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Study of The Distribution of Hepatitis C Virus Genotypes in Patients With Chronic Hepatitis C Infection in Moradabad (U.P) India

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ABSTRACT

Hepatitis C virus (HCV), an important cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, shows a considerable genetic heterogeneity among hepatitis C virus isolates from all over the world. At least six main groups of sequence variants are recognized. The natural history of disease and response to treatment may be related to the genotype of HCV in a particular patient. Antigenic differences between genotypes also have implications for optimal design of serological sequencing and confirmatory assays for HCV. The present study was undertaken with the objective to find out various genotypes of hepatitis C virus prevalent in Indian patients with chronic hepatitis C infection. 360 consecutive newly diagnosed patients with chronic hepatitis C infection were included in the study. Of the 360 patients, genotype 3 was found in 240 (66.6%). Of these 240 patients, 3a was seen in 50 patients (13.8%), 3b in 20 (5.5%) and mixed subtype 3a and 3b in 170 patients (47.2%). Genotype 1 was found in 50 patients (13.8%). 20 patients (5.5%) were infected with genotype 2 (subtype 2a and mixed subtype 2a, 2b respectively). Ten (2.7%) was infected with genotype 4 (4a). Mixed genotype infection was found in 40 patients (11.1%). The present findings showed that genotype 3 of hepatitis C virus was the most prevalent genotype in patients with chronic hepatitis C in Moradabad (U.P) India

KEYWORDS :Genotypes – 3, Hepatitis C virus, Line probe assay.

Introduction

Hepatitis C virus (HCV) is a hepatotropic virus of family Flaviviridae and genus Hepacivirus having single strandard RNA of positive polarity as genomic material. A large number of genotypes have been identified among hepatitis C virus isolates from all over the world. Presently six main groups of sequence variants have been characterized corresponding to types 1-6; each group containing a number of more closely related subtypes (a, b, c, *etc*.). Hepatitis C virus can cause chronic hepatitis in about 80 per cent of cases. Although most chronic HCV patients have mild chronic hepatitis, it is a progressive disease and can lead to cirrhosis or hepatocellular carcinoma. Without treatment, 33 per cent patients have an expected median time to cirrhosis of less than 20 yr. It has been suggested that different genotypes have different clinical outcomes with regard to disease severity and response to interferon therapy.

Very few studies have been done on the distribution of various hepatitis C virus genotypes in India Recently a study also hinted towards geographic variation in the prevalence of various HCV genotypes in India. We took up this study to find out the prevalence of various genotypes of hepatitis C virus in the patients with chronic hepatitis C infection.

Material & Methods

360 consecutive newly diagnosed patients with chronic hepatitis C infection (*i.e.*, anti-HCV positive by third generation ELISA) who attended outpatient department of Teerthanker Mahaveer medical College Hospital, Moradabad during 2013-2016 were included in this study. The serum samples were taken for extraction of viral RNA and determination of viral genotypes by reverse transcriptase polymerase chain reaction (RT-PCR) combined with line probe assay. *RNA extraction*: Serum was mixed with lysis solution containing guanidinium thiocyanate, sarcosyl and beta-mercaptoethanol. RNA was then extracted with phenol and chloroform followed by precipitation with isopropanol at -70ºC. From the RNA thus isolated, complementary DNA (cDNA) was made using avian myeloblastosis virus-reverse transcriptase (AMV-RT, Genei, Bangalore). Nested PCR was carried out using two set of primers (Innogenetics, Belgium) from highly conserved 5′ non-coding region of the HCV genome, as recommended in line probe assay. In brief, the cDNA was introduced in a reagent mixture containing an excess of deoxyribonucleotides, outer primers and thermostable Taq DNA polymerase (Genei, Bangalore). This reagent mixture was put in thermal cycler (Perkin Elmer Gene Amp PCR System 2400, Norwalk, USA) in which the cDNA got amplified by polymerase chain reaction. This was followed by repeat PCR using inner primers having their 5′ end labeled with biotin. *HCV genotyping*: The genotype of the amplified cDNA was determined using the principle of reverse hybridization in a line probe assay (INNO-LiPA HCV II kit, Innogenetics, Belgium). For this the DNA segment obtained after nested PCR was denatured and incubated, under stringent conditions, with strips having probes corresponding to various HCV genotypes. The resulting bands were then made visible with streptavidin alkaline phosphatase immunochemical reaction using nitroblue tetrazolium and bromo chloro indolyl phosphate (NBT/ BCIP) as substrate. The genotypes were identified using the chart provided by the manufacturer. The INNO-LiPA assay strips contain 15 probe lines to identify HCV genotypes and subtypes, according to the Simmonds classification.

