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A Clinical Study on Correlation of Helicobacter Pylori in Cholelithiasis in All Patients Presenting to Tertiary Care Centre

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Abstract: ***Introduction: Gallstones are a common gastrointestinal issue with various risk factors, including obesity, pregnancy, poor nutrition, and certain medical conditions. The prevalence of gallstones is higher in women, particularly in their 30s and 40s. The presence of culturable bacteria in bile, known as bactibilia, is associated with biliary system diseases. This study focuses on the role of Helicobacter pylori in gallstone formation and its presence in the gallbladder mucosa and bile. Objectives: 1) Assess the presence of H. pylori in gallbladder mucosa and bile. 2) Evaluate the risk of gallstone formation due to H. pylori. Methods: Patients undergoing cholecystectomy at Saraswati Institute of Medical Science were included. Data were collected through informed consent, history taking, and relevant investigations. Bile was aspirated during cholecystectomy and subjected to smearing, staining, and culture sensitivity testing. Gallbladder tissues were examined using Giemsa stain for H. pylori presence. Results: The study evaluated 100 patients for H. pylori using bile culture and staining techniques. The majority of patients were aged 51 - 60 years, with a higher prevalence in females. Most patients were overweight, and pigmented stones were common. Bile culture was positive in 45.5% of samples, with black and brown stones showing higher culture positivity. Helicobacter - like organisms were found in seven gallbladder mucosa samples. Conclusion: The study suggests a potential link between H. pylori and gallbladder diseases, particularly gallstone formation. Pigmented stones are more associated with bacterial presence, highlighting the need for further research on bacterial colonization in gallstone pathogenesis.***

Keywords: Gallstones; Helicobacter pylori; Bile culture; Gallbladder mucosa; Cholecystectomy; Biliary system; Bactibilia; Pigmented stones; Chronic cholecystitis; Gallstone formation

1. Introduction

Gallstone disease, medically termed cholelithiasis, is a common and significant health issue with wide - ranging implications for individuals and healthcare systems globally. This condition, marked by the formation of solid particles in the gallbladder or bile ducts, often leads to gastrointestinal distress and complications such as inflammation, infection, and even malignancy. Gallstone disease disproportionately affects certain populations, particularly women in their 30s and 40s, and exhibits regional and dietary variations, emphasizing its multifactorial nature.

In India, cholelithiasis remains a primary reason for abdominal surgeries, with a prevalence ranging between 3% and 6% among the population. The disease is notably more common in the northern regions compared to the southern parts, attributed to differences in diet, genetics, and environmental factors. Cholesterol stones dominate in northern India, whereas pigment stones are more prevalent in the south. Understanding the epidemiology of gallstones in the Indian context is essential for addressing their healthcare burden.

The pathogenesis of gallstone disease is multifaceted, involving genetic predisposition, metabolic imbalances, biliary stasis, and, increasingly, bacterial infections. The role of Helicobacter species, particularly Helicobacter pylori, in the formation and progression of gallstones has garnered interest in recent years. Helicobacter spp. are gram - negative bacteria traditionally linked to gastric conditions like ulcers and gastritis, but emerging evidence suggests their involvement in biliary diseases, including gallstones and gallbladder cancer.

This study explores the bacterial component of gallstone disease, with a specific focus on Helicobacter spp., to elucidate its role in the pathogenesis of benign gallbladder conditions. Insights from this research could pave the way for novel diagnostic and therapeutic approaches, ultimately reducing the disease's impact on patients and healthcare systems.

Background of the Study

Gallstones form due to imbalances in the composition of bile, a fluid composed of bile salts, cholesterol, phospholipids, and bilirubin. Cholesterol stones, mixed stones, and pigment stones represent the primary types, with variations in prevalence and composition influenced by geography, diet, and bacterial colonization.

Risk factors for gallstone formation include obesity, poor nutrition, certain medical conditions (e. g., Crohn's disease, thalassemia), and medications that increase cholesterol levels in bile. Additionally, biliary stasis and infections can further contribute to stone formation by altering bile composition and promoting bacterial growth.

Bacterial infections are increasingly recognized as key players in gallstone pathogenesis, particularly pigment stones. Helicobacter species, such as H. pylori, have been detected in bile and gallstones, suggesting their role in altering bile

biochemistry and promoting stone formation. While bile is typically sterile, bacterial colonization is more likely in cases of biliary obstruction or inflammation, leading to complications like acute or chronic cholecystitis.

