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RAPID COMMUNICATION

H pylori are associated with chronic cholecystitis

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Abstract

AIM: To study whether *H pylori* are associated with chronic cholecystitis.

METHODS: The subjects were divided into three groups: *H pylori*-infected cholecystitis group, *H pylori*-negative cholecystitis group and control group. Pathologic changes of the gallbladder were observed by optic and electronic microscopes and the levels of interleukin-1, 6 and 8 (IL-1, 6 and 8) were detected by radioimmunoassay.

RESULTS: Histological evidence of chronic cholecystitis including degeneration, necrosis, inflammatory cell infiltration, were found in the region where *H pylori* colonized. Levels of IL-1, 6 and 8 in gallbladder mucosa homogenates were significantly higher in *H pylori*-infected cholecystitis group than those in *H pylori*-negative cholecystitis group and control group.

CONCLUSION: *H pylori* infection may be related to cholecystitis.

Key words: *H pylori*; Chronic cholecystitis; Interleukin; Colonization; Gallbladder mucosa

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INTRODUCTION

H pylori have definite pathogenic action and related to gastritis, peptic ulcer and gastric carcinoma[1-3]. Previous studies have demonstrated that *H pylori* correlate with diseases of the extra-gastrointestine, the liver and the cholecyst[4-8]. We have isolated *H pylori* from the gallbladder and cultured *H pylori*, and preliminarily proved that there exist live *H pylori* in the gallbladder. In the present study, we carried out electron microscopic observation and immunohistochemistry to the relationship between *H pylori* and chronic cholecystitis.

MATERIALS AND METHODS

Subjects

A total of 81 cases with chronic cholecystitis were divided into *H pylori*-negative group (n = 59) and *H pylori*-infected group (n = 22), based on previous studies, PCR amplification and culture results for *H pylori*. Besides, a control group was used including another 20 cases who were proved to have no obvious inflammation in the gallbladder mucosa except polyps or *H pylori* infection after a cholecystectomy due to a gallbladder polyp.

Investigation of gastric mucosa metaplasia of epithelial cells of gallbladder mucosa

By histochemical staining of AB/PAS mucus, and based on histology of mucosal epithelial cells and characteristics of cells secreting mucus, an observation was made on gastric mucosa metaplasia of epithelial cells of gallbladder mucosa and colonization of *H pylori* in the epithelial cells of gallbladder mucosa. The relationship between *H pylori* and the epithelial cells of the gallbladder was observed by optic microscopy, W-S silver stain and immunohistochemical stain using anti-*H pylori* antibodies. The resected gallbladder specimens from cases with chronic cholecystitis were immobilized with 3% glutaral, embedded and sliced for transmission electron microscopic investigation.

Relationship between H pylori and cholecystitis

Inflammatory changes of epithelial cells of the gallbladder mucosa in regions where *H pylori* colonized were observed by optic microscopy and ultrastructural changes of epithelial cells of the gallbladder were observed by electron microscopy.

Assay of interleukins in homogenates of gallbladder mucosa

The radio-immune analytical reagent kits including IL-1, IL-6 and IL-8 were purchased from Dongya Research Institute of Biotechnology, Beijing. About 1 g of resected

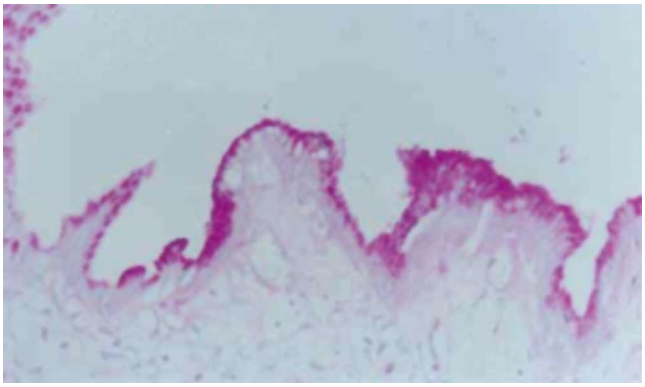

Figure 1 Specimens of chronic cholecystitis. Many positive PAS materials appear in epithelial cells of gallbladder mucosa (PAS × 200).

