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RESEARCH ARTICLE

INCIDENCE OF PRESENCE OF H. PYLORI IN CASES OF CHOLECYSTITIS AND CHOLELITHIASIS IN A RURAL MEDICAL COLLEGE & HOSPITALPradeep Kumar Chaudhary¹, Subhash Goyal¹, Nanak Chand Mahajan², Saurabh Kansal³, Priti Sinha⁴¹Deptt. of General Surgery, Maharishi Markandeshwar Institute of Medical Sci & Research, Mullana, Haryana, India²Deptt. of Pathology, Maharishi Markandeshwar Institute of Medical Sci & Research, Mullana, Haryana, India³Deptt. of Pharmacology, Subharti Medical College, Meerut, U.P., India⁴Deptt. of Anatomy, Subharti Medical College, Meerut, U.P., India©Corresponding Author's Email: kansalsaurabh513@gmail.com

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ABSTRACT

Cholecystitis is defined as inflammation of the gallbladder. Ninety percent of cases involve stones in the cystic duct and gall bladder, (i.e., calculus cholecystitis) with the other 10% of cases representing acalculus cholecystitis.⁽¹⁾ The association of gallstones with *Helicobacter pylori* has been investigated but not clearly demonstrated. In this study, the presence of *H. pylori* in the gallbladder mucosa of patients with symptomatic cholecystitis and cholelithiasis was investigated. The study included 50 cases of cholecystitis and cholelithiasis randomly selected who were admitted to the surgical wards of M.M.I.M.S.R, Mullana in last 2 years. All patients underwent cholecystectomy and gall bladder mucosa was histopathologically examined by giemsa staining (silver slide test). We concluded that no gall bladder mucosa exhibits presence of *H. Pylori*.

Keywords: Cholelithiasis; Cholecystitis; *H. pylori*; Giemsa**INTRODUCTION**

Cholecystitis is defined as inflammation of the gall bladder. Ninety percent of cases involve stones in the cystic duct and gall bladder, (i.e., calculus cholecystitis) with the other 10% of cases representing acalculus cholecystitis.¹ The gallbladder mucosa secretes hydrogen ions and is covered by mucus. The environmental conditions for bacterial colonization are similar to those in the stomach. Gallbladder stones often contain DNA from enteric bacteria, but no compelling evidence demonstrates that *Helicobacter* spp. have been present. Gallstone disease is one of the most common problems affecting the digestive tract where autopsy reports show a prevalence of 11–36%. The prevalence of gallstones is related to many factors including age, gender, and ethnic background. Women are three times more likely to develop gallstones than men and first-degree relatives have a twofold increased prevalence. The association between *Helicobacter pylori* (*H. pylori*) and gallstones has been investigated but not clearly demonstrated. *H. pylori* closely correlates with chronic gastritis, peptic ulcer, gastric carcinoma and malignant lymphoma of gastric mucosa-related lymphoid tissues (MALToma). Recently, it has been found that *H. pylori* have certain relationship to some diseases in the organs besides the stomach and duodenum. However, it is still unclear whether *H. pylori* have any correlation with adjacent structures of the stomach, the liver and the

gallbladder. There is little doubt that bacteria, including *H. pylori*, play a significant role in pigment gallstone formation. Bacterial genetic material has also been identified in pure and mixed cholesterol gallstones. Although, recent animal studies did not confirm any association between *H. pylori* infection and cholesterol gallstone formation, clinical studies have reached contradictory results, providing data in favour of or against the possible role of *H. pylori* as predisposing factor for cholesterol gallstone formation.² The present study evaluates the incidence of presence of *H. pylori* in cases of cholecystitis and cholelithiasis in a rural setup.

MATERIAL AND METHOD

- The study included 50 cases of symptomatic cholecystitis and cholelithiasis, admitted to surgical wards of MMIMSR, Mullana (Ambala), Haryana, India in between October 2012 to October 2014. The cases were selected randomly and were proven cases of cholecystitis and cholelithiasis preoperatively. Preoperative biochemical investigations and ultrasound scanning were done to rule out any associated liver or extra-hepatic biliary disorder. Patients with Chronic pancreatitis, inflammatory bowel disease, Liver cirrhosis, Underlying malignancy, Familial hypercholesterolaemia were excluded. Patient

Cases underwent laproscopic cholecystectomy and open cholecystectomy wherever indicated. Postoperative gall bladder specimen mounted in 10% formelin and send for histopathological examination. Gall bladder mucosa stained by GIEMSA (Silver Slide Test) and observed under microscope.

