RESEARCH PAPER

A Prospective Comparative Study on the Association of Helicobacter pylori Infection with Cholelithiasis and Disease Severity in Patients with H. pylori–Associated Gastritis

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ABSTRACT

Background: Cholelithiasis is a common biliary disorder with a multifactorial etiology, and emerging evidence suggests a possible role of infectious agents in its pathogenesis. Helicobacter pylori, a well-established gastric pathogen, has been proposed as a potential contributor to gallbladder disease; however, existing data remain inconsistent.

Aim: To evaluate the association between Helicobacter pylori infection and cholelithiasis in patients undergoing cholecystectomy.

Methods: This case–control study was conducted at a tertiary care center over a defined study period. Patients diagnosed with cholelithiasis based on ultrasonographic findings were enrolled. Preoperative endoscopic biopsies were obtained, and gallbladder tissue samples were collected following cholecystectomy. Detection of H. pylori was performed using histopathological examination with Giemsa staining and molecular identification through polymerase chain reaction targeting the 16S rRNA gene. Participants were categorized into case and control groups based on the presence or absence of H. pylori. Comparative analysis was carried out to assess the association between H. pylori infection and cholelithiasis, including its clinical severity.

Results: The study demonstrated the presence of H. pylori in gallbladder tissue among a subset of patients with cholelithiasis. A comparative evaluation between the study groups indicated an association between H. pylori infection and cholelithiasis, with differences observed in disease presentation based on infection status.

Conclusion: The findings suggest a possible association between Helicobacter pylori infection and cholelithiasis. The use of combined histopathological and molecular diagnostic techniques enhances detection accuracy and supports further investigation into the pathogenic role of H. pylori in gallstone disease.

Keywords: Cholelithiasis, Helicobacter pylori, Cholecystectomy, Gallbladder Diseases, Polymerase Chain Reaction

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1. INTRODUCTION

1.1 Clinical and Epidemiological Relevance of Cholelithiasis

Cholelithiasis is one of the most common disorders of the biliary tract and represents a significant global health burden. Gallstone disease affects a substantial proportion of the adult population worldwide, with prevalence rates varying widely based on geographic region, ethnicity, dietary habits, and genetic predisposition [1,2]. In India and other developing countries, changing lifestyle patterns and increasing metabolic risk factors have contributed to a rising incidence of gallstone disease [3].

Clinically, cholelithiasis exhibits a broad spectrum ranging from asymptomatic gallstones detected incidentally to symptomatic disease presenting with biliary colic, acute cholecystitis, choledocholithiasis, or pancreatitis [4]. Although many individuals remain asymptomatic, a considerable proportion eventually require surgical intervention, making cholecystectomy one of the most frequently performed abdominal surgeries worldwide [5]. Despite extensive research, the etiopathogenesis of gallstone formation remains multifactorial and incompletely understood, prompting continued investigation into additional contributory factors.

1.2 Pathogenesis of Cholelithiasis and the Role of Inflammation

The pathogenesis of cholelithiasis is complex and multifactorial, involving an interplay of biochemical, mechanical, and inflammatory mechanisms. Cholesterol gallstone formation is primarily attributed to cholesterol supersaturation of bile, nucleation of cholesterol crystals, impaired gallbladder motility, and prolonged bile stasis within the gallbladder lumen [6,7]. Pigment stones, although less common, are associated with altered bilirubin metabolism and chronic hemolytic states [8]. However, these classical mechanisms do not fully account for the wide variability observed in disease occurrence and clinical presentation.

Increasing evidence suggests that chronic inflammation of the gallbladder mucosa plays a crucial role in gallstone formation and progression. Inflammatory changes can alter bile composition, promote mucin hypersecretion, and facilitate crystal nucleation, thereby accelerating stone formation [9]. Gallbladder epithelial injury and inflammatory cell infiltration have also been shown to impair gallbladder contractility, further contributing to bile stasis and stone growth [10].