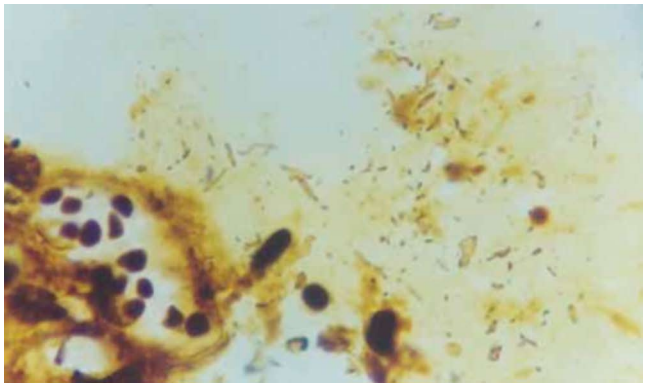

Figure 2 Helicobacter-like bacteria and inflammatory cells in mucus on gallbladder mucosa (WS × 200).

Table 1 Gastric metaplasia of gallbladder mucosa in cases with chronic cholecystitis

Group	*n*	Gastric metaplasia positive (*n*)	Gastric metaplasia negative (*n*)
Control	20	0	20
Chronic cholecystitis			
Positive *H pylori*	22	18	4
Negative *H pylori*	59	7	52

gallbladder mucosa was added into ultrapure water (Center of Molecular Biology, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing) and homogenized in an IS-l homogenizer (Medical Machine Factory, Zhejiang) and the homogenates were centrifuged at 4000 r/min for l5 min, after which the supernatants were collected and frozen at -70℃. The EC-l200 radio-immune auto-γ counting device (Zhongjia Corporation of ChinaAcademy of Sciences) was employed for radio-immune assay and a fully automatic biochemical assay device of Beckman Synchron CX (USA) was used for quantification of proteins of gallbladder mucosa homogenates.

Statistical analysis

Data were expressed as mean ± SD and processed with Chi-square test and Student's t-test. $P < 0.05$ was considered significant.

RESULTS

Metaplasia of gastric mucosa in gallbladder

In cases with a gallbladder polyp, no mucus stained positive for PAS was found in gallbladder mucosa in the region beyond the polyp, nor was positive substance of PAS, i.e., metaplasia of gastric mucosa, found in epithelial cells of mucosa. In cases with chronic lithic cholecystitis, the epithelium of gallbladder mucosa was column-like. AB/PAS stain showed that neutral mucus was positive, with metaplasia of epithelial cells of gastric mucosa (Figure 1).

Gastric metaplasia of gallbladder mucosa appeared in 25 cases, accounting for 30.86% (25/81) of all cases with chronic cholecystitis, however, it was not found in the control group (Table 1). This suggested that gallbladder mucosa was apt to gastric metaplasia in cases with chronic lithiasis cholecystitis, especially in those with *H pylori* infection in the gallbladder, of gastric metaplasia of gallbladder mucosa (18/22, 81.82%) was significantly higher than that in cases with negative *H pylori* (7/59, 11.86%, $P < 0.01$). It indicated that gastric metaplasia of gallbladder mucosa might relate to *H pylori* infection in the gallbladder.

