

# *Helicobacter pylori* is not a contributing factor in gallbladder polyps or gallstones: a case-control matching study of Chinese individuals

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## Abstract

**Objective:** To investigate the relationship between *Helicobacter pylori* (*H. pylori*) infection and gallstones or gallbladder polyps.

**Methods:** This retrospective analysis included 27,881 individuals who underwent health examinations that included a *H. pylori* test and an abdominal ultrasound scan. Patients were divided into four groups: gallbladder polyp (P group), gallstone (S group), gallstone and gallbladder polyp (SP group), and no gallbladder disease (N group). Case-control matching was used to select the participants in the control group.

**Results:** The mean ages of participants in the P, S, and SP groups were all significantly higher than the mean age of participants in the N group. The proportions of participants with each type of body mass index significantly differed between the N and P groups, and between the N and S groups. In total 45.7% of participants exhibited *H. pylori* infection. After case-control matching, the proportion of participants with *H. pylori* infection did not significantly differ according to the presence or absence of gallbladder polyps. Similar results were observed regarding gallstones, as well as gallstones and gallbladder polyps.

**Conclusion:** *H. pylori* infection might not be related to gallbladder polyps or gallstones.

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## Keywords

*Helicobacter pylori*, gallstone, gallbladder polyp, body mass index, gallbladder disease, case-control study

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## Introduction

Approximately half of the world's population is infected with *Helicobacter pylori* (*H. pylori*), and the rate of infection is reportedly 55% in China.<sup>1</sup> There is evidence that *H. pylori* is closely associated with gastric disease, but increasing numbers of studies have suggested that *H. pylori* is involved in extragastric diseases, such as obesity,<sup>2</sup> osteoporosis,<sup>3</sup> diabetes mellitus,<sup>4</sup> chronic obstructive pulmonary disease,<sup>5</sup> and chronic kidney disease.<sup>6</sup>

The incidence of gallbladder polyps has been reported to range from 3% to 7%, as determined by abdominal ultrasound examination.<sup>7–9</sup> Although they are generally considered benign, approximately 2% of gallbladder polyps are reportedly related to gallbladder cancer.<sup>10</sup> The main cause of gallbladder polyp formation is regarded as fibrosis due to cholesterol deposition in the gallbladder wall and chronic inflammation of the mucosa.<sup>11,12</sup> Approximately 10% to 20% of the adult population exhibits gallstone disease.<sup>13</sup> The formation of gallstones is primarily related to metabolic abnormalities.<sup>14</sup>

To the best of our knowledge, no study has focused on the relationship between *H. pylori* and gallbladder polyps. Notably, some studies have shown that *H. pylori* is a causative factor for gallstones, based on the finding of *H. pylori* DNA in the gallbladder mucosa or cholesterol gallstones.<sup>15–18</sup> However, outcomes based on assessment of immunoglobulin G antibodies against *H. pylori* have been

inconsistent.<sup>19,20</sup> In this study, we investigated the relationship between *H. pylori* infection (determined by the <sup>13</sup>C or <sup>14</sup>C urease breath test) and the presence of gallbladder polyps or gallstones.

## Methods

### Participants and data collection

This retrospective analysis screened all individuals who underwent health examinations (including *H. pylori* infection test and abdominal ultrasound examination) in the health management center of Taizhou Hospital (Linhai, China) during the period from January 2017 to December 2019. Patients were excluded if they had invalid data (missing data for one or more study outcomes) or a history of cholecystectomy. *H. pylori* infection was determined by using a <sup>13</sup>C or <sup>14</sup>C urease breath test (Shenzhen Zhonghe Headway Bio-Sci & Tech Co., Ltd., Shenzhen, China). Body mass index (BMI) was calculated as weight/height squared (kg/m<sup>2</sup>). In accordance with Chinese standards,<sup>2</sup> BMI values were categorized into the following four levels: underweight (<18.5 kg/m<sup>2</sup>), normal weight (18.5–23.9 kg/m<sup>2</sup>), overweight (24.0–27.9 kg/m<sup>2</sup>), and obesity (≥28.0 kg/m<sup>2</sup>). Gallbladder disease findings, as confirmed by abdominal ultrasound, were used to divide participants into the following four groups: gallbladder polyp (P group), gallstone (S group), gallstone and gallbladder

polyp (SP group), and no gallbladder disease (N group).

