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Original Research Article

Association of Helicobacter Pylori Infection with Cholelithiasis: A Histopathological Study in Patients Undergoing Cholecystectomy

S. N. Gole[1], Vijayalaxmi Kosma[2]

[1,2]Department of General Surgery, Pt. Jawaharlal Nehru Memorial Medical College and Dr. Bhim Rao Ambedkar Memorial Hospital, Raipur, Chhattisgarh, India

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Corresponding Author: Dr. S. N. Gole
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Abstract:

Background: Gallstones are among the most common digestive and hepatobiliary diseases. Chronic cholecystitis, cholelithiasis, or symptomatic gallbladder disease refers to a long-term mechanical or functional impairment in gallbladder emptying. Approximately 75% of gallstones are cholesterol-based, with 25% being pigment stones made up of bilirubin and calcium, which are important bile ingredients. Ultrasonography, CT scans, ERCP, liver function tests, and pancreatic enzyme evaluations are used to make the diagnosis. The presence of H. pylori in the biliary epithelium among hepatobiliary disease patients has been investigated intermittently. Helicobacter pylori (H. pylori) is a gram-negative, spiral-shaped, microaerophilic bacterium. Evidence suggests H. pylori DNA in bile, gallbladder tissue, and cholesterol gallstones. The link between gallstones and Helicobacter pylori has been examined by various researchers but remains inconclusive.

Methods: This study sought to evaluate Helicobacter pylori infection as a potential risk factor for gallstone disease. Gallbladder mucosa histopathological samples were stained using modified Giemsa, hematoxylin and eosin, and Warthin-Starry methods.

Results: The study identified H. pylori in two cases, lacking statistical significance. Furthermore, reliance on H&E stains alone necessitates confirmation via immunohistochemistry and PCR. Additional research is essential to confirm a causal link between Helicobacter pylori infection and gallstone development.

Conclusions: The role of Helicobacter pylori as a gallstone risk factor remains unconfirmed.

Keywords: Biliary sludge, Cholecystectomy, Cholecystitis, Cholelithiasis, H. pylori.

Introduction

Gallstones are one of the most prevalent digestive and hepatobiliary disorders, and they are frequently surgically treated. Chronic cholecystitis, cholelithiasis, or symptomatic gallbladder refers to prolonged mechanical or functional gallbladder emptying failure. The symptoms range from moderate, vague complaints to severe right upper quadrant pain. The majority of patients experience recurring biliary colic, although pain lasting more than 24 hours necessitates immediate surgery; otherwise, elective cholecystectomy is recommended after conservative treatment. [1-2] Gallstone development is influenced by several factors, including chemical composition, structure, morphology, and microbiology. [3-4] Gallstones form as solid particles settle in bile, generating sediment. The major solutes in bile include bilirubin, bile salts, phospholipids, and cholesterol. [5] Approximately 75% of gallstones are cholesterol, 25% pigment stones including bilirubin and calcium from bile. [6-7]

Clinical manifestations range from nausea, vomiting, and dyspepsia to severe right hypochondrial/epigastric pain, jaundice, fever, and shock. Diagnosis uses ultrasonography, CT, ERCP, liver tests, and pancreatic enzymes. [8-9] The presence of H. pylori in biliary epithelium for hepatobiliary conditions has been investigated sporadically. [10-11]

Figure 1: 3D image of Helicobacter pylori. https://commons.wikimedia.org/wiki/ File:Helicobacter_pylori_3D.png

H. pylori is a gram-negative, spiral, microaerophilic bacterium. [4] It resides in the stomach, linked to acute/chronic gastritis, gastric/duodenal ulcers, gastric/pancreatic adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. [12-13] Literature indicates H. pylori DNA in bile, gallbladder, and cholesterol gallstones. [14-15]

Monstein et al. suggested H. pylori DNA in cholesterol gallstones might indicate normal flora or predisposition via biliary colonization. [4] Recent research has linked H. pylori to extra digestive disorders. DNA from bile-tolerant Helicobacter spp. can be found in human bile, populating the biliary tract. [17] Most H. pylori infections are detected by immunological assays and urease breath tests, rather than molecular approaches, and there is no cultural isolation. [18] The link between gallstones and H. pylori is investigated but not confirmed.

