Original Research Article

Gallbladder colonization by *Helicobacter pylori* in patients with symptomatic gall stone disease

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A B S T R A C T

Bacterial infection is accepted as a precipitating factor in gallstone formation, and recent studies have revealed the presence of *H. pylori* in the hepatobiliary system; still causal relationship could not be established till now. This study aimed to detect the presence of *H. pylori* antigen in bile and stool of patient with gallstone. Also it evaluates the colonization of gallbladder by *H. pylori* in patients with symptomatic gallstone disease, and to find a possible causal relationship between them. The study enrolled (73) patients undergoing laparoscopic cholecystectomy for gallstones. Bile and stool samples were taken from all patients and subjected to rapid antigen detection test for *H. pylori* utilizing polyclonal anti *H. pylori* capture antibody meridian diagnostic kit (CTK biotech Inc.). The data were tested by applying chi-square at a level of significance (p< or = 0.05) using SPSS version19. *H. pylori* antigen was detected in the stool of 16 (21.9%) patients, 14 were females and 2 were males, and it was also detected in gallbladder bile of 14(19.2%) patients, 13 females and one male. A positive test was found in both bile and stool in 7(9.6%) of patients, all of them were females, the test was negative in both samples in 36(49.3%) of patients. It has been proposed that the presence of *H. pylori* antigen in the bile may represent an increased risk of gallstones formation. This study concluded that *H. pylori* antigen may be detected in the bile of many patients with gall stones. Consequently, gallbladder colonization by *H. pylori* might serve as initiating factor in development of gallstones. Nonetheless, whether eradication of *H. pylori* may or may not reduce future gallstone formation is yet not settled down.

**Keywords**

*H. pylori*; cholecystectomy; gall stones; bile and stool.

Introduction

*Helicobacter pylori* are a gram-negative microaerophilic curved spiral bacterium, with a rapid corkscrew motility resulting from multiple polar flagella. It was identified in 1982 by Barry Marshal and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcer (Blaser, 2006).

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal. Infection is more
prevailing in developing countries, and incidence is decreasing in Western countries (Yamaoka and Yoshio, 2008; Brown, 2000). Over 80% of people infected with *H.pylori* show no symptoms (Boyanovo, 2011).

Recent studies have revealed the presence of *H.pylori* in hepatobiliary system (Fox et al., 1998; Lin et al., 1995; Nilsson et al., 2000; Nilsson et al., 2000; Rocha et al., 2005). The presence of *H.pylori* DNA in gallstones was established by polymerase chain reaction (PCR) in several reports (Monstein et al., 2002; Abayli et al., 2005). Together with the discovery of *H.pylori* antigen in bile juice (Lin et al., 1995; Monti et al., 1999; Neri et al., 2005) This has led to the suggestion that Helicobacter species might be an etiological agent in gallstone formation.

**Pathogenesis:** Transmission of *H.pylori* is thought to be person to person by either the oro-oral or feco-oral routes (Brown, 2000). The organism survives in the mucosal layer that coats the epithelium and causes chronic infection. Although it is non-invasive, it recruits and activates inflammatory cells as neutrophils, macrophages, and plasma cells. Urea that is normally filtered from plasma into GIT mucosal surfaces is broken down by urease enzyme into CO2 and ammonia. The latter is converted into ammonium by accepting (H+) which leads to neutralization of acidic media in the vicinity of organism; the survival of *H.pylori* in the acidic media of stomach is dependent on urease. Ammonia also causes injury and potentiates effects of cytotoxins produced by *H.pylori* (Schreiber et al., 2004; Petersen and Krogfelt, 2003; Liver et al., 1998; Smoot, 1997). It has multiple flagella at one end which allow it to burrow and live deep beneath the mucosal layer closely adherent to the epithelial surface. *H. pylori* uses an adhesive molecule (BabA) to bind to the Lewis b antigen uniquely expressed by only gastric epithelial cells (as in stomach or Meckle's diverticulum ectopic gastric cells) or other epithelial cells which undergo gastric metaplasia as in duodenum metaplasia (Dumreese et al., 2009).