Despite well-established metabolic and mechanical risk factors, cholelithiasis has been observed in individuals without significant predisposition, indicating the involvement of additional pathogenic contributors. This has led to growing interest in the potential role of infectious agents in gallstone disease, particularly organisms capable of inducing chronic inflammation within the biliary system [11].

1.3 *Helicobacter pylori* – Beyond Gastric Disease and Biological Plausibility

Helicobacter pylori is a gram-negative, microaerophilic bacterium that chronically colonizes the human gastric mucosa and is well established as a causative agent in chronic gastritis, peptic ulcer disease, and gastric malignancy [12,13]. Owing to its ability to persist in hostile environments and induce sustained inflammatory responses, *H. pylori* infection has also been implicated in several extra-gastric disorders, including hepatobiliary and pancreatic diseases [14].

The pathogenic potential of *H. pylori* extends beyond the stomach due to its virulence factors, such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), which promote epithelial injury, immune dysregulation, and chronic inflammation [15]. These mechanisms provide biological plausibility for the organism's survival and pathogenicity in extra-gastric sites. Experimental and clinical studies have suggested that *Helicobacter* species may reach the biliary system via the duodenum or portal circulation, where they may colonize bile, gallbladder mucosa, or gallstones [16].

The biliary environment, once considered sterile, is now recognized as a potential niche for microbial colonization under pathological conditions. The presence of chronic inflammation and bile stasis in gallbladder disease may further facilitate bacterial persistence, thereby contributing to local inflammatory changes and disease progression [17]. These observations have led to increasing interest in the possible role of *H. pylori* as a contributing factor in the pathogenesis of cholelithiasis.

1.4 Evidence Linking *Helicobacter pylori* and Cholelithiasis

Several studies have explored the potential association between *H. pylori* infection and gallstone disease, with varying and often conflicting results. Early investigations demonstrated the presence of *Helicobacter* species in bile, gallbladder mucosa, and gallstones, suggesting a possible role in the initiation or progression of cholelithiasis [18,19]. These findings supported the hypothesis that *H. pylori*–induced chronic inflammation may contribute to gallstone formation by altering bile composition and promoting gallbladder mucosal injury.

Subsequent clinical and molecular studies have reported differing prevalence rates of *H. pylori* detection in gallbladder specimens from patients with cholelithiasis. While some authors have demonstrated a significant association between *H. pylori* infection and gallstone disease using histopathological staining and polymerase chain reaction (PCR) techniques, others have failed to establish a consistent causal relationship [20–22]. Variations in study design, geographic population, sample size, and detection methodology have been cited as major factors contributing to these discrepancies.

Importantly, molecular techniques such as polymerase chain reaction (PCR) are regarded as highly sensitive methods for the detection of *Helicobacter* DNA in tissue specimens when compared to conventional histological techniques, particularly in cases of low organism density [23].This has raised concerns that reliance on a single diagnostic modality may underestimate the true prevalence of *H. pylori* in gallbladder tissue. Furthermore, limited data are available correlating *H. pylori* presence with the clinical severity or presentation of cholelithiasis, underscoring the need for well-designed comparative studies using combined diagnostic approaches.

1.5 Knowledge Gap, Rationale, Aim, and Objectives of the Present Study

Despite growing interest in the possible association between *H. pylori* infection and cholelithiasis, the existing literature remains inconclusive. Variability in study designs, geographic populations, sample sizes, and diagnostic methodologies has resulted in inconsistent findings regarding the presence and pathogenic significance of *H. pylori* in gallbladder disease. In particular, many studies have relied on a single detection technique, potentially underestimating the true prevalence of *Helicobacter* species within gallbladder tissue.

Furthermore, limited data are available from prospective case–control studies that employ both histopathological and molecular diagnostic approaches to evaluate *H. pylori* infection in patients with cholelithiasis. The relationship between *H. pylori* presence and the clinical severity of cholelithiasis also remains insufficiently explored. These gaps highlight the need for well-designed studies integrating multiple diagnostic modalities to better clarify the potential role of *H. pylori* in gallstone disease.

In this context, the present study was undertaken to evaluate the association between *Helicobacter pylori* infection and cholelithiasis in patients undergoing cholecystectomy, using a combined histopathological and molecular approach.

