

Role of Detection of Helicobacter Pylori in Cholecystectomy Specimens by Using Giemsa Stain

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ABSTRACT

Background and Aim: Helicobacter pylori is a gram negative and microaerophilic organism. A dominant human pathogen, Helicobacter pylori (H. pylori) is related to the development of gastric adenocarcinoma and MALT lymphoma in the stomach. H. pylori is also a causative agent of gallbladder cancer by causing chronic cholecystitis due to gall stones and later developing dysplasia, metaplasia and carcinoma. The aim of the present study was to report the Helicobacter pylori(H-Pylori) in gall bladder lesions of cholecystectomy specimens in two years in our pathology department.

Materials and Methods: The present study is a prospective study undertaken at the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar over a period of two years. A Total number of 55 cholecystectomy specimens have been studied, out of which 5 were confirmed to be positive for Helicobacter pylori by Giemsa Stain.

Results: The study is conducted to identify the gross and microscopic morphological features of cholecystectomy specimens and to find Helicobacter pylori (H-pylori) positive cases by using simple Giemsa stain. Most of the Helicobacter pylori (H-pylori) positive cases are seen in chronic calculous cholecystitis and Females are more commonly affected.

Conclusion: The present study shows Gall bladder pathology is more common in females than in males and affects people mainly in the third to fifth decades of life. Early detection of Helicobacter pylori in cholecystectomy specimens raises chances of potential prevention of gall bladder cancer by eradication of H. pylori through medical therapy.

Keywords: Helicobacter pylori, Cholecystectomy, gastric adenocarcinoma, MALT lymphoma.

INTRODUCTION

The gallbladder may be affected by a variety of pathological processes that have specific clinical correlates. Disorders of the biliary tract affect a significant portion of the world's population. Gallstones afflict 10% to 20% of adult populations in developed countries.^[1]

Helicobacter pylori is a gram negative and microaerophilic organism. A dominant human pathogen is now recognized as the first member of an ultra-structurally diverse genus. The predominant

morphological form of this genus is curved to spiral; however, some members have either a short or tapered rod shape. All helicobacters are motile by means of flagella. These basic characteristics of morphology and motility are thought to be advantageous to these organisms due to their localization in the mucous layer of the gastrointestinal tracts of humans and a variety of animals. H pylori in vivo and under optimum in vitro conditions is an S-shaped bacterium with 1 to 3 turns, 0.5 × 5 µm in length, with a tuft of 5 to 7 polar sheathed flagella.

H pylori transmitted from person to person through the feco-oral route and colonizes the stomach. The organism persists in the healthy host in equilibrium, which is disturbed at times, leading to formation of peptic ulcer and gastric cancer. Presence of H. pylori in the biliary tree of a small proportion of individuals has led to the conjecture that it is a potential cause of gallbladder disease.

The exact pathway of how the organism reaches the gallbladder is unclear. It could reach the gallbladder directly from the stomach or through the portal blood circulation. In contrast to the stomach, colonization of the gallbladder is unlikely due to the alkaline pH of bile, H. pylori being known to thrive at the low pH of gastric juice.^[2]

Kawaguchi et al first detected H-pylori in gallbladder's mucosa of a patient with calculus cholecystitis in 1996.^[3] Two-thirds of the world population is infected with H-pylori.^[4] Presence of Helicobacter in the gallbladder indicates that it is in fact invading the gallbladder.

In general, Helicobacter is demonstrated by at least one of the methods of examination in one-third of cases of gallstone disease and about one half of those with gallbladder cancer.

Moreover many of the 32 known species of Helicobacter are being isolated from the gallbladder such as: H. aurati, H. pullorum, H. bizzozeronii, H. canis, H. felis, H. cholecystus, H. ganmani, H. hepaticus and H. bilis.^[5]

H. pylori is related to the development of gastric adenocarcinoma and MALT lymphoma in the stomach.^[6] Recent work demonstrated the presence of H. pylori and H. bilis both in the bile and gallbladder of more than 75% of patients with gallbladder's cancer and more than 50% of patients with chronic cholecystitis undergoing surgery.^[7]

Demonstration of the bacillus on histology of gallbladder is by Giemsa stain. Molecular tests identify the genes of H. pylori in the tissue. Immunological evidence of H. pylori infection is provided by presence of H. pylori-specific IgG and IgM antibodies in the blood. Demonstration of H. pylori in gallbladder has been attempted directly by culture of Gallbladder bile or gallbladder mucosal scrapings.^[8]

