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Detection of *Helicobacter rodentium*-like DNA in the liver tissue of patients with chronic liver diseases by polymerase chain reaction–denaturing gradient gel electrophoresis and DNA sequence analysis

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Abstract

Many *Helicobacter* spp. were isolated from the stomach, intestinal tract, and liver of different animals and humans. The association between *Helicobacter* spp. and hepatobiliary diseases, including hepatocellular carcinoma, was thoroughly examined, indicating a potential role of the bacteria in the progression toward cancer. In our work, we screened 97 liver biopsies from patients with chronic liver diseases for the presence of *Helicobacter* spp. DNA. With the use of genus-specific polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE) and subsequent sequencing, we found that the majority of *Helicobacter* spp. DNA detected was similar to *Helicobacter rodentium* DNA (71%). The DNA of other detected *Helicobacter* spp. was similar to *Helicobacter pylori* DNA. This is the first indication of *H. rodentium*-like DNA presence in human liver tissue. We also conclude that PCR–DGGE is a useful screening method for assigning species designation and heterogeneity.

Keywords: *Helicobacter*; Chronic liver diseases; PCR–DGGE

1. Introduction

Since the discovery of *Helicobacter pylori*, the number of species included in the genus *Helicobacter* has constantly grown. Nowadays, it comprises 32 formally named species and several putative *Helicobacter* spp., which are currently under investigation (http://www.bacterio.cict.fr/). The DNA of a number of *Helicobacter* spp., which was isolated from the stomach, intestinal tract, and liver of a variety of animals, was also detected in human bile and liver samples (On et al., 2002; O'Rourke et al., 2001). *Helicobacter*-like DNA has been found in the liver of patients with chronic liver disease (CLD) (Nilsson et al., 1999, 2000; Stalke et al., 2005), cholangiocarcinoma, and hepatocellular carcinoma (HCC) (Al-Soud et al., 2008; Avenaud et al., 2000; Nilsson et al., 2001; Ponzetto et al., 2000). Fox et al. (1998) have researched frozen bile samples and gallbladder-resected tissue from patients with chronic cholecystitis and identified *Helicobacter* spp. DNA (*Helicobacter bilis*, *Helicobacter rappini*, *Helicobacter pullorum*), although cultivation of the bacteria was not successful. The possible significance of *Helicobacter* spp. in pediatric patients with miscellaneous liver diseases was stated (Tolia et al., 2004). The results were based on DNA sequencing following polymerase chain reaction (PCR) analysis with *Helicobacter* genus-specific primers. It was not possible to correlate the presence of

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Helicobacter DNA with biochemical markers and a particular disease, although such attempts were made. In a more recent study, only 2 out of 75 liver biopsy samples were typed as *H. pylori*, indicating that such an infection is much less prevalent in benign liver diseases (Cindoruk et al., 2008). Taken together, these data do not show nonambiguously the correlation between *Helicobacter* spp. presence and etiology of CLD.

The aim of the present study was to determine the prevalence of *Helicobacter* spp. in liver specimens in patients with CLDs from Northern Poland with PCR–denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis. Because *Helicobacter* spp. are difficult to detect and culture by standard methods, a PCR–DGGE technique was used in this study for the detection and identification of *Helicobacter* spp. as previously described (Al-Soud et al., 2003).

2. Materials and methods

Ninety-seven patients (46 females, 51 males) aged 18 to 66 years (mean, 41 ± 1) were admitted to the Department of Infectious Diseases, Medical University of Gdansk, Gdansk, Poland, because of CLDs. The etiology of liver disease was chronic viral infections (Hepatitis B virus [HBV] and Hepatitis C virus [HCV]), autoimmune hepatitis, and toxic liver damage. There were no end-stage liver disease patients in the studied group (no liver cirrhosis and HCC). During hospitalization, needle biopsy was done and samples of liver tissue were collected from all patients. The local ethics committee accepted the protocol of this study, and all of the patients provided written informed consent. The collected liver samples were directly frozen at −20 °C before further handling.

