Helicobacter Pylori Status in Explant Livers of Liver Transplant Recipients in a European Patient Cohort

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Abstract
Background and study aims
Helicobacter pylori (H. pylori) are known to be associated with several gastrointestinal and extra-intestinal diseases such as peptic ulcers, mucosa-associated lymphoid tissue lymphoma, gastric cancer and/or idiopathic thrombocytopenic purpura. Recently, H. pylori genomic DNA has been detected in liver tissue of patients with chronic liver diseases. Furthermore, some studies suggested that detection of H. pylori is associated with progressive liver disease. However, the influence of this organism on the progression of chronic liver diseases remains unclear. The aim of this study was to evaluate the role of H. pylori in patients undergoing Liver Transplantation (LT) owing to end-stage liver disease.

Patients and methods
This retrospective study enrolled 50 explant liver tissue from consecutive patients undergoing LT at the University Hospital of Muenster between January 2011 and December 2012. H. pylori DNA was detected in specimens by PCR.

Results
We successfully extracted DNA from every liver tissue sample; however, H. pylori were not detectable in any liver specimen tested.

Conclusion
In our patient cohort, H. pylori were not detectable in explant liver. Based on these results, H. pylori do not appear to have a significant impact on the progression of liver disease in our patient cohort.

Introduction
Helicobacter pylori (H. pylori) are a gram negative, spiral, microaerophilic bacterium, which infects more than 50% of human stomachs[1,2]. Infection with H. pylori plays a crucial role in the pathogenesis of several gastric disorders such as peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer. Elimination of this microorganism is therefore an important goal in the treatment and healing of these disorders[3,4]. Furthermore, H. pylori is also known to favour the emergence of extra-intestinal diseases such as hepatic encephalopathy[5] and idiopathic thrombocytopenic purpura, sideropenic anemia, and

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vitamin B₁₂ deficiency⁶. Additionally, *H. pylori* have also been considered as a potential trigger of autoimmune disorders⁷. In a recently published literature review, Feng et al., found a significantly high prevalence of *H. pylori* infection in patients with liver cirrhosis⁸. The same review showed that prevalence of this infection in patients with primary biliary or viral cirrhosis was higher compared with alcohol-induced cirrhosis of the liver. One study found an association between *H. Pylori* seropositivity and hepatocyte ballooning, suggesting that *H. pylori* infection may play a role in the progression of non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis⁹. Other studies have also indicated that detection of *H. pylori* in liver tissue was associated with a higher incidence and progression of liver diseases such as Hepatocellular Carcinoma (HCC), hepatitis C-infection or autoimmune liver disorders¹⁰. A study from Nilsson et al., found that the detection of helicobacter is more frequent in the liver of patients with cholestatic liver diseases, compared with patients suffering from non-cholestatic diseases or healthy subjects¹¹. Therefore, in primary sclerosing cholangitis, *H. pylori* may play some role in the development and/or progression of the disease in certain patients¹². Furthermore, *H. pylori* has also been reported to induce hepatotoxicity in vitro¹³.

Conversely, a study from Esmat et al., found no association between *H. pylori* DNA and quantitative hepatitis C virus RNA. Furthermore, no significant difference in Child-Pugh stage was found between the helicobacter PCR-positive group and the *H. pylori*-negative group¹⁴. A further study could not find any significant association between *H. pylori* infections and NAFLD¹⁵.

The aim of our study was to evaluate the prevalence of *H. pylori* in explant livers and the role of this microorganism in development and progression of liver disorders leading to end-stage liver diseases in patients undergoing liver transplantation at our centre.

**Material and Methods**

In this retrospective study, we included consecutive patients who underwent LT between January 2011 and September 2012. DNA was extracted from formalin-fixed explant livers and was analysed to detect the presence of *H. pylori*-specific DNA. This study was conducted in accordance with the guidelines of the Declaration of Helsinki after consulting the local institutional review board of the University of Münster.

**DNA extraction and PCR**

For DNA extraction from FFPE, 10 µM sections were used. After standard de-waxing and overnight Proteinase K digestion, DNA was extracted using a Qiagen instrument and recommended reagents (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Standard PCR (40 cycles) was performed in duplicate in a volume of 20 µl with 100 ng and 10 ng of DNA as template and 2.8 pmol of each primer. For *H. pylori* 16S, 23S and GyRA assays, a second round of nested PCR (20 cycles) employing 2 µl of the first PCR product were performed to increase sensitivity and specificity. Primers used were:

- **16S**: 5´-ACGACAGCCGTGCAGCAC-3´, 5´-GTCTTAGTCTCTCCAGTAAGGCTAACG-3´, nested: 5´-TGCGCAAGCAGCACCTCCCA-3´, 5´-GCTTAGTCTCCAGTAAGGCTAACG-3´.
- **23S**: 5´-CATAAGAGCCAGGCCCATTACTCCAAGGC-3´, 5´-CCTTGTCGGTTAAATACCGACCTGC-3´, nested: 5´-CCTTACTTCAAGCCTCCACCTATCC-3´, 5´-CCTTGTCGGTTAAATACCGACCTGC-3´.
- **GyRA**: 5´-GCATGAAATTAGGCCTTACTTCCAGGCAGTGCG-3´, 5´-GCTCTTCTATTGGCCTCCAGCTTG-3´, nested: 5´-GCATGAAATTAGGCCTTACTTCCAGGCAGTGCG-3´, 5´-GCAGGCGTTATGCGCCCAATAGACG-3´.

Suitable positive and negative controls were included in each PCR assay. To confirm the integrity of the DNA, an additional beta-globin PCR was performed as described above using the primers 5´-GAAGAGCCAAGGACAGGTAC-3´ and 5´-CAACTTCATCCACGTTCACC-3´. Products were analysed on 2% agarose gels with expected sizes of 200 bp (16S), 201 bp (23S), 198 bp (GyRA), and 268 bp (beta-globin). PCR products were analysed by Sanger Sequencing.

To determine the sensitivity of the assay, we included a titration experiment with a positive control of *H. pylori* infected gastric mucosa with a (total) DNA concentration of 31 ng/µl. The sample was serially diluted 1:5 in five steps, i.e. the final range of DNA concentration employed as template in the PCR assays was 31 ng/µl – 0.01 ng/µl. PCRs were performed in a nested approach as described above. The detection limit of *H. pylori* after two rounds of PCR was 0.05 ng/µl (total DNA concentration). Figure 1 show testing of sensitivity of *H. pylori* PCRs.

![Figure 1: Testing of sensitivity of *H. pylori* PCRs.](www.ommegaonline.org)
Results and Discussion

A total of 50 explant livers fixed in formalin and embedded in paraffin wax, were included in the study. The mean age of patients at the time of transplantation was 50.8 ± 11.1 years; range, 24 – 73 years. The most common diagnosis leading to LT was HCC, followed by alcoholism and chronic viral hepatitis. Clinical data and demographic characteristics of patients are shown in Table 1. Following PCR analysis, no H. pylori-specific DNA was detectable in the liver tissue from any of our 50 patients. All patients underwent gastroscopy during their clinical course, with 45 also undergoing gastric tissue biopsy. Four of the 45 patients with biopsy material were PCR-positive for H. pylori, three of which were detected prior to LT and therefore received helicobacter eradication treatment, while infection in the fourth patient was detected after transplantation. Multiple studies have implicated H. pylori in the development and/or worsening of several liver diseases. However, the role of this infection as a risk factor in the pathogenesis and progression of liver disorders is still controversial. Since there is evidence that H. pylori DNA can be isolated from formalin-fixed paraffin-embedded liver tissue (FFPET)[16,17], we consecutively analysed explant livers of patients who underwent LT during the time under consideration. In each case, we were able to isolate DNA from FFPET. However, H. pylori DNA could not be detected in any of the cases in our study population. Our results suggest that this microorganism does not play any decisive role in the pathogenesis and progression of liver disease in our western European patient cohort. An explanation for this finding may be the low prevalence of H. pylori infection in the study population. Only four of the 45 patients (8.9%) who underwent gastroscopy with biopsy during their clinical course were positive for H. pylori. This result is consistent with the increasing evidence showing a significant and quick decrease of H. pylori prevalence in Northern European countries, whereas the prevalence of H. pylori in Southern Europe (for example, in Turkey and in Portugal) and in Asian and African countries still remains very high[18,19]. Thus, it is not surprising that much of the data showing H. pylori to be a significant influence on the course of liver diseases have come from Asia, Southern Europe and Egypt, where the prevalence of infection is known to be high[5,20].

<table>
<thead>
<tr>
<th>Underlying disease*</th>
<th>Number (percent):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular carcinoma</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Hepatitis B/C</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Primary sclerosing cirrhosis</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Polycystic liver disease</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Secondary sclerosing cholangitis</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver disease</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Others</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

* Some patients presented with more than one diagnosis

Conclusion

H. pylori infection does not seem to play a decisive role in the development and progression of chronic liver disorders in a Western European patient cohort. This fact may be because of the low prevalence of H. pylori in this study population. Further multicenter studies, encompassing different ethnic and geographical cohorts, are important for evaluation of the true role played by this bacterium in liver diseases.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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