

## Original Research Article

# Diagnosis of *Helicobacter pylori* in cholelithiasis and cholecystitis: by histology and serological association

Srinivasan Doraiswamy, Dharmendra Kumar\*, P. N. Sreeramulu, Suresh

Department of Surgery, R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka, India

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### \*Correspondence:

Dr. Dharmendra Kumar,

E-mail: [dharmendra2606@gmail.com](mailto:dharmendra2606@gmail.com)

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## ABSTRACT

**Background:** Biliary calculus disease is one of the most common disorders affecting the gastrointestinal tract. It is an important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west. In India, it is more common in northern states than in South India. Incidence of gallstones increases with age with a male to female ratio of 1:4. About 50% of these patients are asymptomatic.

**Methods:** An analytical prospective study on patients diagnosed with cholecystitis and/or cholelithiasis was conducted in R. L. Jalappa Hospital Tamaka, Kolar. 58 patients were included in the study between June 2018 and May 2019. Data was collected and analysed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions.

**Results:** The age incidence was found to be highest between 31 to 40 years. The incidence of cholelithiasis was more in females. All patients presented with pain abdomen. Calculous cholecystitis was the most common mode of presentation. Open cholecystectomy was commonly done for cholelithiasis in our set up. Chronic calculous cholecystitis is the commonest histopathology.

**Conclusions:** According to our findings evidence of recent and chronic infection *Helicobacter pylori* as shown by demonstration of IgM and IgG class of antibodies respectively to the organism was found in most of patients and histological evidence of the patients with cholecystitis and cholelithiasis. Thus, the frequency of helicobacter infection seems to be low in the patient population studied.

**Keywords:** Cholelithiasis, Cholecystectomy, *H. pylori*

## INTRODUCTION

Biliary calculus disease is one of the most common disorders affecting the gastrointestinal tract. It is an important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west during the past century. In the UK, USA and Australia, the prevalence rates vary from 15-25%. In India, it is more common in northern states than in South India. Incidence of gallstones increases with age. It is more commonly presented in females than males with a male to female ratio of 1:4. About 50% of these patients are asymptomatic.<sup>1</sup>

The pathogenesis of gall stones is multifactorial. It varies according to the type of gallstones. Primarily gallstones can be divided into two major groups.<sup>2</sup> First is pure gallstones contributing to 10% of gallstones while Mixed and combined gallstone accounts for 90% of gallstones. Mixed gallstones are frequently associated with cholecystitis.<sup>2</sup>

*Helicobacter pylori* is a gram negative and micro aerophilic microorganism that can cause chronic gastritis, gastric and duodenal ulcers and gastric adenocarcinoma.<sup>3</sup> In the last few years, the scientists have been interested in studying the relationship between *H. pylori* infection and

various extra digestive diseases. Bile acids are generally known to have inhibitory effects on the adherence and growth of *H. pylori in-vitro*. The *in-vitro* bacteriostatic effect of bile has not been demonstrated to the same degree *in-vivo*, suggesting the adaptive conditioning of *H. pylori*. The survival of *H. pylori* is documented to be more conducive in low pH.<sup>4</sup>

The aim of the present study was to find the histopathological association and sero-positivity of *H. pylori* species in patients with cholecystitis and/or cholelithiasis.

## METHODS

An analytical prospective study on patients diagnosed with cholecystitis and/or cholelithiasis was conducted in the Department of General Surgery of R. L. Jalappa Hospital over a period of 1 year between June 2018 and May 2019. 58 patients were included in the study met criteria in fix time. Data was collected from the previous records and was analysed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions.

Gall bladder tissue was collected by cholecystectomy and blood collected was investigated. Serum IgM and serum IgG levels (IgG levels was also included) for *Helicobacter* species were measured. Gall bladder tissue for haematoxylin and eosin, Giemsa staining was done for mucosal study. Urease test was done for *H. pylori*.

Fifty eight patients presenting with clinical features of cholelithiasis and/or cholecystitis to the Department of Surgery during the fixed time of the project were included.

### Inclusion criteria

All cases that underwent surgery for cholecystitis and/or cholelithiasis were included.

