

Helicobacter pylori in gall bladder disease

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Abstract

In recent years, *H. pylori* has been detected in bile, liver and biliary epithelium obtained from patients with hepatobiliary diseases and is thought to be associated with these diseases. We, therefore, planned the present case control study to find the role of *H. pylori* in gall bladder disease through culture and serological studies. Serum and gall bladder tissue were obtained from 75 patients and only serum from 40 controls. Tissue was processed for smear and culture and sera were tested for presence of antibodies quantitatively by ELISA. *H. pylori* was not detected by smear or culture from any sample, but statistically significant difference was observed between patients and controls in positivity (80 % vs. 47.5%, p value < .05) as well as titres for antibodies against *H. pylori* (p value < .001). Statistically significant difference was observed between antibody levels in patients and controls. Further studies are required, to elucidate the role of *Helicobacter* in hepatobiliary diseases, especially with regard to the effective culture of the organisms from the biliary tree.

Key Words: *Helicobacter pylori*, Gall bladder, ELISA

Accepted June 27 2010

Introduction

Helicobacter pylori is a newly discovered gram negative bacteria which causes chronic active gastric inflammation. Infection seems to be life-long, unless eradicated by antibiotics, and is strongly associated with duodenal ulceration, gastric ulceration and gastric cancer. Recently, the bacterium has been implicated as a risk factor for various extraintestinal diseases including hepatobiliary diseases ranging from chronic cholecystitis and primary biliary sclerosing cholangitis to gall bladder cancer and primary hepatic carcinomas. In recent years, *H. pylori* has been detected in bile, liver and biliary epithelium obtained from patients with hepatobiliary diseases [1-4]. Many researchers have demonstrated the presence of *Helicobacter* DNA in gall bladder of patients with gall stone disease [5-8], though none were able to isolate *H. pylori*. No such studies appear to have been done in India. We, therefore, planned the present case control study to find the role of *H. pylori* in gall bladder disease through culture and serological studies.

Material and Methods

1. Patients and controls: seventy five patients undergoing cholecystectomy because of cholelithiasis or cholecystitis and 40 controls which were healthy volunteers without any complaints or history of dyspep-

sia or gastritis were included in the study. A detailed relevant clinical history was recorded, with special emphasis on the presence of symptoms associated with *H. pylori* infection on a proforma from both the groups.

2. Specimens :

a) 3-4 ml of venous blood was collected from each patient a day before operation, and from controls. The serum was separated and sera were stored at -20°C in sterile vials till further testing.

b) A small piece of gall bladder sample was collected from the operation theatre with sterile precautions in a vial containing five ml normal saline and rest of the gall bladder tissue put in 10% formalin and sent for histopathological examination by the surgery team, and not included in the study.

3. Specimen processing :

a) Gall bladder sample was crushed in a sterile mortar and pestle in one ml saline and the tissue homogenate used for rapid urease test, gram smear and culture as described earlier [9,10].

b) Quantitative ELISA for IgG antibodies was performed in duplicate by using the kit manufactured by NovaTec Immunodiagnostica GmbH, Germany. The instructions given in the Users' Manual with the kit were strictly followed. Titre of 20 NTU/ ml was given as cut-off, thus any titre > 20 was considered positive.

4. Quality control :

- a) Each batch of urease test and culture media was tested with previously isolated *H. pylori* and were also simultaneously used for gastric biopsies.
- b) Controls supplied with the kit were used for ELISA and the runs which were not giving defined values were rejected and repeated. Mismatching pairs of sera were also repeated again in duplicate.
5. Statistical Analysis: Statistical analysis was done using Z – test for proportions to find the significance of differences.

Observations

Maximum number of patients (34.7%) was in the age group 21-30 years, followed by 29.3% patients in the age group 31-40 years. Females and males constituted 88.0% and 12.0% of the study group, respectively. The most frequent complaint was pain in right hypochondrium in 72 (96%), followed by vomiting in 59 (78.7%), dyspepsia in 41 (54.7%) and epigastric discomfort in 35 (46.7%) patients.

All 75 (100%) patients in the study group had taken broad-spectrum antibiotics, 30 (40%) took metronidazole, 50 (66.7%) took NSAIDS and 25 (33.3%) antacids.

Rapid urease test, though meticulously prepared, was found to immediately turn alkaline upon addition of tissue homogenate due to bile, and therefore discarded and not done after twenty specimens.

No specimen was found to be positive in direct microscopy or culture. The same batches of media were giving good growth of previously isolated *H. pylori* and from fresh gastric biopsies.

Serology for IgG antibody was done on all 75 samples, and this was found to be positive in 60 (80%) samples, while 19 controls were positive (Table 1).

Statistically significant difference was observed between positive cases and controls in all the age groups (p -value $< .05$) except in age < 20 , where sample size was very low. A reverse positivity (statistically significant) was seen in age group 41-50 yrs.

Table 2 and 3 show *H. pylori* IgG antibody titers in relation to age of patients and controls in the study group respectively. The patients were having higher titers than the respective control groups, specially in lower age groups. These differences in titers were statistically significant (p value $< .001$) for most of the ranges.

