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"Presence of Helicobacter pylori infection in resected specimens of gallbladder with different lesions at a tertiary care centre"

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Abstract

Background: The burden of chronic cholecystitis and cholelithiasis extends beyond individual health outcomes and poses a considerable medical and economic burden on society. The costs associated with diagnosis, treatment, and potential complications of gallstone diseases contribute significantly to healthcare expenditures.

Material & methods: This study adopts an Observational Analytical Study design, aiming to investigate the presence of *H. pylori* infection in resected gallbladder specimens with various lesions, including chronic inflammation, dysplastic changes, and malignant lesions with the help of warthin starry stain.

Results: The data presented highlighted the association between Helicobacter pylori (H. pylori) infection status and the occurrence of non-neoplastic and neoplastic lesions. Among individuals with H. pylori positivity, non-neoplastic lesions were observed in approximately 21% of cases, while neoplastic lesions were identified in 4% of cases.

Conclusion: The intricate relationship between H. pylori infection, gut microbiota composition, and gall bladder diseases investigates the potential impact of microbiome alterations on gallstone formation, chronic inflammation, and neoplastic transformations, paving the way for microbiome-targeted interventions.

Key words: Helicobacter pylori, cholecystitis .

INTRODUCTION

Helicobacter pylori (*H. pylori*), a microaerophilic rod with 4-7 flagella, is widely recognized as one of the most prevalent chronic bacterial infections globally, affecting around half of the world's population [1]. Its discovery through histological examination of gastric biopsies and subsequent isolation by Warren and Marshall in 1983 marked a significant breakthrough in the management of various gastro-duodenal disorders [2].

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The colonization of the gallbladder by *H. pylori* could indeed lead to chronic inflammation, similar to how *H. pylori* are associated with chronic gastric inflammation [3]. Studies by Moricz and Chen have highlighted this association. Moricz noted a high prevalence of Helicobacter species in patients with chronic cholecystitis and cholecystolithiasis, suggesting a potential pathological mechanism linked to bacterial infection [4]. Chen's research demonstrated an association between gastric metaplasia of the gallbladder mucosa and chronic cholecystitis, which may be related to *H. pylori* infection in the gallbladder. *H. pylori* can damage gallbladder mucosa epithelial cells by triggering inflammation and immune reactions, contributing to the development of chronic inflammation in the gallbladder.

Bile reflux and regurgitation are linked to chronic cholecystitis, and H. pylori is sensitive to bile salts due to their toxic effects on the bacterium. However, in patients with chronic lithic cholecystitis and bile reflux, *H. pylori* were found in the stomach, indicating a high incidence of *H. pylori* infection alongside bile reflux [5]. This suggests the presence of a potentially resistant strain of H. pylori to bile salts. The reflux of bile may contribute to selecting and promoting the survival of this resistant strain, which could worsen gastritis and potentially enter the gallbladder through a reverse route, exacerbating gastric mucosa injury.

When it comes to detecting Helicobacter species DNA in bile, Polymerase Chain Reaction (PCR) methods, particularly targeting sequences specific to H. pylori (such as 16S rRNA and cagA), are effective [6]. These sequences can be frequently found in bile samples, although isolating the bacterium in culture medium is challenging [7]. One explanation for this difficulty could be the bacterium's ability to transition from a viable helical form to a non-viable coccid in bile. Additionally, the low numbers of bacteria may be suppressed by the unfavorable bile environment [8]. Several studies have proposed a potential role of H. pylori infection in the development of gallbladder diseases. If this association is confirmed, treating H. pylori infections could significantly reduce the incidence of gallbladder diseases, along with their associated morbidity and mortality rates [9].

Chronic cholecystitis and cholelithiasis, commonly known as gallstone diseases, are prevalent conditions worldwide with significant geographical and ethnic variations in their occurrence. The prevalence rates of these conditions differ markedly between Western and Eastern populations, ranging from 4% to 74% in various regions. This disparity can be attributed to differences in dietary habits, lifestyle factors, genetic predispositions, and environmental influences among different ethnic groups and geographical locations [10].

AIMS & OBJECTIVES:

To study presence of H. pylori infection in resected specimens of gallbladder showing chronic inflammation, dysplastic and malignant lesions.

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MATERIAL AND METHODS

Study Design

This study adopts an Observational Analytical Study design, aiming to investigate the presence of *H. pylori* infection in resected gallbladder specimens with various lesions, including chronic inflammation, dysplastic changes, and malignant lesions. The study will be conducted at the Department of Pathology, Integral Institute of Medical Science and Research (IIMSR) in Lucknow, spanning from September 2022 to April 2024.

Study Population

The study population includes patients who have undergone gallbladder resection and whose specimens exhibit chronic inflammatory, metaplastic/dysplastic, or malignant changes. Specimens will be collected post-operatively from the Department of General Surgery at IIMSR.

