

Helicobacter in Biliary Calculus Disease: Histopathological and Serological Association in a Rural Population of Southern India



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Submission: March 23, 2017; Published: March 27, 2017

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Abstract

Introduction: Biliary calculus disease is one of the most common disorders and is an important cause of morbidity world over. In USA and Europe, the prevalence of biliary calculus disease ranges from 7% to 33% among general population. Though accurate estimates are not available, gall stones disease forms an important cause of suffering in India. In recent years, *Helicobacter pylori* has been detected in the human bile and gall bladder tissues. This has evoked considerable interest in the possibilities of colonization and causation of biliary calculus disease by this organism. Here is the study for investigating the evidence of *Helicobacter* infection by histopathological and serological methods at R.L. Jalappa hospital, Kolar, Karnataka which caters mainly for rural population.

Materials and methods: During the period November 2011 to August 2013, 65 patients with clinical features of biliary calculus disease admitted at R.L. Jalappa hospital, Kolar were included in the study. After performing cholecystectomy on the patients selected as above, gall bladder tissues were fixed in 10% formaldehyde and were subjected to histopathological examination. Patients who were treated for *Helicobacter pylori* infections in the past 4 weeks and those with clinical features of gastritis were excluded from the study. A 5 ml blood sample was collected prior to surgery from all the above patients, serum was separated and tested for IgM antibodies to *Helicobacter pylori* using a commercially available ELISA kit.

Results: Sixty five patients of calculus cholecystitis were included in the study. Among them, 27(41.5%) were males and 38 (58.5%) were females. The mean age of overall study population was 41.78± 8.8 years. The youngest patient was 14 years old and the oldest patient was 77 years old. Among the patients studied, all patients had pain in the right hypochondrium, in addition, 20 (30.7%) had dyspepsia and 9(13.8%) had fever. There were no patients with jaundice or mass per abdomen

Conclusion: In the study reported here from southern India, we found that *Helicobacter* species in gall bladder tissue could be detected in 3(4.6%) of 65 patients with Biliary calculus disease. These findings suggest that *Helicobacter pylori* infection in biliary calculus disease is less common among the rural population from southern India. IgM antibody against *Helicobacter pylori* could act as a serological marker for associating *Helicobacter* species with biliary calculus disease.

Keywords: *Helicobacter pylori*; Biliary Calculus Disease; Rural setup

Introduction

Biliary calculus disease is one of the most common disorders and is an important cause of morbidity world over. In USA and Europe, the prevalence of biliary calculus disease ranges from 7% to 33% among general population. Though accurate estimates are not available, gall stones disease forms an important cause of suffering in India. It is thought to be more common in northern than the southern parts of the country [1-3]. In recent years, *Helicobacter pylori* has been detected in the human bile and gall bladder tissues. This has evoked considerable interest in the

possibilities of colonization and causation of biliary calculus disease by this organism [4]. In addition to *Helicobacter pylori*, other *Helicobacter* species such *H. bilis*, *H. pullorum* and others have been isolated from the bile specimens of humans and animals [5-7]. There are very few studies which have investigated the role of *Helicobacter* species in gall bladder disease from India. We investigated the patients with gall stone disease for evidence of *Helicobacter* infection by histopathological and serological methods at R.L. Jalappa hospital, Kolar, Karnataka which caters mainly for rural population.

Materials and Methods

Subjects

During the period November 2011 to August 2013, 65 patients with clinical features of biliary calculus disease admitted at R.L.Jalappa hospital, Kolar were included in the study. A diagnosis of calculus cholecystitis was made in these patients based on detailed clinical examination and relevant laboratory and radiological investigations. Patients who were treated for *Helicobacter pylori* infections in the past 4 weeks and those with clinical features of gastritis were excluded from the study.

Histopathology

After performing cholecystectomy on the patients selected as above, gall bladder tissues were fixed in 10% formaldehyde and were subjected to histopathological examination. Sections from the sample were stained by Haematoxylin and Eosin (H & E) stain and Giemsa stains and examined under microscope for evidence of organisms morphologically resembling *Helicobacter*.

Serological testing

A 5 ml blood sample was collected prior to surgery from all the above patients, serum was separated and tested for IgM antibodies to *Helicobacter pylori* using a commercially available ELISA kit (calbiotech, California. USA). The kit detects Cag-A antibodies against an 128 kd antigen. The results were expressed in antibody units by dividing the optical density value (OD value) of the sample by the cut-off value as per the instructions from the manufacturer. Samples that showed an antibody index of more than 1.1 were considered to be positive for IgM antibodies for *Helicobacter*.

Results

Table 1: Demographics and clinical findings in patient with biliary calculus studied.