### **Ethics approval**

This study was approved by the Institutional Medical Ethics Review Board of Taizhou Hospital. The review board waived the requirement for informed consent because this was a retrospective study that did not include identifying information for any participant.

### **Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean  $\pm$  standard deviation. Associations involving parametric data were analyzed using Student's *t*-test. One-way analysis of variance or chi-squared tests were used for comparisons among groups. A 1:1 case-control matching protocol was implemented to match the essential confounding parameters (sex, match tolerance, 0; age, match tolerances, 2; BMI level, match tolerance, 0) between the P and N groups, between the S and residual N groups, and between the SP and residual N groups in a sequential manner.  $P < 0.05$  was considered statistically significant.

## **Results**

### **Participant characteristics**

During the study period, 151,629 individuals (aged 7–103 years) underwent health examinations in our hospital. Following application of exclusion criteria, 27,881 individuals were included in the study; no individuals under the age of 18 years exhibited gallstones or gallbladder polyps. The numbers of participants in the N, P, S, and SP groups were 24,104 (86.5%), 2764

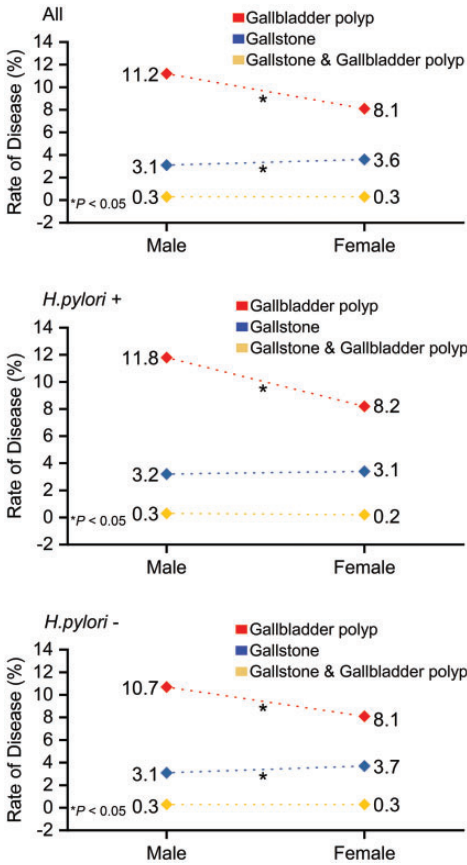
(9.9%), 926 (3.3%), and 87 (0.3%), respectively.

### **Comparisons of gallbladder disease status according to sex and age**

The incidence of gallbladder polyps was higher in men than in women (11.2% vs. 8.1%,  $P < 0.001$ ), while the incidence of gallstones was lower in men than in women (3.1% vs. 3.6%,  $P = 0.031$ ) (Figure 1). Among men in this study, the mean ages in the P, S, and SP groups were  $46.7 \pm 11.9$  years,  $54.0 \pm 12.7$  years, and  $50.5 \pm 9.4$  years, respectively; these significantly differed from the mean age ( $45.5 \pm 13.8$  years) of men in the N group ( $P = 0.003$ ,  $P < 0.001$ , and  $P = 0.016$ , respectively). The mean ages of women in the P, S, and SP groups were  $46.5 \pm 11.5$  years,  $53.6 \pm 12.7$  years, and  $50.6 \pm 10.5$  years, respectively; these significantly differed from the mean age ( $45.0 \pm 13.3$  years) of women in the N group ( $P = 0.001$ ,  $P < 0.001$ , and  $P = 0.014$ , respectively) (Figure 2). Furthermore, the mean ages significantly differed between the P and S groups in men ( $P < 0.001$ ) and women ( $P < 0.001$ ).