India's gallstone disease burden highlights stark regional differences. Northern states exhibit higher rates of cholesterol and mixed stones, while pigment stones are more common in the south. This variability underscores the need for localized studies to understand the factors influencing gallstone formation, including bacterial involvement.

Need for the Study

Despite advances in understanding gallstone disease, the exact mechanisms underlying stone formation, particularly the role of bacteria, remain unclear. Gallstone - related complications, including acute cholecystitis and chronic inflammation, contribute significantly to healthcare challenges in India. Investigating bacterial involvement in gallstone disease could provide insights into its pathogenesis, enabling earlier detection, targeted interventions, and more effective management strategies.

Helicobacter spp., long associated with gastric diseases, have emerged as potential contributors to gallstone formation and gallbladder pathology. Studies identifying Helicobacter DNA in gallstones and bile suggest a pathogenic link, though findings vary by geography and methodology. This underscores the need for region - specific research, particularly in high - prevalence areas like India.

Statement of the Problem

This study seeks to determine the role of bacterial infections, with a focus on Helicobacter spp., in the development of benign gallbladder disease. By clarifying this relationship, the research aims to contribute to improved diagnostic, preventive, and therapeutic strategies, ultimately enhancing patient outcomes and reducing healthcare costs associated with gallstone disease.

Rationale of the Study

The rationale for investigating Helicobacter spp. in gallstone disease stems from the growing evidence of bacterial involvement in biliary pathologies. Helicobacter spp. are adept at colonizing microaerophilic environments, such as the gallbladder, and may contribute to bile composition changes, promoting stone formation. Their association with pigment stones, which are rich in bilirubin and calcium salts, is particularly notable.

This research aims to provide a comprehensive understanding of bacterial contributions to gallstone pathogenesis in India. By elucidating the role of Helicobacter spp., the study seeks to inform clinical practices, improve diagnostics, and guide the development of targeted therapies. The findings could also enhance preventive strategies, such as addressing modifiable risk factors and identifying high - risk individuals for early intervention.

Aim

1) To study the correlation of helicobacter pylori in cholelithiasis

Objectives:

1) To assess the presence of *H. pylori* in gall bladder mucosa and bile.
2) To assess the risk of gall stone formation due to *H pylori*

2. Material and Methods

Source of data: This study included patient who were admitted to the Department of General Surgery at Saraswati Institute of Medical Science in Hapur, Uttar Pradesh, undergoing cholecystectomy

Study Design: A prospective time - bound study

Sample Size: 100

Period of Study: The study took place from july2022 and June 2024

Sample Selection:

Inclusion Criteria

Patients undergoing cholecystectomy for cholelithiasis

Exclusion criteria:

People who were on antibiotics for H. pylori during the last six months

Methodology:

Method of collection of data:

- Subjects hospitalized to the GENERAL SURGERY department for cholecystectomy and participated in the research.
- Informed consent taken from the patient.
- After detailed history recorded with relevant investigation, patient undergone cholecystectomy.
- Patients planned for cholecystectomy approached and informed consent was taken.
- Bile was aspirated during cholecystectomy and subjected to smearing and staining by various staining techniques and various identification methods
- Histopathological assessment of the gall bladder after cholecystectomy has done using the Giemsa stain to look for the presence of H. Pylori.
- Data analyzed using appropriate statistical methods.
- Demographic data and complaints were noted.

Following reports noted in pre op period–

- Liver function test
- Ultrasonography of Abdomen
- Mrcp if indicated

Following reports noted in post op period -

- Histopathology report Giemsa staining for Histopathology specimen carried out and the presence or absence of Helicobacter species noted
- Giemsa staining of bile had done to note for Helicobacter species
- Bile had sent for culture sensitivity pattern

Conflict of Interest: There was no conflict of interest

Statistical analysis
Quantitative variables has been expressed as mean ± SD and categorical variables has been expressed as frequency and proportion. Data has been collected and analyzed statistically by using t - test and Chi square test. If P value was < 0.05, it has been considered as statistically significant.

3. Results

The present investigation evaluated 100 patients for Helicobacter pylori using bile culture and staining techniques on gallbladder and bile tissue.

Age Distribution:
The maximum age groups ranging from 51 to 60 years old accounted for 36.0% of the total, followed by 41 to 50 years old at 29.0%, next 31 to 40 years old at 20%, and 21 to 30 years old at 11%.

