Seroprevalence, Risk Factors and Genotyping of Hepatitis C Virus

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Abstract

Introduction: Parenterally transmitted viruses are a major cause of mortality and morbidity in the health care system of the modern world and the most sinister, though not the most common is the Hepatitis C virus (HCV). HCV is a major cause of liver disease worldwide and an emerging infection in India. HCV is known to have marked genetic heterogeneity. HCV genotypes exhibit different profiles of pathogenicity, infectivity and response to antiviral therapy.

Aims and objectives: The study was aimed at detecting HCV seropositivity, probable risk factors, HIV/HBV co-infections and common genotype prevalence in a tertiary care hospital in South India.

Materials and Methodology: This Cross sectional study was conducted from September 2011 to August 2013 on adult patients clinically suspected of hepatitis, blood donors attending blood bank, pregnant women attending antenatal care (ANC) clinic, renal dialysis patients and patients whose blood samples were received for pre-operative evaluation. Serum was subjected to ELISA for anti HCV antibody detection. Plasma collected from seropositives, genotyped by Real Time PCR.

Results: Out of the 3581 serum samples that were screened, 32 were anti-HCV antibody positive (Seroprevalence - 0.89%). Highest number of HCV seropositivity were found between 41-50 years of age group. Blood transfusion was found to be highly significant risk factor (p = 0.004). Rate of HCV-HBV co-infection was 6.25% (2/32) and HCV-HIV co-infection was 3.12% (1/32). No concomitant HCV, HIV and HBV infection was detected. 12 samples were HCV Genotype 1 (48%), 8 were Genotype 3 (32%) and 5 (20%) were untypeable.

Conclusion: The study showed that seroprevalence of HCV is similar to most of the other studies in India. Blood transfusion is a major risk factor for HCV infection. HCV genotype 1 is widespread in this region of southern India along with, and perhaps predominating over, HCV genotype 3.

Key words: Hepatitis C virus, HCV genotypes, seroprevalence, Real time PCR.

I. Introduction

Parenterally transmitted viruses are a major cause of mortality and morbidity in the health care system of the modern world and the most sinister, though not the most common is the Hepatitis C virus (HCV). HCV is a RNA virus belonging to Flaviviridae family and genus Hepacivirus. It is a major cause of liver disease worldwide and an emerging infection in India. HCV infection causes an indolent and slowly progressive liver disease that is asymptomatic until development of decompensated liver disease and often liver cancer. The development of a protective vaccine remains a distant prospect.¹

The common modalities of spread of hepatitis C infection are blood transfusion, injection drug use, unsafe therapeutic injections and health care related procedures, sexual and vertical transmissions. In developed countries the predominant route of hepatitis C infection is IV drug use, whereas in India blood transfusions and unsafe therapeutic injections are the predominant modalities of transmission of hepatitis C virus.²

HCV is known to have marked genetic heterogeneity. Presently, HCV can be classified into at least 6 major types (1, 2, 3, 4, 5, 6) and series of subtypes. Genotype 1a is common worldwide. In India the most prevalent genotype is 3.³

II. Objectives of the study

1. Detection of HCV seropositivity among adult patients in the study population
2. Detection of common HCV genotype prevalent in this region among HCV seropositive patients.
3. Detecting co-existing HIV/HBV infection
4. To find the association of probable risk factors with HCV seropositivity.

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III. Materials and Methodology

This is a Cross sectional study done between September 2011 to August 2013 that describes HCV seropositivity and genotype distribution in a tertiary care hospital in South India.

Inclusion criteria
- Adult patients with clinical suspicion of hepatitis
- Voluntary blood donors
- Pregnant women attending ANC clinic
- Patients undergoing renal dialysis
- Patients whose blood samples were received for pre-operative evaluation.

Exclusion criteria
- Children and adolescents (0-18 years).

Procedure: Two ml of venous blood sample was collected in a plain vacutainer from the subjects. Serum was separated and tested for anti HCV antibody by ELISA using 3rd generation HCV kit (Biomerieux France).

Another 2ml of venous blood sample was collected in EDTA vacutainer from patients who tested positive for anti HCV antibody and plasma was separated and stored at -70°C for genotyping.

Demographic data and clinical history of the patients who were seropositive was recorded in the proforma and risk factors for the infection were taken note of, haematological tests (TC, DC, ESR) and liver function tests(LFT) values were noted.

Viral RNA was extracted from plasma samples of seropositive patients using QIAamp Viral RNA extraction kit, Qiagen Real Time Polymerase Chain Reaction (RT PCR) was performed for genotype determination using HCV Genotyping 1/2/3/4 by Real Time PCR Kit (Genome Diagnostics Pvt Ltd).

