An observational study to assess the \textit{Helicobacter pylori} infection rates in patients with cholelithiasis

Jeevan Kankaria, Santosh Madagond*, Akshita Bhat

Department of General Surgery, SMS Medical College, Jaipur, Rajasthan, India

Received: 11 April 2023
Revised: 10 May 2023
Accepted: 20 May 2023

*Correspondence:
Dr. Santosh Madagond,
E-mail: santoshmadagond@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Cholelithiasis and chronic cholecystitis cholelithiasis is fairly common all around the world. Since chronic inflammation causes repeated trauma to the gallbladder mucosa and DNA damage led by high or abnormal bile acid exposure, gallstones are closely related to the development of hepatobiliary cancers. After cholecystectomy, some patients experience ongoing upper abdomen pain, which is distressing for the surgeons and raises the possibility of a concomitant upper gastrointestinal issue, which in most cases turned out to be peptic ulcer disease. Numerous studies have found various Helicobacter species in the gallbladder tissue, gallstones, and bile taken from the gallbladder. We conducted this study to assess the relationship between cholelithiasis and \textit{H. pylori} infection in the stomach. Ascending infection of \textit{H. pylori} from the stomach and duodenum to biliary tree may have function in development of gall stone production and hepatobiliary carcinomas.

Methods: Hospital based cross sectional observational study on patients admitted in surgical wards of SMS Hospital Jaipur for laparoscopic cholecystectomy. All patients underwent UGI and gastric antral biopsy followed by laparoscopic cholecystectomy, gastric mucosa and GB mucosa were subjected to Warthin starry silver stain for detection of \textit{H. pylori}.

Results: Out of 116 patients who underwent upper GI endoscopy followed by laparoscopic cholecystectomy, 29 (25%) patients were positive for \textit{H. pylori} infection.

Conclusions: This study shows significant positive co-relation between \textit{H. pylori} infection in stomach, gall bladder to chronic cholecystitis and cholelithiasis, which signifies gastric colonisation of \textit{H. pylori} has it’s role in biliary pathologies through ascending infection, the effectiveness of \textit{H. pylori} eradication therapy in preventing these pathologies need to be studied further.

Keywords: Cholelithiasis, \textit{Helicobacter pylori}, Laparoscopic cholecystectomy, Upper GI endoscopy, Warthin starry silver stain

INTRODUCTION

A spiral-shaped, gram-negative bacteria known as Helicobacter pylori (\textit{H. pylori}) has been shown to be the primary pathogenic factor in the onset and progression of chronic gastritis, gastric ulcers, and duodenal ulcers\(^1\). Additionally, \textit{H. pylori} is linked to a higher risk of both gastric cancer and malignant lymphoma of the mucosa-associated lymphoid tissue (MALT)\(^2,4\) The proportion of helicobacter species infections in the digestive tract varies depending on the population, indicating epidemiological variations in the bacillus' dispersion across diverse nations and areas of India. Concerns regarding the role of \textit{Helicobacter pylori} in the...
pathophysiology of chronic cholecystitis and cholelithiasis are raised by the high prevalence of H. pylori in a group of patients with cholecystitis and cholelithiasis. Cholelithiasis and chronic cholecystitis cholelithiasis is fairly common all around the world. Gallstone illnesses are more common in certain geographic areas than others, particularly among certain ethnic groups. Between 4% and 74%, the prevalence rate in the west is higher than that in the east, and among adults in India, it is 6.12%.\(^5\) since chronic inflammation causes repeated trauma to the gallbladder mucosa and DNA damage led by high or abnormal bile acid exposure, gallstones are closely related to the development of hepatobiliary cancers, including gallbladder cancer.\(^5\) Therefore, chronic cholecystitis and cholelithiasis are major medical issues that place a heavy strain on society.

Since the last ten years, there has been a noticeable increase in the prevalence of gallstones in India, with the shift in lifestyle and the availability of more advanced imaging techniques being the most likely causes. Laparoscopic cholecystectomy is becoming common as minimal access surgery technology advances. After cholecystectomy, some patients experience ongoing upper abdomen pain, which is distressing for the surgeons and raises the possibility of a concomitant upper gastrointestinal issue, which in most cases turned out to be peptic ulcer disease. Numerous studies have found various Helicobacter species, including H. pylori, H. bilis, H. hepaticus, H. pullorum, and H. ganimani, in the gallbladder tissue, gallstones, and bile taken from the gallbladder since the unintentional discovery of H. pylori in the mucosa of a patient with cholecystitis in 1996.\(^7\)-\(^9\) H. pylori is the most prevalent type of Helicobacter among these diverse species. The DNA of H. pylori, a well-known human pathogen, has been found in bile, liver, and biliary epithelium taken from individuals with hepatobiliary disease in recent years.\(^10\)-\(^12\)

We conducted this study to assess the relationship between cholelithiasis and H. pylori infection in the stomach. Ascending infection of H. pylori from the stomach and duodenum to biliary tree may have function in development of gall stone production and hepatobiliary carcinomas.