- This study has been well approved by institutional ethical committee and all the clinical studies included in this research work has been standardized according to the guidelines of ICMR,

RESULTS:

A total of 50 patients operated had 38 (76%) females and 12 (24%) males. The majority of patients 28(56%) were in the age group of 40 to 60 years. All 50 patients went through either open cholecystectomy or laproscopic cholecystectomy, Pain abdomen, nausea, vomiting, fever and dyspepsia were observed in 100%, 18% , 10%, 18%, 16% respectively. On histopathological examination no gall bladder mucosa exhibits H.pylori in GIEMSA Staining . All 50 patients were negative by Silver Slide Test.

Table 1: Gender Distribution of patients

MALE	FEMALE	TOTAL
12	38	50

Table 2: Results of silver slide test according to gender

Result of silver slide test	MALE	FEMALE
Positive	00	00

Table 3: Overall reasult of silver slide test

Positive	Negative	Overall positive
00	50	00

DISCUSSION

Cholecystitis is defined as inflammation of the gallbladder. Ninety percent of cases involve stones in the cystic duct and gall bladder, (i.e., calculus cholecystitis) with the other 10% of cases representing acalculus cholecystitis.⁽¹⁾ The association of gallstones with *Helicobacter pylori* has been investigated but not clearly demonstrated. *H. pylori* present in human bile samples might represent a risk factor for gallstone formation. These reports stimulated interest as to whether these organisms colonize the biliary tract of humans and cause hepatobiliary diseases. The evidence, however, concerning the presence of *Helicobacter* in the bile and biliary tissue of human beings with biliary diseases is controversial³⁻⁶. *H. pylori* may be present in the bile when there are certain environmental changes, such as lowered pH. However, *H. Pylori* does not colonize the bile duct epithelium. They could find no pathogenetic role for *H. pylori* in the formation of hepatolithiasis.⁷ *Helicobacter* species are commonly present in the gallbladder of patients with gallstone diseases and in controls, implying that *Helicobacter* infection alone may not play a significant role in the formation of gallstones. However, their results do not exclude the possibility of *Helicobacter* infection as a cofactor in the development of gallstones.⁸ *Helicobacter* in gallstones of Iranian patients with biliary disease, gallstone and bile samples from 33 patients were

subjected to rapid urease test, culture and Multiplex PCR. *H. pylori* was detected in stone samples of gallstones but not sure of their viability.⁹ The presence of few bacteria, the geographic distribution of *H. pylori* strains, and the bile milieu inhibitory effect might be some of the reasons for growth failure. It concluded that although *H. pylori* was detected by PCR in gallbladder tissue of patients with cholecystitis, a clinical correlation with biliary disease could not be established because several conditions were difficult to meet.¹⁰ Gallbladder tissue and bile specimens from subjects with *H. pylori*-positive gastritis with cholelithiasis. The presence of *H. pylori* in antrum biopsies was confirmed by rapid urease test and/or histopathological examination. *Helicobacter* was detected in the gallbladder tissue and bile of 28% and 18% respectively of the patients, but was not detected in any of the gallstones. These results do not rule out the possibility of *Helicobacter* infection as a contributing agent or cofactor in the development of biliary diseases.¹¹ Bostanoğlu E, Karahan ZC, Bostanoğlu A, Savaş B (2010) also did a study with aim to evaluate the presence of *Helicobacter* species in the gallbladder tissue, bile and gallstones of Turkish patients with cholelithiasis. Forty-seven patients with calculous cholecystitis and 3 controls were evaluated for the presence of *Helicobacter* spp. by culture, polymerase

