INTRODUCTION

Hepatitis C virus (HCV), first identified in 1989, causes a slowly progressive disease affecting about 170 million (3%) people worldwide. 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. The prevalence of HCV and distribution of HCV genotypes in Sri Lanka in comparison with the rest of Asia is not well known. Objectives: The objective of the study was to investigate the presence of HCV and to genotype HCV in a group of Sri Lankan patients suspected to have liver disease. Methods: A total of 1933 samples were screened for HCV antibodies using ELISA and HCV RNA with RT-PCR methods. RNA positive samples were genotyped by type specific amplification and by DNA sequencing. Results: Out of the 1933 liver disease patients tested 219 (11.33%) were detected to be positive for anti-HCV antibodies, out of which, 54 (24.66%) were positive for HCV RNA. Furthermore out of 49 positively tested patients, 24 (48.97%) were found to be categorised as HCV genotype 1. Conclusion: This result confirms previous observations that the contribution of HCV as a causative virus in liver disease patients is low in Sri Lanka. HCV genotype 1 was found to be the most predominant genotype in studied cohort of Sri Lankan liver disease patients.

Key words: Sri Lanka, Liver diseases, Hepacivirus, Genotype

Molecular epidemiology of Hepatitis C Virus (HCV) in liver disease patients in Sri Lanka

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ABSTRACT

Background: Globally, hepatitis C virus (HCV) is an important cause of chronic liver disease. Genotypes of HCV are associated with different profiles of pathogenicity, infectivity, and antiviral therapy. The prevalence of HCV and distribution of HCV genotypes in Sri Lanka in comparison with the rest of Asia is not well known. Objective: The objective of the study was to investigate the presence of HCV and to genotype HCV in a group of Sri Lankan patients suspected to have liver disease. Methods: A total of 1933 samples were screened for HCV antibodies using ELISA and HCV RNA with RT-PCR methods. RNA positive samples were genotyped by type specific amplification and by DNA sequencing. Results: Out of the 1933 liver disease patients tested 219 (11.33%) were detected to be positive for anti-HCV antibodies, out of which, 54 (24.66%) were positive for HCV RNA. Furthermore out of 49 positively tested patients, 24 (48.97%) were found to be categorised as HCV genotype 1. Conclusion: This result confirms previous observations that the contribution of HCV as a causative virus in liver disease patients is low in Sri Lanka. HCV genotype 1 was found to be the most predominant genotype in studied cohort of Sri Lankan liver disease patients.

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than type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt and central Africa. Type 5 is almost exclusively found in South Africa, and genotypes 6-11 are distributed in Asia. In Sri Lanka, studies which were completed with low number of cases showed that genotype 1 and 3 are prevalent in liver disease patients and blood donors respectively.10,11

The objective of the study was to investigate the presence of HCV and to genotype HCV in a cohort of Sri Lankan patients suspected to have liver disease.

MATERIALS AND METHODS

A total of 1933 blood samples from patients who have various types of liver diseases were taken for this study. The samples were collected from the Apollo Hospital (now Lanka Hospitals), Colombo, Sri Lanka during the period of July 2006 to August 2010. There were no specific sampling methods and all the patients who were suspected to have liver diseases (patients with abnormal liver enzymes, fatty liver, acute hepatitis, chronic hepatitis, cirrhosis and hepatocellular carcinoma) were taken for this study. Ethical clearance was obtained from the University of Peradeniya, Sri Lanka.

Serum samples were tested by using an Enzyme Immuno Assay (EIA) for immunoglobulin G antibody to hepatitis C (VITROS ECi assay, Ortho-clinical diagnostics, Raritan, NJ) according to the manufacturer’s instructions. The samples which showed a wide range of antibody titre ranging from ‘marginally positive’ to ‘strongly positive’ were taken as seropositives for this study. Repeat reactivity for HCV antibodies was not tested and all the antibody positive samples were tested for HCV RNA. Guanidium thiocyanate/Silica RNA extraction was done as previously described by Boom et al12 and HCV RNA was detected by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using primers derived from the highly conserved 5’untranslated (5’-UTR) genomic region as previously described.13