Parameter	Number(percentage)
Male	27(41.5%)
Female	38(58.5%)
Mean age	41.78 years
Pain	65(100%)
Dyspepsia	20(30.7%)
Fever	9(13.8%)

Sex	Our Study
Male	27
Female	58
Total	65

Sixty five patients of calculus cholecystitis were included in the study. Among them, 27(41.5%) were males and 38 (58.5%) were females. The mean age of overall study population was 41.78± 8.8 years. The youngest patient was 14 years old and the oldest patient was 77 years old. Among the patients studied, all

patients had pain in the right hypochondrium, in addition, 20 (30.7%) had dyspepsia and 9 (13.8%) had fever. There were no patients with jaundice or mass per abdomen (Table 1) (Figure 1). Among the above 65 patients, 42 patients underwent open cholecystectomy and 23 underwent laparoscopic cholecystectomy. 2 patients of laparoscopic cholecystectomy had to be converted to open cholecystectomy due to dense adhesions (Figure 2).

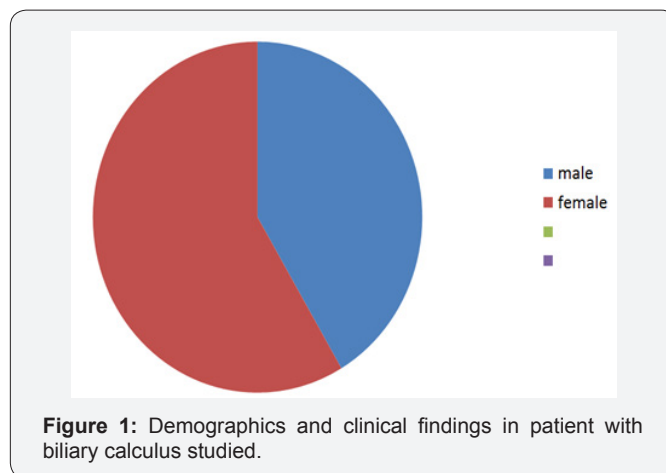


Figure 1: Demographics and clinical findings in patient with biliary calculus studied.

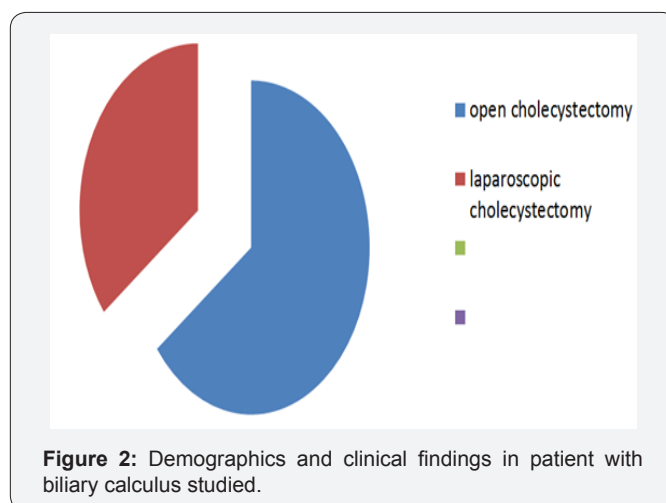


Figure 2: Demographics and clinical findings in patient with biliary calculus studied.

On histopathological examination, 61 (94%) of the cholecystectomy specimens showed features of calculus cholecystitis and 4 (6%) patients showed features of acalculus chronic cholecystitis. H & E stained sections showed diffuse infiltration of gall bladder wall by lymphocytes, plasma cells, histiocytes and perimyceal fibrosis. In three cases H & E and Giemsa stain showed organisms morphologically resembling *H.pylori* (Figures 3 & 4). The IgM antibodies against *Helicobacter* Cag-A antigen could be demonstrated in the five (7.6%) of 65 patients. The antibody index varied between 1.10 to 4.27. The serological findings along with histopathological observations are presented in Table 2. It was observed that organisms resembling *Helicobacter pylori* were detected in the gall bladder tissues of all the 3 patients who showed an antibody index of 4.02 and above.

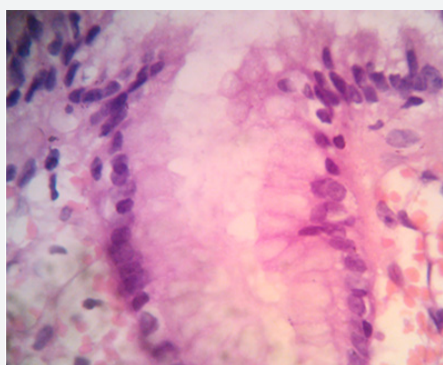


Figure 3: Microphotograph showing an organism morphologically resembling *H. pylori* in gall bladder tissue with Giemsa stain (arrow).

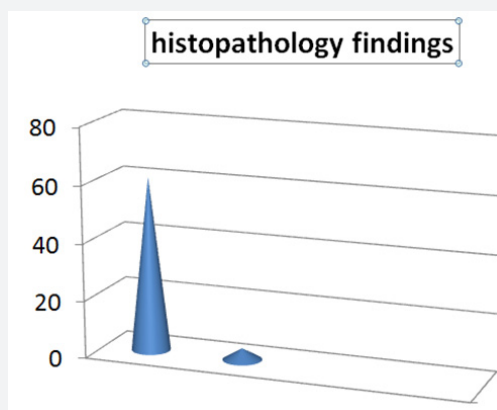


Figure 4: Histopathology Findings.