Since then, more and more studies reported the existence of H. pylori in the bile, tissue, and stones of patients with chronic cholecystitis/ cholelithiasis and tried to explain the relationship between them.^[9]

It is hypothesized that a relationship like that of H. pylori and gastric carcinoma exists in the gall bladder. According

to this, H. pylori is a causative agent of gallbladder cancer by causing chronic cholecystitis due to gall stones and later developing dysplasia, metaplasia and carcinoma. It raises chances of potential prevention of this disease by eradication of H. pylori through medical therapy. The highest incidence of gallbladder cancer is found in women living in India, Pakistan and Ecuador.^[10]

Aim and Objectives:

1. The aim of the present study is to report the H-pylori by using Giemsa stain in gall bladder lesions of cholecystectomy specimens.
2. To study the gross and microscopic features of gallbladder received after cholecystectomy.

MATERIALS AND METHODS

This is a prospective study undertaken at the Department of Pathology, Chalmeda Anand Rao institute of medical sciences, Karimnagar over a period of two years.

Inclusion criteria

All the specimens of the gallbladder obtained by open cholecystectomy and laparoscopy received were included.

Exclusion criteria

Poorly preserved cholecystectomy specimens were excluded from the study.

Ethical approval

The study was approved by the Institute Ethics Committee, CAIMS, Karimnagar.

STATISTICAL ANALYSIS

All the data were entered in MS Excel and analysed by using spssinc, Chicago, IL, USA, version 16.

RESULTS

The study is conducted to identify the gross and microscopic morphological features of cholecystectomy specimens and to find H-pylori positive cases.

Incidence: The study is done over a period of two years. A total of 55 cholecystectomy specimens were received.

Sex distribution: Out of the 55 cholecystectomies, 42 belonged to females (76.3%) and 13 belonged to males (23.6%)(Table 1).

There was a female preponderance with Male: Female ratio being 1: 2.65

Table: 1 Sex Distribution

Total	Male	Female
55	13(23.6%)	42(76.3%)

Type of cases

Type of cases in relation to H.pylori infection detected on Giemsa stain. Of the 55 specimens, acute cholecystitis (7.4%), chronic cholecystitis (25.4%), acute calculous cholecystitis (3.6%), chronic calculous cholecystitis (60%), acute on Chronic cholecystitis (3.6%). (table 2)

Most of the H-pylori positives cases are seen in chronic calculous cholecystitis and females are more commonly affected.

Table 2: Type of Cases

		H-PYLORI	
		Positive	Negative
Acute Cholecystitis	04	00	04
Chronic Cholecystitis	14	01	13
Acute Calculous Cholecystitis	02	01	01
Chronic Calculous Cholecystitis	33	03	30
Acute on Chronic Cholecystitis	02	00	02
Total	55	05	50

Out of 55 cases, H.pylori is positive in 5 cases (chart 1)

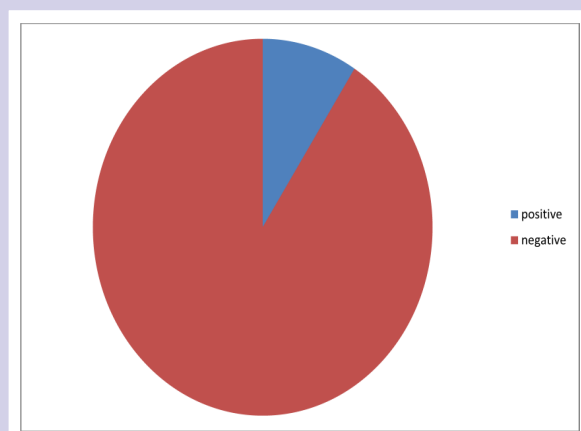
**Chart 1: Number of H-pylori positive cases****Figure 1: Dilated gallbladder****Figure 2: Gallbladder containing stones black pigment stone, having a characteristic jet black color****Figure 3: Gallbladder with mixed stones.**



Figure 4: Gallbladder with thickened wall

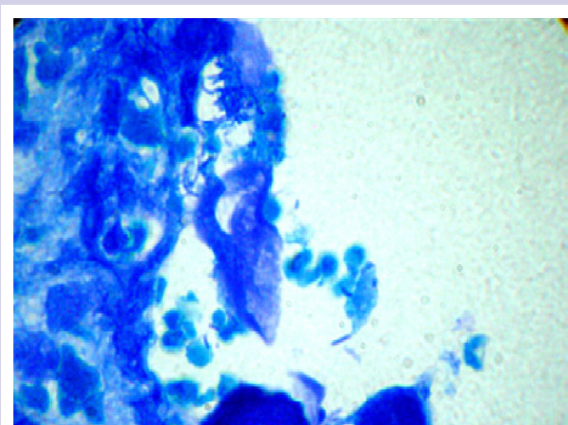


Figure 7: Giemsa stain H.pylori bacilli inside the mucosa(100x)

