

Study of *Helicobacter pylori* in China

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In China, the prevalence of *Helicobacter pylori* (*H. pylori*) infection and gastric cancer is high and the geographic variation is great.

1, Biology

In 1985, Zhang ZH¹ et al. first isolated *H. pylori* in China. Zhou DY et al. found that *H. pylori* might change from helical bacillary form to coccoid form (CF), thread-like, giant coccoid, glomerule and spheroplast after incubation in vitro. These variants were able to grow and reproduce under microaerobic conditions in vitro except CF, and they, especially CF, were associated with recurrent infection, transmission and resistance to drugs. Zhou LY² et al. observed that the subinhibitory concentration (SIC) of metronidazole could induce gastric *H. pylori* CF transformation. Using PCR based random amplified polymorphic DNA (RAPD), Hua JS³ et al. analyzed DNA finger printing of *H. pylori*, indicating that recrudescence was the main reason of relapse of *H. pylori* infection. Cui Yi et al. reported that the reinfection rate in adult was as low in developing countries as in western developed countries. Xiang ZY et al.⁴ reported that the clinical isolates of *H. pylori* could be divided into two major types: type I (VacA+, CagA+) and type II (VacA-, CagA-), representing 56% and 16% respectively. CagA is not necessary for the expression of the vacuolating cytotoxin. Yan XJ et al [5] established a method of quantitative detecting *H. pylori* CagA gene. Zhang XY et al. found that VacA+cagA+ *H. pylori* strains were detected more frequently in peptic ulcer (PU) patients than in gastritis ones, and the detecting rates linked to the severity of disease. But in Shanghai, an investigation of *H. pylori* isolated from Chinese patients of gastritis and duodenal ulcer showed that the prevalence of CagA+ gene was almost 100%. Zheng PY et al.⁶ found lipopolysaccharide (LPS) displayed the antigenic variants that might increase the heterogeneity of *H. pylori*. Ras, c-met, c-erb-2, P16 and P53 genes etc were reported to undertake mutations and anomalous expressions in *H. pylori*-related diseases⁷⁻¹⁰. Liang HJ et al.¹¹ found that *H. pylori* extract could induce human gastric mucosal cells acquiring some malignant phenotypes. Liu WZ and Chen SY et al found that *H. pylori* infection increased the gastric epithelial proliferation, apoptosis, metaplasia and dysplasia. The results supported that the sequence of phenotypes and genotypic changes ultimately led to intestinal type of gastric cancer, which was put forward by Goldstone and Dixon. The process includes the following steps: non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and ultimately cancer^{12, 13}.

By sequencing 16sRNA gene of *Helicobacter*

heilmannii (Hh), formerly named *Gastrospirillum hominis* (Gh), Chen Ye et al.¹⁴ found out Hh was over 93.5% homologous to all other *Helicobacter* species and was most similar to *H. pylori* and *H. felis*. i.e. 97.1% and 96.5% similarities respectively.

2. Epidemiology

In 1996, Xiao SD reported that the prevalence of *H. pylori* infection was 21 - 93% in the common Chinese, 50% in the people in cities and 68.8% in the people in suburb. In 1987, Jiang SJ (Shanghai) first reported *H. pylori* detecting rates in endoscopic cases in China. Li YY¹⁵ reported that *H. pylori* infectious rates in duodenal ulcer (DU), gastric ulcer (GU) and chronic gastritis were 74.1%, 71.9% and 63.6% in a meta-analysis of Chinese Journals from 1985 to 1989. Hu PJ¹⁶ reported *H. pylori* infection was evident in 98.9% of patients with DU, 100% with GU, 83.3% with gastric cancer (GC) and 57.4% with functional dyspepsia (FD) in 1006 consecutive endoscopy cases. In this study, patients with antimicrobial medicine intake history were excluded from the analysis. Serological survey of population on large scale was carried out in Guangzhou, Lanzhou and Shanghai separately. The studies showed that the overall prevalence of *H. pylori* infection was 38.6 - 61.6%, which paralleled with age. But it is different from that seen in Western countries: the age-infection distribution curve showed infection rate markedly increased in the group of 1 - 5 years old, slowly increased in the group of 6 - 19 years old, and was up to or almost up to the peak over 19 years old and the difference of infection rates in different areas was generally reflected in the age range of 1 - 5 years. The studies clearly indicated that early period of childhood was much susceptible to *H. pylori* infection¹⁷.

Chen JJ et al. found out different strains of *H. pylori* in different regions. Using SDS-PAGE protein profiles and immunoblot patterns. An early serum assay of *H. pylori* infection carried out in Kunming showed several epidemic factors: source of drinking water, diet habit, economic condition and drug abusing¹⁸. Family aggregation of *H. pylori* infection has been demonstrated in several studies. Faculties in the department of Gastroenterology have a higher risk of *H. pylori* infection than the other groups in hospitals. All the studies above indicate a person-to-person transmission mode of *H. pylori*.