Remaining patients were either > 61 years (3%) or < 20 years (1%)

Age (Years)	Number	Percentages (%)
<= 20	1	1.00%
21 – 30	11	11.00%
31 – 40	20	20.00%
41 – 50	29	29.00%
51 – 60	36	36.00%
61+	3	3.00%

Sex Distribution:
Out of 100 patients in this study, 67 (67.0%) were female and 33 (33.1%) were male, suggesting that gallstones are more common in females".

Table 2: Showing number of patients according to Sex Distribution

Sex	Number	Percentages (%)
F	67	67.00%
M	33	33.00%

Sex Based Age Distribution:
In our study we found that females presented at a later age than males. Our oldest patient was 67 years old, and the majority of the female patients were between the ages of 51 and 60. However, most of our patients were between the ages of 21 and 50, and men tended to appear at a younger age.

Table 3: Showing sex based Age Distribution

Age (in Years)	Sex		Total
	Females	Male	
<= 20	1	0	1
21 – 30	10	1	11
31 – 40	12	8	20
41 – 50	19	10	29
51 – 60	24	12	36
61+	1	2	3
Total	67	33	100

Index Distribution:
In our study we found 63.6% of the males were overweight while 73.7% females were overweight. No cases of obesity were noted in this study.

Table 4: Showing distribution of Body Mass Index

	Age (in Years)	Sex		Total
		Females	Male	
BMI	18.5- 24.9 (normal weight)	15 (26.3%)	12 (36.4%)	27
	25 – 29.9 (Overweight)	52 (73.7%)	21 (63.6%)	73
	Total	67	33	100

Colour of Stone Distribution:
We identified 55 pigmented stones in the current research; 31 (31%) of them were brown, 24 (24%) were black, and 22 (22%) were yellow. We found no stones in 23 cases (23%)

Table 5: Showing number distribution of different colour stones.

Colour	Number	Percentage (%)
Black	24	24.0%
Brown	31	31.0%
No stone	23	23.0%
Yellow	22	22.0%

Bile Culture Study
The research found that 45.5% of the 90 bile samples were positive for culture, whereas 54.5% were negative. In ten cases, we failed to get bile.

Table 6: Showing percentage of Bile Culture

Bile Culture	Number	Percentage (%)
Positive	41	45.5%
Negative	49	54.5%
Total	90	100%

Organism Distribution in Bile Culture Positive Cases:
In this investigation, the following microorganisms were found to be growing in bile cultures. Of the organisms detected, E. coli accounted for 68.2%, Klebsiella for 26.9%, and Enterococcus for 4.9%.

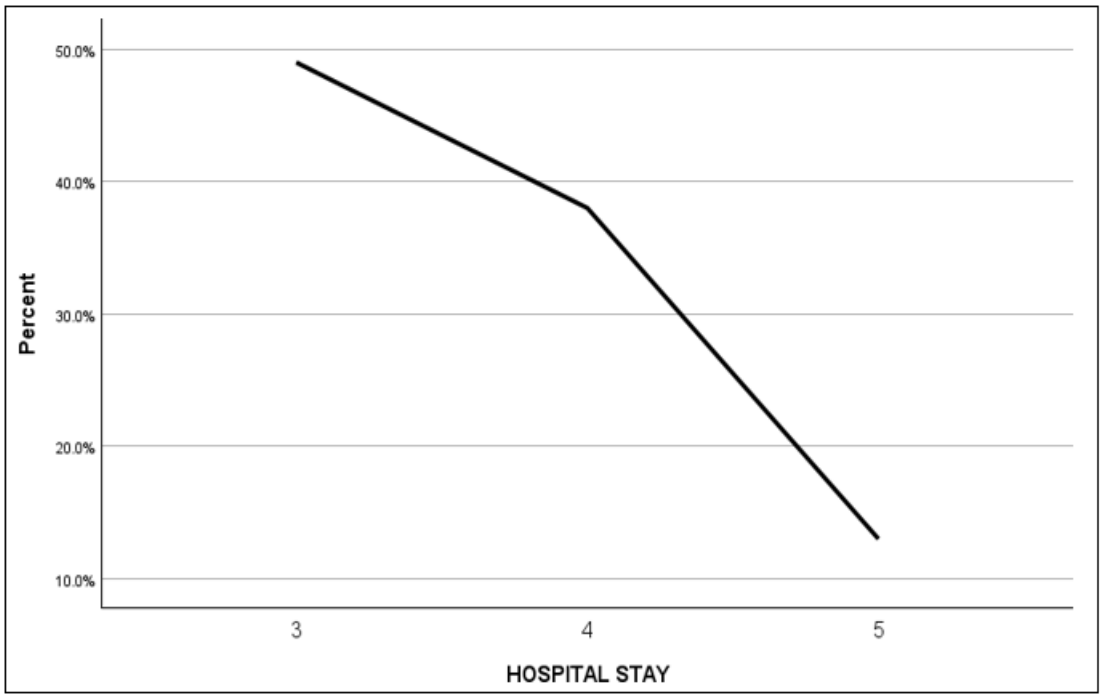
Table 7: Shows percentage of the growth of Organisms

