

Lack of association between seroprevalence of *Helicobacter pylori* infection and primary biliary cirrhosis

Marilena Durazzo, Floriano Rosina, Alberto Premoli, Enrico Morello, Sharmila Fagoonee, Rosaria Innarella, Enrico Solerio, Rinaldo Pellicano, Mario Rizzetto

Marilena Durazzo, Alberto Premoli, Enrico Morello, Department of Internal Medicine, University of Turin, Turin, Italy
Floriano Rosina, Rosaria Innarella, Enrico Solerio, Department of Gastroenterology, Gradenigo Hospital, Turin, Italy
Sharmila Fagoonee, Department of Biology, Biochemistry and Genetics, University of Turin, Turin, Italy
Rinaldo Pellicano, Mario Rizzetto, Department of Gastroenterology, Molinette Hospital, Turin, Italy
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Correspondence to: Professor Marilena Durazzo, Department of Internal Medicine, Corso A.M.Dogliotti 14, 10126 Turin, Italy. marilena.durazzo@unito.it

Telephone: +39-11-6336040 **Fax:** +39-11-6634751

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Abstract

AIM: To determine the association between seroprevalence of *Helicobacter pylori* (*H. pylori*) infection and primary biliary cirrhosis (PBC).

METHODS: In this case-control study, 149 consecutive patients (10 males, 139 females, mean age 58.2±11 years, range 26-82 years) suffering from PBC and 619 consecutive healthy volunteer blood donors (523 males, 96 females, mean age 47±5.3 years, range 18-65 years) attending the Hospital Blood Bank and residing in the same area were recruited. A commercial enzyme linked immunosorbent assay was used to detect anti-*H. pylori* (IgG) antibodies in serum.

RESULTS: Antibodies to *H. pylori* were present in 78 (52.3%) out of 149 PBC-patients and in 291 (47%) out of 619 volunteers ($P = 0.24$, OR 1.24, 95% CI 0.85-1.80). In the subjects less than 60 years old, the prevalence of *H. pylori* infection among PBC-patients (40/79) was slightly higher than in controls (50.6% vs 46.2%) $P = 0.46$, OR = 1.19, 95% CI: 0.72-1.95). In those over 60 years, the prevalence of *H. pylori* infection was similar between PBC-patients and controls (54.2% vs 57.8%, $P = 0.7$, OR 0.86, 95% CI 0.36-2.07).

CONCLUSION: There is no association between seroprevalence of *H. pylori* infection and primary biliary cirrhosis.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is a chronic one. In most instances, it is acquired during childhood, and is often associated with low socio-economic class. The presence of

the bacterium has been established as the main cause of several gastroduodenal diseases, including peptic ulcer disease^[1,2], gastric carcinoma^[3], and gastric MALT lymphoma^[4].

Since the latest decade, several studies have reported on the link between chronic *H. pylori* or *Helicobacter species* (*H. species*) infections and a variety of extragastric manifestations. These include ischaemic heart disease (IHD), liver diseases, skin diseases, blood disorders and others^[5]. However, the hypothesis of an etiological role has not yet been fully investigated.

Epidemiological studies have frequently involved control selection bias, population of small sizes, and presence of confounders, like age and socio-economic conditions.

Non randomised, long-period and large studies on the follow-up of *H. pylori* eradication in extragastric diseases are lacking.

Several *H. species*, such as *H. bilis*, are capable of colonising different anatomical regions of the gastrointestinal tract in humans, including choledochus, gallbladder, intrahepatic bile ducts and liver^[6].

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease in which intra-hepatic bile ducts are progressively destroyed. The etiology of PBC is unknown, but immunological mechanisms may play a part in the pathogenesis. A causal role of infectious agents has been proposed but the data are inconclusive. Recently, Bogdanos and coworkers have shown that microbial mimics, comprising that of *H. pylori*, are major targets of crossreactivity with human pyruvate dehydrogenase in PBC, strengthening the fact that microbial exposure may be instrumental to the appearance and/or maintenance of anti-mitochondrial antibody responses by a cross-reactive mechanism^[7]. These data indicate that a relationship between *H. species* and the biliary tract of humans might exist, where the bacteria could be potentially involved in inflammatory changes or other pathologic manifestations. However, these findings have not been confirmed in other studies^[8].

The prevalence of peptic ulcer is higher in cirrhotics than in control population and the risk is increased by the presence of *H. pylori* infection^[9]. However, in some groups of patients affected from liver disease, as in the case of PBC, the seroprevalence of *H. pylori* infection is still undetermined.