### Exclusion criteria

Patients treated for *H. pylori* within 4 weeks.

### Method of data collection

Fifty eight patients with cholelithiasis and/or cholecystitis were included in the study. A detailed history was elicited followed by general and systemic examination. Investigations as per the porforma made for the study were performed.

### Pathological examination to detect *H. pylori*

The specimen of gall bladder after cholecystectomy was collected in sterile bottle with 10% formalin. The bottle was then sealed and sent for histopathological examination. Gall Bladder specimen was subjected to

haematoxylin and eosin, Giemsa staining for mucosal study and Urease method to detect *H. pylori*.

### Serological testing

Serum IgM and IgG antibodies against *H. pylori* will be detected using the IgM and IgG commercial kit (SD *H. pylori* antibody test kit). For this purpose, 3 ml of blood was collected prior to surgery before starting the patients on antibiotics (proforma enclosed).

Blood samples was allowed to clot at room temperature and centrifuge at 2500 rpm for 5 minutes in REMI centrifuge after separated and stored frozen until they were tested as per the instruction of the manufacturer.

## RESULTS

Fifty eight patients presenting with clinical features of cholelithiasis and cholecystitis to department of surgery at R. L. Jalappa Hospital, Tamaka, Kolar during the six months of study were included in the study.

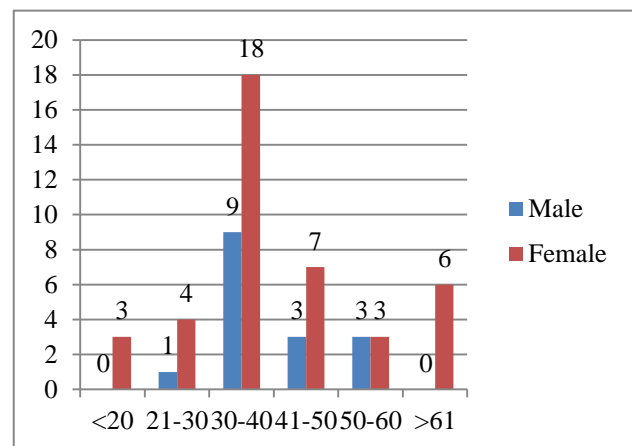


Figure 1: Age distribution.

The youngest patient was 17 years old and the oldest patient was 70 years old. The mean age was 42.76. Bulk of the disease presented in the age group of 31-40 years.

In this study, 29.31% were male, and 70.68% were females, showing female predominance in sex distribution of biliary calculi.

Pain was the most common persisting symptom whereas tenderness in the right hypochondrium was the most commonly elicited sign in these cases. All patients had pain in right hypochondrium; few were associated with pain in epigastrium as well. Jaundice was absent in all the patients involved in the study.

44 patients presented with dyspepsia, 44 patients had fever, out of which 13 were associated with chills and rigors. These patients had septic focus in gall bladder or biliary tract.

Pain was the commonest symptom of biliary calculi universally. Pain was due to luminal obstruction from an impacted stone which is characteristically colicky or from inflammation which is burning type of pain. Biliary dyspepsia was present in up to 75.86% of patients.

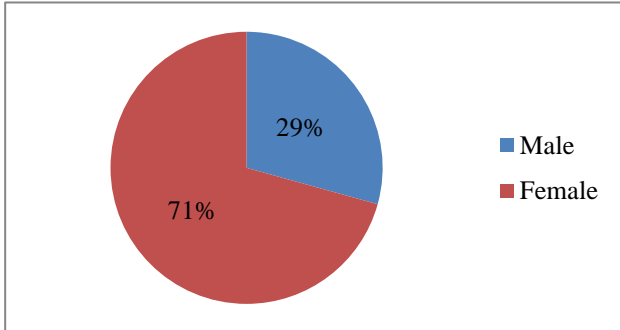


Figure 2: Sex distribution.