1.5.1 Aim

To assess the association between *H. pylori* infection and cholelithiasis in patients undergoing cholecystectomy.

1.5.2 Objectives

1. To detect the presence of *H. pylori* in gallbladder tissue using histopathological examination and molecular techniques.
2. To compare the prevalence of *H. pylori* infection between cases and controls.
3. To evaluate the relationship between *H. pylori* infection and the severity of cholelithiasis.

2. MATERIALS AND METHODS

2.1 Study Design

This case-control study was conducted to investigate the association between *H. pylori* infection and cholelithiasis in patients undergoing cholecystectomy. The study was carried out at Chettinad Hospital and Research Institute, Kelambakkam, over a period from August 2021 to June 2023.

2.2 Inclusion Criteria

Patients diagnosed with cholelithiasis, including both symptomatic and asymptomatic cases as confirmed by ultrasonography, were considered eligible for inclusion. Preoperative endoscopic biopsies were obtained from all consenting participants.

2.3 Sample Size

A total of 60 patients were enrolled, with 30 individuals assigned to each study group.

2.4 Data Collection

1. Preoperative Biopsy: Endoscopic biopsies were collected from patients diagnosed with cholelithiasis prior to undergoing cholecystectomy.
2. Cholecystectomy and Tissue Collection: Following cholecystectomy, gallbladder tissue samples were harvested for further analysis.
3. Histopathological Examination: Gallbladder specimens were subjected to histopathological evaluation. The presence of *H. pylori* was assessed using Giemsa staining. In addition, polymerase chain reaction (PCR) amplification targeting the 16S rRNA gene with *Helicobacter* genus-specific primers was performed for molecular confirmation of *H. pylori*.
4. Study Groups: Participants were categorized into two groups:
   - Case Group: Patients with evidence of *H. pylori* association.
   - Control Group: Patients with cholelithiasis but without *H. pylori* infection.
5. Comparative Analysis: A prospective comparative approach was employed to evaluate the potential role of *H. pylori* as a causative agent in cholelithiasis. The severity of cholelithiasis was analyzed in relation to the presence or absence of *H. pylori* infection.

2.5 Significance of the Study

This study aims to provide insights into the potential relationship between *H. pylori* infection and cholelithiasis, elucidating its possible role in disease pathogenesis and severity. The combination of histopathological and molecular diagnostic methods ensures accurate detection of *H. pylori*.

2.6 Statistical Analysis

Descriptive statistics were reported as mean ± standard deviation (SD) for continuous variables and as frequencies (percentages) for categorical variables. Associations between categorical variables were evaluated using the Chi-square test, while independent t-tests were applied for comparisons of continuous variables between groups. All analyses were conducted using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Chicago, IL).

3. RESULTS

3.1 Baseline Demographic and Clinical Characteristics of the Study Population

The baseline demographic and clinical characteristics of the study participants are summarised in Table 1. A total of 60 individuals were included in the analysis, comprising 30 cases and 30 controls.

Table 1. Baseline demographic and clinical characteristics of the study participants (N = 60)

Variable	Cases (n = 30)	Controls (n = 30)	Test statistic	p-value
Age (years)	38.97 ± 9.96	41.30 ± 8.84	t = −0.96	0.34
Gender				
• Male	15 (50%)	12 (40%)	χ^2 (1) = 0.61	0.43
• Female	15 (50%)	18 (60%)		
Residence				
• Rural	15 (50%)	15 (50%)	χ^2 (1) = 0.00	1.00
• Urban	15 (50%)	15 (50%)		
Socioeconomic status				
• Upper	7 (23.3%)	10 (33.3%)	χ^2 (3) = 2.67	0.44
• Upper middle	8 (26.7%)	4 (13.3%)		
• Upper lower	7 (23.3%)	10 (33.3%)		
• Lower	8 (26.7%)	6 (20.0%)		

Values are expressed as mean ± standard deviation or number (percentage). Continuous variables were compared using independent sample t-test and categorical variables using Chi-square test. A p-value <0.05 was considered statistically significant.