Colonization of H pylori in gallbladder and its relation to cholecystitis

The optic microscopy showed that *H pylori* were scattered or aggregated on, or located within certain distance from the epithelial cells of gallbladder mucosa and that individual *H pylori* distributed inside epithelial cells or existed in intercellular space. At the regions with *H pylori,* column-like cells secreted neutral mucus by AB/PAS stain, indicating that the gallbladder mucosa had gastric metaplasia and that gastric mucosa were absent in some regions where *H pylori* located. Electron microscopy showed that *H pylori* were located on, stuck to, or entered the epithelial cells of gallbladder mucosa, where, however, no tight junction or adhesiveness could be seen. Moreover, optic microscopy revealed degeneration of the epithelial cells of gallbladder mucosa, infiltration and exudation of inflammatory cells, exfoliation of the epithelial cells, or chronic inflammation such as mucous layer shrinkage, decrease or even disappearance of epithelial cells and glands at sites where *H pylori* were present. Sometimes, there could be seen that inflammatory cells aggregated around *H pylori* and the latter were swallowed (Figure 2). On the other hand, only a few inflammatory cells infiltrated the mucosa, with intact epithelial cells, in most cases with chronic cholecystitis without *H pylori* infection. Exceptionally, even in these cases, there emerged acute inflammatory manifestations including infiltration of large numbers of inflammatory cells, degeneration, apoptosis and exudation of epithelial cells or chronic inflammatory manifestations including atrophy of glands. In the gallbladder epithelial cells that were proved to have

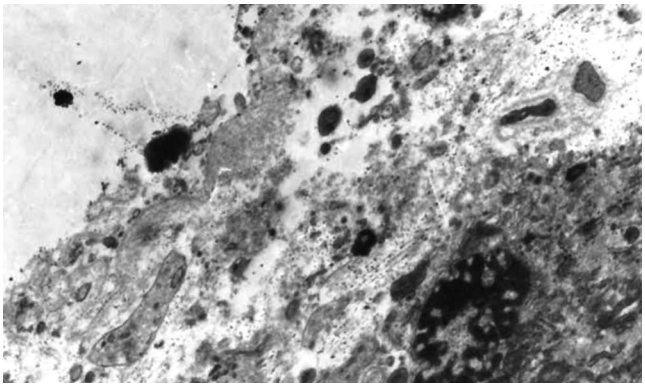

Figure 3 Electron microscopic images of *H pylori* on the epithelial cells of gallbladder (× 6000).

H pylori infection by electron microscopy, changes such as destructed epithelial cell membranes, loose cellular connection, dilatation of mitochondria, decrease or disappearance of crest, and dilatation of endoplasmic reticulum could be seen, which were severer compared with those without *H pylori* infection (Figure 3). The levels of IL-l, IL-6 and IL-8 of gallbladder mucosa homogenates were expressed as ng/g protein, and are shown in Table 2.

In cases with chronic cholecystitis, the levels of IL-l, IL-6 and IL-8 in gallbladders in both negative *H pylori* group and positive *H pylori* group were significantly higher than those in control group ($P < 0.01$). Moreover, there was a significant difference between positive *H pylori* group and negative *H pylori* group in levels of IL-1, IL-6 and IL-8 in the gallbladder ($P < 0.01$).

Table 2 Levels of IL-l, IL-6 and IL-8 of gallbladder mucosa homogenates (ng/g protein)

Groups	*n*	IL-1	IL-6	IL-8
Control	20	21.65 ± 4.28	77.10 ± 10.56	101.35 ± 19.39
Chronic cholecystitis				
Negative *H pylori*	59	68.76 ± 15.08[b]	159.54 ± 37.65[b]	152.10 ± 46.57
Positive *H pylori*	22	142.68 ± 25.41[b,d]	241.50 ± 80.60[b,d]	593.18 ± 93.59[b,d]

[b]$P < 0.01$ *vs* control group; [d]$P < 0.01$ *vs* negative *H pylori* group.