In accordance with the prevalence of seropositivity the same number of age and gender matched controls were randomly selected and analysis done for association with risk factors using chi square test.

IV. Results

During the study period 3581 serum samples were screened for anti-HCV antibody by ELISA and among them 32 samples tested positive (95% CI varying from 30.441 to 33.559). The seroprevalence of HCV infection was 0.89%. Highest number of HCV seropositive patients were in the age group 41-50 years [13/32 (40.62%)], followed by patients between 51-60 years [7/32 (21.87%)] (Table 1). There were 22 males (68.75%) and 10 females (31.25%) among the total number of seropositives (Table 2).

Table 1: Age distribution

<table>
<thead>
<tr>
<th>Age category(years)</th>
<th>Seropositives (%)</th>
<th>Seronegatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>5(15.6)</td>
<td>12(37.5)</td>
</tr>
<tr>
<td>31-40</td>
<td>6(18.8)</td>
<td>11(34.4)</td>
</tr>
<tr>
<td>41-50</td>
<td>13(40.6)</td>
<td>6(18.8)</td>
</tr>
<tr>
<td>51-60</td>
<td>7(21.9)</td>
<td>1(3.1)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>1(3.1)</td>
<td>2(6.3)</td>
</tr>
<tr>
<td>Total</td>
<td>32(100)</td>
<td>32(100)</td>
</tr>
</tbody>
</table>

Chi square -11.765 P value - 0.019(Significant)

Table 2: Gender distribution

<table>
<thead>
<tr>
<th>Gender</th>
<th>Seropositives (%)</th>
<th>Seronegatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22(68.8)</td>
<td>21(65.6)</td>
</tr>
<tr>
<td>Female</td>
<td>10(31.3)</td>
<td>11(34.4)</td>
</tr>
<tr>
<td>Total</td>
<td>32(100)</td>
<td>32(100)</td>
</tr>
</tbody>
</table>

Chi square - 0.071 P value- 0.790(Not Significant)

In the study group comprising of totally 3581 subjects, 649 were blood donors and 198 were antenatal; HCV infection was not detected among these group. In addition, 749 out of the total samples had been submitted to the laboratory as part of routine pre-operative workup and none of them were reactive. There were 104 patients on renal dialysis among whom 11 (10.57%) were seropositive and all of them had received blood transfusion on one or several occasions. Of the total no of patients, 1881 were clinically suspected of hepatitis and 21 in this group were HCV seropositive (Table 3).
### Table 3: HCV Seropositivity - Clinical Categories

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Samples screened</th>
<th>Number of Seropositives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal cases</td>
<td>198</td>
<td>0</td>
</tr>
<tr>
<td>Blood donors</td>
<td>649</td>
<td>0</td>
</tr>
<tr>
<td>Clinically suspected hepatitis</td>
<td>1881</td>
<td>21(1.11%)</td>
</tr>
<tr>
<td>Dialysis</td>
<td>104</td>
<td>11(10.37%)</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>249</td>
<td>0</td>
</tr>
</tbody>
</table>

The different risk factors for HCV infection noted in our study were blood transfusion (17/32), renal dialysis (11/32), tattooing (1/32) and sexual transmission (1/32).

History of tattooing without any other risk factor could be elicited from one of the seropositives. In another patient without other obvious risk factors, sexual partner was HCV reactive. The sibling of the couple was HCV non-reactive.

### Table 4: History of blood transfusion

<table>
<thead>
<tr>
<th>History of Blood transfusion</th>
<th>Seropositives (%)</th>
<th>Seronegatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>17(53.1)</td>
<td>6(18.8)</td>
</tr>
<tr>
<td>Absent</td>
<td>15(46.9)</td>
<td>26(81.3)</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

Chi square - 8.212  P value - 0.004 (Highly Significant)  
Odds ratio - 4.911 (95% CI: 1.591, 15.157)

### Table 5: Seropositivity among dialysis patients

<table>
<thead>
<tr>
<th>Category</th>
<th>Seropositives (%)</th>
<th>Seronegatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis</td>
<td>11(34.4)</td>
<td>7(21.9)</td>
</tr>
<tr>
<td>Non Dialysis</td>
<td>21(65.6)</td>
<td>25(78.1)</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

Chi square - 1.237  P value - 0.266 (Not Significant)  
Odds Ratio - 1.871 (95% CI: 0.616, 5.683)