Nested RT-PCR was performed with type specific primers on HCV RNA positive specimens to determine the genotype status of HCV as previously described by Huang et al14,15 to detect type 1b, 2a, 2b, and 1a, 3a respectively. However, some of the type 1b PCR products were the result of cross-annealing of type 1b primers with a cDNA template for type 1a. Therefore restriction enzyme digestion with restriction enzyme AccI was performed to confirm sub-type 1b.16

A total of 20 HCV RNA positive specimens (representing different types and considering availability of the specimens) were further characterized by sequencing parts of the Core/E1 and NS5B regions of the HCV genome. Briefly, purified RNA was used to generate cDNA by reverse transcription. Nested RT-PCR was performed with sets of published primers to amplify DNA from Core/E1 or NS5B regions.3 The amplified products were separated in an agarose gel and purified with the Promega Wizard® PCR preps DNA purification system (Promega, Madison, WI, USA). DNA sequencing was performed at Eton Bio-Science, USA. The sequences were aligned in the BioEdit sequence alignment editor version 7.0.9.017 by using ClustalW Multiple alignment.18 Phylogenetic tree for HCV which was based on Core/E1 sequence and genetic distances were calculated with MEGA software version 4 using the Maximum Likelihood model.19 The sequences of Core/E1 and NS5B of HCV strains in Sri Lanka were deposited in NCBI GenBank under the accession numbers given in Table 1.

RESULTS

Of 1933 liver disease patients only 219 (11.33%) were positive for anti-HCV antibodies and of that, 54 (24.66%) were positive for HCV RNA by RT-PCR. If we assume antibody negative samples are true negatives, of 1933 samples only 54 (2.79%) were positive for HCV RNA.

Out of 54 HCV RNA positive samples, 48 samples were genotyped by combining two methods: Type specific PCR assay and DNA sequencing. The majority of the samples belonged to type 1b (13, 27.08%), followed by type 1a (11, 22.91%), type 3a (8, 16.66%), type 2a (6, 12.50%), type 2b (6, 12.50%), type 4a (2, 4.17%), type 3b (1, 2.08%), and mixed infection of genotypes 1b and 2b (1, 2.08%). Therefore, it was found that genotype 1 is the predominant (24 cases out of 48, 50%) genotype among the studied cohort of liver disease patients.

DISCUSSION

Here, we present the occurrence of HCV in a cohort of liver disease patients in Sri Lanka and their genetic diversity. In Egypt, the country that has recorded the highest prevalence of HCV, it was found 73.5% had antibodies to hepatitis C among the liver disease patients. The HCV RNA was detected in 15.5% of patients with chronic liver disease. Therefore our data suggests that the occurrence of HCV among the liver disease patients is relatively low in Sri Lanka.
We detected 219 HCV antibody positive specimens but only 54 (24.66%) of these were confirmed by RT-PCR. These results may indicate that false-positive ELISA results for HCV are frequent. False-positive results could be due to nonspecific antibody binding or to cross-reactivity with other circulating organisms. Alternatively; perhaps true antibody positive participants did not have positive RT-PCR results because they had cleared HCV viremia. Out of 20 specimens which were further characterised by sequencing, 18 and 7 were amplified and sequenced with Core/E1 and NS5B primers respectively. Based on their relative positions on the phylogenetic tree (Figure 1), and their relative distance from known reference types, it was possible to assign subtypes to the specimens in this study. Therefore, genotyping results of the two typing methods could be compared (Table 1) and concordant results were obtained for all the tested samples except the sample which gave mix infection of type 1b and 2b with type specific primers. Sequence analysis of the NS5B region was able to detect it as type 2b and Core/E1 region failed to amplify. Theoretically, in true mixed infections, one of the genotypes might be represented in an extremely large proportion with respect to the others. However, type specific PCR was found to be most sensitive method to detect mix infection of HCV.

The profile of liver disease was obtained for the HCV RNA positive patients and it is compared with the genotype of respective isolates (Figure 2). It was found that there was no clear relationship between the profile of the disease and the genotype. However, due to the low numbers of samples in each category, a statistical analysis could not be performed to find out the statistical significance of this observation.

A separate phylogenetic analysis was performed with specimens which belonged to genotype 1. In the Core/E1 phylogeny of genotype 1, there was a one sequence (EU849140) which grouped with global sub-type 1b references with a higher bootstrap values over 1000 replicates. However, there was another Sri Lankan isolate (EU849141) which fell outside the sub-type 1b cluster with a low bootstrap support. The low bootstrap value can be due to limited phylogenetic information in relation to the particular sequence. The highest similarity scores for the isolate EU849141 was 77% with an isolate (QC 156/EF115770) found from Canada, classified as sub-type 1h. The second highest similarity was observed with an isolate (I91/FJ207711) from USA which belonged to sub-type 1b. Because of these observations and very low homology with already available sequences, it could be assumed that it may belong to a previously unrecognized sub-type. However, according to the criteria proposed by Simmonds et al to assign a new-sub-type there should be at least 3 strains with the new sequence and also at least two regions within the coding sequence should be sequenced. Because it was not possible to find more isolates in this study with this new variant and also because the NS5B region could not be sequenced for the particular isolate, a new sub-type assignment was not attempted. However, this could be a new subtype of HCV genotype 1.

### Table 1: The comparison of genotyping results of two genotyping methods: Type specific PCR and DNA sequencing

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Genotypes with Type-Specific PCR</th>
<th>Genotypes with DNA sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core/E</td>
<td>Accession no.</td>
</tr>
<tr>
<td>HCV 1</td>
<td>1a</td>
<td>GU075876</td>
</tr>
<tr>
<td>HCV 2</td>
<td>1a</td>
<td>EU849146</td>
</tr>
<tr>
<td>HCV 3</td>
<td>1a</td>
<td>EU849147</td>
</tr>
<tr>
<td>HCV 4</td>
<td>1a</td>
<td>EU849148</td>
</tr>
<tr>
<td>HCV 5</td>
<td>1a</td>
<td>EU849149</td>
</tr>
<tr>
<td>HCV 6</td>
<td>1a</td>
<td>EU849150</td>
</tr>
<tr>
<td>HCV 7</td>
<td>1a</td>
<td>EU849151</td>
</tr>
<tr>
<td>HCV 8</td>
<td>1a</td>
<td>EU849139</td>
</tr>
<tr>
<td>HCV 9</td>
<td>1b</td>
<td>EU849140</td>
</tr>
<tr>
<td>HCV 10</td>
<td>1b</td>
<td>EU849141</td>
</tr>
<tr>
<td>HCV 11</td>
<td>2a</td>
<td>EU849142</td>
</tr>
<tr>
<td>HCV 12</td>
<td>2a</td>
<td>EU849143</td>
</tr>
<tr>
<td>HCV 13</td>
<td>2a</td>
<td>EU849144</td>
</tr>
<tr>
<td>HCV 14</td>
<td>UT</td>
<td>GU075875</td>
</tr>
<tr>
<td>HCV 15</td>
<td>2b</td>
<td>-</td>
</tr>
<tr>
<td>HCV 16</td>
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<tr>
<td>HCV 17</td>
<td>3a</td>
<td>GU075873</td>
</tr>
<tr>
<td>HCV 18</td>
<td>1b &amp; 2b</td>
<td>-</td>
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<tr>
<td>HCV 19</td>
<td>UT</td>
<td>FJ236904</td>
</tr>
<tr>
<td>HCV 20</td>
<td>UT</td>
<td>GU075874</td>
</tr>
</tbody>
</table>

Where NA: Not Amplified, UT: Un Typed
Genetic distances between isolates of a virus could give an indication of the epidemiology of that infection. Low genetic distance between isolates is indicative of recent spreading of the virus from local sources, whereas greater genetic distances indicate either regionalized spreading within a country or the introduction of isolates from external sources. The mean genetic distance measurements among the Sri Lankan genotype 1 Core/E1 region was comparatively low (0.085) and this low genetic distance being indicative of internal transmission of the virus. It shows that although Sri Lanka has the low number of intravenous drug users as well as efficient blood screening system before transfusion, still there may be some gaps to be filled.

CONCLUSION

The result confirms previous observations that the contribution of HCV as a causative virus in liver disease patients is low in Sri Lanka. HCV genotype 1 was found to be the most predominant genotype in studied cohort of Sri Lankan liver disease patients.

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REFERENCE


Authors Contribution:
SDB, WY & TP − Collection of specimens, conduct experiments and data collection; SDB, SJ, SA & FND − Literature search, concept, design and manuscript preparation of the study.

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