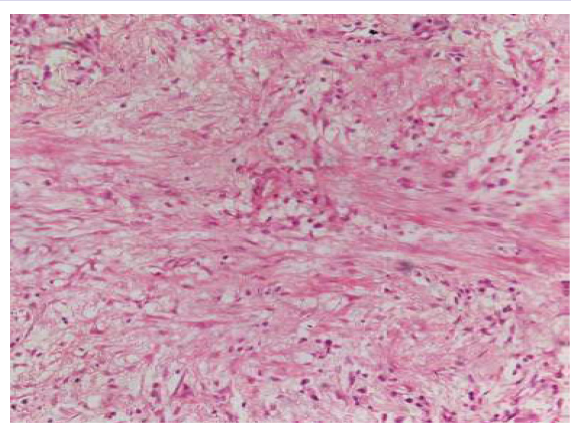


Figure 5: Acute cholecystitis superimposed on chronic. (H & E, 40X)

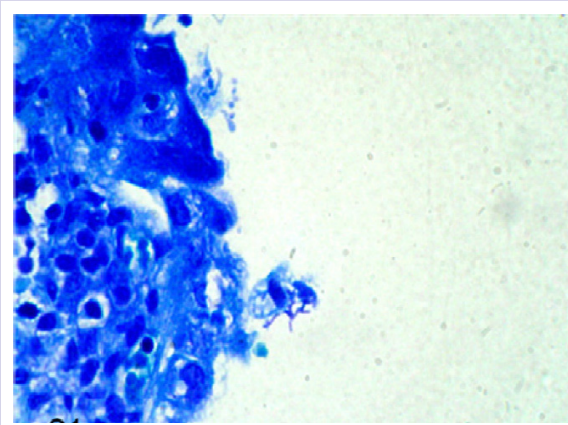


Figure 8: Giemsa stain H. pylori bacilli in the mucus close to the Surface (100x)

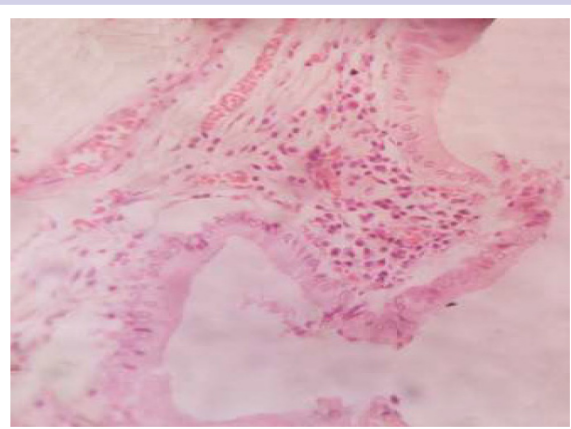


Figure 6: Chronic cholecystitis-the dense lymphoplasmacytic infiltrate in the lamina propria (H & E, 40X)

DISCUSSION

H. pylori has been proven to be the chief pathogen responsible for various gastrointestinal diseases, especially the pathogenic development of gastric cancer.^[11]

Recently, more and more studies reported the existence of *Helicobacter* species in the gallbladder, including *H. hepaticus*, *H. bilis*, *H. Ganmani*, and *H pullorum*. Among those diverse *Helicobacter* species, *H. pylori* is the most disquisitive one.^[12,13]

However, researches performed so far have failed to establish a definite relationship of the *H. pylori* infection of the gall bladder and chronic cholecystitis/cholelithiasis. In addition, there is inadequate evidence to determine the exact pathway of *H. pylori* to the

hepatobiliary system through the rising duodenum infection or portal system circulation way.^[12,13]

In Indian subcontinent, a high prevalence of *H.pylori* is reported in nearly all gastroduodenal diseases.^[14,15]

South Indian study stated that *H.pylori* colonization is present in more than half of the Indian population. In many cases it is present even without any associated abdominal symptoms.^[14,15]

Recently presence of *H.pylori* is frequently documented in various extra-gastric sites. The evidence of *H.pylori* colonization has been found in the gallbladder, ears, nose, skin, and even eyes. Whether *H.pylori* is a primary cause of disease in these locations or colonizes only after other agents initiate disease is not known.^[16]