DISCUSSION

The gallbladder and stomach are originated from endoblasts and have similar tissue structures, with the mucosa covered with a slime layer [9-11]. AB/PAS stain showed that the epithelial cells of gallbladder mucosa secreted neutral mucus, with gastric metaplasia, in about 31% (25/81) of cases with cholecystolithiasis. Caselli *et al*[12-14] also demonstrated that the epithelial cells of gallbladder mucosa had gastric metaplasia in cases with cholecystolithiasis. Roa *et al*[15,16] found that pepsinogen I , Ⅱ were expressed in the epithelial cells of the gallbladder. The significance of gastric metaplasia of gallbladder mucosa lies in that the gastric metaplasia provides conditions for *H pylori* colonization in the gallbladder. The results of our study showed that compared with cases without *H pylori* infection in the gallbladder, there was a significantly higher incidence rate of gastric metaplasia in epithelial cells of gallbladder mucosa in cases with *H pylori* infection. It also proved that the gastric metaplasia of gallbladder mucosa closely correlated with *H pylori* infection in the gallbladder. Nevertheless, there were cases with *H pylori* infection but without gastric metaplasia in the gallbladder; meanwhile, there were cases with gastric metaplasia but without *H pylori* infection. These findings indicate that there is no absolute causality between gastric metaplasia and *H pylori* infection in the gallbladder. We hypothesize that many epithelial cells of the gastrointestinal tract have receptors for *H pylori* colonization factors. Therefore, *H pylori* can colonize on the epithelial cells of the gallbladder mucosa with no gastric metaplasia[17].

We also found that *H pylori* were separated from or adhered to the epithelial cells of gallbladder mucosa and that some *H pylori* penetrated through epithelial cells of the gallbladder. It that *H pylori* had a weak ability to pass through cells. At sites where *H pylori* aggregated, the epithelial cells of gallbladder mucosa were degenerated, erosive and even apoptotic. In some parts, inflammatory cells infiltrated, which became more obvious with increases in the number of *H pylori*. Electron microscopy revealed that at sites infected with *H pylori*, the integrity of the cell membrane of epithelial cells was destructed, with swelling of mitochondria and dilatation of endoplasmic reticulum. It showed that colonization of *H pylori* in the gallbladder cause inflammation of the gallbladder, mainly chronic nonsuppurative inflammation, which in turn provides an important condition for *H pylori* as one of the etiological factors leading to cholecystitis. Damage of the epithelial cells of gallbladder mucosa caused by *H pylori* may relate to specific virulence factors of *H pylori* such as cytotoxin-associated protein (CagA) and vacuoles toxin (VacA) , as well as urease, lipopolysaccharides and mucus enzyme of *H pylori*[18].

H pylori can also damage the epithelial cells of gallbladder mucosa through mediating inflammation and immunoreaction. The levels of IL-l, IL-6 and IL-8 in gallbladder mucosa homogenates in both *H pylori* negative and positive groups were significantly higher than those in control group ($P < 0.01$). It indicates that chronic lithic cholecystitis is associated with these three cytokines. We also found that in cholecystitis specimens with or without *H pylori* infection, levels of IL-1, IL-6 and IL-8 were significantly higher than those in control group, indicating that these interleukins may participate in pathogenesis of chronic cholecystitis. This may accord with the function of IL in *H pylori*-related gastritis and gastric ulcer[19,20]. After infection with *H pylori*, the urease, lipase and heat shock proteins secreted by *H pylori* can activate regional epithelial cells of mucosa and vascular endothelial cells expressing IL-1, IL-6 and other cytokines such as ICAM-I, hence stimulating and chemotaxy intravascular lymphocytes and monocytes to shift to *H pylori*-infected sites. IL-6 can activate and induce differentiation of T cells through other cytokines and enhance the function of

monocytes and NK cells, resulting in inflammation and injury at sites infected with *H pylori*. Our study verified that CagA of *H pylori* exerted strong action in stimulating epithelial cells and other cells expressing IL-8, which can activate and chemotactic neutrophils and lymphocytes[21]. In cases with chronic cholecystitis infected with *H pylori*, levels of IL-1, IL-6 and IL-8 of gallbladder mucosa were significantly higher than those in cases without *H pylori* infection. It suggests that *H pylori* participate in and aggravate cholecystitis, destruction of epithelial cells of the gallbladder and atrophy of the gallbladder[22]. Taken together, our study indicates that *H pylori* infection in the gallbladder may be one of the etiological factors leading to cholecystitis. The precise mechanism requires further verifications.

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