H. pylori's biliary entrance mechanisms are uncertain, but may involve duodenal translocation through the Oddi sphincter or hematogenous liver spread and bile excretion. [20] The purpose of this study was to determine whether Helicobacter pylori was a risk factor for gallstones. H. pylori is a curved gram-negative rod colonizing the stomach, tied to peptic ulcers and gastric cancer. Aims and objectives is to determine if Helicobacter pylori risks gallstone disease patients and any association.

Study Design and Setting: This hospital-based observational study was conducted in the Department of General Surgery, Pt. J.N.M. Medical College and associated Dr. B.R.A.M. Hospital, Raipur, Chhattisgarh, India, over a period of 12 months from July 2021 to July 2022.

Study Population: Patients diagnosed with gallstone disease, cholecystitis, biliary sludge, or cholelithiasis and planned for elective cholecystectomy were considered for inclusion.

Inclusion Criteria

- Patients with confirmed gallbladder pathology (gallstone disease, cholecystitis, biliary sludge, or cholelithiasis)
- Patients fit for elective cholecystectomy after routine clinical and laboratory evaluation
- Patients providing informed written consent

Exclusion Criteria

- Patients who did not provide consent
- Patients deemed unfit for cholecystectomy

Sample Size: A total of 50 consecutive eligible patients undergoing cholecystectomy during the study period were included.

Specimen Collection and Processing: Tissue sections were prepared from representative areas of each specimen. Gallbladder specimens obtained after cholecystectomy were submitted to the Department of Pathology for histopathological examination.

Histopathological Examination: All 50 specimens were examined microscopically for the

presence or absence of H. pylori. Sections were stained using the following methods to detect Helicobacter pylori: Hematoxylin and eosin (H&E) stain, Modified Giemsa stain, Warthin–Starry silver stain

Outcome Measure: Results were recorded as presence or absence based on histopathological staining. The primary outcome was the detection of H. pylori in gallbladder tissue.

Ethical Considerations: The study was conducted after obtaining approval from the Institutional Ethics Committee of Pt. J.N.M. Medical College, Raipur. Written informed consent was obtained from all participants prior to inclusion.

Results

Patient Demographics: A total of 50 patients undergoing elective cholecystectomy for gallstone disease were included in the study. The mean age of the study subjects was 46.5 ± 13.8 years, reflecting a middle-aged population commonly affected by cholelithiasis. The age distribution showed a bimodal pattern, with the highest number of cases in the 41-50 years age group (28%, n=14), followed closely by the 51-60 years group (24%, n=12). Younger patients (≤40 years) accounted for 20% (n=10), while those aged 61-70 years and >70 years represented 18% (n=9) and 10% (n=5), respectively. This distribution aligns with the known epidemiology of gallstone disease, which peaks in the fourth to sixth decades of life due to cumulative risk factors such as obesity, diet, and hormonal influences.

Gender analysis revealed a clear female predominance, with females comprising 64% (n=32) of the cohort and males 36% (n=18). This female-to-male ratio of approximately 1.8:1 is consistent with global trends, where estrogen-related factors (e.g., multiparity, oral contraceptive use) contribute to higher bile cholesterol saturation and gallstone formation in women.