### **Comparison of BMI among groups**

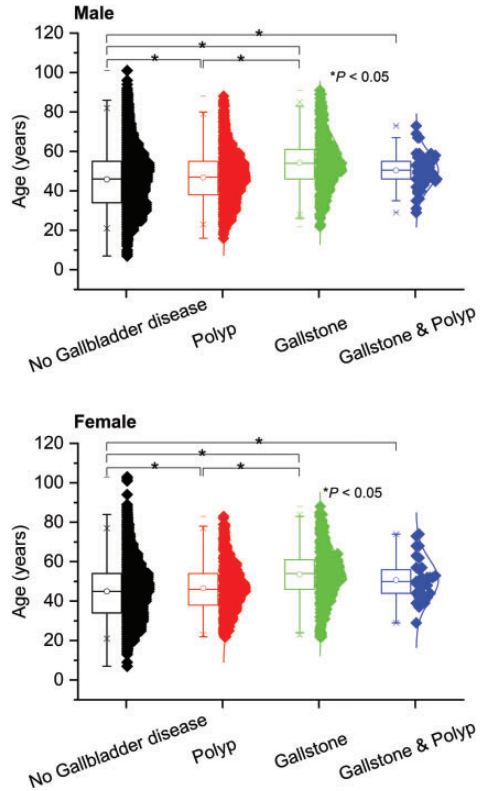
BMI data were missing for 10 participants (11.5%) in the SP group, 68 participants (7.3%) in the S group, 211 participants (7.6%) in the P group, and 1722 participants (7.1%) in the N group. According to the recorded BMI data, the proportions of participants with each BMI level were significantly different between the N and P groups ( $P < 0.001$ ), and between the N and S groups ( $P < 0.001$ ). However, the proportions of participants with each BMI level did not significantly differ between the N and SP groups (Figure 3).



**Figure 1.** Incidences of gallbladder polyps, gallstones, and gallstones and gallbladder polyps in men and women according to the status of *Helicobacter pylori* infection (top, all patients; middle, patients with *H. pylori* infection; bottom, patients without *H. pylori* infection). Red diamonds, gallbladder polyp group; blue diamonds, gallstone group; yellow diamonds, gallstone and gallbladder polyp group. \* $P < 0.05$ .

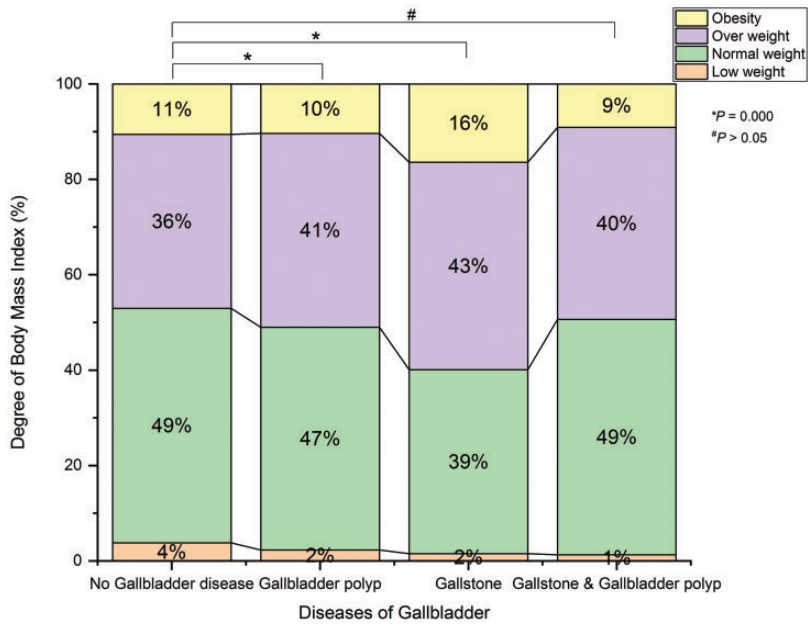
**Comparisons of *H. pylori* infection according to gallbladder disease status, sex, and age**

In total, 45.7% (12735/27881) of participants exhibited *H. pylori* infection. The proportion of participants with *H. pylori* infection was higher in the P group than in the N group (47.5% vs. 45.5%,



**Figure 2.** Mean ages of participants with gallbladder polyps, gallstones, or gallstones and gallbladder polyps, compared with participants without gallbladder disease, stratified according to sex. \* $P < 0.05$ .

$P = 0.048$ ); however, this proportion did not significantly differ between the S group (42.5%) and the N group, or between the SP group (45.5%) and the N group. The mean age was higher among participants with *H. pylori* infection than among participants without *H. pylori* infection ( $46.5 \pm 13.5$  years vs.  $45.4 \pm 13.8$  years,  $P < 0.001$ ). The mean BMI values were higher among participants with *H. pylori* infection than among participants without *H. pylori* infection ( $24.0 \pm 3.3$  kg/m<sup>2</sup> vs.  $23.9 \pm 3.3$  kg/m<sup>2</sup>,  $P = 0.001$ ) (Figure 4). Among participants with *H. pylori* infection, the incidence of gallbladder polyps was higher in men than