### Table 6: Seropositivity among clinically suspected hepatitis

<table>
<thead>
<tr>
<th>Category</th>
<th>Seropositives (%)</th>
<th>Seronegatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically suspected hepatitis</td>
<td>21(65.6)</td>
<td>10(31.3)</td>
</tr>
<tr>
<td>Non hepatitis</td>
<td>11(34.4)</td>
<td>22(68.8)</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

Chi square - 6.275  P value - 0.012 (Significant)  
Odds ratio - 3.667 (95% CI: 1.303, 10.321)

Among the HCV seropositives 2 samples were also positive for HBsAg and a different subject was reactive for HIV. Thus, the rate of HCV-HBV coinfection in the present study was 6.25% (2/32) and that of HCV-HIV coinfection was 3.12% (1/32). No concomitant HCV, HIV and HBV infection was detected.

Out of the 32 HCV seropositive samples, RNA could be extracted from 25. They were further genotyped by Real Time PCR. 12 samples were characterised as belonging to HCV Genotype 1 (48%) and 8 as HCV Genotype 3 (32%) and 5 (20%) were untypeable.

### V. Discussion

Hepatitis C virus is one of the major causes of parenterally acquired hepatitis. It is a leading cause of chronic hepatitis and primary hepatocellular carcinoma (HCC) in most parts of the world. In the developing countries of Asia and Africa, though hepatitis B virus (HBV) infection is the commonest cause of chronic liver disease, HCV is fast evolving as an equally important infection among these populations.

In India about 20 million people are known to have HCV infection and a quarter of them expected to develop chronic liver disease in the next 10-15 years. The impact of this infection has just started to emerge in India. In the absence of efficient screening in our country, the HCV infection from various sources will continue to add.

Today, worldwide HCV is the commonest cause of post-transfusion hepatitis. The seroprevalence among the blood donor population in India is 1.8% - 2.5%, and the community seroprevalence has been reported to be 0.87%. HCV is the etiological agent in about 20% of patients with chronic hepatitis in northern India.

In our study the seroprevalence of HCV infection was 0.89%. This is the common rate of HCV infection seen in many hospitals in India. The seroprevalence was similar to other reports. Seroprevalence 0.22% in South India, 0.3% in Western India, 1.8% in Central India and 1.9% in North India. In the present study highest number of HCV seropositivity was found in patients between 41-50 years age (40.62%), followed by patients between 51-60 years age group (21.87%). As the age increased the seropositivity was observed to be increasing and statistically significant (p value < 0.019). In the developing world, unsafe
therapeutic injections and blood transfusions are likely to be the major modes of transmission, especially in countries where age-specific seroprevalence rates suggest on-going increased risk of HCV infection\textsuperscript{16}

HCV seropositivity was high in males (68.75\%) in comparison with females (31.25\%). In epidemiological reviews relating to disease accelerating co-factors among HCV infected people male sex has been quoted as one such factor\textsuperscript{15}, similar correlation was observed in our study also. Statistically there was no significance with respect to seropositivity among males and females in our study.

Acquisition of HCV infection through perinatal transmission is estimated to occur in 2.7–8.4\% of infants born to HCV infected mothers, and a higher proportion of infants born to HIV/HCV coinfected mothers.\textsuperscript{13} There were no positives detected among the 198 antenatal women in our study. Kumar et al\textsuperscript{14} in a study from Delhi reported a prevalence of 1.03 \% in ANC population while the results of other studies done at Shimla\textsuperscript{15} and Vellore\textsuperscript{2} are concurrent with that of our study.

Out of the 649 blood donors that were screened for HCV infection in the current study, none of them were seropositive. This could be due to the fact that commercial blood donation is discouraged in our blood bank and most of the voluntary blood donations are by the relatives of the patients. Screening of blood and blood products is stringently followed in our blood bank.

Patients undergoing haemodialysis (HD) are at high risk of acquiring blood-borne pathogen, since HCV is efficiently transmitted by the parenteral route. In addition, infected patients have an increased tendency to develop chronic hepatitis and to be also a potential reservoir for its transmission, possibly contributing to the nosocomial spread of HCV in dialysis centres and explaining the high prevalence of HCV infection among haemodialysis patients.\textsuperscript{16}

In our study, 11 dialysis patients were HCV positive out of 104 (10.15\%). The sample size was not sufficient for analysing risk of dialysis. In our study all seropositive dialysis patients (11) received blood transfusion on one or several occasions. 6 non dialysis patients also received blood transfusion. Blood transfusion was found to be highly significant risk factor with a p value of 0.004 (Odds ratio 4.91 varying from 1.59 to 15.15).