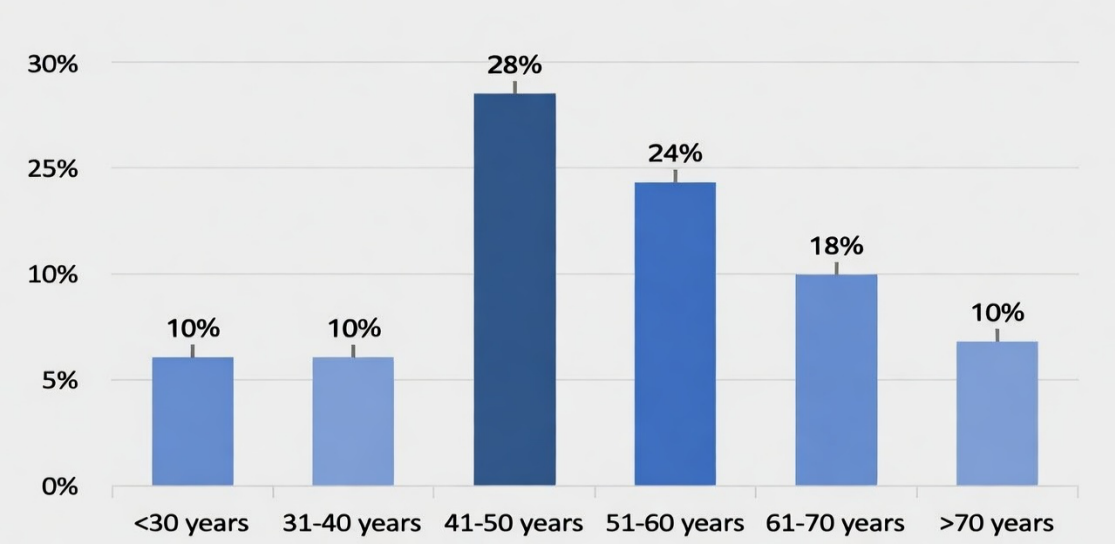

Figure 2: Age distribution among study subjects. (Bar chart illustrating the percentage of patients in each age group: ≤30 years: 10%; 31-40: 10%; 41-50: 28%; 51-60: 24%; 61-70: 18%; >70: 10%.

The chart highlights the peak in mid-life, with error bars representing standard deviation where applicable.)

Clinical Presentation: All patients (100%, n=50) presented with abdominal pain as the primary symptom, underscoring its universal role in symptomatic gallstone disease.

The duration of pain varied from 1 to 9 months, with the maximum number of patients reporting symptoms lasting 1-3 months (48%, n=24). Shorter durations (<1 month) were noted in 20% (n=10), 4-6 months in 18% (n=9), and prolonged symptoms (>6 months) in 14% (n=7). This pattern suggests that most patients sought medical attention during acute or subacute exacerbations, potentially delaying diagnosis in chronic cases.

Associated symptoms included vomiting in 58% (n=29) of cases, often accompanying pain episodes and indicative of biliary colic or complications like cholecystitis. Other less frequent symptoms, such as nausea (not quantified in detail but noted in clinical records), fever, or jaundice, were reported in a minority, emphasizing the nonspecific nature of early presentations.

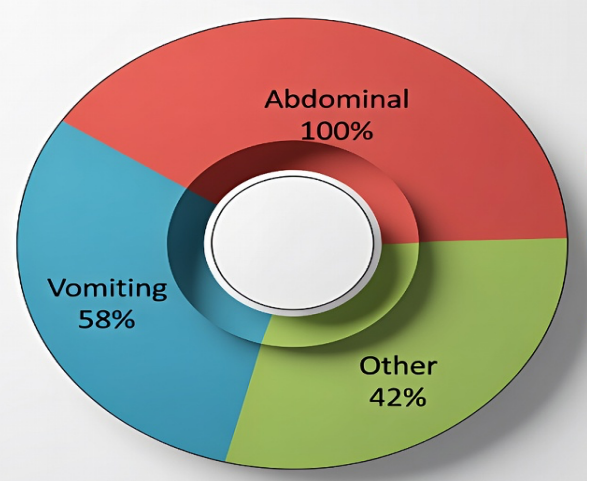

Figure 3: Clinical features/presentation. (Pie chart depicting the distribution of symptoms: Abdominal pain: 100%; Vomiting: 58%; Other (e.g., nausea, fever).

Comorbidities and Risk Factors: Comorbidities were assessed to evaluate potential confounding factors in gallstone pathogenesis. A significant majority were non-diabetic (84%, n=42), with only 16% (n=8) having diabetes mellitus, which is a known risk factor for cholesterol gallstones due to insulin resistance and altered bile composition. Similarly, 78% (n=39) were non-hypertensive, while 22% (n=11) had hypertension, another metabolic factor linked to gallstone formation through obesity and dyslipidemia. No significant correlations were observed between these comorbidities and H. pylori detection in this cohort, though larger studies may be needed to explore interactions.

Gallstone Characteristics: Gallstone types were classified based on gross and histopathological examination. Mixed gallstones predominated, present in 68% (n=34) of cases, followed by pure cholesterol stones in 26% (n=13) and pigmented stones in 6% (n=3). This distribution reflects the typical Western and Indian patterns, where cholesterol supersaturation drives most formations, with pigmented stones more common in hemolytic or infectious contexts.