The present study attempted to highlight on the seroprevalence of antibodies against *H. pylori* in a cohort of patients suffering from PBC in comparison to a group of volunteers attending the Blood Bank of the San Giovanni Battista Hospital (Molinette) in Torino, Italy.

MATERIALS AND METHODS

The presence of anti-*H. pylori* antibodies was evaluated in 149 consecutive subjects (10 males, 139 females, mean age 58.2±11 years, range 26-82 years) suffering from PBC.

Patients were considered to have PBC if they fulfilled at least 2 of the following criteria: positive anti-mitochondrial antibody (AMA) at a titer higher than 1/40, abnormal liver function tests (alkaline phosphatase, gamma glutamyl transpeptidase, bilirubin, transaminase level) or liver histology diagnostic or consistent with PBC^[10].

AMA was evaluated by immunofluorescence using rat

stomach and kidney as substrate^[10].

Other causes of liver disease, such as viral (hepatitis B virus, hepatitis C virus), autoimmune (anti-nuclear, anti-smooth muscle and anti-microsome antibodies) or metabolic (serum iron, percentage of transferrin saturation, ferritin, ceruloplasmin, alpha-1 antitrypsin) hepatitis, were ruled out.

The controls were 619 consecutive volunteer blood donors (523 males, 96 females, mean age 47±5.3 years, range 18-65 years) attending the Hospital Blood Bank and residing in the same area^[11].

A commercial enzyme linked immunosorbent assay (ELISA, Helori-test® Eurospital, Trieste Italy) was used to detect anti-*H pylori* (IgG) antibodies in serum. The assay sensitivity and specificity versus histology were 70.6% and 90.5% respectively, positive predictive value was 87.2% and negative predictive value 77.0%^[12].

Briefly, calibrators, positive controls, negative controls and diluted (1:200) serum samples were added to wells coated with purified *H pylori* group-specific antigens. Plates were incubated for 60 min at 37 °C. The plate was then washed thrice and anti-IgG conjugate was pipetted into each well and the plate was incubated again for 60 min at 37 °C. The washing step was repeated, chromogenic substrate was added to each well, followed by incubation for 30 min at 37 °C. The reaction was then stopped. Reading was performed at 405 nm and the mean optical density was expressed as a percentage of the optical density of the positive control serum assayed on the same plate.

A commercial enzyme immunoassay (Helori®-CTX Eurospital, Trieste, Italy) was used to detect serum IgG antibodies against more virulent strains of the bacterium, expressing cytotoxin-associated gene product A (CagA). The manufacturer's instructions were followed. The assay sensitivity and specificity given by the manufacturer were 94.1% and 97.9% respectively.

The seroprevalence of *H pylori* infection in cases and controls was compared using the chi-square test (χ^2) by means of 2×2 contingency table. Fisher's exact test was used for small sample size. Results were considered statistically significant when $P < 0.05$.

RESULTS

Mean age between patients and controls was not statistically different.

The prevalence of antibodies to *H pylori* was 52.3% (78/149) in the patients with PBC compared to 47% (291/619) in the controls ($P = 0.24$, OR 1.24, 95% CI 0.85-1.80).

When the patients were subdivided into age-groups (<60 and ≥60 years), the difference was as follows. In the youngest age group (less than 60 years old), the prevalence of *H pylori* infection among CBP-patients (50.6%, 40/79) was higher than in controls (46.2%, 269/581), but this was not significant ($P = 0.46$, OR 1.19, 95% CI 0.72-1.95). In the group over 60 years, the prevalence of *H pylori* infection was lower in CBP-patients (38/70, 54.2%) than in controls (22/38, 57.8%) ($P = 0.7$, OR 0.86, 95% CI 0.36-2.07) (Table 1).

Table 1 Seroprevalence of anti-*Helicobacter pylori* antibodies among patients with primary biliary cirrhosis [PBC] and controls

Age (yr)	Patients with PBC Hp (+)/tot (%)		Controls Hp (+)/tot (%)		P
<60	(50.6)	40/79	269/581	(46.2)	0.46
≥60	(54.2)	38/70	22/38	(57.8)	0.7
Total	(52.3)	78/149	291/619	(47)	0.24

The anti-CagA antibodies were detected in 28.1% of patients with PBC (42/149) and in 44.8% of those with seropositivity for

anti-*H pylori* (35/78). In our population, anti-CagA antibodies were present in 61.8% of a general population admitted to the Emergency Care Unit, as published elsewhere^[13].

Out of the 10 patients with a past history of peptic ulcer, 7 had anti-*H pylori* antibodies in circulation. In 29 cases, the signs of portal hypertension [varices or congestive gastropathy] were shown by upper GI endoscopy while in 111 patients there were no abnormalities.