**METHODS**

This was a hospital based cross sectional observational study. This study was conducted at Department of General Surgery, SMS Medical College and Hospital, Jaipur from April 2021 to November 2022. Patients admitted in surgical wards of SMS Hospital Jaipur for laparoscopic cholecystectomy after taking written informed consent.

**Sample size calculation**

Sample size 116 is calculated at confidence interval of 95% and with absolute error of 5% to verify the proportion of 18% of *Helicobacter pylori* infection in patients with cholelithiasis.\(^28\)

**Public health importance of the study**

Since H. pylori infection promotes the formation of gallstones, we consider whether H. pylori eradication therapy can prevent the formation of gallstones hence the morbidity and mortality associated with gall stone pathology can be prevented.

**Figure 1:** Warthin starry stain showing *H. pylori* bacilli.

**Figure 2:** HPE of antral biopsy showing gastric mucosa.

**Inclusion criteria**

Age - 18-75 years, all patients with upper abdominal pain more than 6 month with cholelithiasis and who given informed consent was included.

**Exclusion criteria**

Alcoholic patients, patients taking chronic NSAIDS, acute abdominal pain and immunocompromised patient were excluded.

A prior informed consent from the participants was taken. A pre-tested and pre-designed pro-forma was used to collect the data among the study population. On admission to hospital presenting complaints, detailed
history and examination findings were recorded and routine blood investigation were conducted. Ultrasonography Whole abdomen to look for GB wall thickness and CBD diameter was performed. Upper GI endoscopy with antral biopsy followed by laparoscopic/open cholecystectomy was performed. Findings of UGI were noted, KSS (Kankaria scoring system) was followed for performing safe laparoscopic cholecystectomy. Antral biopsy and GB mucosa were subjected to Warthin starry silver stain for detection of Helicobacter pylori. H. pylori triple regimen was started for all H. pylori positive cases.

**Outcome variables**

USG findings, UGI findings of gastric mucosa, HPE of gall bladder, H. pylori infection of stomach in patients with cholelithiasis, Helicobacter pylori infection of gall bladder mucosa outcomes were assessed.

**Data analysis**

The Data was compiled in MS Excel, and analyzed by SPSS version 29.0.0.0. The data is presented in table and graph whichever applicable. The data was analysed as per objectives. Inference is drawn with appropriate significant level.

**RESULTS**

**Demographic variables**

A total of 116 patients were studied, include 79 (68%) female and 37 (32%) male patients. Mean age (in years) of study group is 43.98±14.70, mean age (in years) of male patients is 45.97±14.70, mean age (in years) of female patients is 43.05±15.85. Maximum cases lie in age group 20-30 years (26 patients) accounting for 23% of study population. 25 patients including 7 male and 18 female patients in study group are diabetic, accounting for 21.5% of study group with p value of 0.004 indicating increased incidence of cholelithiasis in diabetic patients (Table 1, and 2).

### Table 1: Sex wise distribution of study group.

<table>
<thead>
<tr>
<th>Sex</th>
<th>H. pylori positive (%)</th>
<th>H. pylori negative (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>12 (32.4)</td>
<td>25</td>
<td>37 (32)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (21.5)</td>
<td>62</td>
<td>79 (68)</td>
</tr>
</tbody>
</table>

### Table 2: Distribution of diabetic patients in study group.

<table>
<thead>
<tr>
<th>Diabetes mellitus</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>7 (6)</td>
<td>18 (15.5)</td>
<td>25 (21.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Absent</td>
<td>30 (26)</td>
<td>61 (52.5)</td>
<td>91 (78.5)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

### Duration of symptoms

Mean duration of symptoms is 18.69 with SD of 6.07 which vary among Male patients (23.16±5.2) and female patients (16.6±5.2) with significant p-value 0.01 to positive H. pylori infection in chronic symptomatic cases, which infers association of H. pylori in chronic symptomatic cases. Mean duration of presentation in male patients is higher than female patients can be due to higher pain tolerance in male patients (Table 3).

