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Diagnosis and Genotyping of Hepatitis C Virus by Polymerase Chain Reaction in Chronic Liver Disease Patients

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Abstract: All 50 blood samples from Chronic Liver Disease (CLD) patients were screened for (Antibodies against hepatitis C virus) Anti HCV by 3rd generation (Enzyme Linked Immuno-Sorbant Assay) ELISA for diagnosis and genotyping of HCV by (Polymerase Chain Reaction) PCR. Samples were screened by slot blot hybridization using P³² ATP labelled genotype specific oligonucleotide probes indicating prevalence of genotype HCV^{-1b} which was further confirmed by southern hybridization and direct sequencing. Positivity by ELISA was 37% while with that of PCR was 42% in hepatitis patients indicating its sensitivity and reliability and can be used to determine the carrier state and hence its spread can be checked through blood screening.

Key words: HCV, genotyping, screening

Introduction

Liver is the target organ in hepatitis which is effected by a number of unrelated viruses. Recently discovered, contributing large portion of viral hepatitis is HCV which has 6-8 weeks incubation period (Stanford and Shulman, 1992).

There are six million chronic viral hepatitis carriers in united states only and about sixteen thousands die each year with cirrhosis or liver cancer (Justin, 1995). Infection becomes chronic in more than eighty percent of the infected people hence the disease is an important public health and economic problem (Dusheiko, 1994).

General characteristics of disease are, persistent infection in majority of exposed individuals, associated with wide spectrum of histological damage and fluctuating biochemical expression, low tendency towards spontaneous recovery, high tendency to progress to cirrhosis, high prevalence to auto immune phenomenon and weak immunity against reinfection (Camps *et al.*, 1995).

What has been done includes complete characterization of HCV genome with its genetic variability. Development of four generations of diagnostic antibody tests, better understanding of epidemiology and introduction of antiviral treatment (Van der Poel *et al.*, 1994).

Materials and Methods

RNA isolation and polymerase chain reaction: 1 ml blood from each patients was collected in tubes containing ethylene diamine tetra-acetate (EDTA). Plasma was obtained by centrifugation of blood at 3000 rpm for 10 minutes. RNA was extracted according to Petrelli *et al.* (1994).

For cDNA synthesis by reverse transcriptase the protocol of Tisminetzky *et al.* (1994) was used. Primers were synthesized at nuclear institute for biology and genetic engineering (NIBGE). PCR amplification was performed in a DNA Thermal cyclor by using specific primers complementary to '5 NCR of HCV genome. For N-PCR, 1 ml of regular PCR product was used as template and amplified by using internal S² and AS² primers.

The amplified products of regular and nested PCR were analyzed in 1.5% agarose gel following Davis *et al.* (1994) then visualized by ethidium bromide (EtBr) staining by exposing to UV light.

In southern blot hybridization each amplified cDNA sample was electrophoresed and gel was photographed. cDNA was denatured by soaking the gel in denaturing solution. Then

soaked in neutralizing solution. Transfer of denatured cDNA from gel to filter takes place overnight. cDNA was fixed to filter by baking and vacuum drying. Hybridization was performed in hybridization solution containing G₁ probes. Autoradiography was done overnight at -70°C.

DNA sequencing and Auto radiography: For the direct sequencing of amplified PCR products the protocol of Perkin elmer kit no (8080175) was used. Autoradiography was performed to visualize signals of radioactive nucleic acids in slot blot. Blot or dried gel was placed in x-ray cassette in dark room, after desired time of exposure, film was dipped in developer and then in fixer and washed with tap water.

Results

Blood samples were screened for HBV and HCV by 3rd generation ELISA. Results indicated 23% positivity for HBV and 71% negativity while 6% were on borderline. HCV prevalence was 37%, negativity in 60% while border line were 3%. In case of regular PCR external S¹ and AS¹ primers were used while in N-PCR internal primers and 1 µl of regular PCR product as template was used. There was 92% positivity by regular PCR and 94% positivity by N-PCR indicating its high sensitivity. In slot blot hybridization only G₁ probe which was specific for HCV genotype showed hybridization indicating its prevalence in the samples. Southern blot hybridization also confirmed the results of slot blot hybridization. Sequence analysis revealed the prevalence of HCV^{1b} type in the samples studied.

Discussion

HCV genome is the only marker for the diagnosis of HCV infection in the detection of HCV RNA (Zekri *et al.*, 1995) HCV is present in blood at extremely low level therefore molecular methods are the only way for its detection. Eleven percent positivity by ELISA for both HBV and HCV indicated co-infection or cross reactivity. Co-infection plays an important role in fulminant hepatitis and development of HCC (Alberti *et al.*, 1994) even though two viruses seems to inhibit each other at molecular level while their cytopathic effect is enhanced. Low sensitivity of ELISA may be due to low level of antibodies or difference in antigenic region of local isolates from the ones used in the ELISA kit. Antibodies against the envelope region of HCV are only recognized if glycosylated antigens of the viral envelope are used. In case of immuno suppressed individuals

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patients have antibodies against non structural or envelope region therefore even 3rd generation Anti HCV test may miss cases of HCV infection with antibodies against single components of the HCV (Hess, 1994).

R-PCR product of HCV genome may be below the level of detection by EtBr staining, is amplified in N-PCR and can be easily detected. This was reflected by an increase in positivity by N-PCR. Slot blot and southern blot hybridization assays using allele specific oligonucleotide probes (ASO) reducing the time required for direct sequence analysis (Stuyver *et al.*, 1993; Tisminetzky *et al.*, 1994).

Samples with negative RT-PCR showing no hybridization in slot blot or southern hybridization may have unknown genotype. Confirmation of genotype was done by direct sequencing of HCV isolates. Found in many samples HCV^{-1b} type which was associated with complication in the course of disease, severe progression in chronic hepatitis C severe graft damage and resistant to interferon and ribavirin treatment (Gane *et al.*, 1996).

Investigation concerning the source of sporadic infection of HCV deserves merit and suggests that individuals that are asymptomatic are in fact the carriers of HCV and suggests the necessity for the screening of blood donors.

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