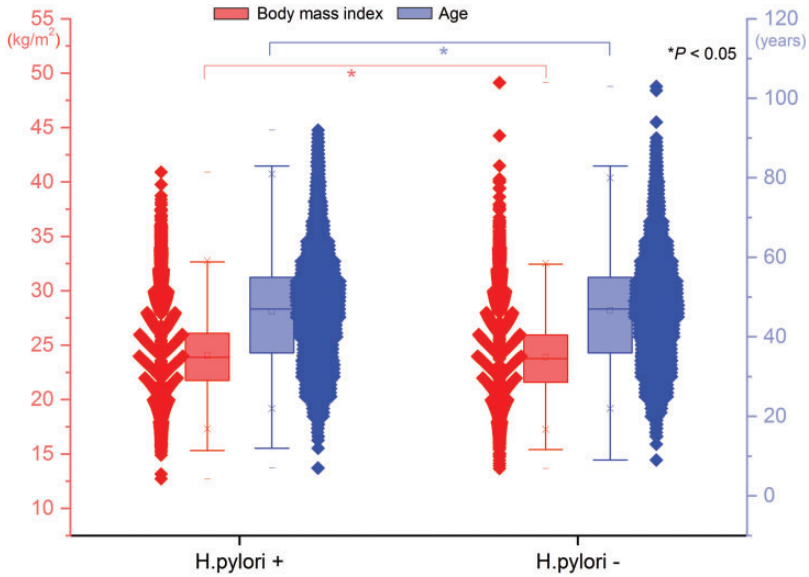
**Figure 3.** Body mass index levels, based on Chinese standards, among participants with gallbladder polyps, gallstones, or gallstones and gallbladder polyps, compared with participants without gallbladder disease. Yellow indicates BMI in obesity range; purple indicates BMI in overweight range; green indicates BMI in normal weight range; orange indicates BMI in underweight range. \* $P < 0.001$ . BMI, body mass index; n.s., not significant.

in women (11.8% vs. 8.2%,  $P < 0.001$ ); in contrast, there were no significant sex-related differences in the incidences of gallstones or gallstones and gallbladder polyps (Figure 1). Among participants without *H. pylori* infection, the incidence of gallbladder polyps was higher in men than in women (10.7% vs. 8.1%,  $P < 0.001$ ), whereas the incidence of gallstones was lower in men than in women (3.1% vs. 3.7%,  $P = 0.021$ ).

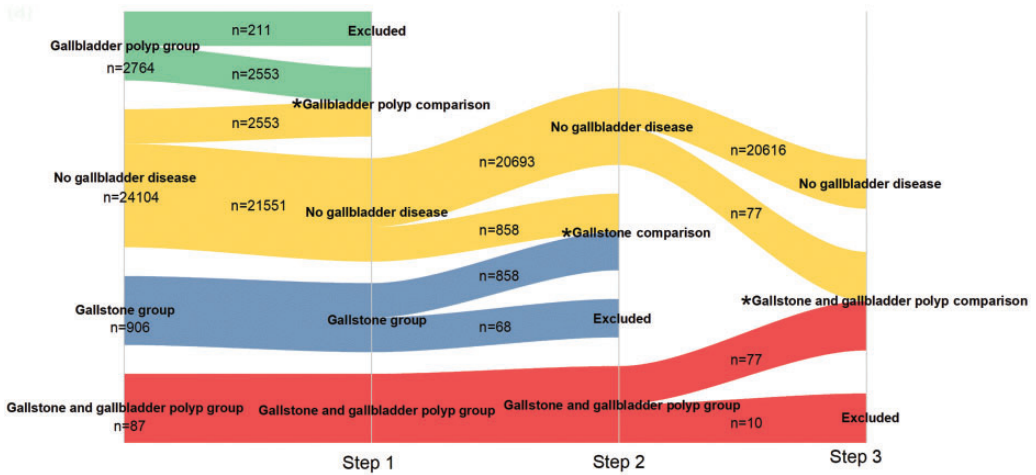
#### Case-control matching analysis of *H. pylori* infection according to gallbladder disease status

Based on the above outcomes, a 1:1 case-control matching protocol was implemented to match the essential confounding parameters among the P, S, and SP groups in a sequential manner. In step 1, there were