**Clinical significance:** Patients with gallstones may be asymptomatic or presented with recurrent abdominal pain which has three notable characteristics, localization to right hypochondrium, episodic occurrence and relationship to fatty meal. However some patients have atypical symptoms as vomiting, or chronic dyspepsia (Zaliekas and Manson, 2008; Heuman and Moore, 1996). A gallstone is a crystalline material formed within gallbladder by concretion of bile components, occasionally with amorphous materials from mucosal surfaces. On the basis of composition, gall stones can be divided into either cholesterol or pigment stones.

**Cholesterol stones** are single or multiple, varying in color from light yellow to dark green, usually their size range from small granules to large stones exceeding 3cm in diameter. They often have a tiny dark central spot. To be classified as such they must be at least 80% cholesterol by weight. The main two factors for cholesterol stones formation are:

A-the amount of cholesterol secreted by liver relative to lecithine and bile salt.

B-the degree of concentration and extent of bile stasis in gall bladder.
Pigment stones: contain <20% cholesterol and are dark because of the presence of calcium bilirabinate otherwise black and brown pigment stones have little in common and should be considered as separate entities.

Black pigment stones are usually small, brittle and black. They are formed by supersaturation of calcium bilirubinate, carbonate and phosphate, most often secondary to hemolytic disorders such as hereditary spherocytosis and sickle cell disease.

Brown pigment stones are usually <1 cm in diameter, brownish-yellow, soft. They may form either in the gall bladder or in the bile ducts, usually secondary to bacterial infection caused by bile stasis.

Diagnosis

The current available options for diagnosis of *H. pylori* infection are mainly of two categories; invasive which require endoscopy and sometime tissue biopsy and non-invasive methods which include blood for detection of antibodies, stool antigen detection and carbon urea breath test in which the patient drink 14C or 13C-labeled urea. In the latter, the bacterium metabolizes urea producing labeled CO2, that can be detected in the breath of the patient (Table 1). However the most reliable methods is tissue biopsy through endoscopy with rapid urease test, histological examination and microbial culture(23). There is also a urine ELISA test with 90% sensitivity and 79% specificity.

Materials and Methods

A prospective cross sectional study was carried on in the general surgical department of AL-Sader teaching medical city hospital in Najaf, Iraq, during the period between March 2012 to August 2012. Inclusion criteria include any patient with gallstone(s) who is symptomatic and Those with atypical symptoms underwent esophagogastroduodenoscopy (OGD) examination and if negative, are scheduled for surgery. Those with asymptomatic gallstones or those undergoing cholecystectomy for reasons other than gallstone disease were excluded from the study.

A total of seventy three (73) patients (63 women and 10 men) who were diagnosed to have symptomatic gall stones, were enrolled in this study.

Age range was (28-63) with median age of 41 years. Routine demographic data had been collected from all patients with full clinical examination and routine preoperative evaluations. Stool specimen have been taken from all patients for rapid antigen detection test pre-operatively. Patients were admitted at the same day of the surgery. Perioperative antibiotic in form of metronidazole 500 mg and a third generation cephalosporin (ceftriaxone) 1 g were given to all patients. Patients who are allergic to cephalosporin were given an aminoglycoside agent. All patients underwent laparoscopic cholecystectomy and cholecystic bile (2-3) ml was obtained during surgery and sent for *H. pylori* antigen test in the same day.

*H. pylori* Ag rapid test is a sandwich lateral flow chromatographic immunoassay. *H. pylori* antigens detection in the stool of all patients was also done. The trade name of the kit which were used in this study is CTK biotech inc. 10110 Mea rim rood Sandiego, CA 92121; USA [e-mail: info@ctkbitech.com].