chain reaction, and histological and immunohistochemistry methods. *Escherichia coli* (10.6%), *Enterobacter amnigenus* (6.3%), *Klebsiella planticola* (2.1%), and *Klebsiella ozaenae* (2.1%) were isolated from the sample cultures of 8 patients. No other microorganisms, including *H. pylori* and other *Helicobacter* spp., were detected. Polymerase chain reaction was negative for *Helicobacter* spp. and *H. pylori*. There was no association between the presence of *Helicobacter* spp. and development of cholelithiasis in this study group. The microorganisms found in the samples did not reveal any significant association with the underlying disease.¹² Figura et al. (1998) who proposed that *H. pylori* present in human bile. In some studies, the presence of intestinal *Helicobacter* spp. *H. pylori* was detected in bile and/or gallbladder. The role of *Helicobacter* in the pathogenesis of gallbladder disease in humans, or even its presence in gallbladder tissue, therefore remains unclear.¹³ Bansal et al 2012 examined patients having mean (SD) age of 42.4 (11.1) years. Urea breath test was positive in 17 (34.6%) cases. Rapid urease test was negative in all the cases. There was no evidence of *H. pylori* infection of gallbladder on histopathological examination using H&E, Giemsa and Warthin Starry stains. *H. pylori* DNA were detected in 16 patients (32.6%) and none of the 12 controls by PCR analysis ($p=0.025$). The presence of *H. pylori* DNA in bile and/or gallbladder was associated with positive urea breath test, ($p<0.0001$). Other factors like age, gender, jaundice and cholestasis were not associated with *H. pylori* infection of gallbladder and bile.¹⁴ Yakoob J, Khan MR et al (2011) *Helicobacter* species colonise the biliary tract and therefore this study explores the relationship between of *Helicobacter pylori* and cholecystitis. Bile and gall bladder tissue samples were obtained from 144 patients who underwent cholecystectomy. Of these, 89 had chronic cholecystitis with cholelithiasis, 44 had gall bladder carcinoma and 11 had gall bladder polyps. Histopathology examination included special staining and immunohistochemistry (IHC), while *Helicobacter* species (*H. pylori*, *H. bilis* and *H. hepaticus*) were detected by the polymerase chain reaction (PCR). Sequencing and BLAST query of PCR products was undertaken and samples were considered to contain *H. pylori* if both PCR and IHC were positive. Immunohistochemistry for *H. pylori* was positive in 22 (25%) cases compared to five (9%) in the control group ($P=0.02$). Testing (PCR) for 16S rDNA was positive in 23 (26%) cases compared to six (11%) controls ($P=0.03$). Negative PCR results were obtained for *H. bilis* and *H. hepaticus*. Twenty-four (89%) were positive by both 16S rDNA PCR and IHC for *H. pylori* ($P<0.001$). Both PCR for 16S rDNA and IHC were positive in 21 (24%) cases compared to five (9%) controls ($P=0.03$). Sequencing of 16S rRNA and glmM PCR products were consistent with *H. pylori*. In

conclusion, *H. pylori* DNA was demonstrated in cases of chronic cholecystitis and gall bladder carcinoma associated with cholelithiasis, but this association requires further study.¹⁵ Xiao et al 2014 said that since the discovery of *Helicobacter* species in human biliary system, the association between *Helicobacter* species infection and cholangiocarcinoma is under debate. This meta-analysis aims to explore this issue. Literature search was carried out to identify all eligible articles. We performed overall meta-analysis of all included studies and subgroup analysis based on regional distribution. Subgroup analysis in the light of detection methods and specimens was also conducted. Ten case-control studies were included. Overall meta-analysis favoured a significant association between *Helicobacter* species infection and cholangiocarcinoma (cumulative OR 8.88, 95% CI 3.67–21.49). Subgroup analysis based on geographic distribution indicated that *Helicobacter* species infection may serve as a risk factor not only in a region with high cholangiocarcinoma incidence (Asia, OR 6.68, 95% CI 2.29–19.49) but also in low incidence region (Europe, OR 14.90, 95% CI 4.79–46.35). The other subgroup analysis showed that PCR was the most effective and efficient method to detect *Helicobacter* species in surgically rejected tissue and bile. There was significant heterogeneity among studies and obvious publication bias. They concluded that our meta-analysis supports the possible association between *Helicobacter* species infection and cholangiocarcinoma. Further investigations are required to clarify the role of *Helicobacter* species in this malignancy¹⁶.

Detection of the presence of *H. pylori* in bile can be done with different in the bile of Canadian patients with biliary disorders, whereas a completely methods, techniques used may be responsible for the difference in the reported studies which are far from being perfect. The best way to show *H. pylori* is to grow *H. pylori* in cultures, but *H. pylori* are extremely hard to culture due to the microaerophilic properties of this microorganism, which die when they contact air. In another study it was shown that various PCR techniques could be used as a method for detection of *H. pylori* DNA in bile. Fallone et al. failed to find DNA of the genus *Helicobacter* contrary result was described by Silva et al. (who found bacterial nucleotide sequences in most Brazilian subjects with similar diseases). Regional differences due to variable rates of infection and the changing sensitivity of the various PCR¹⁷⁻¹⁸.

CONCLUSION:

The study did not depict presence of *H. pylori* in gall bladder mucosa by GIEMSA staining alone. Further investigations are required to clarify the presence of *Helicobacter* species in cases of cholecystitis and cholelithiasis.

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