2764 participants in the P group. After matching, 211 participants were excluded from the P group; 2553 participants remained as the case group. In addition, 2553 participants from the N group were selected as the control group. At the end of the step 1, the remaining 21,551 participants in the N group were used as candidates for step 2. The same matching approach was used for the S and SP groups; the procedure is shown in Figure 5. Participants with missing BMI values were excluded from all groups. Participant characteristics in each group are shown in Table 1. The proportion of participants with *H. pylori* infection did not significantly differ between those with gallbladder polyps and those without gallbladder polyps. Similar results were observed concerning gallstones and



**Figure 4.** Ages and body mass indexes of participants with and without *Helicobacter pylori* infection. Red boxes, body mass index; blue boxes, age. Lines inside boxes indicate median values, boxes indicate inter-quartile ranges, and whiskers indicate minimum and maximum values. \* $P < 0.05$ .



**Figure 5.** Schematic of 1:1 case-control matching protocol performed to match the essential confounding parameters for the gallbladder polyp, gallstone, and gallstone and gallbladder polyp groups. \*indicates outcome of each step.

gallstones and gallbladder polyps. These results did not show significant relationships between *H. pylori* infection and the presence of gallbladder polyps or gallstones.

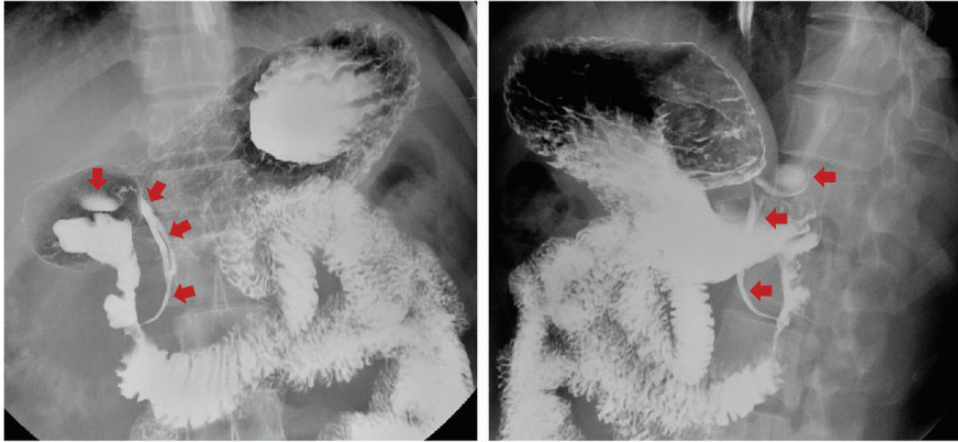
### Discussion

Multiple studies have shown that *H. pylori* species are present in patients with gallbladder diseases. Most studies involving