This study was approved by the ethics committee of each institution and
Table 1 Methods for the diagnosis of Helicobacter Pylori infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-invasive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>Rapid office kits available</td>
<td>Lacks sensitivity and specificity , and cannot differentiate current from postinfection</td>
</tr>
<tr>
<td></td>
<td>Good for population studies</td>
<td></td>
</tr>
<tr>
<td>$^{13}$C-urea breath test</td>
<td>High sensitivity and specificity</td>
<td>Requires expensive mass spectrometer</td>
</tr>
<tr>
<td>Fecal antigen test</td>
<td>Cheap, specific (&gt;95%)</td>
<td>Acceptability</td>
</tr>
<tr>
<td><strong>Invasive (Endoscopic biopsy)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>Sensitivity and specificity</td>
<td>False negatives occur takes several days to process</td>
</tr>
<tr>
<td>Rapid urease tests</td>
<td>Cheap, quick specific (&gt;95%)</td>
<td>Sensitivity 85%</td>
</tr>
<tr>
<td>Microbiological culture</td>
<td>'Gold standard'</td>
<td>Slow and laborious</td>
</tr>
<tr>
<td></td>
<td>Defines antibiotic sensitivity</td>
<td>Lacks sensitivity</td>
</tr>
</tbody>
</table>

Informed consent was obtained from all patients. Differences between groups were statistically tested by applying chi-square test at a level of significance (P<0.05) using SPSS version 19 software program.

**Result and Discussion**

In this study, a total of 73 patients diagnosed with symptomatic gallstones have been admitted for laparoscopic cholecystectomy where a sample from stool and from bile were collected and tested for the presence of H. pylori antigens for all patients. There were 63 female (86.3%) and 10 (13.7%) males with age ranging from 28-63 years, mean age 41 (SD11.3) years. Twenty three patients (31.5%) have positive H. pylori antigen in their stool samples, while 50 patients (68.5%) have negative test. Twenty one patients (28.8%) have positive H. pylori antigen in their bile samples, while 52 patients (71.2%) have negative test. This shows the biliary colonization by H. pylori in patients with symptomatic gallstones, (Table 2).

Subgroup analysis revealed that sixteen patients (21.9%) have positive test for H. pylori antigen in their stool, but are bile-negative, and fourteen patients (19.2%) are positive for
Table 2 Results of HPSA test in bile and stool samples in patients who underwent laparoscopic cholecystectomy.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antigen positive</th>
<th>Antigen negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td>21(28.8%)</td>
<td>52(71.2%)</td>
<td>73 (100%)</td>
</tr>
<tr>
<td>Stool</td>
<td>23(31.5%)</td>
<td>50(68.5%)</td>
<td>73 (100%)</td>
</tr>
</tbody>
</table>

Table 3 Subgroup analysis of patients with gallstones who underwent laparoscopic cholecystectomy according to their bile and stool HPSA results.

<table>
<thead>
<tr>
<th>Result of HPSA test</th>
<th>Number of patients (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> antigen positive stool and negative bile samples</td>
<td>16</td>
<td>(21.9%)</td>
</tr>
<tr>
<td><em>H. pylori</em> antigen negative stool and positive bile</td>
<td>14</td>
<td>(19.2%)</td>
</tr>
<tr>
<td>Both samples positive</td>
<td>7</td>
<td>(9.6%)</td>
</tr>
<tr>
<td>Both samples negative</td>
<td>36</td>
<td>(49.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

Chi-square = 46.448, DF (degree of freedom) = 3
P-value 0.0002

Table 4 Gender distribution of *H. pylori* antigen test in (73) patients underwent laparoscopic cholecystectomy for gall bladder disease

<table>
<thead>
<tr>
<th>Sex of patients</th>
<th>Bile positive</th>
<th>Stool positive</th>
<th>Both positive</th>
<th>Both negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>13(17.8%)</td>
<td>14(19.2%)</td>
<td>7(9.6%)</td>
<td>29(39.7%)</td>
<td>63</td>
</tr>
<tr>
<td>Male</td>
<td>1(1.3%)</td>
<td>2(2.7%)</td>
<td>Zero(0%)</td>
<td>7(9.6%)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>14(19.2%)</td>
<td>16(21.9%)</td>
<td>7(9.6%)</td>
<td>36(49.3%)</td>
<td>73</td>
</tr>
</tbody>
</table>

P-value 0.449
*H.pylori* antigen in their bile, but are stool-negative. In contrast, only 7 patients (9.6%) revealed positive result in both specimens (stool and bile), with a P-value of 0.0002 which is highly significant (Table 3).