Results

Genotype 3 was observed in 240 of the 360 patients (66.6%). Of these 240 patients, 50 showed infection with subtype 3a (13.8%), 20 had subtype 3b (5.5%) and 170 showed both 3a and 3b infection (47.2%). Genotype 1 was seen in 50 patients comprising 13.8 per cent of all cases – one of these had subtype 1b and four had subtype 1a. 20 patients (5.5%) showed infection with genotype 2 (one each with subtype 2a and mixed subtype 2a, 2b).

Discussion

With the discovery of hepatitis C virus by Choo *et al*, a large number of patients belonging to the non-A, non-B hepatitis could be etiologically defined and definitely categorized. The virus was identified by cDNA cloning and sequencing of RNA genome. A comparison of HCV genome sequences from various geographical regions of the world has shown substantial heterogeneity of nucleotide sequences within several regions of viral genome. On the basis of these genomic differences HCV has been classified into various genotypes. Six major genotypes with several subtypes were identified and a nomenclature for these was given following a consensus proposal. Subsequently several new genotypes and subtypes have been reported from different parts of the world. In one study atleast 12 genotypes were predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. But currently, the existence of six major HCV types is generally accepted, and scientific debate is ongoing about whether types 7-11 are distinct types or subtypes of types 3 and 6. Of various HCV genotypes in India. HCV types 1a, 1b, 2a, 3a, 3b, and 3g have been identified in the earlier studies from northern and western India and genotype 1 predominated over genotype 3 in southern India. Recently a study on samples from many parts of India also found high prevalence of genotype 3. Our analysis of HCV infection in 36 patients with chronic hepatitis C demonstrated genotype 3 as the predominant

genotype, most of our cases with genotype 3 infection showed presence of mixed subtype 3a and 3b. Genotype 1 was the second most common genotype, with most cases showing infection by HCV subtype 1a. Other genotypes seen included genotype 2, 4 and 5. The earlier studies as well as the present study indicated that genotype 3 was the most prevalent genotype in north India. From other parts of the world studies reveal that genotype 3 is prevalent in South East Asia whereas genotype 1 is common in USA and Western Europe. These geographical differences may help in predicting the origin of HCV virus. The remarkable heterogeneity of virus is the major limitation for developing a vaccine for HCV infection. It has been suggested that the degree of sequence variability of HCV is sufficient to significantly alter the antigenic and biologic properties of the virus. Antigenic differences between genotypes have implications for optimal design of serological sequencing and confirmatory assays for HCV. Diminished sensitivity to genotype 3 was seen in recombinant immunoblot assay-2 (RIBA-2) and to a lesser extent in RIBA-3, and this might also be reflected in the sensitivity of screening ELISAs. Future tests will need to incorporate antigens from various genotypes to ensure an optimal sensitivity for all, especially genotype 3, which has been reported as the commonest genotype in our country as well as South East Asian countries. The severity of disease, its progression, and response to therapy may vary according to the genotype. A number of studies have reported that severe liver disease occurs in relation to type 1 infection (especially type 1b) and that cirrhotic patients infected with HCV type 1b carry a significantly higher risk of developing hepatocellular carcinoma as compared to those infected with other HCV types. But the results of various studies are conflicting. It is known that genotype 1 is the second most common genotype reported from various parts of our country, as also seen in the present study. Thus knowledge on the distribution of various genotypes in our country is essential for its prognostic implications in chronic hepatitis C infection.

Conclusion

In our study, Genotype 3 was found to be the most prevalent genotype in patients with chronic hepatitis C in Moradabad (U.P) India. The natural history of disease and response to treatment may be related to the genotype of HCV in a particular patient. Antigenic differences between genotypes also have implications for optimal design of serological sequencing and confirmatory assays for HCV

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