**Table 1.** Participant characteristics after case-control matching, stratified among gallbladder diseases.

	Gallstone and gallbladder polyp disease			Gallstone disease			Gallbladder polyp disease		
	Case group (n = 77)	Control group (n = 77)	P	Case group (n = 858)	Control group (n = 858)	P	Case group (n = 2553)	Control group (n = 2553)	P
	Age, years (mean ± standard deviation)	50.5 ± 9.6	50.1 ± 10.1	0.769	54.1 ± 12.6	54 ± 12.9	0.876	46.8 ± 11.9	46.7 ± 12.1
Sex, female (%)	25 (32.5)	25 (32.5)	1.000	373 (43.5)	373 (43.5)	1.000	842 (33.0)	842 (33.0)	1.000
<i>H. pylori</i> infection	30 (39.0)	33 (42.9)	0.623	398 (46.4)	420 (49.0)	0.288	1214 (47.6)	1157 (45.3)	0.110
Hypertension	19 (24.7)	10 (13.0)	0.064	269 (31.4)	217 (25.3)	0.005	570 (22.3)	484 (19.0)	0.003
Diabetes	2 (2.6)	5 (6.5)	0.246	55 (6.4)	49 (5.7)	0.544	74 (2.9)	78 (3.1)	0.742
Body mass index (kg/m <sup>2</sup> )	24.1 ± 2.9	22.8 ± 2.8	<0.001	25 ± 3.2	23.3 ± 3.1	<0.001	24.3 ± 3	22.2 ± 3.1	<0.001
Underweight	1 (1.3)	1 (1.3)	1.000	13 (1.5)	13 (1.5)	1.000	59 (2.3)	59 (2.3)	1.000
Normal weight	38 (49.4)	38 (49.4)		331 (38.6)	331 (38.6)		1192 (46.7)	1192 (46.7)	
Overweight	31 (40.3)	31 (40.3)		373 (43.5)	373 (43.5)		1038 (40.7)	1038 (40.7)	
Obesity	7 (9.1)	7 (9.1)		141 (16.4)	141 (16.4)		264 (10.3)	264 (10.3)	
Total bilirubin (μmol/L)	13.1 ± 4.0	14.4 ± 6.7	0.134	14.4 ± 6.3	14.4 ± 5.9	0.793	14.4 ± 5.9	14.5 ± 5.8	0.711
Direct bilirubin (μmol/L)	3.8 ± 1.2	4.3 ± 2.1	0.069	4.2 ± 2.0	4.2 ± 1.8	0.948	4.2 ± 1.7	4.3 ± 1.7	0.559
Indirect bilirubin (μmol/L)	9.3 ± 3.1	10.1 ± 5.0	0.211	10.2 ± 4.6	10.2 ± 4.4	0.743	10.2 ± 4.4	10.2 ± 4.3	0.791
Total bile acid (μmol/L)	4.9 ± 4.7	3.6 ± 2.4	0.130	4.9 ± 5.0	4.5 ± 3.3	0.214	4.7 ± 6.7	4.5 ± 4.1	0.410
Alanine aminotransferase (U/L)	25.3 ± 16.8	26.7 ± 36.5	0.760	26.7 ± 23.4	23.1 ± 19.3	0.001	25.1 ± 23.3	22.7 ± 17.6	<0.001
Aspartate aminotransferase (U/L)	22.7 ± 7.9	24.2 ± 17.2	0.498	24.8 ± 13.1	24.1 ± 11.9	0.258	23.3 ± 14.1	23.1 ± 9.4	0.494
Alkaline phosphatase (U/L)	30.8 ± 24.0	35.1 ± 57.6	0.540	36.6 ± 34	31.8 ± 36.8	0.005	32.2 ± 31.6	30.9 ± 37	0.188
Triglyceride (mmol/L)	1.8 ± 1.0	1.8 ± 1.6	0.998	2.0 ± 1.7	1.7 ± 1.9	0.007	1.8 ± 1.7	1.6 ± 1.4	<0.001
Total cholesterol (mmol/L)	5.1 ± 0.9	5.1 ± 1.1	0.998	5.1 ± 1.0	5.1 ± 1.0	0.383	5.0 ± 1.0	5.0 ± 0.9	0.642
High-density lipoprotein (mmol/L)	1.4 ± 0.3	1.4 ± 0.3	0.533	1.4 ± 0.3	1.5 ± 0.3	<0.001	1.4 ± 0.3	1.5 ± 0.3	<0.001
Low-density lipoprotein (mmol/L)	2.8 ± 0.7	2.7 ± 0.8	0.824	2.7 ± 0.7	2.6 ± 0.7	0.167	2.6 ± 0.7	2.6 ± 0.7	0.005
Leukocyte count (*10 <sup>9</sup> /L)	6.6 ± 1.5	6.4 ± 1.6	0.376	6.5 ± 2.1	6.1 ± 1.6	<0.001	6.3 ± 1.6	6.2 ± 1.6	0.014
Neutrophil granulocyte count (*10 <sup>9</sup> /L)	3.9 ± 1.2	3.7 ± 1.3	0.289	3.8 ± 1.3	3.6 ± 1.2	<0.001	3.7 ± 1.2	3.6 ± 1.2	0.015
Platelet count (*10 <sup>9</sup> /L)	242.5 ± 56.1	238.6 ± 51.6	0.649	232.5 ± 55.8	228.8 ± 54.7	0.174	237.3 ± 53.1	233.9 ± 51.9	0.022
Hemoglobin (g/L)	147.8 ± 17.8	146.6 ± 13.2	0.631	143.8 ± 15.2	142.5 ± 15.1	0.076	146.3 ± 15.5	145.4 ± 15.1	0.036

Data are shown as n (%), except where otherwise indicated



**Figure 6.** Barium reflux from the duodenum to the gallbladder in a 32-year-old man with duodenal ulcer due to *Helicobacter pylori* infection (red arrows).