There was no correlation between the presence of *H.pylori* antigen in stool and bile with the sex of the patients with P-value =0.449 (Table 4).

This study showed the biliary colonization by *H. pylori* in patients with symptomatic gallstones was (28.8%), although it is an unusual anatomical site for *H. pylori* colonization. This is similar to Farshad et al., (2004) who reported the presence of DNA but not antigen in 18.1% of gallstones and suggested that *H.pylori* infection may serve as initiating factor in development of gall stones (Farshad et al., 2004; Fox et al., 1998; Bulajic et al., 1946; Sheta et al., Pandey, 2007; Figura et al., 1998).

The role of *H.pylori* infection in formation of different types of gallstones is still unclear. Although human biliary system is thought to be sterile, this can be broken through an ascending infection via duodenal papillary sphincter and descending through portal system (Dye et al., 1978). Although the exact mechanism is not known, bacterial biofilm composed of glycocalyx is suggested to play a role as a nucleation factor. Changes of bile juice composition by beta-glucuronidase and phospholipase produced by bacteria, excessive mucin production of gall bladder epithelial cells triggered by lipopolysaccharides produced by bacteria and promotion of nucleation process through activation of immune system by bacterial itself (Stanley et al., 1993).

In fact, there is no evidence of viable organism in the bile and biliary tract tissue and all recent published studies are based on DNA and antigen detection techniques. Nevertheless, positive presences of bacterial DNA and antigen in bile have been significantly associated with the presence of inflamed gallbladder and cholelithiasis (Kuroki et al., 2002).

It may be argued the same prototype of bacterium present in both intestine and cholecystic bile, therefore; the intestine represent the source of biliary contagion. However, most patients were harboring the microorganism in their bile, but not their stool. This study may suggest that gastrointestinal infection with *H. pylori* may increase the risk for biliary colonization with *H.pylori* as the P-value is highly significant, this agreed with the study which had been done in southern Italy for detection of both the bacteria DNA and the specific antigen (*H.pylori* stool antigen) identified in the stools of 33 consecutive patients undergoing laparoscopic cholecystectomy for gall stones in Foggai University Hospital which concluded that *H.pylori* DNA and protein antigens may be found in gall bladder bile of patients with gall stones especially in the presence of a marked gastro-duodenal colonization by the bacterium. Nevertheless, it does not clarify whether bacterial DNA and/or protein antigens may be suggestive of the presence of viable organisms playing an active role in the pathogenesis of lithiasis and/or cholecystitis (Neri and Margiotta, 2005).

However another explanation for findings in this study may be represented by the presence of residual material from
bacterium which has been damaged by bile.

It has been proposed that the presence of *H. pylori* in bile may represent an increased risk of gall stone formation (Figura et al., 1998). A possible consequence of colonization by *H. pylori* is chronic inflammation of gall bladder mucosa, which may impair gall bladder acid secretion and acidification of content, reducing the solubility of calcium salts in the bile and increasing the risk of their precipitation in gall bladder lumen (Cetta, 1991). Together with the discovery of *H. pylori* in bile juice (Petersen and Krogfelt, 2003), this has led to the suggestion that Helicobacter species are potential etiological agents in gallstone formation.

This study concluded that
1. *H. pylori* antigen may be detected in the bile of many patients with gall stones.
2. Gallbladder colonization by *H. pylori* might serve as initiating factor in development of gallstones.
3. Whether eradication of *H. pylori* may or may not reduce future gallstone formation is yet not settled down.

**Recommendations**

1. Further studies with larger samples of patients are needed to confirm a causal relationship between *H. pylori* infection and gallstone formation and other hepatobiliary diseases, especially if held in prospective way in asymptomatic patients who are harboring *H. pylori*, yet have normal gallbladder.
2. Although it is not cost-effective, use of PCR to detect *H. pylori* DNA in bile as well as in gallstones themselves is worthy to try in further studies.

From this available data it seems that *H. pylori* stool test represents highly accurate diagnostic tool. In addition it is simple, noninvasive, cheap, and can be widely used, so it has the potential to become the preferred diagnostic tool for *H. pylori* infection.

**References**


