Original Article

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Surgery Section

Prevalence of *Helicobacter pylori* Infection in Gallbladder Specimens of Patients with Calculous Cholecystitis: A Cross-sectional Study

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ABSTRACT

Introduction: *Helicobacter pylori* (*H. pylori*) infection is highly prevalent in developing countries and is well known for its role in gastroduodenal diseases. In recent years, its possible association with extra-gastric disorders, including hepatobiliary diseases, has been increasingly studied. Gallstone disease is a common surgical condition with multifactorial aetiology, and the contribution of chronic infection to its pathogenesis remains unclear.

Aim: To determine the prevalence of *H. pylori* infection in gallbladder specimens of patients with calculous cholecystitis and to evaluate its association with clinicopathological parameters.

Materials and Methods: This hospital-based cross-sectional study was conducted in the Department of General Surgery in collaboration with the Department of Pathology, in tertiary hospital, Chennai, Tamil Nadu, India, between July 2024 and February 2026. A total of 85 patients aged 18-75 years with clinically and radiologically confirmed calculous cholecystitis who underwent laparoscopic or open cholecystectomy were included by consecutive sampling. Gallbladder specimens were examined histopathologically for detection of *H. pylori* and associated mucosal changes. Data were analysed using Statistical Package for the Social Sciences (SPSS) version 26.0. Categorical variables were compared using Chi-square test/ Fisher's exact test as appropriate and mean age was compared using student t-test. A p-value <0.05 was considered statistically significant.

Results: The mean age of the study population was 44.8±11.6 years. Females constituted 53/85 (62.4%) patients. Multiple gallstones were present in 58/85 (68.2%) patients. *H. pylori* infection was detected in 52/85 gallbladder specimens, giving a prevalence of 61.2%. *H. pylori* positivity was significantly associated with multiple gallstones (44/58, 75.9%) compared with single gallstones (8/27, 29.6%; p-value <0.001). Positivity was also higher in acute-on-chronic cholecystitis (10/12, 83.3%) than in chronic cholecystitis (42/73, 57.5%; p-value=0.046). No significant association was observed with age (p-value=0.160) or sex (p-value=0.470).

Conclusion: A considerable proportion of patients diagnosed with calculous cholecystitis exhibited infection with *H. pylori*. The observed association between this infection and an increased incidence of gallstones, coupled with the presence of inflammatory alterations, implies a potential role for *H. pylori* in the pathogenesis of gallstone disease.

Keywords: Biliary tract diseases, Cholecystectomy, Cross-sectional studies, Histological techniques, Inflammation

INTRODUCTION

Helicobacter pylori infection remains one of the most prevalent chronic bacterial infections worldwide, with a particularly high burden in developing countries. Although the organism is primarily known for its role in gastritis and peptic ulcer disease, recent studies have suggested that its effects may extend beyond the stomach and involve extra-gastric organs, including the hepatobiliary system [1,2]. Persistent infection can induce chronic inflammatory responses and metabolic alterations, which may influence disease processes in the biliary tract [3].

Gallstone disease is one of the most common hepatobiliary disorders encountered in clinical practice. The formation of gallstones is a multifactorial process involving cholesterol supersaturation, impaired gallbladder motility, bile stasis, and chronic inflammation [4]. Recent studies have suggested that infectious agents may also contribute to gallstone formation by altering bile composition and promoting inflammatory changes within the gallbladder wall [5-7].

Helicobacter species have been proposed as possible contributors to gallbladder disease because of their ability to survive in adverse environments and produce urease, which can modify the local biochemical milieu. Detection of *Helicobacter* organisms in bile, gallstones, and gallbladder mucosa have been reported in several investigations, suggesting that colonisation of the biliary tract may be associated with gallstone formation [8,9]. Systematic review and meta-analyses data had demonstrated a higher prevalence of *H. pylori* infection in patients with gallbladder disease, although the strength of association varies across different studies [4,10].

Recent clinical evidence has further supported a possible relationship between *H. pylori* infection and gallstone disease. Cen L et al., reported a potential bidirectional association between infection and gallstone formation through mechanisms involving chronic inflammation and altered bile composition [5]. A recent multicentre study by Yao SY et al., showed that individuals with *H. pylori* infection had a significantly higher prevalence of gallstones compared with uninfected individuals [8]. Studies from the Indian population have also demonstrated a considerable frequency of *H. pylori* detection in gallbladder specimens of patients with calculous cholecystitis [11,12].