### Table 3: Significance of H. pylori infection with duration of symptoms in male and female patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of symptoms</td>
<td>23.16±5.2</td>
<td>16.63±5.2</td>
<td>18.69±6.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Table 4: Co-relation of UGI findings with H. pylori infection rate.

<table>
<thead>
<tr>
<th>UGI findings</th>
<th>H. pylori positive (%)</th>
<th>H. pylori negative (%)</th>
<th>Total cases (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric ulcer</td>
<td>4 (22.2)</td>
<td>14 (77.7)</td>
<td>18 (15.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>Gastritis</td>
<td>9 (22.5)</td>
<td>31 (77.5)</td>
<td>40 (34.5)</td>
<td>0.45</td>
</tr>
<tr>
<td>Duodenitis</td>
<td>2 (40)</td>
<td>3 (60)</td>
<td>5 (4.5)</td>
<td>0.00</td>
</tr>
<tr>
<td>Normal study</td>
<td>14 (26.4)</td>
<td>39 (73.6)</td>
<td>53 (45.5)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### Table 5: Co-relation of USG findings to H. pylori infection rate.

<table>
<thead>
<tr>
<th>USG findings</th>
<th>H. pylori positive (%)</th>
<th>H. pylori negative (%)</th>
<th>Total (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholelithias</td>
<td>13 (19.4)</td>
<td>54 (80.6)</td>
<td>67 (57.5)</td>
<td>0.48</td>
</tr>
<tr>
<td>Chronic calculus</td>
<td>13 (34.2)</td>
<td>25 (65.8)</td>
<td>38 (33)</td>
<td>0.007</td>
</tr>
<tr>
<td>Acute calculus</td>
<td>1 (12.5)</td>
<td>7 (87.5)</td>
<td>8 (7)</td>
<td>0.68</td>
</tr>
<tr>
<td>Gb polyp</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>3 (2.5)</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Upper GI endoscopy findings

No significant findings are noted on upper GI endoscopy in 45.5% (53) patients, which include 36 (31%) female patients and 14.5% (17) male patients. Gastric ulcers are found in 18 (15.5%) patients, 40 (34.5%) patients got gastritis and 5 (4.5%) patients got duodenitis (Table 4).

When compared upper GI endoscopic positive findings with Warthin starry stain findings of antrum (P=0.413) and GB mucosa (P=0.868), no significant association found. Suggesting H. pylori colonisation in otherwise normal mucosa of stomach.

USG findings

Cholelithiasis is the most common (57.5%) finding in USG. There is significant association found between USG findings of chronic calculus cholecystitis 38 (33%) and H. pylori infection in gastric antrum and GB mucosa with p value of 0.007, there is no association with other findings. Cholelithiasis (P=0.48), acute calculus cholecystitis (P=0.68), GB polyp (P=0.38) (Table 5).

HPE of gall bladder

Significant association between HPE finding of chronic cholecystitis and H. pylori in Antrum and GB mucosa with P value of 0.021 (Table 7). Chronic cholecystitis is the most common finding (56%) in HPE of gall bladder followed by cholelithiasis (22.5%), acute on chronic cholecystitis (15.5%), acute cholecystitis (3.5%), GB polyp (2.5%) (Table 6).

| Table 7: Correlation of HPE findings of GB with H. pylori infection rate. |
|-------------------|-----------------|-----------------|-----------------|---------------|
| HPE findings      | H. pylori positive (%) | H. pylori negative (%) | Total (%)     | p-value       |
| Cholelithiasis    | 6 (23.0)         | 20 (77.0)        | 26 (22.5)      | 0.25          |
| Chronic cholecystitis | 17 (26.2)   | 48 (73.8)        | 65 (56)        | 0.16          |
| Acute on chronic cholecystitis | 3 (16.7) | 15 (83.3)        | 18 (15.5)      | 0.021         |
| Acute cholecystitis     | 1 (25)         | 3 (75)           | 4 (3.5)        | 0.00          |
| GB polyp            | 2 (66.7)         | 1 (33.3)         | 3 (2.5)        | 0.38          |

Table 8: Results of warthin starry stain.

| Warthin starry stain | Gb mucosa |                  |               |               |
|---------------------|-----------|-----------------|---------------|
|                     | Positive  | Negative        | Total         |
| Gastric antral mucosa | 5         | 19              | 24            |
| Positive            | 5         | 87              | 92            |
| Negative            | 10        | 106             | 116           |

Warthin starry stain for H. pylori results

There is significant association (P=0.017) between H. pylori infection in stomach antrum to H. pylori in GB mucosa. With rate of H. pylori infection in cholelithiasis being 25%. It suggest that there is significant association between H. pylori infection in stomach and GB.