Bile Culture		
Organism	Number	Percentage (%)
E. Coli	28	68.2%
Enterococcus	2	4.9%
Klebsiella pneumoniae	11	26.9%

Postoperative Stay
Post operative stay in majority of the patients was median of 4 days.

Table 8: Number of days of hospital stay of patients

Days	Number	Percentage (%)
3	49	49.0%
4	38	38.0%
5	13	13.0%
Total	100	100%



Graph 8: Number of days of hospital stay of patients.

Bile Culture Positivity in various types of Stones

In our study we found black and brown colour stones were more associated with culture positivity. Black stones (31.7%) and brown stones (39.0%), followed by yellow stones (29.3%).

Table 9: Culture positivity among different type of stones

TYPE OF STONE * CULTURE POSITIVITY									
Culture Positivity		Negative		Not collected		Positive		Total	
		N	%	N	%	N	%	N	%
Type of Stone	Black	8	16.30%	3	30.00%	13	31.70%	24	24.00%
	Brown	10	20.40%	5	50.00%	16	39.00%	31	31.00%
	No stone	23	46.90%	0	0.00%	0	0.00%	23	23.00%
	Yellow	8	16.30%	2	20.00%	12	29.30%	22	22.00%
Total		49	100.00%	10	100.00%	41	100.00%	100	100.00%

Helicobacter Pylori Evaluation with GIEMSA Staining of both Bile and Gallbladder Tissue

Gall bladder tissue and bile collected after cholecystectomy were examined for Helicobacter pylori - like forms using Giemsa stain.

Among all the cases assessed for Helicobacter pylori via Giemsa staining, seven cases we were able to visualize Helicobacter pylori like organisms in the gallbladder mucosa. (see Figure 16 & 17) In the other specimens both bile and gallbladder tissue were found to be negative

Table 10: Showing distribution of Helicobacter pylori in bile stained with Giemsa stain.

BILE Smear	Results	Percentage%
Negative	90	90.00%
Not Collected	10	10.00%
Positive	0	0%

Table 11: showing distribution of Helicobacter pylori in gallbladder mucosa with Giemsa stain

HPE STAIN	N	%
Negative	93	93.00%
Positive	7	7.00%
Total	100	100%

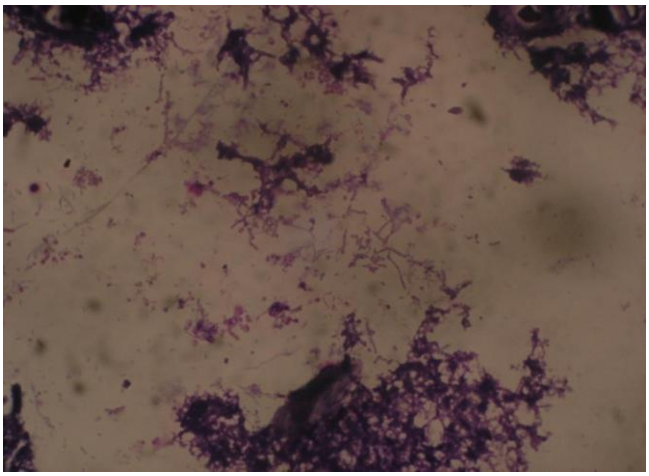


Figure 17: Microphotograph showing slender bacteria resembling Helicobacter species (Giemsa stain 100X)

4. Discussion

Among the several benign biliary system diseases that might need abdominal surgery in India, gallstone disease ranks high. Chronic cholecystitis is the most common gallstone complication, occurring in around 4% of cases.[13] Surgical antibiotic prophylaxis is crucial. The gold standard for

treating gallstones that cause symptoms is laparoscopic cholecystectomy. The negligible stress caused by laparoscopic cholecystectomy is believed to be the reason why fewer than 2% of patients get postoperative infections after the procedure.