The fact of *H. pylori* positive in dental spots and saliva supports oral-oral transmission pattern. Wu LJ et al. had detected *H. pylori* in foul water by PCR. However, there were not significant differences between people drinking water from rivers or wells in one region of Guangzhou. Using the antibody to hepatitis A virus (HAV)

as a marker of fecal-oral exposure, seroprevalence data from 1501 subjects in rural and urban areas in Guangzhou, suggested that community wide fecal-oral spread of *H. pylori* might be of limited importance in the transmission of *H. pylori* in China¹⁹. Cat is found to be vertebrate host of *H. pylori*.

3. Animal model

Sun GH et al. established the "Chinese No.1 Pig" model infected with *H. pylori*. Wang JD et al.²⁰ inoculated SPF Balb/c mice with *Helicobacter felis* (Hf). Chronic gastritis characterized by the infiltration of lymphoid cell and formation of lymphoid follicles (LF) could be found in 20 - 40 weeks after infection. Acute gastritis features could be seen lately. Xu KQ et al. set up a rat model where *H. pylori* could cause allergy. In wistar rat infected with *H. pylori* (CagA+/VacA+), the bacteria could colonize in gastric gland mucosa 3 months later, and the colony's densities increased as time went by. Chin MH et al.²¹ immunized SPF Balb/c mice orally with *H. felis* sonicate plus cholera toxin, and succeeded in preventing infection of *H. felis*. First in the world, they proved intervention against *H. pylori* was a real possibility. Animal inoculated with Hh offered us a new clue to carry out comparative studies on *H. pylori*.

4. Mechanism

In vitro, *H. pylori* adhere to the gastric-type epithelial cells specially. Only in gastric mucosa that *H. pylori* receptor like materials exist. *H. pylori* infection decreased the gastroduodenal mucosal blood flow (GDMBF), increased the release of histamine and enhanced the mast cell degranulation²². Higher PGE2 and lower EGF can be seen in *H. pylori* positive group. Shi Li et al. recently confirmed the synergy of *H. pylori*'s urease and ammonia²³. The enhanced NO synthase activity in *H. pylori* infection suggested that NO, as a free radical, may be involved in gastric mucosal ROM damage²⁴. Chen MH et al. found that *H. pylori* especially CagA+ strains, might induce gastric epithelial secretions of IL-8, IL-6, TNF- α ²⁵.

Nie SH et al. found that as a consequence of *H. pylori* inflammation, delicate gastrin/somatostatin (SS) balance was broken, leading to hypergastrinemia and decreasing of SS. Chen JX et al. found that the levels of ascorbic acid and Copper Zinc superoxide dismutase (CuZnSOD) in gastric juice were significantly lower in *H. pylori* positive patients than *H. pylori* negative ones and normal controls ($P < 0.05$). The degree of decrease in the two items was in the decreasing order of patients with superficial gastritis, atrophic gastritis and gastric cancer²⁶.

5. *H. pylori* associated diseases

5.1 gastritis and peptic ulcer

Many groups have confirmed the strong association between *H. pylori* and gastritis, and peptic ulcer.

5.2 gastric carcinoma (GC)

GC is estimated to be the world's second most common cancer. In 1994, infection with *H. pylori* has been classified as the group 1 (definite carcinogen) carcinogenic

exposure. In China, GC mortality ranks the first in the cancer death, accounting for 23.02% of all malignant tumors. The generous *H. pylori* infection of Chinese is 60-80%, higher than the 30% of American, whose GC mortality is only 20/100,000. Both *H. pylori* infection and GC are prone to increase along with age. Studies to compare the seroprevalence of *H. pylori* in areas with high or low risks for GC (mortality 40/100,000 vs. 5/100,000) showed that in children under 5 years old; the prevalence of *H. pylori* infection in the area with high GC mortality (Lanzhou) was 45.1%, significantly higher than the 15.1% with low GC mortality (Guangzhou), however in children over 5 years old, the increase in prevalence of *H. pylori* infection was almost the same in the two areas. The peak age of infection in the former was in the group of 30 - 39 years old, i.e. 10 years younger than the latter, and moderate-severe intestinal metaplasia (IM) and atrophy emerged earlier in the former area (Fig. 1 - 3). The result strongly suggested the relationship between early *H. pylori* infection and GC. Chen MH et al.¹⁷ stated that low mortality of GC could be found in the area with low *H. pylori* infection rate, while its uncertain high mortality of GC exists along with a high *H. pylori* incidence. The result suggested the pathologic consequence of *H. pylori* infection would depend on other factors other than *H. pylori* itself.