History of tattooing without any other risk factor could be elicited from one of the seropositives. Khaja et al found a significantly higher rate of transmission of HCV among people exposed to tattooing (2.8\%).\textsuperscript{17}

Sex with an infected partner and with multiple partners have been identified as risk factors for HCV transmission, but sexual transmission of HCV is far less efficient than that of other sexually transmitted viruses. Among people in long-term monogamous relationships in particular, the risk of sexual transmission of HCV is extremely low.\textsuperscript{14} Sexual transmission of HCV is not as common as it is with hepatitis B virus.\textsuperscript{18} In the present study, for one of the seropositives, without other obvious risk factors the sexual partner was HCV reactive. The sibling of the couple was HCV non-reactive, ruling out vertical transmission. The prevalence of HCV infection in individuals with STDs in a tertiary care hospital in South India was 6\%.\textsuperscript{19}

Co-infection with HIV and HCV is emerging as an important and frequent finding in patients seeking therapy for one or the other disease. The fact that both viruses share a similar route of transmission and mechanisms of epidemic spread appears to be the most important reason for the growing nature of the co-infection. The highest prevalence of co-infection is occurring in patients with a current or previous history of injecting drug use.\textsuperscript{20}

The rate of HCV-HIV coinfection in the present study was 3.12 \% (1/32). In comparison with western data\textsuperscript{20}, this was very low. This could be due to high risk group of IV drug users in the West. Recent studies on HCV-HIV coinfection from India have reported a prevalence of 3.02\% in Andhra Pradesh\textsuperscript{21}, 2.2\% in Tamil Nadu\textsuperscript{22}, 1.6\% in Lucknow\textsuperscript{13} and 1.06\% in Vellore\textsuperscript{2}. Our results were also in the same range.

The proportion of HCV-infected people who also have chronic HBV infection will have an impact on the overall burden of chronic liver disease. HCV and HBV coinfection in chronic hepatitis patients has been associated with clinically and histologically more severe liver disease than that of chronic hepatitis patients with HCV infection alone. A meta-analysis found HBV/HCV coinfection to be more strongly associated with HCC than either infection alone, suggesting a synergistic effect between the two viruses in the carcinogenic process of HCC.\textsuperscript{13} The rate of HCV-HBV coinfection in the present study was 6.25\% (2/32). 3.7\% prevalence of dual infection in haemodialysis patients was observed in a study conducted by Reddy et al\textsuperscript{21} and 4.4\% by Kosaraju et al.\textsuperscript{24}

Genotyping and subtyping of HCV is relevant to the epidemiology of HCV, vaccine development, clinical management and assessment of the risk benefit ratio of therapeutic measures against chronic HCV infection. Genotype 1 in particular cannot be treated efficiently with IFN-alpha, while genotypes 2 and 3 respond favourably. Moreover, genotype 1 infection may proceed more rapidly to severe forms of chronic hepatitis, cirrhosis and hepatocellular carcinoma, when compared with genotype 2 and 3.\textsuperscript{23}

Out of the 32 HCV seropositive samples, RNA could be extracted from 25 and they were further genotyped by Real Time reverse transcription PCR. 12 samples were characterised as belonging to HCV Genotype 1 (48\%) and 8 as HCV Genotype 3 (32\%) and 5 (20\%) were untypeable. RNA could not be extracted.
Seroprevalence, risk factors and genotyping of Hepatitis C virus

from other 7 samples. This could be due to denaturation of nucleic acid during storage or its inadequate concentration in the samples. Untypeability of 5 PCR runs could be attributed to presence of other genotypes, which were not detectable by the kit in use (i.e. genotype 5 and 6) or low quantity of extracted RNA.

In our study, genotype 1 was predominant, which is similar to that reported by other workers from Southern India. In Northern and Western India, genotype 3 was found to be predominant, whereas it was second commonly detected in our study. In the United States, HCV genotype 1 accounted for the majority (74%) of infections followed by genotype 2 (15%), genotype 3 (6%), and genotype 4 (1%).

Assessment of treatment outcome could not be done in our study as none of the patients were started on specific therapy due to economic constraints.

VI. Conclusion

The study showed that seroprevalence of HCV is similar to most of the other studies in India. As renal dialysis and blood transfusion were observed to be the major risk factors in our study, it may be concluded that prevention strategies must be targeted towards this population of patients. HCV genotype 1 is widespread in this region of southern India along with, and perhaps predominating over, HCV genotype 3.

Ethics: Institutional ethical committee clearance obtained.

Source of Support: Nil.

Conflict of Interest: None declared.

References