Total 116 patients of chronic abdominal pain with cholelithiasis of which 37 (32%) were male and 79 (68%) were females. Mean age group of the study was 43.98±14.7. 25 (21.5%) patients were diabetic and 91 (78.5%) patients were non-diabetic. Mean duration of symptoms was 18.69±6.07, in male it was 23.16±5.2 and in female it was 16.63±5.2. Mean BMI of study group was 23.58±2.63. On upper GI endoscopy gastric ulcer was detected in 18 (15.5%) patients, gastritis was detected in 40 (34.5%) patients, duodenitis was detected in 5 (4.5%) patients, only normal findings was found in 53 (45.5%) patients. 67 (57.5%) patients were diagnosed as cholelithiasis, 38 (33%) patients were diagnosed as chronic calculus cholecystitis patients, 8 (7%) patients were diagnosed as acute calculus cholecystitis patients, 3 (2.5%) patients were diagnosed as GB polyp patients on basis of USG. Chronic cholecystitis was found in 65 (56%) cases, acute on chronic cholecystitis was found in 18 (15.5%) patients, acute cholecystitis was found in 4 (3.5%) patients, GB polyp was found in 3 (2%) patients on histopathological examination of gall bladder. H. pylori was detected on warthin starry stain in 29 (25%) patients of cholelithiasis of which 20.68% (24) detected in gastric antral mucosa, 8.6% (10) detected in GB mucosa, in 5 (4.3%) cases H. pylori detected in both GB mucosa and antral mucosa.
DISCUSSION

Gallstones are partly caused by *H. pylori*.

Due to inconsistent studies and ambiguous findings, the connection between *H. pylori* and gallbladder problems, particularly gallstones, is still up for debate. The danger of gallstones that resemble cholesterol is increased, however, according to sufficient data provided by the *H. pylori* bacterial population. There are numerous causes behind this illness, but current research has focused on the part played by *H. pylori*.

A study in the World Journal of Surgery Oncology claims that *H. pylori* releases a protein that functions similarly to an aminopeptidase enzyme and that this protein creates the conditions for gallstone development.\(^{14}\) This enzyme has the capacity to encourage the crystallisation of cholesterol. Consequently, the presence of *H. pylori* can cause gallstones and act as a catalyst for infection in the area surrounding them. As a result, the existence of *H. pylori* can influence the development of gallstones and act as a catalyst for infection around which a stone can form. In addition to releasing proteins, it also creates soluble antigens that may disrupt the conjugated bile acid cycle. This could cause aberrant bile acid transit times.

In addition to the previously described causes, *H. pylori*’s effects on the immune system as a whole are thought to indirectly contribute to cholelithiasis or stone development. *H. Pylori* makes the inflammation in the gallbladder worse.

Chronic cholecystitis has a wide range of recognised causes. A bacterial infection in the biliary system is one of them.\(^{10}\) Through the same method that it contributes to the emergence of several gastrointestinal disorders, *H. pylori* has been linked to gallbladder inflammation, according to numerous studies.

Laboratory investigations demonstrate that the cells lining an *H. pylori*-infected gallbladder are damaged, along with enlarged mitochondria and dilated endoplasmic reticulum. These are vital cell components required for the generation and transportation of proteins, as well as the production of energy. The results of *H. pylori* infection include a rapid decline in cell division, cell rupture, and cell death. The toxic components of *H. pylori* can activate elements that prevent cell proliferation, which ultimately results in cell death. Gallbladder cells exposed to *H. pylori* also induce the activation of inflammatory cells through cellular immunity, humoral immunity, and autoimmune.

**Gallbladder cancer and tumour development are made more likely by *H. pylori***

Due to the bacteria's function in inflammation, *H. pylori* can also cause tumours and gallbladder cancer, which is another gallbladder issue. The greater prevalence of adenomyomatosis and the formation of benign tumours are thought to be significantly influenced by *H. pylori*. Adenomyomatous hyperplasia of the gallbladder, is characterised by gallbladder wall thickening, cholesterol buildup, cholesterol crystallisation, and/or gallbladder enlargement. Even though adenomyomatosis is typically asymptomatic, it might be the first sign of gallbladder cancer.

The chronic inflammation caused by *H. pylori* in gallbladder cancer, on the other hand, is a hallmark of the disease. DNA is harmed as a result, and enzyme activity are also altered. *Helicobacter pylori* infection has been linked to the emergence of stomach cancer, according to a 1994 report from the International Agency for Research on Cancer. Non-Hodgkin's lymphoma has also been connected to *H. pylori*, in addition to stomach and gallbladder cancer.