DISCUSSION

The prevalence of *H pylori* infection in patients with liver disease needed to be studied on the basis of clinical and experimental considerations.

From a clinical point of view, the medical history of cirrhotic patients was punctuated by frequent and recurrent hospitalisations due to high rate of complications. Among the most relevant of them, peptic ulcer and upper GI hemorrhage were of peculiar relevance, being life-threatening for the patient and of high cost for Health Care Services, requiring both emergency care and subsequent long hospital stay^[14]. By a multivariate analysis, Calvet *et al.* found that male sex and *H pylori* seropositivity (OR 1.7, 95% CI 1.02-2.81) were variables independently related to peptic ulcer in cirrhotics with different etiologies^[15]. Moreover, in the case of hemorrhage from peptic ulcer, the presence of cirrhosis was independently associated with increased mortality ($P < 0.001$)^[16]. Little information is available on the prevalence of peptic ulcer in subjects suffering from PBC or primary sclerosing cholangitis (PSC). The prevalence of duodenal ulcer (DU) in male cirrhotics was investigated by Rabinovitz *et al.* in 216 subjects. Occurrence of DU amounted to 7.8% in patients and 2.2% in controls ($P < 0.005$). When the patients were subdivided according to etiology, DU prevalence was observed in 9.4% of HBV-related chronic hepatitis patients, 12.2% of alcohol-related chronic hepatitis patients, 3.5% of cryptogenic, 6.6% of autoimmune cirrhosis, 9.5% of PSC and none of the patients with PBC. However, in the latter case only 9 patients were included^[17].

Since peptic ulcer is related to the presence of *H pylori* infection in non-cirrhotic patients, it is logical to suppose a similar role for the bacterium also in subjects with cirrhosis.

A high prevalence of anti-*H pylori* antibodies in HCV-infected cirrhotic patients has been reported in several North Italian towns^[18,19]. On the contrary, there was no increased seroprevalence in patients suffering from autoimmune hepatitis^[20]. Fan *et al.* demonstrated a higher seroprevalence of *H pylori* in Chinese patients with HBV-related chronic hepatitis than in controls matched for age and socio-economic status^[21]. In Taiwan, Chen *et al.* found no association between peptic ulcer and *H pylori* infection in cirrhotic patients^[22]. Selection biases and methods of diagnosis were possible sources of the heterogeneity of the studies. Contrasting results might arise from the choice of tests used for the diagnosis of *H pylori* infection. Indeed, when the diagnosis relies solely on histological examination of gastric biopsies, sampling error is the source of severe misdiagnosis. The European *H pylori* Study Group has recommended to search for IgG anti-*H pylori* when performing an epidemiological investigation^[23].

Regarding populations of subjects suffering from PBC, Floreani *et al.* showed that *H pylori* colonization was significantly more frequent in controls than in patients but IgG anti-*H pylori* were detected in the same percentage in the two groups^[24]. Thus, the latter finding is in agreement with those of our study. Dohmen and coworkers in Japan, have found that *H pylori* is a possible pathogenic factor in atrophic corpus gastritis in PBC-patients. Furthermore, a positive correlation between the titers of anti-pyruvate dehydrogenase antibody and anti-*H pylori* was confirmed^[25]. However, in this investigation,

no comparison with a control population has been made.

From an experimental point of view, infection of healthy A/JCr male mice with *Hepaticus* could result in chronic hepatitis and liver cancer in a short time^[26]. Since this report, several other *H. species* have been subsequently found in the liver and biliary tract of cats and dogs suffering from hepatitis and hepatocellular carcinoma. *H. species* have been demonstrated both in the bile and in the gallbladder mucosa of Chilean patients with chronic gallbladder inflammation, raising the question as to whether the frequent finding of gallbladder cancer in Chile might arise from such an infection^[6]. By polymerase chain reaction, hybridisation and partial DNA sequencing in human liver of patients with PBC or PSC, Nilsson *et al.* found the positivity for *Helicobacter* genus-specific primers in 11 out of 12 samples of PBC-subjects and in 9 out of 12 samples of individuals suffering from PSC^[27].

In conclusion, we found that the seroprevalence of *H pylori* in subjects suffering from primary biliary cirrhosis was not more frequent than in controls, suggesting that the putative role of *H pylori* in triggering organ-specific autoimmunity does not hold true for PBC.

On the other hand, although these data do not provide proof for the association between *H pylori* infection and PBC, a type II statistical error, i.e. the probability of accepting the *null hypothesis* when it is false, cannot be ruled out.

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