The mean age of participants in the case group was 38.97 ± 9.96 years, while the control group had a mean age of 41.30 ± 8.84 years. Statistical comparison using an independent sample t-test demonstrated no significant difference in age distribution between the two groups (t = −0.96, p = 0.34), indicating that cases and controls were age-matched.

Gender distribution was comparable across both groups. Among cases, males and females were equally represented, each accounting for 50% of participants. In the control group, females constituted 60% and males 40% of participants. This difference was not statistically significant (χ^2 = 0.61, p = 0.43), suggesting no gender-based imbalance between the groups.

Residence status showed complete comparability between cases and controls. Both groups had an equal distribution of rural and urban participants, with 50% residing in rural areas and 50% in urban areas. No statistically significant difference was observed in residence distribution (χ^2 = 0.00, p = 1.00).

Socioeconomic status was assessed across four categories namely upper, upper middle, upper lower, and lower and demonstrated a similar pattern of distribution between the two groups. Among cases, participants were distributed relatively evenly across the socioeconomic strata, with 23.3% classified as upper, 26.7% as upper middle, 23.3% as upper lower, and 26.7% as lower socioeconomic status. A comparable distribution was observed among controls, and the overall difference between groups was not statistically significant (χ^2 = 2.67, p = 0.44).

Collectively, these findings indicate that the case and control groups were well matched with respect to age, gender, residence, and socioeconomic status, thereby minimising potential confounding due to baseline demographic and clinical variables.

3.2 Gallstone Characteristics and Familial Predisposition

3.2.1 Number of Gallstones

The distribution of gallstones among cases and controls is presented in Table 2. Single gallstones were identified in 16 cases (53.3%) and 11 controls (36.7%), while multiple gallstones were observed in 14 cases (46.7%) and 19 controls (63.3%). Although multiple gallstones were more frequently observed among controls and single gallstones were more common among cases, this difference did not reach statistical significance ($\chi^2 = 1.68$, $p = 0.19$). The findings indicate that the number of gallstones was comparable between the two study groups.

3.2.2 Family History of Gallstone Disease

The distribution of gallstone number did not differ significantly between cases and controls. Although single gallstones were more frequently observed among cases (53.3%) compared to controls (36.7%), and multiple gallstones were more common among controls (63.3%) than cases (46.7%), this difference did not reach statistical significance ($\chi^2 = 1.68$, $p = 0.19$). These findings suggest that the number of gallstones, whether single or multiple, was not independently associated with case status in the present study. The distribution of gallstone number did not differ significantly between cases and controls (Figure 1).

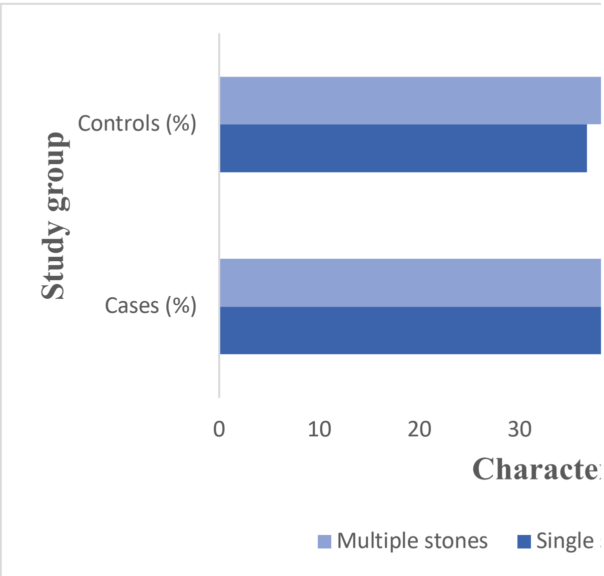

Fig. 1 Distribution of gallstone number among cases and controls. Values are expressed as percentages. Comparison was performed using the Chi-square test.