However, findings across different studies are not uniform, and variations in diagnostic methods, population characteristics, and geographic distribution of infection may explain these inconsistencies. Therefore, institution-based studies using histopathological evaluation are required to better understand the role of *H. pylori* in gallstone disease.

Hence, the present study was undertaken to determine the prevalence of *H. pylori* infection in gallbladder specimens of patients with calculous cholecystitis undergoing cholecystectomy and to evaluate its association with clinicopathological parameters.

MATERIALS AND METHODS

This was a single-centre, hospital-based, cross-sectional study conducted in the Department of General Surgery in collaboration with the Department of Pathology, Chennai, Tamil Nadu, India, between July 2024 and February 2026. The study was conducted after obtaining approval from the Institutional Ethics Committee (IEC Approval No: 002/SBMCH/IHEC/2024/2240). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional committee and the Declaration of Helsinki, 1975, as revised in 2013. Written informed consent was obtained from all participants before enrolment, and confidentiality of patient information was maintained throughout the study.

Inclusion criteria: Patients aged 18-75 years of either sex with clinically and radiologically confirmed calculous cholecystitis who underwent laparoscopic or open cholecystectomy during the study period were included in the study.

Exclusion criteria: Patients with a previous history of *H. pylori* eradication therapy, patients deemed unfit for surgery and patients unwilling to participate were excluded from the study.

Sample size: The sample size was calculated using the formula for estimating a single proportion: $n=Z^2\times P\times(1-P)/d^2$, where Z is the standard normal deviate at 95% confidence (1.96), P is the expected prevalence of *H. pylori* positivity in gallstone disease and d is the absolute precision. Taking an expected prevalence of 5.6% from previous study by Raza DM et al., and an absolute precision of 5%, the calculated sample size was $n=(1.96^2\times0.056\times0.944)/(0.05^2)=81.28$ [13]. This was rounded to 85, and a total of 85 eligible patients were enrolled by consecutive sampling.

Study Procedure

Eligible patients underwent detailed clinical evaluation, routine preoperative investigations and abdominal ultrasonography to confirm gallstones and document single or multiple stones. Depending on clinical indication and operative feasibility, patients underwent laparoscopic or open cholecystectomy. Resected gallbladder specimens were fixed in formalin and sent to the Department of Pathology. Sections from the gallbladder mucosa were examined using routine haematoxylin and eosin staining, and special staining methods as per institutional pathology protocol were used wherever required to identify *H. pylori*-like organisms. Histopathological diagnosis was recorded as chronic cholecystitis or acute-on-chronic cholecystitis. The use of routine histopathology with special staining was chosen because it was feasible, cost-effective and available within the institutional diagnostic workflow for all cholecystectomy specimens; however, the absence of immunohistochemistry or polymerase chain reaction confirmation was considered while interpreting the findings.

STATISTICAL ANALYSIS

Data regarding age, sex, ultrasonographic findings, type of surgery, histopathological diagnosis and *H. pylori* status were entered in Microsoft Excel and analysed using SPSS version 26.0. Categorical variables were expressed as frequency and percentage. Association between categorical variables was analysed using Chi-square test or Fisher's exact test as appropriate. Mean age between *H. pylori*-positive and negative patients were compared using Student's t-test. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 85 patients diagnosed with calculous cholecystitis and undergoing cholecystectomy were included in the study. The mean age of the study population was 44.8±11.6 years. The most frequent age group was 41-50 years (23/85, 27.1%), followed by 31-40 years (21/85, 24.7%). Females constituted 53/85 (62.4%) of the study population, showing a female predominance [Table/Fig-1].

Age group (years)	Male n	Female n	n (%)
18-30	5	7	12 (14.1)
31-40	7	14	21 (24.7)
41-50	9	14	23 (27.1)
51-60	7	12	19 (22.4)
>60	4	6	10 (11.7)
Total	32	53	85 (100.0)

[Table/Fig-1]: Demographic characteristics of the study population (n=85).

On ultrasonography, multiple gallstones were present in 58/85 (68.2%) patients, while 27/85 (31.8%) had a single gallstone. Laparoscopic cholecystectomy was performed in 73/85 (85.9%) patients and open cholecystectomy in 12/85 (14.1%). [Table/Fig-2].