The list of diseases associated with *H.pylori* is also expanding day by day.^[16,17,18] Ischemic heart disease, idiopathic thrombocytopenic purpura, iron-deficiency anaemia are to name a few from a long list.^[19] There is sufficient evidence of presence of other *Helicobacter* species like "*H. heilmanni*" in the stomach, intestine, and biliary tract.^[16]

They appear to cause the same diseases as *H.pylori*.¹⁶ *H.pylori* has a number of virulence factors which influence bacterial colonization and disease severity. One of the most important and best-studied virulence factor is CagA, encoded by cytotoxin-associated gene A (cagA).^[16] Another toxin, vacuolating toxin A (VacA), facilitates nutrient acquisition thereby improving the ability of *H.pylori* to colonize the gastric epithelium.^[16,20]

The availability of an array of diagnostic methods for the identification of *H. pylori* denotes the incompetency of any one test to pinpoint the diagnosis. Non-invasive diagnostic tests included urea breath test, stool antigen test and serology include, positive result even if fewer bacteria are present in the sample, faster results and no need for special processing or transportation. Test material for RTPCR can be taken from tissue in paraffin blocks. Also, PCR detects specific mutations and virulence factors, such as CagA and VacA. This helps to understand the variation in clinical presentation with different strains of *H. pylori*.

Many studies demonstrated that the presence of virulence factors, such as CagA and VacA gene, are associated with severe inflammation of the gastric mucosa and higher prevalence of peptic ulcer disease and gastric cancer.^[21]

There are several studies, from different parts of the world, which have documented the presence of *H.pylori* in gall bladder.^[22] Zhou D et al., reported isolation of *Helicobacter pylori* from 20.55% of chronic cholecystitis specimens.^[23]

In their study, patients with *H.pylori* positive gallbladders had higher incidences of premalignant lesions including adenomyomatosis and metaplasia and increased levels of iNO.^[16,23]

The study by Chen DF et al., opined that levels of interleukin-1, 6 and 8 (IL-1, 6 and 8) in gallbladder mucosa homogenates were significantly higher in *H.pylori*-infected cholecystitis group than *H.pylori*-negative cholecystitis group and control group.^[24]

According to them, *H.pylori* participates in and aggravates cholecystitis, causes destruction of epithelial cells and atrophy of the gallbladder. They opined that *H.pylori* infection in the gallbladder may be one of the etiological factors leading to cholecystitis.^[24]

They have noted inflammatory cell infiltrates and fibrosis in most of the cases, the degree of which varied from case to case. The chronic inflammatory cell infiltrate included mostly lymphocytes admixed with plasma cells.^[24]

Chen DF et al., observed that in 7.1% patients with chronic calculus cholecystitis, *H.pylori* was positive by immunohistochemistry using anti-*H.pylori* antibodies. They explained that this rather low level in gall bladder may be due to the poisonous effect of unconjugated bile salts on *H.pylori*. *H.pylori* is sensitive to bile salts, and cannot live in an environment with bile salts.^[25]

In contrast, a study from Egypt by Helaly GF et al., showed 73.3% and 66.7% positivity among gall bladder neck and body biopsies respectively by immunohistochemistry. They observed a significant association between gastric and gall bladder *H.pylori* positivity. The authors have concluded that *H.pylori* infection may be an etiological factor leading to cholecystitis.^[26]

The source for gall bladder infection may be gastric colonization with *H.pylori* and this may act as a lithogenic component, especially in the presence of pure pigmented gallstones.^[26]

A recent Indian study noted the presence of *H.pylori* DNA in the gallbladder of carcinoma gallbladder patients. This study reported significant elevation of circulating levels of cytokines like interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in the *H.pylori* positive gall bladder carcinoma patients.^[27]

CONCLUSION

Gall bladder pathology is more common in females than in males and affects people mainly in the third to fifth decades of life. Cholecystitis is a common pathology. It has a wide histomorphological spectrum of acute acalculus cholecystitis, acute calculus cholecystitis, chronic calculus cholecystitis, chronic acalculus

cholecystitis.

The peak age group for cholelithiasis was found to be 31 to 40 years, with female preponderance. Regarding gallstones, mixed types and black pigment types were the commonest to be observed.

Though gallbladder may appear normal grossly, there can be a spectrum of histopathological lesions microscopically. Hence, histopathological examination should always be carried out on all resected cholecystectomy specimens with Giemsa stain along with regular H&E stain. The present study has documented *H. pylori* in the gallbladder.

The ability of *H. pylori* colonization in the gallbladder to give rise to cholelithiasis, gastric metaplasia, gallbladder cancer is possible so far, as studied from the analysis above.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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