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Rapid Communication

Detection of hepatitis C virus genomes from patient's plasma using PCR method.

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Recently, the genome of putative hepatitis C virus (HCV) was cloned and the assay system of anti-HCV antibody was developed (1). However, there is no method to detect HCV from a small amount of blood. We tried to detect HCV genomes directly from plasma using polymerase chain reaction (PCR).

MATERIALS AND METHODS: Two oligonucleotide primers (HCV1: sense primer, HCV4: antisense primer) were synthesized based on the sequence of NS5 domain of the prototype HCV of Chiron (2). The 5'end of HCV1 is 400 nucleotides upstream from the 5'end of HCV4. RNA was extracted from one ml of plasma samples, and cDNA was synthesized by reverse transcriptase using HCV4 as the primer. The cDNA product was amplified by PCR with HCV1 and HCV4. After amplification, the one-tenth PCR products were subjected to electrophoresis through 4 % polyacrylamide gel, and the gel was stained with ethidium bromide to check the amplification of 400 base pairs (bp) DNA fragments which implicates the presence of HCV genomes in the plasma. The nucleotide sequence of the PCR product was determined by the dideoxy chain termination method.

RESULTS: From the plasma of 5 patients with non-A non-B (NANB) hepatitis, 400 bp DNA fragments were clearly amplified by PCR (Fig.). These fragments were not amplified without reverse transcription, indicating that the PCR products were derived from plasma RNA. On the other hand, 400 bp DNA fragments were not amplified from the plasma of the patients with type B hepatitis or healthy control subjects. The sequence of one PCR product showed 80 % homology at the nucleotide level and 85 % homology at the amino acid level, compared with the prototype HCV.

CONCLUSION: This 400 bp DNA fragment was specifically amplified from patients with NANB hepatitis and was homologous to the prototype HCV, indicating that the HCV-RNA genomes were detected by our PCR method. By this method, the HCV genomes could be detected during the acute phase of NANB hepatitis, suggesting that the present PCR method is the only method to diagnose acute type C hepatitis at the acute stage.

Key Words: HCV, HCV-RNA, PCR

1) Kuo G, et al: Science 1989;244:362-364
2) Houghton M, et al: EP-A-0 318 216 A1

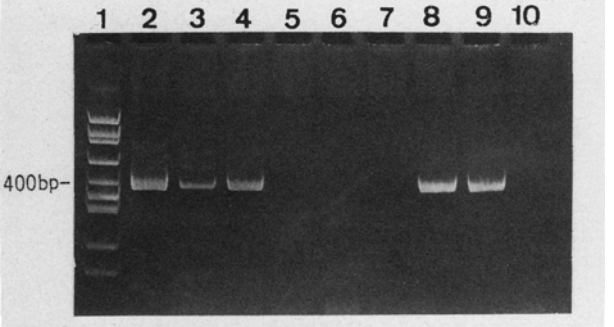

Fig. 4% Polyacrylamide gel electrophoresis of PCR products. lane 1:molecular weight marker, lane 2:NANB-CAH, lane 3:post-transfusional NANB hepatitis in acute phase, lane 4:NANB-CAH, lane 5:B-LC, lane 6:B-CAH, lane 7:Healthy control, lane 8:post-transfusional NANB-CAH , lane 9:NANB-LC, lane 10:PCR of the same sample as lane 9 without reverse transcription. CAH:chronic active hepatitis, LC:liver cirrhosis.

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