease test. It is recommended that the collected biopsy specimens first be used for culture, before being used for histopathological examination.

Rapid urease test

The CLOtest (Delta West Ltd, Bentley, Australia) was the first commercial biopsy urease test developed by Marshal.[1] The test

consists of gall agar containing urea and phenol red. The biopsy specimen is placed on the agar and in the presence of urease (produced by *H.pylori*), the urea undergoes hydrolysis, which effects a change in the pH of the agar (the latter becoming alkaline) such that there is a color change of the phenol red. This test can be read up to 24 hours after placing the biopsy tissue on the agar gel.

In addition to the CLOtest, other commercially available biopsy urease tests are "Hpfast" and "PyloriTek" (as a strip test). All three tests have almost identical sensitivities and specificities (sensitivity 88-93%; specificity 99-100%).[7,8] The results of the "PyloriTek" may be read in one hour, but the test has a lower sensitivity, namely 66-71%.

Culture

H.pylori is a slow-growing organism that requires complex growth factors. Culture of *H.pylori* has two principal advantages (i) antibiotic sensitivity testing can be performed; (ii) the culture isolate can be subjected to further detailed characterization. Culture may be performed on biopsy specimens, gastric juice and feces, but the most common specimens yielding high sensitivity are gastric biopsies.

Transport of biopsy specimens is an important factor in the successful culture of *H.pylori*. The problem in this case is that gastroenterologists have a negative view of bacteriological culture systems, as errors in specimen transport lead to negative results. It should be noted that *H.pylori* is an extremely fragile organism that has to be protected from drying and from contact with oxygen and room temperature. After the biopsy specimen has been collected, it is placed in normal saline (0.1 ml NaCl-0.85%) and stored at a low temperature (in a coolbox, 4^0C) prior to and during transport to the laboratory. Normal saline is a simple transport medium used for transport times of <6 hours. For longer tranport times a more complex medium is utilized (transport medium), usualy in the form of semisolid agar. A commercially available transport medium for this purpose is Portagerm *pylori* (bioMerieux, France).[13] In the transport medium the culture specimen can survive for 24 hours at the temperature of 4^0C used for its transport. If this condition cannot be met or if long-term storage is required, the biopsy specimen can be frozen and stored in sterile conditions at a temperature of -70^0C.[14] Culture is performed on a nonselective medium (blood agar 5-10%), such as Columbia agar or Brucella agar with the addition of 5-10% blood, and also on a selective medium for increasing the sensitivity of the culture. Useful selective media are (i) blood agar + vancomycin, trimethoprim, amphotericin B and polymyxin B (Skirrow); or (ii) blood agar + vancomycin, trimethoprim, amphotericin B and cefsulodin. *H.pylori* requires microaerophilic culture conditions (N_2-80%; CO_2-5-10%) (Campypak microaerophilic system, Columbia Diagnostics), a temperature of $35\text{-}37^0$C, high humidity and an incubation time of 7-10 days. In positive cultures growth occurs within 3-5 days as translucent and clear colonies on the agar plates, with the microscopic picture of gram-negative curved rods that are positive for urease, catalase, and oxidase.

Culture of biopsies is the gold standard for detecting *H.pylori* infection. In the case of a negative culture, a combination of the rapid urease test and histological results may be used. Culture has a sensitivity of 95%.[1,2,15]

Urea breath test (UBT)

This test is based on the substantial urease activity of *H.pylori* in the stomach and its sensitivity depends on the bacterial load. The urea breath test qualitatively detects active infection with a sensitivity and specificity of over 90%. The principle of UBT is identical to that of other urease tests. Urea as the substrate in UBT is administered as ingested [^{13}C]urea. or [^{14}C]urea. In the presence of *H.pylori,* urea is hydrolyzed by the bacterial urease, leading to absorption of labeled CO_2 by the blood and elimination of the CO_2 from

the respiratory tract by exhalation. The test accuracy depends on several factors, i.e. collection time of the breath, gastric emptying time, and distribution pattern of urea (tablets vs liquids). A commercially available test is the UBT Kit (Meretek Diagnostics). UBT is indicated for initial diagnosis of *H.pylori* infection and for follow-up of eradication therapy. In the latter case, the urea breath test should not be performed until after a time interval of 4 weeks has elapsed, to prevent false negative test results. UBT is valid for children over 6 years old, while for younger children the test has not been definitively validated.

Serum antibody test

H.pylori infections of the gastric mucosa result in local and systemic immune responses, including raised specific serum IgG and IgA and gastric IgA and IgM levels. This allows the detection of bacteria by serological tests. In general, serologic testing is performed by means of Enzyme-linked immunosorbent assay (ELISA) which has a reported sensitivity and specificity of 85% and 79%, respectively. Without therapeutic intervention the antibody levels are persistently high, perhaps for the lifetime of the patient, and are used as a measure of the duration of infection. Following eradication of *H.pylori,* the specific IgG and IgA levels tend to decrease within approximately 6 months.

