Biotecnologia Aplicada, ISSN: 0684-4551
Elfos Scientiae


REPORTE CORTO/SHORT REPORT

Presented in the Congress Biotecnologia Habana 94. La Habana, Cuba, Nov. 28 - Dec. 3, 1994

PRESENCE OF HEPATITIS C VIRAL RNA IN SERUM OF PATIENTS WITH NON-A, NON-B HEPATITIS IN CUBA DETECTION BY PCR

Juan Morales-Grillo, Mario Barro, Ariel Vina, Cesar R. Gomez, Ciro Garcia, Odalys Garcia.

Vaccine Department. Center for Genetic Engineering and Biotechnology. P.O. Box. 6162. C.P.10600. Havana. Cuba(53- 7)218070.

Code Number: BA95027
File Sizes:
   Text: 6K
   No associated graphics

INTRODUCTION

Hepatitis C is the major causative agent of post transfusion and sporadic non-A, non-B Hepatitis in the world (1). Although the commercial kits containing several epitopes of the Hepatitis C virus are available for the screening of HCV infection, they only reflect the immune response and do not indicate active viremia. There is a need for a reliable marker that differentiates ongoing HCV infection with viremia from resolved or past infections. To discriminate between these possibilities, a direct test for the presence of HCV would be invaluable. Due to the relatively small quantities of viral RNA found in serum and liver
tissues of infected individuals it can not be detected by the conventional hybridization techniques (2).

Recently, HCV RNA detection by PCR reaction has been used to diagnose Hepatitis C in patients with non-A, non-B Hepatitis (3). The aim of this study was to detect the presence of HCV RNA in serum of patients with chronic non-A, non-B Hepatitis in Cuba.

MATERIALS AND METHODS

The 18 samples used in this study were previously tested using UBI HCV EIA and LIA TEK HCV, commercial kits (supplied by Organon Teknika B.V., Oss, The Netherlands). All patients were seropositive for both anti-HCV assays (4). The primers were derived from the highly conserved 5'-noncoding region (5), based on alignment of previously published HCV sequence (6). RNA was prepared by the proteinase K-SDS method described previously (3). For cDNA synthesis, the RNA and random primers were denatured together at 95 C for 3 min and incubated at 42 C for 1 h with avian Myeloblastosis virus reverse transcriptase (Boehringer) as described by Sambrook et al. (7).

The PCR reaction was performed in 100 uL mixture containing 5 uL of specific cDNA and 50 picomole of each outer primer. The PCR reaction was carry out in 35 cycles of 94 C for 1 min, 60 C for 1 min and 72 C for 1 min., followed by a 5 min final extension at 72 C. For the nested amplification, 10 ul from the first reaction were added to a similar PCR reaction mixture as the former, but with the inner primers. PCR was carried out for 35 cycles as described for the first amplifications but the annealing temperature was 65 C.

Ten microlitres of nested PCR final reaction was analyzed by electrophoresis through 1.5% agarose gel and by Southern blot using the internal probe labelled with 32P (7). To avoid contamination the precautions recommended by Kwok et al. (8) were used.

RESULTS AND DISCUSSION

All the 18 patients were positive for HCV RNA. The observed size of the RT-PCR products was around 230 bp in the ethidium bromide- stained agarose gel and was consistent with the expected length of 229 bp between the two primers used for nested PCR. The specificity of these products was confirmed by hybridization to radiolabelled internal oligonucleotide used as probe in the Southern blot. No additional bands were seen in the agarose gel or in the autoradiogram. The HCL RNA was detected in all patients positive to antibodies anti-HCV and previously diagnosed with a chronic hepatitis. It was not detected in the healthy individual used as negative control who was also seronegative for anti-HCV antibodies.
In our study, the HCV RNA was detected in all Cuban patients, with chronic Hepatitis C, seropositive for anti-HCV assays and revealed that viremia was highly associated with anti-HCV antibodies. The same good correlation was reported by others (9, 10).

The detection of HCV RNA using the 5'UT region primers in the 18 samples confirm the importance of using primers from this region, which is the most well conserved of the genome among all strains of HCV isolated up to date (6). We conclude that our RT-PCR is a highly sensitive assay for detection of HCV sequences in serum. It could be useful as a confirmatory test for the diagnosis of acute Hepatitis C infection and for identifying viremia in the chronic disease.

REFERENCES


Copyright 1995 Sociedad Iberolatinoamericana de Biotecnologia Aplicada a la Salud

Contact: Biotecnologia Aplicada