H. Pylori has been proved to be the main cause for peptic ulcer disease. Helicobacter Pylori has stepped into the limelight of being a causative agent of diseases of the gall bladder. Several studies around the world have identified Helicobacter species via PCR studies among patients with gall bladder diseases successfully. Helicobacter species including H. pylori, H. bilis, 'Flexispira rappini, ' and H. pullorum have been found in the human hepatobiliary system according to many investigators, who used genetic and histological approaches to confirm their findings. Consequently, a wide variety of hepatobiliary illnesses, including chronic cholecystitis, primary sclerosing cholangitis, and gallbladder cancer, were thought to be caused by these species.[3, 8]

Several methods have been used to directly demonstrate the presence of H. Pylori in the gall bladder, such as gall bladder mucosal scrapings, bile or gall bladder cultures, or histology of the gall bladder, however these approaches have had conflicting results [4 - 6]. Gall stones, bile, and gall bladder tissue from specimens showing Cholelithiasis may all contain the same fastidious spiral or rod - shaped gram - negative bacteria, Helicobacter spp.[7]

Our study included 100 patients who underwent cholecystectomy at our center, and with their consent bile and tissue of gallbladder was studied for Helicobacter pylori with the help of staining techniques.

Age and Sex Prevalence

In our study we found that females presented at a later age than males. Our oldest patient was 67 years old, and the majority of the female patients were between the ages of 51 and 60 when they first presented. However, most of our patients were between the ages of 21 and 50, and men tended to appear at a younger age.

According to research by Nagaraj S K et al.[89], cholelithiasis is most common in those between the ages of 41 and 50. The ratio of males to females was 2.57 to 1.

The research by Pradhan SB et al.[23] indicated that the age range of 30–39 years was the most affected by cholelithiasis (32.5%), and that females constituted the majority (M: F=1: 3.2).

Gallstones are more common in women than in males, according to research by Rajani Sharma et al.[90]. The male - to - female patient ratio was around 1: 3. The majority of the cases (72.6%) were found in the 21–50 age group.

We found a female dominance with a male - to - female ratio of 1: 1.73 and, as in previous research, the largest age group was between 31 and 60 years old (83.4%). However, our Female population seemed to be higher than male like other studies.

Body Mass Index:

Cholelithiasis risk factors include obesity. In contrast to the abundance of research attempting to link obesity with cholelithiasis, the problem of cholelithiasis in otherwise normal - weight individuals has received surprisingly little study attention.

In our study we found our patients to have a mean BMI of 26.2.73.7% of our patients were found to be overweight, comparable in both males and females.

This was found similar to findings noted by Liu T et al [91] in their study where they found that the mean BMI was 25.07, comparable in both sexes among a large study population of 88, 947.

Independent risk factors for new - onset gallstone disease in both sexes were found to be a higher body mass index (BMI) and waist circumference. Bikram Kharga et al[86] also showed a mean BMI of 24.93, comparable in both genders similar to our study.

Bile Culture:

Out of 100 individuals who had cholecystectomy, 45.5% tested positive and 54.5% tested sterile. Along with E. coli, Klebsiella, and enterococcus, the most prevalent organisms were found in the following proportions: 68.2% (4 out of 7), 28.5% (2 out of 7), and 14.5% (1 out of 7).

A total of 36% of the 1, 394 patients studied by Chang WT et al.[87] for bacteriology in biliary tract illness had positive bile culture results. For biliary cancer, it was 9%, GB stones were 25%, CBD stones were 66%, IHD stones were 67%, and so on. In the context of gallstone illnesses, gram - negative bacteria, including Escherichia coli and Klebsiella, accounted for 74% of the organisms cultivated. Gram - positive bacteria, including Enterococcus, accounted for 15% of the organisms grown. The organism pattern noted was comparable to our study.

