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ORIGINAL ARTICLE

Survey of association between *Helicobacter pylori* and hepatocellular carcinoma in the specimens derived from health centers of Shahid Beheshti University

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ABSTRACT

Background: Prior investigators demonstrated *Helicobacter pylori* as a risk factor of liver diseases. In this study association between *H. pylori* and hepatocellular carcinoma was investigated.

Patients and methods: Totally, 59 specimens of liver were collected from two health care centers affiliated to Shahid Beheshti University of Medical Sciences, including 22 specimens of hepatocellular carcinoma, 18 specimens of cirrhosis and 19 normal specimens of liver. Polymerase chain reaction was used to determine the presence of *H. pylori* in liver samples using *H. pylori* gene-specific primers.

Results: *16srRNA* of *Helicobacter* genus were found in 31.8% of hepatocellular carcinoma and 16.7% of cirrhotic specimens, however, we could not find this gene in normal samples. Meanwhile, the presence of *ureC* and *cagA* genes specific for *H. pylori* were investigated in positive specimens to confirm the *H. pylori* infection, however, these genes were not detected.

Conclusion: *Helicobacter* infection exists in liver of patients with hepatocellular carcinoma. However, further studies are necessary to confirm this association.

Keywords: *Hepatocellular carcinoma; Cirrhosis; Helicobacter pylori*.
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INTRODUCTION

Helicobacter pylori is one of the most common bacteria worldwide found in more than 50% of human population (1). *H. pylori* is a Gram-negative and microaerophilic microorganism that can cause chronic gastritis, peptic ulcers and gastric adenocarcinoma (2,3). In 1994, *H. pylori* has been classified by the International Agency for Research on Cancer as type I carcinogen (4).

In recent years, the attention has been drawn towards the possible association of *Helicobacter* infections not only with upper gastrointestinal tract disease but also with several extra-gastrointestinal diseases such as liver and biliary disease. *Helicobacter* species have been isolated from the

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liver samples of a variety of mammals, the role of which has been documented in the chronic hepatitis and various types of liver carcinoma (5).

Hepatocellular carcinoma is the third cause of cancer death worldwide (6). Each year, more than 626,000 cases of hepatocellular carcinoma are reported. However, since no proper diagnostic modality has been approached, more than 598,000 people lose their lives (7). This tumor often follows chronic liver inflammation and cirrhosis (8). Persistent hepatitis B (HBV) and hepatitis C virus (HCV) infections and aflatoxins are the main causes of HCC (9,10).

Prior investigators demonstrated an association between *H. pylori* and hepatocellular carcinoma. Therefore, in this study, we investigated the presence of *H. pylori* in the liver of patients with hepatocellular carcinoma.

PATIENTS and METHODS

We collected 59 liver specimens from two health care centers affiliated to Shahid Beheshti University of Medical Sciences, including 22 specimens of hepatocellular carcinoma, 18 specimens of cirrhosis and 19 normal specimens of liver.

DNA extraction: DNA was extracted from liver specimens by using QIAamp DNA mini kit (Qiagen, Germany). Then DNA was stored at -20Cº.

Polymerase Chain Reaction (PCR): Samples were amplified by *Helicobacter* genus specific 16S rRNA primers, the forward 5′-GCT ATG ACG GGT ATC C -3′ and the reverse 5′-GAT TTT ACC CCT ACA CCA -3′ primer (11) . To make sure that the bacteria was *H. pylori*, in positive specimens the presence of *ureC* gene (forward primer 5′-GGA TAA GCT TTT AGG GGT GTT AGG GG-3′ and reverse primer 5′-GCT TAC TTT CTA ACA CTA ACG CGC-3′) and *cagA* gene (forward primer 5′-GAT AAC AGG CAA GCT TTT GAG G -3′ and reverse primer 5′-GCG TCA AAA TAA TTC CAA GG -3′) specific for *H. pylori* were also investigated.

Sequence analysis: The amplified products of 16S rRNA genes were purified using PCR clean up kit (Roche, Germany). The purified PCR products were sequenced by using an automated sequencer. Sequence analysis was performed with the BioEdit (version 5.0.6). The partial nucleotide sequences of the 16S rRNA gene have been submitted to Genbank.

RESULTS

16S rRNA of *Helicobacter* genus were found in 7(31.8%) hepatocellular carcinoma and 3 (16.7%) cirrhotic subjects, however, we could not detect these genes in normal samples. To make sure that the bacteria were *H. pylori*, in positive specimens the presence of *ureC* and *cagA* gene specific for *H. pylori* were also investigated, however, these genes were not detected.

Analysis of the nucleotide sequences of 16S rRNA PCR amplicon showed comparable results with other previously deposited 16S rRNA sequences in GenBank. Hence, the sequence of 16S rRNA ampilcon has been also confirmed (figure 1).

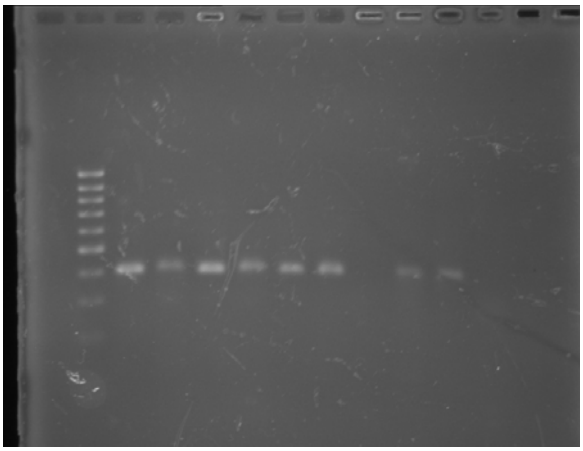

Figure 1. Agarose gel (1.5%) showing PCR products obtained with *Helicobacter* genus specific *16S rRNA* primers. **M**: Marker 100pb, **1**: positive control, **NC:** negative control.

DISCUSSION

Gastrointestinal and liver diseases are among the most common causes of morbidity in Iran resulting in substantial proportion of mortality with enormous consequences for this country (12).

In the present study, *Helicobacter* DNA was detected using *16S rRNA* specific primers in 31.8% (7/22) of liver specimens from patients with HCC and 16.7% (3/18) specimens of patients with cirrhosis. Huang et al. have shown the presence of *Helicobacter* DNA using *16S rRNA* specific primers in eight of 20 liver samples of HCC (13). Similarly, Rocha et al. have demonstrated presence of *16S rRNA* of *Helicobacter spp.* in 90.5% of HCC specimens (11). In another study in Italy, Dore et al. have detected *16S rRNA* of *Helicobacter spp.* in 17% of cirrhotic specimens and 55% of HCC specimens (14).

Ponzetta et al. have reported that *cagA* could be obtained from liver tissue of cirrhotic patients with HCC (15). In China, Xuan et al. have detected *cagA* in 10.7% of 28 specimens from patients with HCC (16). Furthermore, Huang et al. have found *cagA* in 2 samples from 8 samples of *16S rRNA* positive specimens of HCC (13). Nevertheless, we could not find *cagA* from any of HCC and cirrhotic specimens. This could be in part explained by the fact that *cagA* is present in approximately 60% of strains of *H. pylori* (13).

Our results are in agreement with previous studies that have shown the presence of *Helicobacter spp.* in liver of humans. These data confirmed the association between *Helicobacter spp.* with chronic liver diseases. The relationship between *H. pylori* and HCC need further studies.

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