detection of *H. pylori* species have demonstrated that the presence or absence of *H. pylori* is closely related to gallbladder disease status. For example, the proportion of patients with *H. pylori* in the biliary tract has reportedly ranged from 30% to 70%.<sup>21-23</sup> In a meta-analysis that included 15 studies regarding hepatobiliary tract cancers, *H. pylori* species were found in 42.9% of patients with biliary diseases. In that study, specimens were obtained from one or more of the following sites: bile, gallbladder, liver, tumor tissue, and non-tumor tissue; they were assessed by 16s rRNA or DNA-based polymerase chain reaction, histology, genus-specific primers, and/or *H. pylori* primers, suggesting that the results were generalizable to other contexts.<sup>24</sup> Another meta-analysis of 18 studies showed that *H. pylori* infection of the gallbladder was significantly associated with enhanced risks of chronic cholecystitis and cholecystitis.<sup>25</sup> However, Rudi et al.<sup>26</sup> found that no *H. pylori* DNA was present in 73 patients with acute or chronic cholecystitis. Moreover, *H. pylori* DNA was found in only one of 95 patients with chronic cholecystitis.<sup>27</sup> All *H. pylori* isolates were

obtained from patients (not from normal individuals) in those studies.

*H. pylori* can enter the bile duct by reflux from the duodenum when *H. pylori* is present in either gastric or gallbladder mucosa. Bansal et al.<sup>17</sup> found that 81% of patients with *H. pylori*-positive bile had *H. pylori* in the gastric mucosa, based on urea breath test results. Bulajic et al.<sup>21</sup> reported a strong association between the presence of *H. pylori* in the bile and the presence of *H. pylori* in the stomach. In our clinic, we have encountered barium reflux from the duodenum to the gallbladder in patients with duodenal ulcer due to *H. pylori* infection (Figure 6 shows a representative image of this reflux in a 32-year-old man). Hence, *H. pylori* is presumed to be present in the gallbladder or bile in such patients. However, this phenomenon does not indicate that *H. pylori* is a contributing factor in the onset of gallbladder diseases. Several types of bacteria have been detected in the bile duct, and microbial organisms contained in the bile from various biliary diseases are of intestinal origin. These species include isolates of *Enterococcus*, *Klebsiella*, *Escherichia coli*, *Enterobacter*, *Pseudomonas*, *Proteus*, and



*Streptococcus*.<sup>28,29</sup> The best objective evidence was obtained in a prospective study of C57L mice by Maurer et al. The results revealed no significant differences in formation of cholesterol monohydrate crystals, sandy stones, or true gallstones in *H. pylori*-infected mice, compared with uninfected mice, at the end of the 12-week study period. Therefore, the relationship between *H. pylori* and human gallbladder diseases requires further investigation.<sup>30</sup>

In the present study, we investigated the relationship between *H. pylori* and human gallbladder diseases by controlled epidemiological analysis. We found that the incidences of gallstones and gallbladder polyps differed according to sex (Figure 1). Moreover, the mean age and BMI levels significantly differed between participants with gallstones or gallbladder polyps, compared with participants who had no gallbladder diseases (Figures 2 and 3). Furthermore, the mean BMI values and mean age both significantly differed between participants, according to *H. pylori* infection status (Figure 4). The proportions of participants with *H. pylori* infection were similar between the gallbladder polyp and control groups after case-control matching with respect to age, sex, and BMI levels; similar results were observed gallstones and gallstones and gallbladder polyps. Although the proportion of participants with *H. pylori* infection was higher in the P group than in the N group, the P value was only 0.048 before case-control matching. Li et al.<sup>20</sup> reported that the presence of *H. pylori* infection, based on <sup>13</sup>C urease breath test results, was not associated with cholelithiasis in 8749 Chinese individuals.

There were several limitations in our study. First, selection bias was inevitable because this was a retrospective study without a rigorous design. Importantly, case-control matching was performed to reduce bias related to demographic factors.

Second, the urease breath test results only indicated the presence of ongoing infection, so the length of the infection could not be confirmed. Notably, the formation of gallbladder polyps or gallstones is presumed to be a long-term process. Long-term follow-up studies may enable more robust conclusions. The results of our research are contrary to the conclusions of most published articles, possibly because this was a cross-sectional study. Finally, our study did not investigate the underlying mechanism involved in any potential relationship between *H. pylori* and human gallbladder diseases.

Our results suggest that *H. pylori* infection is a contributing factor in the onset of gallbladder polyps or gallstones. Further prospective cohort studies are needed to confirm our findings.

#### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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