H. pylori gave an overall prevalence of 61.2% [Table/Fig-3].

Variables	Category	n (%)
USG finding	Single gallstone	27 (31.8)
	Multiple gallstones	58 (68.2)
Type of surgery	Laparoscopic	73 (85.9)
	Open	12 (14.1)
Histopathology	Chronic cholecystitis	73 (85.9)
	Acute-on-chronic cholecystitis	12 (14.1)

[Table/Fig-2]: Clinical and histopathological profile of the study population (n=85).

H. pylori status	n (%)
Positive	52 (61.2)
Negative	33 (38.8)

[Table/Fig-3]: Prevalence of *H. pylori* infection in gallbladder specimens (n=85).

No statistically significant association was observed between *H. pylori* infection and sex (p-value=0.470) or mean age (p-value=0.160). However, *H. pylori* positivity was significantly more frequent among patients with multiple gallstones than among those with single gallstones (44/58, 75.9% vs 8/27, 29.6%; p-value <0.001). *H. pylori* positivity was also significantly higher among patients with acute-on-chronic cholecystitis compared with chronic cholecystitis (10/12, 83.3% vs 42/73, 57.5%; p-value=0.046) [Table/Fig-4].

Variable†	Category	*H. pylori*+	*H. pylori* –	Total	p-value
Sex†	Male	18	14	32	0.470
	Female	34	19	53	
Gallstones†	Single	8	19	27	<0.001*
	Multiple	44	14	58	
Histopathology††	Chronic cholecystitis	42	31	73	0.046*
	Acute-on-chronic cholecystitis	10	2	12	
Age (years)^ Mean±SD		43.2±10.8	47.1±12.3		0.160

[Table/Fig-4]: Association between *H. pylori* infection and clinicopathological variables.
*p-value <0.05 was considered significant; test of significance was analysed by Chi-square test†, Fisher's-exact test†† and independent t-test^

DISCUSSION

The present study was conducted to determine the prevalence of *H. pylori* infection in gallbladder specimens of patients with calculous cholecystitis and to evaluate its association with clinicopathological parameters. In this study, *H. pylori* was found in 61.2% of the

S. No.	Author and year	Place of study	Sample size	Parameters assessed	Conclusion/comparison
1	Silva CP et al., 2003 [14]	Brazil	54 gallbladder tissue specimens	*Helicobacter* DNA and cholelithiasis	*Helicobacter* DNA was detected in gallbladder tissue, supporting possible biliary colonisation in patients with cholelithiasis.
2	Attaallah W et al., 2013 [9]	Turkey	94 patients	Gallbladder mucosa and gastric *H. pylori* status	Studied gallbladder mucosa in symptomatic gallstone disease and reported evidence supporting the possible association of *H. pylori* with gallstone disease.
3	Geetika KC and Talwar OP, 2014 [15]	Nepal	500 specimens	Histomorphology and *Helicobacter* infection	Reported *Helicobacter*-associated inflammatory changes in gallbladder specimens, supporting a possible role in gallbladder inflammation.
4	Wang L et al., 2021 [4]	Systematic review/ meta-analysis	Published studies	*Helicobacter* infection, cholecystitis and cholelithiasis	Reported a positive association between gallbladder *Helicobacter* infection and cholecystitis/cholelithiasis, although heterogeneity across studies was noted.
5	Raza DM et al., 2022 [13]	India	Calculous cholecystitis patients	Gallbladder *H. pylori* and calculous cholecystitis	Provided Indian evidence supporting an association between *H. pylori* infection and calculous cholecystitis.
6	Yao SY et al., 2024 [8]	China/multicentre and meta-analysis	Multicentre study and pooled studies	*H. pylori* infection and cholelithiasis risk	Reported a positive association between *H. pylori* infection and cholelithiasis, especially in Asian populations.
7	SB Vasanthbalan et al., 2026	India, Chennai	85 patients	*H. pylori* status, stone number and histopathology	*H. pylori* positivity was 61.2%; infection was significantly associated with multiple gallstones and acute-on-chronic cholecystitis, but not with age or sex.

[Table/Fig-5]: Similar studies from the literature [4,8,9,13-15].

gallbladder samples, indicating a high rate of infection in patients with gallstone disease. This finding supports the hypothesis that a long-term infection could contribute to gallbladder problems, rather than being a coincidence. The high prevalence observed in this study was consistent with results of Silva CP et al., suggesting that *Helicobacter* species can be detected in biliary specimens and may be associated with cholelithiasis or chronic cholecystitis [14]. Lim KPK et al., also concluded that *H. pylori* have been reported in cholelithiasis, cholecystitis, gallbladder polyps and biliary tract malignancies, although the causal relationship remains incompletely established [2].