Table 1. *H. pylori* IgG antibodies in relation to age in the cases and controls

Age group (years)	No. of cases	No. of controls	Cases positive for <i>H. pylori</i> IgG	Controls positive for <i>H. pylori</i> IgG
<20	1	1	1 (100)	0
21-30	26	22	23 (88.4)	7 (31.9)
31-40	22	10	18 (81.9)	6 (60)
41-50	19	4	11 (57.9)	4 (100)
>50	7	3	7(100)	2 (66.6)
Total	75	40	60 (80)	19 (47.5)

Figures in parentheses indicate percentage

Table 2. *H. pylori* IgG antibody titers in relation to age of patients in the study group

Antibody titers (NTU/ml)	No. of cases	<20	21-30	31-40	41-50	>50
0-20	15	0	3 (20.0)	4 (26.7)	8 (53.3)	0
21-40	17	0	8 (47.0)	5 (29.4)	2 (11.8)	2 (11.8)
41-60	8	0	3 (37.5)	1 (12.5)	1 (12.5)	3 (37.5)
61-80	10	0	5 (50.0)	2 (20.0)	3 (30.0)	0
81-100	10	1(10.0)	1(10.0)	5 (50.0)	2 (20.0)	1 (10.0)
101-120	3	0	2 (66.7)	0	1 (33.3)	0
121-140	3	0	1(33.3)	1 (33.3)	1 (33.3)	0
0>140	9	0	3 (33.3)	4 (44.4)	1 (11.1)	1 (11.1)
Total	75	1	26	22	19	7

Figures in parentheses indicate percentage

Table 3. *H. pylori* IgG antibody titers in relation to the age of controls in the study group

Antibody titers (NTU/ml)	No. of controls	<20	21-30	31-40	41-50	>50
0-20	21	1(04.8)	15 (71.4)	4 (19.0)	0	1(04.8)
21-40	7	0	3 (42.9)	3 (42.9)	1(14.2)	0
41-60	1	0	1 (100)	0	0	0
61-80	2	0	0	1 (50.0)	0	1(50.0)
81-100	3	0	1 (33.3)	1 (33.3)	1(33.3)	0
101-120	3	0	2(66.7)	0	1(33.3)	0
121-140	2	0	0	1 (50.0)	0	1(50.0)
>140	1	0	0	0	1(100)	0
Total	40	1	22	10	4	3

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 man as well as animals. However, research in this area has been limited by the lack of gold standard in the diagnosis of these organisms in bile. A meta-analysis of some published work has shown strong association between *Helicobacter* and gall bladder disease [11]. We, therefore, attempted to find a relation between *Helicobacter pylori* and gall bladder disease.

Most of the patients in our study were females with cholelithiasis (88%). It is said that a fat, fertile, female of forty is more at risk of developing cholelithiasis. However, we found that a majority of females (34.7%) were in the age group of 21-30 years showing a decreasing trend in the age for development of cholelithiasis. This could probably be attributed to the changes in lifestyle.

Culture remains the definitive investigation to prove the viability of *H. pylori* in bile and gall bladder tissue. Despite prolonged incubation, we were not able to isolate *H. pylori* from any of the patients. This could be due to the fact that all the patients have taken broad spectrum antibiotics and, moreover, 40% of them took metronidazole as well, which constitute a part of eradication therapy for *H. pylori*. It is possible that the number of bacteria remains very low and they may have been partially inhibited by adverse conditions in the biliary milieu. Other possibility is that there is patchy colonization of the biliary epithelium, thus culturing a small piece may not give positive results. Most other workers were also not successful in getting positive cultures, but could demonstrate DNA by PCR [1,5-8]. The lack of recovery has been attributed to the prolonged freezer storage of tissue and bile without

glycerol or other preservatives or that the DNA detected was from non-viable organisms. However, in a recent study, *H. pylori* was isolated from 6 gall bladder samples by culture [12].

Direct microscopy was also found to be negative. A corollary to the same finding is apparent in the work of others [1,13,14]. However, some workers [1,12] detected curved bacteria suggestive, but not diagnostic of *Helicobacter* species, by different histopathological methods (Warthin Starry, Haematoxylin Eosin and Gram staining).

In the present study, statistically significant difference was found between the prevalence of anti *H. pylori* IgG in the study group and the controls. In contrast, some other workers [13,15] found no significant difference in the prevalence of *H. pylori* IgG antibodies in patients with cholelithiasis and controls. However, it has to be emphasized that in most of the studies there was either no control group or very few controls.

Various workers have studied antibodies in healthy individuals [16-18] as well as in patients of dyspepsia [10] and have reported positivity ranging between 49 and 79%. Most of these workers [16,17] have observed increase in positivity for *H. pylori* IgG antibody with increasing age, and the same was observed in the present study among controls. However, we observed that among the patients, positivity was slightly more in the age group of 21-50 years which is also the most common age groups for cholelithiasis.

Some of our patients had symptoms like dyspepsia (54.7%) and epigastric discomfort (46.7%) and it may be suspected that they were suffering with acid-peptic disease as well as with cholecystitis, but as both these symptoms can be present in cholecystitis, they were not explored for the presence of acid-peptic disease.

In the present study, it was observed that 57.3% of the patients had titers of >40 NTU/ml while only 30% of individuals in the control group had titers above 40 NTU/ml. This difference was found to be statistically significant ($p < 0.001$). This is an important information suggesting that majority of patients of cholelithiasis in the study group were positive with higher titers.

These findings highlight the need to improve the conditions for the growth of *Helicobacter* species from the biliary tree and to develop experimental models for studying the role of *Helicobacter* in the genesis of biliary diseases. Further studies are required to elucidate the role of *Helicobacter* in hepatobiliary diseases, especially with regard to the effective culture of the organisms from the biliary tree, the development of accurate tests to identify these bacteria, large-scale autopsy studies and experimental infection of the biliary tract in animal models.

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