The number of stones varied, with multiple stones found in 68% (n=34) and solitary stones in 32% (n=16). Gallbladder wall thickness, an indicator of chronic inflammation, was 4 mm in 38% (n=19), 3 mm in 44% (n=22), and >4 mm (suggesting acute or severe cholecystitis) in the remaining 18% (n=9). These findings highlight the chronic nature of the disease in most patients, with wall thickening potentially exacerbating symptoms.

H. pylori Detection: Histopathological staining revealed H. pylori in only two cases (4%, n=2), with the organism absent in the remaining 96% (n=48). This low detection rate was not statistically significant ($p > 0.05$, chi-square test), suggesting no strong association in this cohort. The positive cases showed characteristic features: sloughing of the gallbladder mucosa, inflammatory cell infiltrates (predominantly lymphocytes and neutrophils) in the lamina propria, and suspicious curved organisms on hematoxylin and eosin (H&E) stain. Confirmation was achieved with modified Giemsa and Warthin-Starry stains, which highlighted H. pylori on the denuded mucosal surface and embedded within mucinous material. No correlations were found between H. pylori positivity and variables such as age, gender, stone type, or comorbidities, further diminishing its role as a primary risk factor.

Table 1: H. pylori infection among study subjects.

H. pylori	Frequency	Percent (%)
Absent	48	96
Present	2	4
Total	50	100

In summary, the results indicate a typical profile of gallstone patients with female predominance, mid-life onset, and mixed stones as the most common type.

The minimal detection of H. pylori underscores the need for advanced diagnostic methods (e.g., PCR) to rule out under-detection due to staining limitations.

Discussion

The Department of General Surgery, Pt. J.N.M. Medical College, Raipur, investigated H. pylori as a gallstone risk factor. Abro et al. examined H.

pylori in calculous cholecystitis; mean age 48.72±8.78. [21] Fikry et al.: Cross-sectional study on H. pylori in chronic calculous cholecystitis; 62.1% females, mean age 39.04±7.3. [22]. Motie et al. studied 84 individuals with cholecystitis and H. pylori, with a mean of 45.19±1.78, and a 67.9% prevalence. Females have similar findings. All had gastrointestinal pain, with 48% experiencing it during the last three months and 58% vomiting. Fikry et al. found that 92% of patients experienced discomfort and 64% reported vomiting. Comorbidities: PUD, diabetes, and hypertension have been documented. [24-26] Complex GSD associated with prediabetic/diabetic individuals. Multiple stones: 68%, single: 32%. Stone size ranges from 6 to 14mm, with a maximum of 10mm (32%). Dhamnetiya et al. found 31.7% single, 68.3% multiple, and 54.2% ≤10mm. [7] Similar in others. [28-29] of 50, two H. pylori-positive. Cases showed mucosal sloughing, infiltrates, organisms on H&E. Abro et al.: 55% H. pylori in 100. Helaly et al.: Gallbladder H. pylori with quiescent gastritis (40.9%); etiological factor. Cen et al.: Meta-analysis; H. pylori linked to cholecystitis/cholelithiasis (OR=3.022). [28] Fatemi et al.: 77 cholecystitis; DNA in eight. [18] Khorsheed et al.: 95 patients; 33% positive. Two positives not significant. Tsuchiya et al., Ari et al.: No difference case-control. [3-] This study shows no relation, suggesting no role in gallstones. Bashir et al.: 150 specimens negative on Giemsa/IHC. Based on H&E/special stains; no IHC/PCR. Larger case-control with ancillaries needed.

Conclusion

Helicobacter pylori's involvement as a risk factor for gallstone disease is unproven. While some PCR-based research imply a link to gallbladder colonization, H. pylori is not the sole cause. Its well-established relationship to stomach pathology (gastritis and malignancy) does not hold true for cholelithiasis. In this investigation, only two positive cases were statistically insignificant. The absence of immunohistochemistry and PCR limits the conclusions. Larger, well-designed studies that eliminate confounders and use advanced techniques (special stains, IHC, PCR) are required to establish any probable causal association.

Ethical approval:

The study was approved by the Institutional Ethics Committee

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