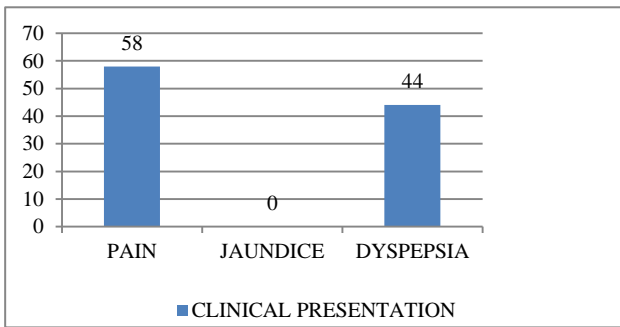


Figure 3: Symptoms of clinical presentations.

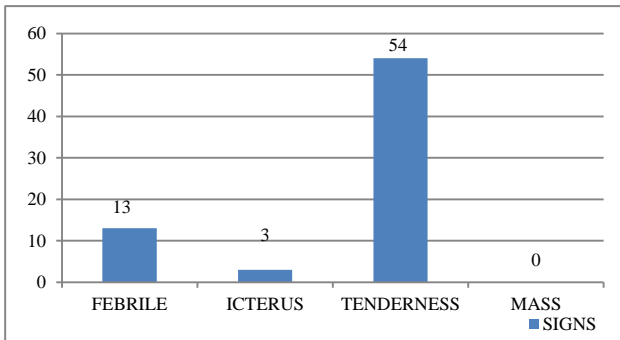


Figure 4: Signs.

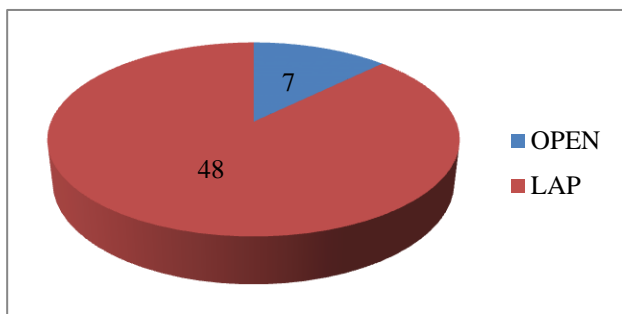


Figure 5: Type of surgery.

7 patients underwent open cholecystectomy and 48 patients underwent laparoscopic cholecystectomy. Patients who underwent open cholecystectomy, Kocher's right subcostal incision were used. Patient taken for laparoscopic cholecystectomy were uncomplicated.

Table 1: Histopathological findings.

Histopathology	Numbers
Calculous cholecystitis	10
Cholelithiasis	48

10 patients had calculous cholecystitis and 48 had chronic cholecystitis without calculi.

Table 2: Serological methods.

Antibody titres (serum IgM) (in units)	Gender		Total
	Male	Female	
<9	-	-	-
9-11	14	38	52
>11	3	3	6

Table 4: Serological methods.

Antibody titres (serum IgG)	Gender		Total
	Male	Female	
<0.75	-	-	-
0.75-0.99	12	32	44
>0.99	5	9	14
Total	17	41	58

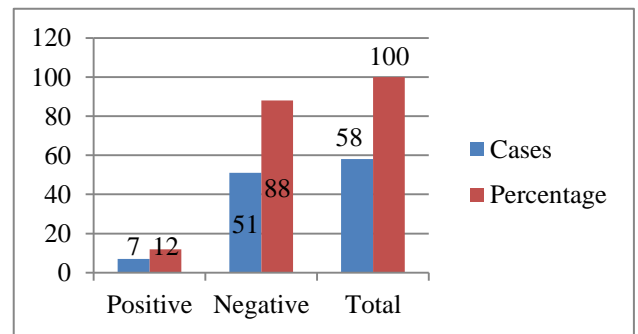


Figure 6: Staining method.

7 cases tested positive for *H. pylori* from cholecystectomy specimen.

