Brief Original Article

*Helicobacter pylori* DNA in gallbladder tissue of patients with cholelithiasis and cholecystitis

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Abstract

Background: Earlier reports on the detection of *H. pylori* DNA in gallbladder tissue of patients with cholelithiasis and cholecystitis gave discordant results. Our aim was to detect the presence of *H. pylori* DNA and to determine whether a correlation can be established with the biliary diseases.

Methodology: The study included a total of 68 patients 20 to 79 years of age. Fifty-three of the participants were females, of whom 33 had cholelithiasis and 20 did not. Out of the 15 male patients, 8 were had cholelithiasis and 7 did not. Gallbladder tissue specimens were taken from all patients undergoing cholecystectomy and processed immediately for histology, culture and PCR.

Results: Histological examination revealed that 36 (68%) of 53 females and 9 (60%) of 15 males had cholecystitis. PCR results detected *H. pylori* DNA in 15 (22%) of 68 samples but no bacteria were isolated in culture. 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.

Conclusions: In this study 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 because several conditions were difficult to meet as discussed in the text.

Key words: *Helicobacter pylori*, cholelithiasis, cholecystitis, PCR


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Introduction

*Helicobacter pylori*, a Gram-negative bacterium that colonizes the human gastric epithelium, infects 50% of the world populations. It is the causative agent of peptic ulcer diseases and a major contributor to gastric cancer. Whether *H. pylori* participates in the pathogenesis of biliary diseases is a question that has been addressed by several investigators. The DNA of *H. pylori* has been identified in the bile, liver, and biliary epithelium of patients with hepatobiliary diseases [1,2,3]. In regard to the biliary diseases, in one study, *H. pylori* specific ureB DNA was detected in the gallbladder tissue of a Japanese patient with gallstone and cholecystitis [4]. Similarly Chen *et al.* [5] found *H. pylori* ureA and ureB DNA in 13.55% of the gallbladders of patients with cholecystitis. However, in a study evaluating the presence of *H. pylori* ureA DNA in the bile by nested PCR, Lin *et al.* [1] observed a negative result in four patients with biliary diseases. Queiroz *et al.* [6] were able to isolate *H. pylori* strain from the liver of a Brazilian patient with cirrhosis, which indicates that *H. pylori* may be viable in the human liver. In studies that included a larger number of patients, discordant results have also been observed. A study from Yugoslavia [7] revealed that the presence of *H. pylori*-specific DNA in the bile was associated with biliary tract carcinoma but no association was seen between patients with gallstone and those without biliary disease. Other studies from Germany [8] and Mexico [9] failed to detect the presence of DNA of *Helicobacter* spp. in bile or gallbladder tissue from patients with biliary diseases. Furthermore, DNA of Campylobacter (rather than that of Helicobacter) was detected in the bile and biliary epithelium of Japanese patients with cholelithiasis [10]. Regional differences may contribute to these discordant results. Furthermore, it has also been emphasized that most of these studies involved few control patients or no control group at all. The objectives of this study were to detect the presence of *H. pylori* DNA in gallbladder tissue, attempt to isolate the bacterium from these tissues, and to determine its association with the biliary diseases.
Table 1. Patient distribution in regards to gender and cholelithiasis

<table>
<thead>
<tr>
<th></th>
<th>with cholelithiasis</th>
<th>without cholelithiasis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>33</td>
<td>20</td>
<td>53</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>27</td>
<td>68</td>
</tr>
</tbody>
</table>

Materials and methods

Patients

A total of 68 patients aged 20 to 79 years (average 52 years) attending the Istanbul Teaching hospital on a consecutive basis were included in this study. Fifty-three of the patients were females. Among the 53 females, 33 had cholelithiasis and 20 did not. Of the 15 male participants, 8 were had cholelithiasis and 7 did not. Only patients who received no antimicrobial drugs for four weeks before surgery were included.

Gallbladder tissue specimens (neck and body) were obtained from each patient undergoing cholecystectomy and then processed immediately for histology and culture or frozen at -20°C for DNA extraction. An informed written consent was obtained from each patient and the study was approved by the hospital’s ethical committee. The GCP principles were followed during the different stages of this study.

Histology

Gallbladder tissue specimens for histology were fixed immediately in 10% buffered formalin and then embedded in paraffin wax. Thin sections were stained with hematoxylin and eosin for histological analysis. The presence of cholecystitis was diagnosed by the presence of mononuclear and polymorphonuclear leukocytes in lamina propria, crypts of epithelium that results from fusion of the mucosal folds, and the presence of sinuses [11].

Microbiology

The gallbladder tissue specimens for culture placed in 0.5 ml of PBS buffer were transported immediately to the lab under cold conditions. The samples were homogenized in an Ultratrax homogenizer (Germany) and inoculated onto freshly prepared Colombia agar medium (Oxoid, UK) with 5% horse blood. Plates were incubated under microaerophilic conditions at 37°C for seven days. In addition, four biopsy specimens were also obtained from the antral part of the stomach of four patients undergoing upper gastroendoscopy to be used as a control for the isolation of H. pylori. The specimens were handled in a similar way to those of the gallbladder specimens.