A positive family history of gallstone disease was reported by 18 cases (60.0%) and 13 controls (43.3%), whereas 12 cases (40.0%) and 17 controls (56.7%) reported no such history. Statistical analysis demonstrated no significant difference in the distribution of family history between cases and controls ($\chi^2 = 0.07$, $p = 0.79$). This suggests that familial predisposition to gallstone disease was similarly distributed across both groups.

Table 2. Gallstone characteristics and family history among the study participants (N = 60)

Variable	Cases (n = 30)	Controls (n = 30)	Test statistic	p-value
Number of gallstones				
• Single	16 (53.3%)	11 (36.7%)	χ^2 (1) = 1.68	0.19
• Multiple	14 (46.7%)	19 (63.3%)		
Family history of gallstone disease				
• Positive	18 (60.0%)	13 (43.3%)	χ^2 (2) = 0.07	0.79
• Negative	12 (40.0%)	17 (56.7%)		

Values are expressed as number (percentage). Comparisons between cases and controls were performed using the Chi-square test. A p-value <0.05 was considered statistically significant.

3.3 Severity of Gastritis Among Study Participants

The severity of gastritis differed markedly between cases and controls, as detailed in Table 3. None of the participants in the case group exhibited mild gastritis, whereas mild gastritis was observed in 16 individuals (53.3%) in the control group. In contrast, moderate gastritis was identified in 16 cases (53.3%) compared to 10 controls (33.3%).

Severe gastritis was present in nearly half of the cases, with 14 participants (46.7%) demonstrating severe disease, while only 4 participants (13.3%) in the control group exhibited severe gastritis. Overall, cases showed a predominance of moderate to severe gastritis, whereas controls were largely characterised by mild to moderate gastritis. The severity of gastritis differed significantly between cases and controls, with a higher proportion of moderate and severe gastritis observed among cases (Figure 2).

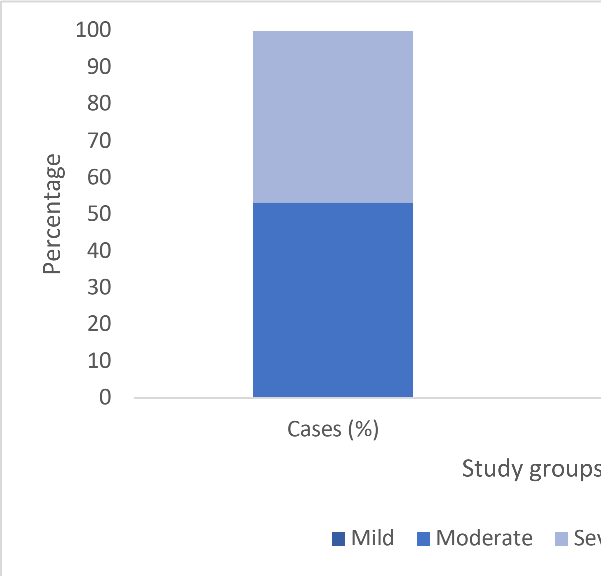

Fig. 2 Distribution of severity of gastritis among cases and controls. Values are expressed as percentages. Comparison between cases and controls was performed using the Chi-square test. A p-value <0.05 was considered statistically significant.

Statistical analysis revealed a highly significant difference in the distribution of gastritis severity between the two groups ($\chi^2 = 22.94$, $p < 0.001$). This finding indicates a substantially higher burden of advanced gastritis among cases compared to controls.

Table 3. Severity of gastritis among cases and controls (N = 60)

Severity of gastritis	Cases (n = 30)	Controls (n = 30)	Test statistic	p-value
Mild	0 (0.0%)	16 (53.3%)	χ^2 (2) = 22.94	**<0.001**
Moderate	16 (53.3%)	10 (33.3%)		
Severe	14 (46.7%)	4 (13.3%)		

Values are expressed as number (percentage). Comparison of gastritis severity between cases and controls was performed using the Chi-square test. A p-value <0.05 was considered statistically significant.

