

HELICOBACTER SPP. DETECTED IN LIVER, PANCREAS IN HUMAN PATIENTS WITH TUMOURS

RUPA MULCHANDANI¹, HANS-OLOF NILSSON², TORSEL WADSTROM³ & PADMA DESHMUKH⁴

^{1,4}Department of Microbiology, Smt. Chandibai Himathmal Mansukhani College of Arts, Science and Commerce,
Affiliated to University of Mumbai, Ulhasnagar Thane, Maharashtra, India

^{1,2,3}Department of Infectious Diseases and Medical Microbiology, Lund University, Sölvegatan 23, 223 62, Lund, Sweden

ABSTRACT

Helicobacter species is now-a-days found commonly in the liver, stomach and intestine of human beings and animals. It has now been detected in pancreas of the human beings also. It is the cause of cancer as it grows into the tissue and is circulated around the body through blood stream and spreads in many organs. Where-ever it finds the niche it grows. In our study, *Helicobacter* species were identified in tissue surrounding liver cancer and pancreatic tumours from patients undergoing pancreatectomi. By DNA sequence analysis of two PCR amplified 16Sr DNAs, homologies were found to be *Helicobacter pylori* and *Helicobacter pullorum* from fresh pancreas tissue, respectively. Also, in one of fresh liver sample *Helicobacter* spp. was confirmed by DNA sequencing.

KEYWORDS: *Helicobacter*, Liver, Pancreas, Human, PCR, Sequencing

Short Running Title: *Helicobacter* spp. in Human Patients with Cancer

INTRODUCTION

Many novel *Helicobacter* species have been isolated and characterized recently. *H. hepaticus* was shown to cause severe liver diseases, such as hepatitis and liver tumour, in laboratory rodents (Wang, 1994). Other new species of urease positive and negative *Helicobacter* have been reported to cause similar diseases in animals such as dog (*H. canis*), hamster (*H. cholecystus*), sheep (*H. rappini*) and poultry (*H. pullorum*) (Fox, 1997). *Helicobacter* species, such as *H. pylori*, *H. bilis*, and *H. pullorum*, were identified in bile, gallbladder and liver of patients with primary sclerosing cholangitis, primary biliary cirrhosis and cholecystitis, hepatocellular and cholangiocarcinomas using PCR and subsequent DNA-sequencing (Nilsson *et al*, 2000, Nilsson *et al*, 2001, Casswall *et al*, 2010). *Helicobacter* species has been identified in pancreas in patients suffering from pancreatic tumours (Nilsson *et al*, 2002, Nilsson *et al*, 2006). The association of *Helicobacter* species and extra-gastric disease prompted us to analyse the liver and pancreas, thus, the objective of this study was to analyse biopsies from liver and pancreatic cancer patients for *Helicobacter* species by PCR and DNA-sequencing.

MATERIALS AND METHODS

Seven liver samples were obtained from patients suffering from hepatocellular carcinomas. Tissue surrounding resected pancreatic tumours from three patients undergoing pancreatectomi, as well as tissue from a non-tumour histologically normal pancreas, were analysed preliminarily. The samples were transported in sterile Stuart's transport medium and immediately sent to the laboratory for the detection of *Helicobacter* spp. Each sample was homogenized and cultured microaerobically on GAB-Camp's medium and Brucella blood agar medium (Jalal *et al*, 2002). For the PCR, DNA was extracted by the Qiagen Tissue DNA kit protocol.

Also, 10 samples of paraffin embedded pancreas tissue surrounding tumours were de-embedded by xylene and ethanol washing and extracted as described above. Similarly, 7 paraffin embedded liver tissue tumours from Kaunas, Lithuania were also treated by the same way. Extracted samples were analysed by PCR using *Helicobacter*-specific 16Sr DNA primers (Fox *et al*, 1998). PCR-products amplified with *Helicobacter* genus-specific primers were purified from 1.5% agarose gels by centrifugation in Ultrafree-DA tubes (Millipore, Bedford, US). DNA sequence analysis was carried out using an Applied Biosystems DNA sequencer according to the protocols of the manufacturer (Perkin Elmer, Applied Biosystems, Foster City, US), using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and confirmed by GeneBank/EMBL database comparison. One gall bladder specimen from a patient with gallstone was also analysed by the same method.

RESULTS

There was no growth obtained after incubating the culture plates for 3-7 days. Thus, culture was found to be negative for *Helicobacter* spp. for the fresh liver and pancreas samples.

4/7 liver samples and 5/7 paraffin-embedded liver samples, 1/10 paraffin-embedded pancreas and 3/3 pancreas, analysed shortly after surgery, were positive for the genus *Helicobacter* by 16Sr DNA-PCR analysis. One sample from a normal pancreas was negative in the same analysis from pancreas. The gall bladder sample was found positive for *Helicobacter* spp.

Helicobacter 16Sr DNA-PCR products were analysed using DNA sequence analysis for liver and pancreas. The liver specimen from one of the 7 fresh liver biopsy samples was confirmed for *Helicobacter* species by sequencing (Table I). By sequence comparison, carried out using the Blast program (GCG, Madison, US) and GenBank/EMBL databases, one of the sequences for pancreas sample showed high homology (at least 98%) to the 16Sr DNA of *Helicobacter* species "liver 3" (GenBank accession number AF142585) and to the 16Sr DNA of *Helicobacter pylori* (Nilsson *et al*, 2001). The other sequence showed high homology to the 16SrRNA of *Helicobacter pullorum* (AF047850), *Helicobacter bilis* (AF047847) and other strains of *H. pullorum* (Fox, 1997), (Table II).

DISCUSSIONS

Helicobacter species were identified by PCR and DNA sequence analysis in tissue surrounding liver and pancreatic tumours. This finding is not surprising given the many recent reports of *Helicobacter* associated with extra-gastric disease in both animal and man (Fox, 1997, Nilsson *et al*, 2000, Fox, *et al*, 1998, Nilsson *et al*, 2001, Nilsson *et al*, 2006). The amplified sequences showed high homology to previously published sequences of *Helicobacter pylori* and *Helicobacter pullorum*. Interestingly, the sequences in the GeneBank database that showed the highest homology to the sequences reported in this study, were not identified or isolated from gastric tissue but from liver, pancreas, gall bladder tissue, suggesting that subspecies of certain *Helicobacter* species, colonizing different abdominal niches, may exist. Thus, it was confirmed that *Helicobacter* infection can occur in the extra-gastric organs. Though our study is based for diagnostic purpose but it confirms that *Helicobacter* infection in Sweden which is a developed country is also possible. This reflects and confirms reports from other studies also. Hence, it is necessary to analyse extra-gastric organs too for detecting the main route cause of infection in developing countries also.

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APPENDICES

Table 1: PCR of Liver Samples

S. No	Fresh Liver Samples 16Sr DNA-PCR	Paraffin-Embedded Liver Samples 16Sr DNA-PCR	Sequencing
1.	+	+	+
2.	+	+	NT
3.	+	+	NT
4.	+	+	NT
5.	-	+	NT
6.	-	-	NT
7.	-	-	NT

NT: Not Tested

Table 2: PCR of Pancreas Samples

S. No	Fresh Pancreas Samples 16Sr DNA-PCR	Sequencing Identification
1.	+	+AF142585 <i>H. pylori</i>
2.	+	+AF047850 <i>H. pullorum</i>
3.	+	+AF047847 <i>H. bilis</i>
4.	-	-