The total frequency of live bacteria in bile samples taken after cholecystectomy was 15.6%, according to Morris GJ et al.9[2]. In 100 instances of cholelithiasis, Sattar I et al.[24] identified common infecting organisms. Out of 36 patients, 36 had positive bile cultures, with the following results: E. coli (17 patients), Klebsiella (9 patients), Pseudomonas (6), Staphylococcus aureus (2), Salmonella (1), and Bacteroides fragilis (1). Our study's overall culture positive rate of 26% is in line with the majority of research.

According to our 26% finding, the 52% obtained by Pushpalata H et al.[23] is much higher. But Ozturk A et al[13] and Morris GJ et al[92] found only 13.1% less than our present study of 26 %. Though our total culture 66 positivity was only comparable to a few studies, we found the organism culture patterns to be similar with most studies.

Colour of the Stones and its Relation with Bile Culture:

The 55 pigmented stones analysed in this research belonged to three distinct colour categories: brown (31%), black (24%), and yellow (22%). In that, we found black and brown colour stones were more associated with culture positivity. Black

stones (31.7%) and brown stones (39.0%), followed by yellow stones (29.3%).

Mixed stones accounted for 78.75% of all stones, with cholesterol stones coming in at 12.5%, brown pigment stones at 7.5%, and black pigment stones at 1.25%, according to Pradhan SB et al.[23].

In 50 instances of chronic calculous cholecystitis, Rakesh BH et al.93 discovered that 60% of the gallstones were of the pigment variety. Based on their morphological examination, Mohan et al.[94] determined that 686 (62.3%) gallstones were of the mixed type, 34 (3.2%) were pigment stones, 182 (17.3%) were cholesterol stones, and 148 (14%).

In our study commonly found stone was pigmented stone (both black and brown) followed by yellow, which was comparable with the other studies. Consistent with previous research, colour stones showed a high rate of organism culture development, with brown stones accounting for 39.0%, black stones for 31.7%, and yellow stones for 29.3%.

Mandal PS et al[95], in their observational study noted that positive culture was more commonly noted in pigmented stones much like ours in comparison to yellow stones. According to their findings, the kind of gallstone is the only characteristic that can be used to determine the frequency of bacterial culture positive. Additionally, it was shown that gallstones have a higher concentration of Gram - negative bacteria.

Shrestha KR et al[96], in their study where they found that culture positivity was the highest in mixed stones (58.5%), pigmented stones (27%) and cholesterol stones (14.5%) out of the 152 cases studies.

Helicobacter Pylori Staining:
Gall bladder mucosa and bile were stained with Giemsa stain and studied for Helicobacter pylori. In our study, seven sample of the gallbladder mucosa showed Helicobacter like organisms. The remaining samples studied showed no such organisms on both bile and gallbladder mucosa.

Kawaguchi et al[45], found a microbe in a 41 - year - old woman's resected gallbladder mucosa that closely resembles Helicobacter pylori, both immunohistologically and genetically. The woman's complaints of fever and right hypochondrial discomfort led to her hospital admission. Gallstones and cholecystitis were the reasons for the cholecystectomy. During the course of the pathology investigation, a bacteria that looked like Helicobacter pylori was found by chance. Also, immunohistochemistry staining revealed that the microbe was present. Genetic analysis using the polymerase chain reaction (PCR) technique revealed an amplification response (urease beta - genes). Based on our research, it seems that H. pylori may infect more than only the gastrointestinal tract.

The purpose of the study conducted by O Rotimi et al.8[8] in 2000 was to compare two newly described staining methods for histological identification of Helicobacter pylori organisms (HpSS methods) to two established techniques (the modified Giemsa and anti - H pylori antibody immunostain) and to find out which one was better in terms of cost, availability, sensitivity, speed, and reproducibility. Based on its sensitivity, low cost, ease of performance, and reproducibility, the modified Giemsa stain was determined to be the gold standard for stomach mucosa Helicobacter pylori detection in their investigation.

In order to determine the function of Helicobacter pylori in gall bladder illness, Jafri D et al.8 conducted a case control study. then processed gallbladder tissue for smear and culture, and then quantitatively analysed serum for the presence of antibodies using an ELISA. Neither the smear nor the culture of any sample revealed the presence of H. pylori.

However, there was a notable disparity in positivity rates (80% vs.47.5%, p value <.05) and titres for antibodies against H. pylori (p value <.001) between the patients and the controls. The levels of antibodies in the control group and the patients were significantly different.