3.4 Detection of *Helicobacter pylori* in Gallbladder Tissue

Detection of *H. pylori* in gallbladder tissue was assessed among cases using both histopathological and molecular methods, as summarised in Table 4. Histopathological evaluation using Giemsa staining demonstrated the presence of *H. pylori* in 24 out of 30 cases (80.0%), while 6 cases (20.0%) showed no evidence of the organism on staining.

Molecular analysis using 16S rRNA PCR identified *H. pylori* positivity in 21 cases (70.0%), whereas 9 cases (30.0%) were negative by this method. Overall, a substantial proportion of cases demonstrated evidence of *H. pylori* on both histopathological and molecular assessment. *H. pylori* were detected in a substantial proportion of cases using both histopathological and molecular methods (Figure 3).

Table 4. Detection of *Helicobacter pylori* in gallbladder tissue among cases (n = 30)

Diagnostic method	Result	n (%)
Giemsa stain	Positive	24 (80.0)
	Negative	6 (20.0)
16S rRNA PCR	Positive	21 (70.0)
	Negative	9 (30.0)

Values are expressed as number (percentage). Giemsa staining represents histopathological detection, while 16S rRNA PCR represents molecular detection of *Helicobacter pylori* in gallbladder tissue.

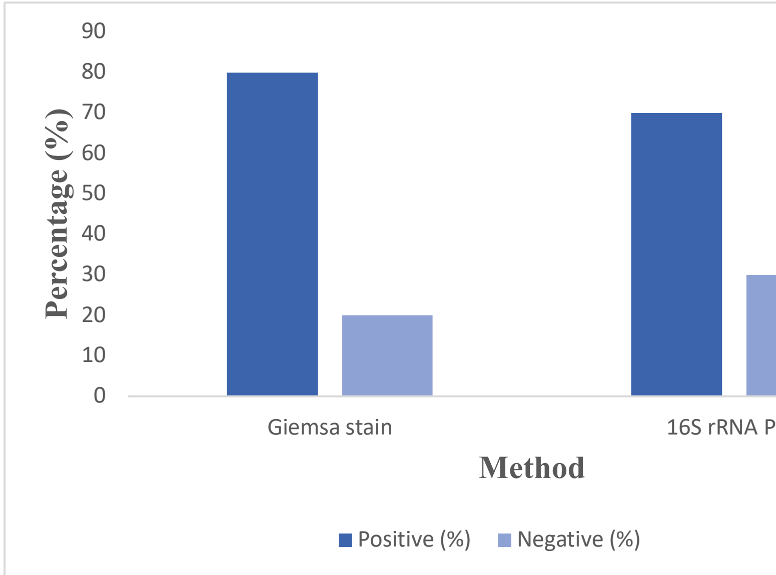

Fig. 3 Detection of *H. pylori* in gallbladder tissue among cases. Detection of *H. pylori* in gallbladder tissue among cases using histopathological (Giemsa stain) and molecular (16S rRNA PCR) methods. Values are expressed as percentages.

4. DISCUSSION

In this prospective case–control study, we investigated the presence of *H. pylori* in gallbladder tissue among patients with cholelithiasis and examined its relationship with gastritis severity and clinical characteristics. A key finding of our study was the significantly higher burden of moderate to severe gastritis among cases compared with controls. Additionally, a substantial proportion of gallbladder tissues from cases tested positive for *H. pylori* by both histopathological and molecular methods. In contrast, gallstone number and family history did not differ significantly between the groups.

4.1 Severity of Gastritis and Its Clinical Implications

Our results demonstrate a significantly greater severity of gastritis among cases compared with controls ($p < 0.001$). None of the cases exhibited mild gastritis, whereas more than half of the controls did. The predominance of moderate and severe gastritis in cases suggests that underlying gastric mucosal inflammation may be more pronounced in individuals with cholelithiasis. This observation is consistent with earlier clinical findings showing that *H. pylori* infection is strongly associated with the degree of gastric mucosal inflammation in patients with gallstone disease. In a study by Stathopoulos et al., moderate to marked gastritis was predominantly linked to *H. pylori* infection in symptomatic gallstone patients, whereas patients with mild or no gastritis seldom exhibited *H. pylori* positivity on histological examination [24].