The multiplication of *H. pylori* can have a negative impact on the liver, pancreas, and other closely connected organs within the biliary system in addition to the digestive tract and gallbladder.

The *Helicobacter* species can result in liver cancer, hepatitis, and serious immune system damage, according to studies in animal models. Pancreatic cancer is also known to be caused by *H. pylori* infection. *H. pylori* has now been linked to numerous problems outside of the biliary system, including skin disorders, coronary artery disease, autoimmune diseases, and infant growth retardation.

Takahashi et al found a statistically significant trend in the reduction of gallstones among the patients who received *H. pylori* eradication.\(^{16}\) However, the drawback is that they used the fasting C urea breath test and serum anti-*Hp* antibody test as detection methods for *H. pylori*.\(^{15}\) Although the two methods are highly specific and sensitive, they cannot locate *H. pylori* infection in the gallbladder, which may undermine the reliability of the study. Besides, the serological test is not a reliable test for evaluating eradication therapy because antibodies can be present in the blood for a long time even after successful eradication. Also, the presence of gallstones in the gallbladder was diagnosed by abdominal ultrasonography, which may lead to a false-negative result. In a word, the exact effect of eradicating *H. pylori* infection to prevent gallstone formation remains to be elucidated.

A comparative study of clinicopathological features between chronic cholecystitis patients with and without Helicobacter pylori infection in gallbladder mucosa by Di Zhou et al showed higher prevalence of acid regurgitation symptoms (p=0.001), more histories of chronic gastritis (p=0.005), gastric ulcer (p=0.042), duodenal ulcer (p = 0.026) and higher presence of *Helicobacter pylori* in the stomach as compared to patients without *Helicobacter*
pylori infection in the gallbladder mucosa and also Helicobacter pylori 16s rRNA in gallbladder and gastric-duodenal mucosa from the same individual patient had identical sequences. As compared to this study in our study H. pylori is identified in 22.2% of gastric ulcer patients, 22.5% of gastritis patients, 40% of duodenitis patients, 26.4% patients with no significant UGI findings. These findings are co-relating with previous study that suggest higher the H. pylori infection in stomach is the possible cause of H. pylori colonisation in biliary tract through ascending infection.

A study conducted by Khorsheed et al to determine the prevalence of helicobacter pylori among patients with cholelithiasis in 2019 using real time PCR technique for detection of H. pylori by UreC gene amplification in Gall bladder samples after cholecystectomy, detected H. pylori in 31 (33%) out of 95 patients. Compared this our study has less Positive results for H. pylori in GB specimen (10 out of 116). It can be due to gene amplification methods has higher H. pylori detection when compared to staining methods.

In this study we attempted to find a relation between Helicobacter pylori and cholelithiasis in patients undergoing cholecystectomy for symptomatic gallbladder stones, investigation by warthin starry silver stain showed that the incidence of H. pylori is 20.68% and 8.6% in gastric mucosa and GB mucosa respectively. In Basra, the results of Helicobacter detection in gallbladder specimens using PCR technique showed 36.23% positive gallbladder specimens and the bile specimens appear 75% positive for Helicobacter.

The presence of Helicobacter DNA was determined by nested polymerase chain reaction assay in Egyptian study, Helicobacter DNA was detected in the gallbladder tissue and bile of 28% and 18% respectively of the patients.

Compared to these studies our study has less incidence of H. pylori in gall bladder mucosa can be because of staining errors and less sensitivity of warthin starry stain compared to PCR technique.

This study has some limitations. Inter observer variations may also interfere with the yield of findings at endoscopy. Technique of staining for H. pylori i.e. warthin starry silver stain may yield false negative results in some samples. Sensitivity of staining technique is less when compared to genetic testing to identify Helicobacter pylori. The size of the sample in the current study is small therefore results can not be applied to the general population.

CONCLUSION

Our study shows significant positive co-relation between H. pylori infection in stomach, gall bladder and chronic cholecystitis with cholelithiastis, also it shows positive co-

relation between H. pylori colonisation in stomach and Gall bladder. Signifying the ascending infection of H. pylori from stomach to biliary tree through various route has a role in biliary colonisation of H. pylori. Colonisation of H. pylori in biliary tree might be a significant factor in development of cholelithiastis and chronic cholecystitis and biliary cancers.

The question of whether or not H. pylori infection eradication therapy can prevent gallstone formation is still in question. The association between them might be made clearer by additional randomised trials in the future, which might also offer clinical proof for the management of chronic cholecystitis/cholelithiasis.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES
