RESEARCH NOTE

GENOTYPES OF HEPATITIS C VIRUS (HCV) IN LIVER DISEASE PATIENTS IN SRI LANKA

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Abstract. A total of 460 samples (serum or plasma) were obtained from clinically diagnosed liver disease patients from January 2006 to December 2007 and subjected to reverse transcription - polymerase chain reaction (RT-PCR) based detection of HCV. Of these, 32 samples (6.9%) were positive for HCV RNA. Samples that were positive for HCV were genotyped with type specific primers. The genotyping assay was validated by DNA sequencing and phylogenetic analysis. The Sri Lankan isolates were comprised predominantly of genotype 1b (46.9%) followed by genotype 2b (21.9%), 2a (15.6%) and mixed infection with genotypes 1b and 2b (3.1%). This is the first report of the distribution of HCV genotypes in Sri Lanka.

INTRODUCTION

Hepatitis C virus (HCV), first identified in 1989, causes a slowly progressive disease affecting about 170 million (3%) people worldwide (Choo *et al*, 1989; WHO, 2000b). More than three million new cases of infection are reported annually, and epidemiological studies indicate a wide variation in its prevalence patterns in different continents and countries (WHO, 2000b).

The genome of HCV is a single-stranded, positive sense RNA molecule of approximately 9.6 kb in length (Choo *et al*, 1989). There is remarkable genetic heterogeneity and divergence among HCV sequences which has led to the categorization of HCV into “genotypes”. HCV genotypes are related to regional distribution (Liselotte *et al*, 2004), clinical manifestation, response to treatment and prognosis (Martinot *et al*, 1999). Phylogenetic evaluation of HCV sequences recovered from multiple geographic regions suggest that there are at least six major genotypes (Huang *et al,* 1999). Genotype 1a (older nomenclature genotype I) (Simmonds, 1995) is the most prevalent globally, and also the commonest type in the USA, Australia and UK. Genotype 1b (older nomenclature genotype II) is more evenly spread over most geographical areas. Genotype 2a (older nomenclature genotype III) is the most prevalent type in both India and Pakistan, but in the Middle East it is genotype 2b (older nomenclature genotype IV) (Roche, 2008). This is the first study of the genotypes of HCV in Sri Lanka.

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MATERIALS AND METHODS

Specimen collection

Samples were requested from consultant physicians who were working in government

and private hospitals. Samples were obtained from 460 patients with a clinical diagnosis of liver disease and subjected to RT-PCR based detection of HCV. Of these, 32 samples positive for HCV RNA were used for genotyping. Ethical clearance was obtained from the University of Peradeniya, Sri Lanka.

RNA extraction

Viral RNA was extracted with the Guanidinium thiocyanate / Silica method using 100 µl of serum or plasma (Gunesekera *et al*, 2003).

Nested RT-PCR for genotyping

RNA was reverse transcribed using reverse primer (5′ ATGTACCCCATGAGGTCGCT 3′; nt 391-410) (Huang *et al,* 1999) and M-MLV-reverse transcriptase. The cDNA was PCR amplified in the same tube using Taq polymerase, forward (5′ CGCGCGACTAGGAA GACTTC 3′; nt 139-158) (Huang *et al,* 1999) and reverse primers. The thermal cycler was programed for 35 cycles of 94ºC for 1 minute, 55ºC for 1.5 minutes, 72ºC for 2 minutes; followed by a final extension at 72ºC for 10 minutes. The first round PCR product was subjected to nested PCR using Taq polymerase with a common forward primer (5′ AGGAA GACTTCCGAGCGGTC 3′; nt 148-167), and reverse primers which are specific to each genotype, genotype 1a (5′ TGCCTTGGGG ATAGGCTGAC 3′; nt 185-204), genotype 1b (5′ GAGCCATCCTGCCCA CCCCA 3′; nt 272 - 291), genotype 2a (5′ CCAAGAGGGACGGG AACCTC 3′; nt 302-321), and genotype 2b (5′ ACCCTCGTTT CCGTACAGAG 3′; nt 251-270) (Huang *et al,*1999). The thermal cycler was programed at 94ºC for 2 minutes, 30 cycles of 94ºC for 45 seconds, 65ºC for 1 minute, 72ºC for 1.5 minutes, and a final extension at 72ºC for 5 minutes.

Detection

PCR products were run in 2% agarose gel with a 50 bp marker in the presence of ethidium bromide (10 µg/ml) (Akahane *et al,* 1994). Electrophoresis was achieved at 100V and amplicons were observed on a UV transilluminator. The expected sizes for each genotype were: 57 bp-genotype 1a, 144 bp - genotype 1b, 174 bp - genotype 2a, 123 bp - genotype 2b.

Validation

DNA sequencing was performed (Eton Bioscience, USA) for the 12 genotype specific amplified products which represented genotype 1b (7 products), genotype 2a (2 products) and genotype 2b (3 products) to validate the assay. Each sequence was entered into the NCBI database using the BLAST search tool and similar sequences were retrieved. In each case the phylogenetic tree that was derived showed that the sequence was closely related to other accessions of the same genotype. The DNA sequences for the amplified products are available in Genbank under the accession numbers EU233421-7 (genotype 1b), EU284704-5 (genotype 2a), EU293542-4 (genotype 2b).

RESULTS

Of 32 samples, 15 (46.9%), 5 (15.6%), 7 (21.9%), 1 (3.1%) and 4 (12.5%) were found to be genotypes 1b, 2a, 2b, mixed infection of genotypes 1b and 2b and none of the genotypes 1a, 1b, 2a or 2b, respectively.

DISCUSSION

In the UK, USA and Australia genotype 1a is the predominant strain, in the Middle East it is genotype 2b, in Pakistan and in India in general, it is genotype 2a (Roche, 2008). In South India, however, the predominant strain is genotype 1b (Khaja *et al*, 2006). Our data shows that the most prevalent HCV genotype among clinically diagnosed liver disease patients in Sri Lanka is also type 1b, which is more evenly spread over most geographical areas (Roche, 2008).

HCV genotypes have been shown to be closely related to the clinical condition and degree of severity of HCV. The clinical manifestations of those infected by HCV type 1b are usually more severe than types 2a and 2b, and are more commonly the cause of hepatocarcinomas (Huang *et al,* 1999). A high prevalence of HCV type 1b is an important indicator of the public health burden caused by this pathogen in Sri Lanka.

We did not detect genotype 1a in any of the samples. A positive control sample for genotype 1a was not available. There is no conclusive proof that genotype 1a is not present in Sri Lanka, especially considering the fact that 4 of the positive samples could not be genotyped with the primers that were used.

Our data suggests that the occurrence of HCV among clinically diagnosed liver disease patients is relatively low in Sri Lanka (6.9%). This supports the observations that alcoholism and hepatitis B are the major causes of chronic liver disease in Sri Lanka (Fernando *et al,* 2001). Two probable reasons for this are that the Sri Lanka National Blood Center screens all blood donors for HCV prior to transfusion and low numbers of injecting drug users (UNDP, 2008) in Sri Lanka.

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