2.1. DNA extraction and amplification conditions

Liver specimens were thawed, and DNA was extracted by a QIAamp DNA Kit (QIAGen, Hilden, Germany) according to the manufacturer's instructions and stored at −20 °C. Amplification was carried out by using a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, Foster City, CA) and *Helicobacter* genus-specific primers for 2 conserved regions: v6–7 and v1–2. For the amplification of the v6–7 region, C98 (TCC CAC ACT CTA GAA TAG T), previously published by Fox et al. (1998), and H.ssp-F (AAC GAT GAA GCT TCT AGC TTG CTA G) and H.ssp-R (GTG CTT ATT CGT TAG ATA CCG TCA T) oligonucleotides, described by Verhoef et al. (2003), were used (Fig. 1).

The reaction mixture for the first PCR (25 μL) contained 0.5 μmol/L concentration of each primer (H.ssp-F and C98), 0.2 mmol/L of each deoxynucleotide triphosphate (dNTP) (Pharmacia Biotech, Uppsala, Sweden), 1× chelating buffer, 2.5 mmol/L $MgCl_2$, 0.4% (w/v) bovine serum albumin (BSA), 1.25 U of rTth DNA polymerase (Applied Biosystems, Foster City, CA), and 5 μL of extracted DNA. Amplification conditions for the first PCR were 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and finally, 72 °C for 5 min.

The reaction mixture for the second PCR (seminested PCR) (25 μL) contained 0.5 μmol/L of each primer (H.ssp-R and H.ssp-F with a GC-clamp: 5′-GCG GCC GCC CGT CCC GCC GCC CCC GCC CCG CCG CGG CCG C-3′),

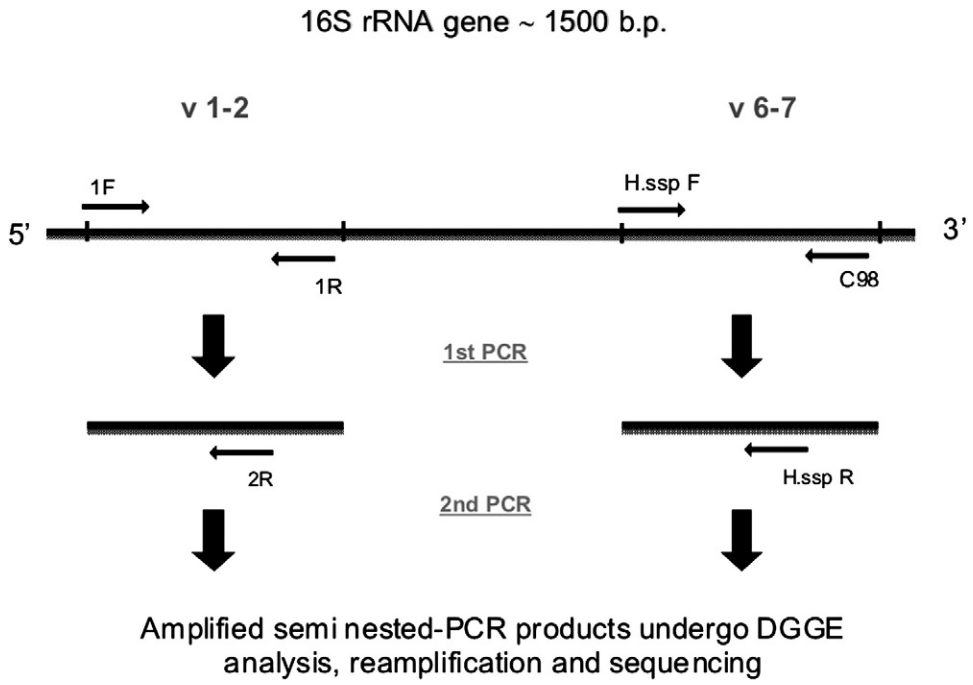

Fig. 1. Scheme of the strategy used to identify *Helicobacter* spp. in the clinical samples with PCR–DGGE. Above the arrows, particular primers are indicated.