James G. Fox et al36 conducted a study among Chilean women, due to the unusually high rates of cancer of the gallbladder among them. Polymerase chain reaction (PCR) analysis was performed using Helicobacter - specific 16S ribosomal RNA primers on bile or resected gallbladder tissue from 46 Chileans who had cholecystectomy for persistent cholecystitis. Helicobacter sp. could not be recovered from specimens that had been frozen. Thirteen out of twenty - three bile samples and nine out of twenty - three gallbladder tissues were positive for Helicobacter when PCR was run. Phylogenetic analysis was performed on eight of the Helicobacter - specific PCR amplicons that had been sequenced. Two strains of "Flexispira rappini" (ATCC 49317), one strain of H. pullorum, and five strains of H. bilis were represented by the sequences. Based on these findings, bile resistant Helicobacter sp. is likely to be associated with gallbladder illness. He reflected as in our study that frozen section alone will not suffice to determine for H. pylori and further PCR analysis is required as an adjunct.

Another study by Abayli B et al[33] examined 77 mixed cholesterol gallstones post cholecystectomy against 20 control specimens. By using PCR amplification, H. pylori was successfully recovered from six gallbladder samples and seven gallstones. Similar histopathologic techniques (Warthin - Starry, hematoxylin - eosin, and gramme staining) also revealed Helicobacter - like organisms in five samples. Helicobacter spp. /H. pylori was detected in just four samples across all tests. Researchers came to the conclusion that some bacteria, such as Helicobacter pylori, may contribute to the development of cholesterol gallstones. Since, their study (77 cholesterol stones + 20 controls) was predominantly concentrated on cholesterol based stones (less in South India) and our study (30 cases) had few cholesterol stones (07), it was less likely that we could demonstrate the presence of H. pylori via Giemsa staining method alone.

In their work, Chaudhary P. K. et al.2 used Giemsa staining to analyse 50 instances of gall bladder mucosa histopathologically. They came to the conclusion that H. Pylori is not present in any gall bladder mucosa. Results from urea breath tests for Helicobacter pylori infection of the gastric antrum were analysed in a research by Bansal V. K. et

al.4 that included 49 patients having laparoscopic or open cholecystectomy for benign biliary tract illnesses. We used a combination of methods, including a quick urease test, histopathology, culture, and polymerase chain reaction (PCR), to look for H. pylori in the gallbladder and bile samples. In seventeen instances (34.6%), the urea breath test came back positive. Every single patient had a negative result from the rapid urease test. H&E, Giemsa, and Warthin Starry stains were used in the histological investigation of the gallbladder, but no signs of H. pylori infection were detected.

The PCR investigation revealed the presence of H. pylori DNA in 16 patients (32.6%), whereas none of the 12 controls showed any such results ($p = 0.025$). A positive urea breath test was linked to the presence of H. pylori DNA in the gallbladder and/or bile ($p < 0.0001$). There was no correlation between H. pylori infection of the gallbladder and other variables such as gender, age, jaundice, or cholestasis. They found that H. pylori DNA could be detected in gallbladder tissue in around 75% of individuals who tested positive for urea breath tests.

Giemsa stain was choosen as an accurate and cost effective means for assessing

H. pylori as noted by other studies predominantly conducted by Kawaguchi. As noted with the other studies, staining studies alone give a poor positive yield for

H. pylori and needs further confirmation by PCR analysisi which is the gold standard.9[7] We found only seven sample of gallbladder mucosa to be positive, accounting to 7%.

5. Conclusion

- Helicobacter pylori, traditionally linked to peptic ulcer disease, showed sporadic presence in gallbladder mucosa in our study using Giemsa staining.
- Our findings suggest a potential but limited role of H. pylori in gallstone pathology.
- Demographically, females presented with gallstone disease at a later age compared to males, with the majority of cases observed between 31 and 60 years.
- Obesity, indicated by a mean BMI of 26.2, was prevalent and consistent with its known association as a risk factor for gallstones.
- Bile culture revealed a 45.5% positivity rate, predominantly with Escherichia coli and Klebsiella pneumoniae.
- Pigmented stones, especially brown and black varieties, correlated with higher culture positivity rates, suggesting a possible link between stone composition and bacterial colonization.
- Limitations include small sample size and reliance on staining techniques, potentially underestimating H. pylori prevalence compared to PCR.

Future research should utilize larger cohorts and advanced molecular techniques to confirm the role of H. pylori and other pathogens in gallbladder diseases, informing targeted diagnostic and therapeutic strategies.

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