Moreover, systematic evidence supports a broader relationship between *H. pylori* presence and hepatobiliary conditions. A meta-analysis of multiple studies found that *H. pylori* infection of the gallbladder is associated with an increased risk of chronic cholecystitis and cholelithiasis, suggesting that bacterial colonisation and associated inflammation may contribute to biliary pathology [25].

While the precise mechanisms underlying these associations remain under investigation, such findings reinforce the concept that *H. pylori*-associated gastritis may be part of a broader inflammatory milieu that influences extra-gastric tissues and could contribute to gallstone-related disease processes.

4.2 Detection of *Helicobacter pylori* in Gallbladder Tissue

In our study, *H. pylori* were detected in 80.0% of cases by Giemsa staining and 70.0% by 16S rRNA PCR, indicating a high prevalence of the organism in gallbladder tissue among patients with cholelithiasis. These detection rates support the hypothesis that *H. pylori* or related Helicobacter species may colonise the biliary tract in the context of gallstone disease. Molecular evidence from a nested PCR analysis in a cohort of cholelithiasis patients showed the presence of *H. pylori* DNA in a subset of gallstones, bile, and gallbladder mucosa specimens, underscoring the potential relevance of Helicobacter species in biliary tissue. In that study, *H. pylori* strains were confirmed by sequencing of 16S rRNA amplicons from multiple hepatobiliary samples, suggesting that these bacteria are not confined to gastric epithelium but can be present in biliary structures as well [26].

Previous investigations report wide variability in the detection of *H. pylori* or Helicobacter DNA in biliary tissues, reflecting differences in methodological sensitivity, population characteristics, and specimen types examined. For example, in a study of symptomatic gallstone patients, *H. pylori* DNA was identified in approximately 37% of gallbladder mucosa samples when assessed with a combination of histochemical and immunohistochemical techniques, a lower rate than observed in the present cohort [27]. Another investigation documented *H. pylori* DNA in bile and gallbladder wall specimens of patients with benign biliary disease, and also demonstrated a significant correlation between gastric and biliary presence of the organism, highlighting the potential for gastrointestinal colonisation to extend into the biliary environment [19]. These discrepancies in detection rates among studies may be attributed to differences in diagnostic modalities (e.g., nested PCR versus conventional PCR, histology, culture, and immunohistochemical assays), regional prevalence of *H. pylori*, and sample handling practices.

Although the demonstration of *H. pylori* DNA through molecular techniques provides evidence of presence, it does not directly prove organism viability or causal involvement in gallstone pathogenesis. Nevertheless, the consistent identification of *H. pylori* and Helicobacter DNA in gallstones, bile, and gallbladder mucosa across diverse settings reinforces the need for further research into the biological significance of these findings and their potential implications in biliary disease mechanisms.

4.3 Gallstone Characteristics and Familial Factors

Despite the substantial detection of *H. pylori* and the marked difference in gastritis severity observed in the present study, no statistically significant difference was found in the distribution of single versus multiple gallstones between cases and controls ($p = 0.19$). Similarly, family history of gallstone disease did not differ significantly between the two groups ($p = 0.79$). These findings suggest that gallstone burden and hereditary predisposition may not be directly associated with *H. pylori* status in this cohort, and that other metabolic or environmental determinants could modulate these relationships.

The association between *H. pylori* infection and gallstone disease has been variably reported in the literature. A large multicentre retrospective study and meta-analysis involving more than 70 000 participants found a positive association between *H. pylori* infection and cholelithiasis, with pooled analyses showing an increased risk particularly in Asian populations [28]. Similarly, cross-sectional evidence from a large Japanese cohort demonstrated that *H. pylori* infection was significantly associated with gallstone prevalence after adjustment for demographic and metabolic risk factors [29]. In contrast, some population-based analyses have reported inconsistent associations between *H. pylori* and gallbladder diseases, including a large retrospective study in China that found differing relationships between *H. pylori* status, gallstones, and related biliary conditions depending on age group and disease phenotype [30].