Sample Collection and Processing

1. **Sample Collection:** Gall bladder specimens will be collected immediately post-surgery and fixed in 10% neutral buffered formalin to preserve tissue architecture and cellular details.

2. **Sample Processing:** The fixed specimens will undergo standard processing in the Pathology department of IIMSR, including dehydration, clearing, infiltration with paraffin wax, embedding, and sectioning into 5 μm thick sections.

   After processing for routine histopathological examination section is stained with H&E staining and specific staining techniques were employed to detect the presence of Helicobacter pylori (H. pylori) organisms within the gall bladder tissue i.e. Warthin-Starry staining method. This silver staining method highlighted the presence of *H. pylori* organisms as black or dark brown structures within the tissue.

RESULTS

Different lesions of Gall bladder

The table 1 summarizes the distribution of different types of lesions in the gall bladder based on a sample size of 100 cases. 85 cases (out of 100) were classified as non-neoplastic lesions and 15 cases (out of 100) were classified as neoplastic lesions.

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Table 1. Different lesions of Gall bladder (N= 100)

Type of lesions	Number
Non-neoplastic	85
Neoplastic	15

The distribution of gall bladder lesions revealed interesting patterns within the studied population. Chronic cholecystitis with dysplasia emerged as the most prevalent lesion, constituting approximately 31.76% of the cases, suggesting a significant occurrence of dysplastic changes in the context of chronic inflammation. Following closely was chronic cholecystitis with pyloric metaplasia (PM), representing around 16.47% of the cases, indicating a notable presence of PM alongside chronic inflammatory processes. Additionally, chronic cholecystitis without specific metaplastic changes (CC) and chronic cholecystitis with intestinal metaplasia (IM) showed similar frequencies, each accounting for about 15.29% of the cases. This suggested that both types of metaplastic changes, pyloric and intestinal, occurred with comparable frequencies in association with chronic cholecystitis. Further, the combination of chronic cholecystitis with cholelithiasis was observed in approximately 14.11% of the cases, indicating a noteworthy association between chronic inflammation and the presence of gallstones. Lastly, chronic cholecystitis with cholesterolosis was the least common, comprising about 7.05% of the cases.(Table-2)

Table 2. Number and percentage of Non-neoplastic lesion (N=85)

Type of lesions	Number	Percentage (%)
Chronic cholecystitis with dysplasia	27	31.76%
Chronic cholecystitis with pyloric metaplasia (PM)	14	16.47 %
Chronic Cholecystitis (CC)	13	15.29%
Chronic cholecystitis with intestinal metaplasia (IM)	13	15.29%
CC with cholelithiasis	12	14.11%
CC with cholesterolosis	6	7.05%

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Neoplastic lesion of GB

The neoplastic lesions of the gall bladder, as detailed in Table 7 with a sample size of 15 cases, revealed a varied spectrum of malignancies. Among these, papillary adenocarcinoma stood out as the most common type, representing 6 cases within the sample. Following closely was adenocarcinoma of the gall bladder, noted in 4 cases. Intracystic papillary neoplasm, accounting for 3 cases, was characterized by papillary growths within cystic structures and was considered a precursor to invasive adenocarcinoma in certain instances. Carcinoma in situ (CIS), observed in 2 cases, represented an early-stage localized cancer confined to the epithelial layer without deeper tissue invasion (Table 3).

Table 3. Neoplastic lesion of GB (N=15)

Type of lesions	Number
Papillary adenocarcinoma	**6**
Adenocarcinoma of GB	**4**
Intracystic papillary neoplasm	**3**
Carcinoma in situ (CIS)	**2**

Warthin-Starry Stain

The data presented highlighted the association between Helicobacter pylori (H. pylori) infection status and the occurrence of non-neoplastic and neoplastic lesions. Among individuals with H. pylori positivity, non-neoplastic lesions were observed in approximately 21% of cases, while neoplastic lesions were identified in 4% of cases. On the other hand, in individuals without H. pylori infection, non-neoplastic lesions were more prevalent, accounting for 64% of cases, with neoplastic lesions found in 11% of cases. These findings suggested a potential correlation between H. pylori infection and the development of neoplastic lesions in the studied population. However, it's crucial to note that correlation did not imply causation, and further research and analysis were needed to establish the causal relationship between H. pylori infection and gall bladder neoplasms. Additionally, factors such as age, gender, and other medical conditions may also have contributed to the observed differences in lesion types among individuals with and without H. pylori infection (Table 4; Fig-1&2).