DNA isolation

Gallbladder tissue DNA was extracted with a QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions.

PCR

PCR amplification of the 16S rRNA in gallbladder tissue specimens was done using the forward primer 16S-rRNA.F 5’ TAAGAGATCGCATATATGTC 3’ and the reverse primer 16S-rRNA.R 5’ TCCCACGCTTTAAGCGCAAT 3’ [12]. The same primers were also used to amplify the DNA from the antral specimens. The setup was a denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1 minute, for 40 cycles and a final extension step at 72°C for 5 minutes using the thermal cycler (Techne, UK).

Statistical analysis

Data were analyzed with a statistical software package, version 10 (SPSS Inc., Chicago, Ill.). A P value of < 0.05 was set as significant.

Results

The distribution of patients according to their gender and the biliary diseases is shown in table 1. The attempt to isolate H. pylori from gallbladder tissue specimens was negative. No H. pylori was grown in culture, even though plates were incubated for up to 14 days. However, we were able to isolate H. pylori in culture from three out of the four stomach antral specimens. Histological examination of tissue specimens revealed that 36 (68%) of 53 females and 9 (60%) of 15 males had cholecystitis. Thin sections of these tissues showed inflammatory cell infiltration, degenerative changes, and necrosis in the region where H. pylori DNA was present or not. However, the severity of the inflammatory changes were more obvious in H. pylori DNA positive samples (P = 0.05). We were able to amplify the H. pylori 16S rRNA gene (amplicon size of 625 bp) even though the gallbladder tissue is not the favoured site for H. pylori as is that of the antral region of the stomach. PCR results showed that H. pylori DNA was detected in 15 (22%) of 68 samples, of these 2 samples were
from patients who also had cholelithiasis. We also detected *H. pylori* DNA in the 3 antral biopsy specimens by PCR which further confirmed the amplification process.

**Discussion**

The presence of *H. pylori* DNA in the gallbladder epithelium of patients with cholelithiasis and cholecystitis has been reported. However, the results were conflicting and some investigators detected the presence of *H. pylori* DNA [11] while others did not [8,13]. This phenomenon might be attributed to the small number of subjects studied, differences in the studied populations, the geographic distribution of *H. pylori*, and the difficulty in obtaining a healthy control group.

The distribution of *H. pylori* in gallbladder tissue is well known to be scarce, a fact that makes the localization and finding of this bacteria a difficult task. Our findings were consistent in terms of the amplified PCR products of *H. pylori* DNA obtained from different gallbladder samples. We were able to detect *H. pylori* DNA in 22% of the samples of patients with cholelithiasis and cholecystitis; however, whether it has a role in pathogenesis is still questionable. On the other hand, samples that turned negative might be the result of the presence of few bacteria, inhibitory effect of the biliary milieu, or needed certain requirements that have to be adopted.

In one recent study Chen *et al.* [5] indicated that *H. pylori* exist in the gallbladders of patients with chronic cholecystitis, and in another they showed [14] that *H. pylori* infection may be related to cholecystitis. While Silva *et al.* [11] reported earlier that even though their findings pointed toward a real association between *H. pylori* infection and cholecystitis, they did not rule out the possibility that *H. pylori* colonized a previously damaged epithelium. Stathopoulos *et al.* [15] have investigated the relation of gallbladder function and *H. pylori* infection to gastric mucosa inflammation in patients with symptomatic gallstones prior to cholecystectomy and reported that the gallbladder function is not related to the degree of gastritis. So it appears that several important factors and requirements have to be met in order to establish a definitive association between the presence of *H. pylori* and biliary diseases. In addition, we were unable to isolate the bacterium in culture as has been reported by other investigators in a previous study [11]. As suggested earlier, the failure to do so could be due to the bacterial conversion from viable helicals to non-viable coccoids in an adverse bile-rich environment. This study demonstrated the existence of *H. pylori* DNA in gallbladder tissue; hence future studies are needed to investigate the exact role of this bacterium in this organ.

In conclusion, in this study although we have detected *H. pylori* DNA by PCR in gallbladder tissue of patients with cholecystitis, a clinical correlation with the biliary disease could not be established because several conditions that are difficult meet limited the investigation. Further studies that include healthy control subjects, patients with biliary diseases that also harbor *H. pylori* in their stomachs, isolation of the bacterium from both locations, and molecular analysis of the isolated strains are required in order to establish a significant correlation.

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**Figure 1.** PCR results showing positive bands of *H. pylori* DNA in gallbladder tissues (lane 1, 2, 3, 4, 5). Lane 6 and 7 were negative. M: molecular marker.
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References

Conflict of Interest: No conflict of interest is declared.

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