H. pylori positivity was significantly higher among patients with multiple gallstones than those with single gallstones. Multiple gall stones may cause increased mucosal irritation, impaired bile flow and larger surface area for *H. pylori* colonisation. Cen L et al., reported a potential bidirectional association between *H. pylori* infection and gallstone disease, while Yao SY et al., observed a positive association between *H. pylori* infection and cholelithiasis risk, particularly in Asian populations [5,8]. The present finding was therefore clinically relevant because multiple gallstones may represent longer disease duration, recurrent bile stasis and repeated inflammatory injury.

The study also demonstrated significantly higher *H. pylori* positivity in acute on chronic cholecystitis compared with chronic cholecystitis alone. This suggests that infection may be related to active inflammatory activity within the gallbladder wall. Similar tissue-level observations have been described by Attaallah W et al., and Geetika KC and Talwar OP who reported detection of *H. pylori* or *Helicobacter*-like organisms in gallbladder specimens with inflammatory changes [9,15].

In the present study, gallstone disease was more common in females and in the fourth to sixth decades of life, which was consistent with the known epidemiological pattern of gallstone disease. However, no statistically significant association was observed between *H. pylori* positivity and age or sex. Similar findings were reported by Raza DM et al., in an Indian study, where infection rates did not differ significantly across demographic groups [13]. These observations suggest that local pathological factors within the gallbladder, such as bile stasis and chronic inflammation, may be more important determinants of bacterial colonisation than demographic variables alone. Similar studies from the literature are presented in [Table/Fig-5] [4,8,9,13-15].

The specific process through which *H. pylori* potentially fosters gallstone development is still not fully understood. Some researchers have posited that the organism's urease activity could cause bile alkalinisation and subsequent precipitation of calcium salts, thus promoting stone formation [16]. Furthermore, the chronic inflammatory response triggered by ongoing infection might compromise gallbladder motility and encourage bile stasis, a critical factor in lithogenesis [5]. Moreover, molecular studies have shown *Helicobacter* DNA in gallbladder tissue, supporting the idea of real biliary colonisation rather than just contamination [17].

In contrast, not all studies have shown a clear association between *H. pylori* infection and the development of gallstone disease. Variability across studies may stem from differences in diagnostic methods, geographical differences in infection rates, and small sample sizes [4]. Therefore, studies done in specific institutions, using consistent histopathological methods, are still important for better understanding the relationship between infection and gallbladder disease.

The present study adds to the existing literature by providing data from a tertiary care centre in India, where both *H. pylori* infection and gallstone disease are highly prevalent. Unlike many previous studies, the present study evaluated the association of *H. pylori* with multiple clinicopathological parameters including gallstone number, histopathological diagnosis, and demographic factors using uniform histopathological examination of gallbladder specimens [9,14,16]. The demonstration of a significant association between *H. pylori* infection, multiple gallstones, and acute on chronic inflammatory changes suggests that the organism may be involved in disease progression rather than merely being an incidental finding. Further studies with larger sample size and molecular diagnostic methods are required to clarify the exact role of *H. pylori* in gallbladder pathology.

Limitation(s)

The present study had certain limitations. The study was conducted at a single centre, which may limit generalisation of the results. Detection of *H. pylori* was based on histopathological examination alone, and molecular methods were not used for confirmation. In addition, the cross-sectional design does not allow establishment of a causal relationship between infection and gallstone formation.

Despite these limitations, the high prevalence of *H. pylori* infection observed in this study suggests that chronic infection may be an additional factor influencing gallbladder inflammation and stone formation, particularly in regions where the organism is endemic. Further multicentric studies using molecular techniques are required to clarify the exact role of *H. pylori* in the pathogenesis of gallstone disease and to determine whether eradication therapy could have any preventive or therapeutic benefit.

CONCLUSION(S)

The present study demonstrated a high prevalence of *H. pylori* infection in patients with calculous cholecystitis. A significant association was observed between *H. pylori* positivity and the presence of multiple gallstones as well as acute on chronic inflammatory changes in the gallbladder. However, no significant relationship was found with age or sex. These findings suggest that *H. pylori* infection may act as a contributory factor in the progression and severity of gallstone disease rather than being a mere incidental finding.

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