Table 2: Serological and histopathological evidence of *Helicobacter* infection in patients with gall stone disease.

Serial number	Patient number	Antibody index in IgM ELISA*	Histopathological diagnosis	Presence of organism resembling <i>Helicobacter</i> in gall bladder tissue
1	M/727258	4.27	calculus cholecystitis	+
2	B/778920	4.16	calculus cholecystitis	+
3	M/785761	4.02	calculus cholecystitis	+
4	J/735399	1.71	calculus cholecystitis	-
5	D/767910	1.10	calculus cholecystitis	-

*The antibody index was calculated by dividing OD value of the sample by the cut off value.

Discussion

In the study described here, we could detect organisms resembling *Helicobacter pylori* in gall bladder tissues in 3 (4.6%) of 65 patients subjected to histopathological examination. We could demonstrate IgM class of antibodies among 5 (7.6%) of the patients studied. However, those patients with IgM antibody index of 4 and above only (high titres), were associated with histopathological evidence of *Helicobacter* like organisms in gall bladder tissues. *Helicobacter* species have been associated with gall stone disease in 1.05% to 55% of patients from different parts of the world. Mexico, Greece, Germany and Korea have reported an association of less than 10% [8-11]. In contrast, Turkey and Pakistan have reported an association of *Helicobacter* 22% and 55% respectively in gall stone disease [12,13].

There are a few studies from India which have explored the possibility of *Helicobacter* infection in gall bladder disease. The study by Bansal et al. [14] conducted on patients at Delhi showed that *Helicobacter* DNA could be detected in 32.6% of 49 gall bladder tissues with gall stone disease. The study at Varanasi by Shukla et al. [5] demonstrated *Helicobacter* in 28% of 54 patients with gall stone disease by histopathology, serology and molecular biological methods. Deeba et al. [15] demonstrated IgG class of antibodies against *Helicobacter* in 80% of 75 patients with gall stone disease studied at Aligarh. To our knowledge

there are no studies examining the role of *Helicobacter* in gall stone disease, to date, from Southern India.

Our study conducted among a rural population with biliary calculus disease shows that 4.6% of patients can be associated with *Helicobacter* infection both by histology and serology. This detection rate is lower compared to that from Northern India. Further investigations are required to confirm the disparity in the association of *Helicobacter* in gall bladder disease from different parts of the country. Detection of IgM class of antibodies against *Helicobacter pylori* with higher antibody index in ELISA in the very same patients who had histopathological evidence of presence of organism in the gall bladder tissue suggests that IgM antibodies against *Helicobacter pylori* can be used as serological a marker to detect the presence of *Helicobacter pylori* in gall bladder disease.

Similar Studies

a. Association of the Presence of *Helicobacter* in Gallbladder Tissue with Cholelithiasis and Cholecystitis. According to this study, results support the hypothesis that *Helicobacter* is associated with the pathogenesis of human cholelithiasis and cholecystitis but it is less significant and further studies on larger group of population are required to confirm the observations.

b. *Helicobacter pylori* DNA in gallbladder tissue of patients with cholelithiasis and cholecystitis. According to this study results, although *H. pylori* DNA was detected by PCR in gallbladder tissue of patients with cholecystitis, a clinical correlation with biliary disease could not be established but further studies are required to confirm the results.

Conclusion

In the study reported here from southern India, we found that *Helicobacter* species in gall bladder tissue could be detected in 3(4.6%) of 65 patients with Biliary calculus disease. All the 3 patients with histopathological evidence of *Helicobacter* in gall bladder tissue had higher antibody index(>4.02) for IgM class of antibodies to *Helicobacter pylori* in ELISA test.

In similarity to the findings of other studies on this subject, we were unable to isolate the bacterium by culture; that may have been due to the fact that the DNA we detected was from nonviable organisms. Conversely, there are different ways to explain our inability to isolate viable bacteria. Firstly, most (85.9%) patients had received antimicrobial therapy before surgery. In addition, the samples were maintained frozen without any protective solution, which may have compromised bacterial viability. Finally, as discussed above, it is possible that the number of bacterium is very few and that they may have been partially inhibited by adverse conditions in the biliary milieu. In addition, we can speculate that these strains might have had some distinct requirements that are as yet unknown. These facts highlight the need to improve the conditions for the growth of *Helicobacter* species from the biliary tree to better characterize the microorganism and to allow the development of experimental models for studying the role of *Helicobacter* in the genesis of biliary diseases.

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DOI: [10.19080/OAJS.2017.03.555609](https://doi.org/10.19080/OAJS.2017.03.555609)

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