These serologic tests are of considerable benefit in the screening of large numbers of individuals such as in epidemiological studies. A commercially available test kit for assessing serum anti-*H.pylori* antibodies is the HM-CAP ELISA (EZ-EM Inc, New York).

Stool antigen test

Tests for fecal *H.pylori* antigens have the advantages of being non-invasive, of using easily obtainable test materials and of being capable of detection of bacteria or bacterial components (DNA, antigen).

Fecal *H.pylori* antigen tests in general use are ELISA with polyclonal antibody reagents such as the HpSA test (Meridian Diagnostics, Cincinnati, OH) or the Amplified-IDEA HpStAR (Dako, Glostrup, Denmark) with monoclonal antibodies. Amplified-IDEA HpStAR reportedly has a higher accuracy[2] and sensitivity than HpSA.

Stool antigen tests have several limitations; e.g. rapid transit time maintains the integrity of the antigens, whereas constipation may increase their degradation.[16]

PCR

This technique has great potential for detection of *H.pylori* as it has high sensitivity and specificity. It can be applied to biopsy specimens as well as specimens obtained by non-invasive means, such as gastric juice, blood, saliva, urine and fecal specimens. However, several factors may affect its accuracy, including selection of DNA primer and target, specimen preparation, and technical procedures.

GERD AND *H.PYLORI* INFECTION: IS THERE ANY ASSOCIATION?

The main lesions in the esophagus, stomach and duodenum are associated with *H. pylori* infection or Gastro-Esophageal Reflux Disease (GERD). When the numbers of *H.pylori* decrease and the organisms disappear from the human stomach, the incidence of gastro-esophageal reflux disease or GERD increases dramatically, particularly in developed countries such as the United States and Europe. GERD has become a problem associated with infection by *H.pylori*.[6,17] *H. pylori* causes a chronic infection and has a profound impact on the gastric mucosa, while GERD occurs as a result of abnormal exposure of the esophagus to gastric acid.

It was hypothesized that *H.pylori* has a beneficial effect by regulating the acidity of

gastric contents and reducing the impact of regurgitation of gastric acid into the esophagus. This hypothesis has not been universally accepted because a number of studies have been unable to demonstrate aggravation of the reflux disease following eradication of *H.pylori*. In patients with chronic atrophic gastritis as a result of *H.pylori* infection, gastric acid is suppressed, and thus is not produced in the critical quantities necessary for the induction of GERD.[6] Is there an actual increase in prevalence of GERD in connection with the decreased prevalence of *H.pylori* infection, or is it the result of more accurate observations of endoscopists in detecting erosive esophagitis?

GERD was long considered to be independent of *H.pylori* colonization or infection. Both in *H.pylori* positive and negative patients, GERD may occur at similar frequencies and severity.

However, this concept was subsequently modified by a group of investigators in connection with numerous study results indicating that *H.pylori* may be protective against development of GERD such that it confers benefits on the subject in question. The hypothesis put forward by this group is that inflammation of the gastric corpus caused by *H.pylori* has a suppressive effect on acid production such that the individual is prevented from developing GERD. Reports from Japan mention that patients with atrophic gastritis have an increased production of gastric acid following eradication of *H.pylori*, thus inducing the development of GERD in a subset of the patients. *H.pylori* infection presumably has a protective effect against GERD.[6,17] Conversely it has been suggested that long-term suppression of gastric acid may aggravate gastritis of the gastric corpus due to *H.pylori* and increase the risk of gastric carcinoma.[6,17] However, European studies have reported that eradication of *H.pylori* did not modify duodenogastro-esophageal reflux and *H.pylori* status in patients with GERD did not affect the degree of exposure of the esophagus to acid. From these data it was concluded that the risk of GERD after *H.pylori* eradication was not high. Currently this conclusion is still the subject of controversy. Many populations of patients have *H.pylori* infection concomitantly with GERD and there is no conclusive evidence that severe GERD is associated with low or decreased prevalence of *H.pylori* infection, or that low infection rates are associated with virulent strains of *H.pylori*.

The sharply differing opinions regarding the role or association of *H.pylori* infection with GERD have not been reconciled and contradictory study results are still widespread. The opinion that the association between GERD and *H.pylori* is a negative one is suggested by studies on endoscopically proven reflux esophagitis rather than symptomatic GERD.[18,19] The majority of the study results from East Asian countries revealed a considerably greater negative association compared with that from West European studies.[18] This negative association is greater in patients infected with cagA-positive strains of *H.pylori*.[20] These data support the opinion that patients with GERD have a low probability of experiencing infection with *H.pylori*, because *H.pylori* infection protects the esophagus against reflux of gastric acid. However, there are still other possibilities to account for the fact that patients with GERD are protected against *H.pylori*, such as the influence of social status in the community.