These discrepant findings likely reflect geographic, methodological, and population differences across studies. Variations in diagnostic criteria for *H. pylori* infection, gallstone detection methods, and the mix of metabolic and genetic risk factors examined may contribute to inconsistent phenotypic associations, such as gallstone number or familial predisposition. Taken together, while *H. pylori* infection appears to be linked with an increased risk of gallstone disease in some settings, its influence on specific gallstone characteristics and hereditary factors remains inconclusive and warrants further rigorous investigation.

4.4 Mechanistic Considerations

While our study was not designed to elucidate mechanisms, the co-occurrence of severe gastritis and *H. pylori* detection in gallbladder tissues raises questions regarding potential pathogenetic pathways. One hypothesis is that chronic gastric colonisation leads to systemic inflammation and altered bile composition, which may facilitate cholesterol crystallisation and gallstone formation. Others posit that ascending infection from the duodenum to the biliary tree may allow direct colonisation of the gallbladder, contributing to local inflammation and mucosal changes. However, definitive mechanistic evidence remains limited and warrants further basic science and translational research.

4.5 Strengths and Limitations

A key strength of the current study is the use of both histopathological and molecular methods to detect *H. pylori*, improving the robustness of detection compared with single modality approaches. The case–control design also allowed direct comparison of gastritis severity and bacterial presence between groups.

However, limitations should be acknowledged. The sample size was modest, which may restrict generalisability. Additionally, the presence of *H. pylori* DNA by PCR does not necessarily indicate live infection or direct causality. Finally, the study did not assess other Helicobacter species, which some research suggests may also be involved in biliary pathology.

4.6 Clinical and Research Implications

The findings underscore the potential importance of evaluating *H. pylori* status in patients with cholelithiasis and significant gastritis. While current evidence does not establish causality, the frequent detection of *H. pylori* in gallbladder tissue and its association with gastritis severity suggest that gastrointestinal microbial factors may contribute to biliary disease phenotypes. Future larger, multicentre studies and mechanistic investigations are needed to clarify whether *H. pylori* eradication could influence gallstone development or clinical outcomes.

5. CONCLUSION

The present study demonstrates a significant association between cholelithiasis and increased severity of gastritis, with cases exhibiting a markedly higher prevalence of moderate to severe gastritis compared to controls. A substantial proportion of gallbladder tissue samples from cases showed evidence of *Helicobacter pylori* on both histopathological examination and molecular analysis, supporting the presence of the organism in the biliary system.

In contrast, the number of gallstones and family history of gallstone disease did not differ significantly between cases and controls, indicating that these factors were not independently associated with *H. pylori* detection or disease grouping in this cohort. These findings suggest that while gallstone burden and hereditary predisposition may contribute to cholelithiasis, they do not appear to influence the association between *H. pylori* and disease severity.

Overall, the study highlights a potential link between *H. pylori* infection and the severity of gastritis in patients with cholelithiasis, reinforcing the need to consider gastrointestinal microbial factors in the clinical evaluation of such patients. Further large-scale and multicentric studies are warranted to clarify the pathogenic role of *H. pylori* in biliary disease and to determine its possible implications for clinical management and preventive strategies.

Statements and Declarations

Competing Interests

The authors declare that they have no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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Ethics Approval

The study was approved by the Institutional Ethics Committee of Chettinad Hospital and Research Institute **(IHEC-I/1768/23)** and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Consent to Participate

Written informed consent was obtained from all participants prior to their inclusion in the study.

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions (CRediT Statement)

Divya Padmakumar: Conceptualization, Investigation, Data curation, Methodology, Writing – original draft preparation. **Harish R:** Conceptualization, Investigation, Data collection, Formal analysis, Writing – original draft preparation, Correspondence and manuscript submission. **Mahadevan Andiyappan:** Methodology, Supervision, Validation, Writing – review and editing. **R. Anantharamakrishnan:** Supervision, Project administration, Conceptual guidance, Writing – review and editing, Final approval of the manuscript. All authors read and approved the final version of the manuscript.

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