Hu PJ et al. reported the prevalence of *H. pylori* in tissue of the cancer, around the cancer, beneath the cancer were 16.1%, 71.4% and 93.6%. The cancer tissue itself does not fit for adhesion of *H. pylori*.

Several groups have confirmed the correlation of *H. pylori* with GC. In the above study, the detecting rate of serum anti-*H. pylori* antibody in advanced GC patients was 92.9%, significantly higher than the 69.6% of healthy individuals ($P < 0.05$). But there is no difference between the intestinal-type and diffuse-type²⁷. Xu SP et al.²⁸ detected *H. pylori* in antral tissue by PCR, and showed that *H. pylori* infection was closely related to GC (82.4 vs. 66.2), particularly in the intestinal-type and non-cardia GC. No remarkable difference was found between early and advanced GC. Correlation rate of natural population infection to GC death is 40% ($p = 0.02$), which is not seen in other tumors.

But in a serological study on large-scale, Zhu YQ et al.²⁹ reported patients with GC or non-digestive ulcer had similar *H. pylori* prevalence. Prospective studies in Mainland and Taiwan showed that matching OR value of GC risk in *H. pylori* patients were only 1.1 (95% CI: 0.6 - 2.1) and 1.6 (95% CI: 0.7 - 2.6).

Zhong WR et al. found that *H. pylori* detecting rate in IM group was statistically higher than that in the group without IM ($P < 0.05$). Yang RK et al.³⁰ reported no significant differences were seen among the four types of IM, *H. pylori* may be ineffective in the formation of IM. Dysplasia was found to be more common in *H. pylori*-related gastritis than in *H. pylori* negative cases.

Several international researches suggested that the worldwide variation in GC mortality might be associated more strongly with the *H. pylori* infection of CagA+ strains than of CagA- strains. But no evidence of correlation of CagA to GC was found so far in studies performed in China³¹.

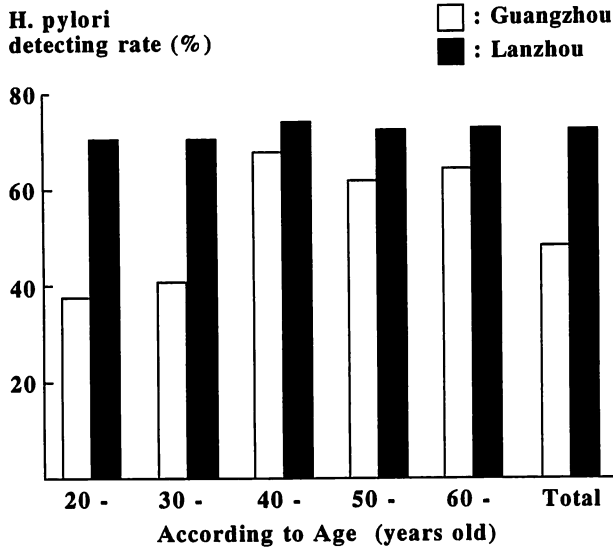


Figure 1. H. pylori detecting rate according to Age in high and low GC mortality

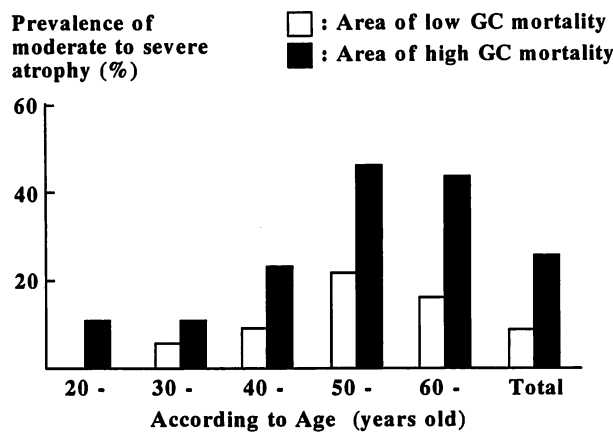


Figure 2. Prevalence of moderate-severe atrophy according to Age in high and low GC mortality areas

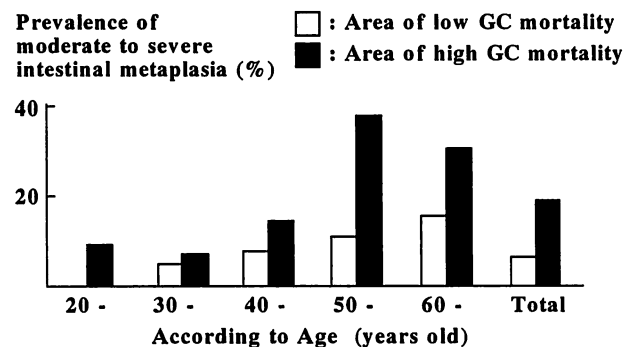


Figure 3. Prevalence of moderate-severe intestinal metaplasia according to Age in high and low GC mortality areas