DISCUSSION

Detection of *Helicobacter* species in human bile has prompted a growing interest as to whether these organisms truly colonize the biliary tract of humans and cause hepatobiliary diseases. Bile resistant *Helicobacter* species such as *H. hepaticus*, *H. bilis* and *H. pullorum*, have been discovered in both man and animals. Due to lack of gold standard in the diagnosis of these organisms in bile, researches in this area are mostly limited. A meta-

analysis of some published work has shown strong association between *Helicobacter* and gall bladder disease. We, therefore, attempted to find a relation between *Helicobacter pylori* and gall bladder disease.<sup>5</sup>

This study was conducted in Department of Surgery of R. L. Jalappa Hospital, Tamaka, Kolar. 58 cases with cholelithiasis and/or cholecystitis in the age group 17-70 years were included in this study. Patients who had received treatment for *H. pylori* were excluded from the study. All these cases were stratified according to age, sex, presenting symptom, elicited signs, type of surgical intervention, histopathology, biochemical tests, staining and serology.

Most of the patients in our study were 31-40 years. Few patients were in younger age group as well, showing a decrease trend in the age for development for cholelithiasis. This could probably be due to change in lifestyle.<sup>6</sup>

Our study was compared with Deeba et al study; mean age group in our study was 42.76 years as opposed to 42.32 years in Deeba et al study.<sup>7</sup> The majority of patients in our study were in the age group of 31-40 years old. In the study, females outnumbered in the ratio 2.4:1 which was comparable to Arshad et al study which had a ratio of 1.38:1.

Pain and tenderness in the right hypochondrium were the common symptom and elicited sign, 44 had biliary dyspepsia. All this data was comparable with Arshad et al study, however biliary dyspepsia was present in more number of patients compared to other study.

No cases of jaundice were reported. 7 cases underwent open cholecystectomy, rest underwent laparoscopic cholecystectomy. Majority of the patients had calculous cholecystitis and few patients had features of chronic cholecystitis without calculi.

**Table 1: Comparison of symptom presentation.**

Symptoms	Our study (%)	Arshad et al study (%)
<b>Pain</b>	100	100
<b>Jaundice</b>	-	-
<b>Dyspepsia</b>	75.86	68

Curved bacteria were detected in 7 cases in direct microscopy with haematoxylin, eosin and gram staining, which was suggestive of *Helicobacter* species. Those cases were confirmed by using urease test.

#### Staining method

In our study, 12.08% cases were positive species by staining methods. Data in our study, showing a low incidence of *Helicobacter* species colonization on the

biliary mucosa, is in agreement with similar studies from different countries such as Turkey, Mexico, Germany, Canada and various other workers.<sup>7</sup>

On the other hand, it is in contrast with findings from other population and workers such as Attallah et al (22%) and Hussain et al (55%), where they found to have higher incidence of *Helicobacter* species on gall bladder mucosa.<sup>8</sup>

This broad variation in the colonization rate cannot be explained only by the difference in sero prevalence of *Helicobacter* species among different populations, also the methodology and sensitivity and specificity of the tests used to detect the organism have to be taken into consideration.

#### Serological methods

In our study, 7 cases were positive for serum IgM and IgG antibodies against *Helicobacter*, compared to other studies the prevalence is low. Few other workers have noted increase in antibody titres levels and in addition to that, our study did not include the controls. Various workers have studied antibodies in healthy individuals as well as in patients of dyspepsia and have reported positivity ranging between 49 and 79%.<sup>9,10</sup>

We found that 7 of the 58 patients screened for IgM and IgG class of antibodies in their serum gave positive results. The gall bladder tissue from these patients also showed spiral organisms suggestive of *Helicobacter*. Thus, there seems to be good correlation between the presence of antibodies in the serum to the *H. pylori* and histological demonstration of the organism in the gall bladder tissue.

Thus, we think in about most of the patients with calculous cholecystitis the disease maybe associated with *Helicobacter*. It is quite possible that in a population there may be individuals who have *Helicobacter* species in the gall bladder mucosa which may not manifest as acute cholecystitis but may go on to have chronic infection.

In this context, one needs to screen a population without gastritis for the presence of IgG and IgM antibodies to *H. pylori* and those were positive should be followed up to understand the natural history of such colonization if found in the gall bladder.

#### CONCLUSION

According to our findings evidence of recent and chronic infection *H. pylori* as shown by demonstration of IgM and IgG class of antibodies respectively to the organism was found in most of patients and histological evidence of the patients with cholecystitis and cholelithiasis. Thus, the frequency of *Helicobacter* infection seems to be low in the patient population studied.

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