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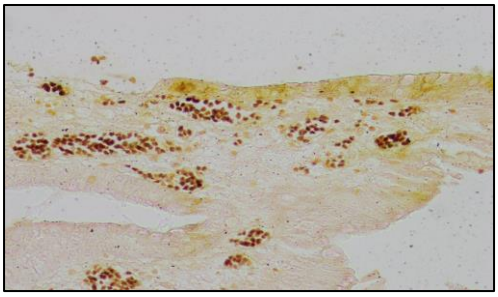
Table 4. Results of Warthin-Starry Stain

Category	Non-neoplastic	Neoplastic
***H. pylori* +**	21	4
***H.pylori* -**	64	11



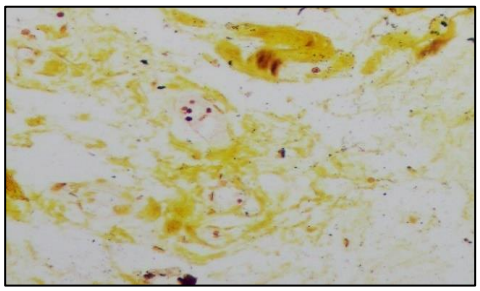


Fig1. H. pylori negative (4x). **Fig 2.** H. Pylori positive (40x).

DISCUSSION

In this study it was found that 85 cases (out of 100) were classified as non-neoplastic lesions and 15 cases (out of 100) were classified as neoplastic lesions. Non-neoplastic lesions, which encompass inflammatory and benign growths, were most commonly observed in individuals aged 61-70, with 22 cases. This age group exhibited the highest incidence of such lesions compared to other age brackets. Notably, the 31-40 and 51-60 age groups also showed considerable numbers of non-neoplastic lesions, with 18 and 15 cases, respectively. Conversely, the youngest age group of 10-20 had the fewest instances of non-neoplastic lesions, amounting to 7 cases. On the other hand, neoplastic lesions, characterized by abnormal cell growth, were notably absent in the youngest age groups of 10-20 and 21-30. However, the incidence of neoplastic lesions began to rise notably in the 31-40 age group, with 2 cases, and increased progressively with age. The 51-60 age group showed the highest occurrence of neoplastic lesions with 7 cases, followed by the 41-50 and 61-70 age groups, each with 3 cases.

In our study it was also reported that the distribution of gall bladder lesions revealed interesting patterns within the studied population. Chronic cholecystitis with dysplasia emerged as the most prevalent lesion, constituting approximately 31.76% of the cases, suggesting a significant occurrence of dysplastic changes in the context of chronic inflammation. Following closely was chronic cholecystitis with pyloric metaplasia (PM), representing around 16.47% of the cases, indicating a notable presence of PM alongside chronic inflammatory processes. Additionally, chronic cholecystitis without specific metaplastic changes (CC) and chronic cholecystitis with intestinal metaplasia (IM) showed similar frequencies, each accounting for about 15.29% of the cases. This

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suggested that both types of metaplastic changes, pyloric and intestinal, occurred with comparable frequencies in association with chronic cholecystitis. Further, the combination of chronic cholecystitis with cholelithiasis was observed in approximately 14.11% of the cases, indicating a noteworthy association between chronic inflammation and the presence of gallstones. Lastly, chronic cholecystitis with cholesterolosis was the least common, comprising about 7.05% of the cases.

Study	H. pylori Infection & Lesion Occurrence	H. pylori Detection Methods & Accuracy
Our Study	H. pylori infection possibly correlated with neoplastic lesions development.	Individuals with H. pylori infection: Non-neoplastic lesions: 21% of cases;Neoplastic lesions: 4% of cases Individuals without H. pylori infection: Non-neoplastic lesions: 64% of cases**;** Neoplastic lesions: 11% of cases
Farouk WI et al. (2018)[11]	WS stain less sensitive in detecting H. pylori.	104 previously WS-stained cases, 24 (23%) were positive, and the remaining 80 (77%) slides were negative.
Rotimi et al.[12]	IHC staining reported 98% sensitivity.	Of the 63 patients, 30 were originally negative in all tests for H pylori infection, 30 were positive, and the remaining three cases had discordant results using a combination of five tests (rapid biopsy urease test, urea breath test, culture, serology, and histology).
Patnayak et al.[13]	IHC referred to as gold standard with 100% sensitivity.	H. pylori was detected in 49 (62%) cases. Routine H and E and special stains like Giemsa and WS detected H. pylori in 26 (32.9%) cases
Mohammed Abdalla et al. (2022)[14]	IHC had a 40% positivity rate and 60% negativity rate for H. pylori detection.	IHC yielded (40%) positive cases while (60%) were negative

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CONCLUSION

The intricate relationship between H. pylori infection, gut microbiota composition, and gall bladder diseases investigates the potential impact of microbiome alterations on gallstone formation, chronic inflammation, and neoplastic transformations, paving the way for microbiome-targeted interventions.

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