One of the opininons advanced in the initial debates about the role of *H.pylori* in connection with GERD is the problem of treatment for eradication of *H.pylori*. Cases of duodenal ulcer reflux esophagitis occur more frequently in patients receiving eradication therapy for *H.pylori* compared with those receiving placebo.[18-20] Nakajima and Hattori[21] recently reviewed the data on development of GERD following eradication of *H.pylori* in patients with peptic ulcer and concluded that there were no results supporting the opinion

that eradication of *H.pylori* results in the development of GERD or symptoms of GERD. Apparently the opinions advanced in various study results are paradoxical in nature. The present situation is therefore such that on one hand there is a negative association between prevalence of *H.pylori* infection and GERD, but on the other hand eradication of *H.pylori* does not induce or aggravate GERD. What lesson is to be learned from these contradictory opinions?

In reality, the incidence of GERD has increased drastically since 1995, whereas in the same time period duodenal ulcers have sharply decreased. Statistical data from developed countries on gastric carcinoma and duodenal ulcer reveal that the decreased prevalence of *H.pylori* is the cause of abovementioned changes. Is it possible that the decrease in *H.pylori* also causes an increase in GERD? GERD is an abnormality due to failure of the gastro-esophageal anti-reflux mechanism, thus allowing gastric acid to damage the esophageal mucosa. Koike et al.[22] have confirmed that there is a high level of gastric acid secretion in patients with esophagitis. This is not surprising since it is the acidity of the refluxate that determines the extent of damage to the esophageal mucosa. According to Axon[18] gastric acid secretion in the Japanese community, both in the elderly and the young, increased substantially in the last decades, indicating that there had been a gradual rise in gastric acid secretion over a number of years. However, it is of interest that the increase in gastric acid secretion did not occur only in *H.pylori*-negative individuals, but also in those with *H.pylori*-positive status. Thus the overall increased gastric acid secretion is not a direct result of decreased incidence of *H.pylori*. It is possible that this increased gastric acid secretion is the cause of the increase in GERD in patients with an abnormal anti-reflux mechanism.

To date there has not been any direct evidence supporting the hypothesis that a high level of gastric acid secretion protects against *H.pylori* infection. The mode of transmission of *H.pylori* is still unclear. However, this organism apparently prefers a hypochloric environment to an achlorhydric one or one with high acid secretion. High acid secretion apparently provides protection against colonization by *H.pylori*, possibly by inhibiting or reducing the colonizing capacity of the microorganism.

The effect of *H.pylori* on acid secretion is complex. In individuals with an high natural acid secretion, *H.pylori* gastritis is limited to the antrum, similar to the pattern of gastritis encountered in patients with duodenal ulcer.[18] In these patients the post-prandial secretion of gastric acid is raised and this abnormality may be partially corrected through treatment of the infection. In contrast, individuals with an inherently low acid secretion tend to suffer from pangastritis, which subsequently results in impairment of the parietal cells in the gastric corpus and furthermore causes further reduction in acid secretion. Successful treatment of the infection in these patients may increase their gastric acid secretion.[18]

The epidemiology of the diseases associated with *H.pylori* has changed dramatically over the last two centuries. Gastric ulcer was prevalent in Europe in the 19th century, being replaced by duodenal ulcer in the 20th century. This condition was followed by a decreased prevalence of *H.pylori*, leading to a lower incidence of duodenal ulcer and gastric carcinoma, but the still increasing acid secretion resulting in an increase in GERD.

Several reports have drawn attention to the presence of a strong negative association between gastritis of the gastric corpus and GERD.[23,24] This observation led to the hypothesis that patients with pangastritis have damaged parietal cells, which secrete lesser amounts of acid, thus there is a low probability of the patients suffering from reflux and therefore they are protected from developing GERD. Actually this is a circular argument as

individuals who develop gastritis of the gastric corpus have at the moment of infection a naturally lower acid secretion anyway and thus a lower probability of developing GERD, whether they are infected or not.

From the reported data it is apparent that eradication of *H.pylori* does not aggravate the symptoms of GERD and also does not induce the development of GERD in the normal population or in patients with duodenal ulcer.[18] Thus arises the question why patients with GERD are not given treatment in the form of *H.pylori* eradication? Pharmacological suppression of acid secretion is reportedly more effective in patients infected with *H.pylori* in comparison to non-infected individuals. Therefore eradication of *H.pylori* theoretically makes pharmacologic control of the symptoms more difficult.[18,24] However, as in other aspects of the connection between GERD and *H.pylori*, the evidence on the efficacy of acid suppression therapy and its association with *H.pylori* infection is still contradictory.

CONCLUSIONS

Apart from the diversity of studies on the relationship between GERD and *H.pylori*, the prevalence of *H.pylori* is significantly lower in patients with GERD compared with those without. However, there a still a great number of mutually contradictory study results on *H.pylori* infection in connection with GERD.

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