5.3 Primary gastric malignant lymphoma (PGML)

PGML is an uncommon cancer, accounting for only 0.93 -2.4% of gastric neoplasm in China³². It remains, however, the most frequent extranodal form of lymphomas. The majority of the tumors derive from mucosa-associated lymphoid tissue (MALT), making for 5% of lymphomas and 51% of all these MALT lymphomas. The incidence is lower than that in western countries, but in recent years, the number is reported to be rising in China³³.

Yi ZH et al.³⁴ found that H. pylori detecting rate of 39 cases with PGML was significantly higher than that of control groups with lymphocytic gastritis and H. pylori unrelated diseases, respectively (87.18 vs. 63.64, 53.13) (P<0.005). MALT type was accounted for 92.31% of PGML and the H. pylori positive rate was 86.11%. The data indicated the association of H. pylori with PGML, which was also supported by another group who detected H. pylori infection in all 15 PGML patients. However, Shao JY et al.³⁵ reported that the H. pylori detecting rate was only 17.65% in Guangdong, obviously lower than the 79.06% in the control with gastritis (P<0.01). The result supported the speculation (Fagioli in 1994, Luppi in 1996, Xu et al. in 1997) that other infectious agents and environmental factors might have a pathogenic role in lymphomagenesis of MALT PGML.

The onset of MALT lymphoma in stomach is preceded by the acquisition of MALT as a result of H. pylori infection. ZhaugWei³⁶ reported that lymphoid follicle (LF) rate in patients with H. pylori infection was 60.01%, remarkably higher than that in patients without H. pylori infection (17.1%), LF rate could decrease after eradication of H. pylori, indicating the presence of LF might directly associated with H. pylori infection.

5.4 Functional dyspepsia (FD)

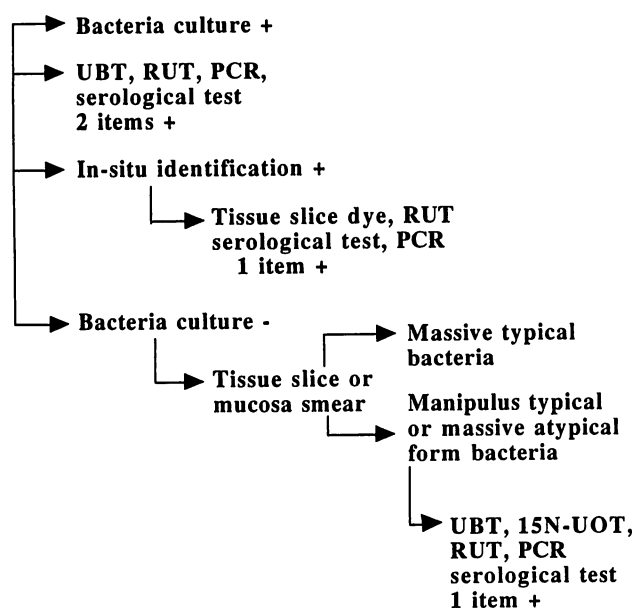
It is still not sure whether or not H. pylori infection and FD are related to each other. Several studies in China have shown that H. pylori infection and H. pylori associated active chronic gastritis have no effect on dyspeptic symptoms and gastric emptying in FD patients, though some patients' symptom can be unreleased or disappear after H. pylori's eradication. The positive rate of H. pylori in patients with FD was 48.2%, significantly lower than the 89.5% in patients with peptic ulcer (P<0.01). H. pylori may play an unimportant role in the pathophysiology of FD³⁷.

6. Diagnosis method³⁸

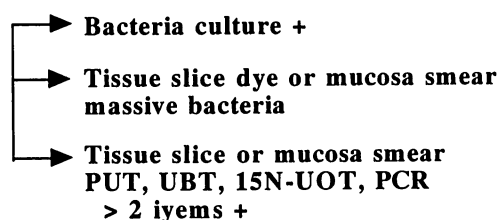
- (1) Urease breath test (UBT)
- (2) Rapid urease test (RUT)
- (3) Serological test: ELISA, purified urease, ultrasound pulverization antigen
- (4) Gastric mucosa biopsy
- (5) Bacteria isolate and culture
- (6) In-situ identification technique
- (7) PCR
- (8) Histological examination

7. Diagnosis criteria

7.1 Research diagnosis standard



7.2 Clinical diagnosis standard



Patients using anti-*H. pylori* drugs in 2 weeks should be excluded from all the tests.

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