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HCV genotype determination in monoinfected and HIV co-infected patients in Cuba

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ABSTRACT

With the aim to characterize the HCV genotype distribution in Cuba, sera were collected from two subgroups: HCV-monoinfected and HCV/HIV co-infected patients. A combination of reverse transcription-PCR using genotype-specific primers, restriction fragment length polymorphism and sequencing was used to determine the genotype of 84 samples. Seventy-nine (94%) showed single infections (10 [12%] were genotype 1a and 69 [82%] genotype 1b) and 5 (6%) samples corresponded to mixed infections (2 [2%] with genotypes 1a/3a and 1 sample [1%] each with 1b/3a, 1b/4a and 1a/1b/3a). HCV/HIV co-infected subjects had a higher frequency of mixed infections (p=0.08), infection with genotype 3a (p=0.18) and for the first time genotype 4a was found. There was no association of any demographic characteristics with any specific genotype although HCV/HIV co-infected patients showed a tendency to have mixed genotypes in those older than 45 years of age (p=0.11). Phylogenetic analysis showed that HCV isolates clustered with subtypes 1b (n=15, maximal genetic distance 2.51%) and 1a (n=2, maximal genetic distance 0.35%). This report presents the prevalence of HCV genotypes in monoinfected and HIV co-infected patients, mixed HCV infections in HCV/HIV co-infected men who have sex with men with high-risk sexual practices and for the first time identifies that the uncommon genotype 4a can be present in a patient co-infected with HIV.

1. Introduction

Hepatitis C virus (HCV) infection is a major global cause of chronic liver disease. The estimated number of HCV infected subjects worldwide is increasing, and now numbers approximately 170 million people.[1] In Cuba,

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the seroprevalence of HCV infection is low in the general population (0.6 to 1.9%)[2,3] and in blood donors (0.7 to 0.8%).[3,4] However, it is higher in risk groups such as people with hemophilia, plasmapheresis blood donors, patients with onco-hematological diseases and multi-transfused hemodialysis patients (59, 47.3, 19.5 and 95.6% respectively).[3,4] The Cuban HIV/AIDS epidemic has the lowest prevalence rate of the Caribbean region, and by the end of 2011, the island had 15 824 patients living with HIV-1 (National Registry of HIV/AIDS, Cuba). Studies in Cuba showed that in HIV-positive patients, 14.2% were positive for anti-HCV antibodies, and of them 77.7% were also positive for HCV-RNA.[5] Risk factors for parenteral exposure to both viruses clearly influence the risk of co-infection. In addition, the accumulated evidence suggests that HCV behaves like an opportunistic infection in people with HIV infection.[6]

The distribution of different HCV genotypes and subtypes varies geographically. Genotypes 1, 2 and 3 are prevalent in Europe, Japan, United States and Latin America; genotype 4 in Central and North Africa and in the Middle East; genotype 5 in South Africa and genotype 6 in Hong Kong and Viet Nam. Each of the six main genotypes is equally divergent from the others, differing at 31–34% of nucleotide positions on pairwise comparison of complete genome sequences and the variation among different subtypes is 15–30%. These differences result in approximately 30% amino acid sequence divergence in the encoded polyproteins. Patients chronically infected with HCV require significantly different durations of therapy and achieve substantially different sustained virologic response rates to therapies, depending on the HCV genotype with which they are infected.[7,8] This fact together with the possibility that different genotypes could affect diagnostics assays and the natural history of the disease has motivated a series of molecular epidemiology studies. However, prevalence rates of genotypes appear to be changing different populations with the emergence of different genotypes in diverse parts of the world, causing differences in the survival rate of the patients.[7–9]

Sequence variation is equally distributed throughout the HCV viral genome, apart from the highly conserved 5′ non-coding region (5′ NCR) and core, and highly variable HVR1 (hypervariable region 1) in E2. Because of its sequence conservation, the 5′ NCR is the target of most HCV detection and quantification assays. This 5′ NCR also contains genotypically variable sequence positions which permit discrimination of all of the major genotypes and many of the subtypes of HCV. Procedures based on 5′ NCR can be considered the most adequate for genotyping in clinical practice. The core region, although less conserved than the 5′NCR, is the most conserved region in the HCV genome open-reading frame and the most conserved protein in different genotypes.[10–12]

Little information regarding HCV genotype circulation and phylogenetic analysis of HCV strains is available from Cuba.[13–15] None of the studies have analyzed HCV infection within different populations and risk groups. Because each group has its own clinical and epidemiological characteristics, it is vital to know the distribution of HCV genotypes for the follow-up of chronic infections.

2. Materials and methods

2.1. Study population

Serum samples from 109 patients who were confirmed as anti-HCV positive by a third generation immunoassay were included. The samples were received in the National Reference Laboratory on Viral Hepatitis for HCV molecular diagnosis and were stored at −20 °C until use. For analysis purposes the population was divided into two main subgroups: HCV-monoinfected (n=63) and HCV/HIV co-infected (n=46) patients. The samples were collected from 2001 to 2005 in the first subgroup, and from 2007 to 2008 in the second. The demographics, epidemiological and clinical characteristics of the study population are summarized in Table 1. Patient data were collected from medical charts.

2.2. Laboratory assays

2.2.1. RNA extraction, reverse transcription, PCR reaction and genotyping by RFLP analysis (n=63)

Total RNA was extracted from 200 μL of serum with Trizol LS (Invitrogen Life Technologies, Carlsbad, CA, USA), followed by chloroform extraction and isopropanol precipitation. The RNA pellet was washed with 70% ethanol (vol/vol) and then briefly air dried and redissolved in 20 μL of RNAse/DNAse free water. The RNA was reverse-transcribed into cDNA using MMLV (200 U/μL) and random hexamer primers (50 μM) during 90 minutes at 37 °C. Following transcription, the cDNAs were amplified by nested-PCR targeting the 5′ NCR using primers 209, 939, 211 and 940.[16] Genotype and subtype determination of HCV strains was done by cleavage of the amplified 5′ NCR using restriction enzymes (*HAE III, RSA I, MVA I, HINF I, BSTU I* and *SCRF I*) that recognize sequence polymorphisms between six HCV genotypes: 1(a and b), 2 (a and b), 3 (a and b), 4, 5 and 6.[17]

2.2.2. PCR reaction and genotyping using specific primers targeting the core region (n=109)

After RNA extraction and cDNA synthesis, a nested PCR was performed using in the first PCR reaction primers 256 and 186 NTER and in the second reaction a mix of 10 (four sense and six antisense) primers specific for the amplification of four different HCV genotypes: 1 (a and b), 2, 3 (a) and 4. This primer combination produces PCR products of the following sizes: 125 bp for genotype 1a; 141 bp for 1b; 75 bp for 2; 87 bp for 3a and 336 bp for 4a.[11]

2.2.3. Amplification of the 5′ NCR and part of the core gene, PCR product purification, sequencing and phylogenetic analysis (n=48)

In addition, the cDNAs were amplified by nested-PCR directed to the 5′ NCR and part of the core gene of the HCV genome (spanning nucleotide positions of 107–452).[18] The amplified PCR fragments were purified using the QiaQuick PCR purification Kit (Qiagen, Valencia, CA, USA) and sequenced in both directions using the dideoxynucleotide terminator method, with the ABI Prism 3100 Genetic