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Seroprevalence of Hepatitis C Antibodies and their Molecular Characterization in Patients Attending at Thanjavur Medical College and Hospital, Tamil Nadu, India

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A B S T R A C T

Hepatitis C viruses are the hepatotropic blood borne viruses of significant global and public health problem especially in developing countries like India. HCV are important causes of Liver cirrhosis and hepatocellular carcinoma. It is transmitted by parenteral, sexual, and perinatal modes. Blood transfusion, transplantation from infectious donors and injecting drug use are the commonest modes of transmission. It causes liver related mortality and morbidity. Worldwide over 350 million have chronic HCV infection. The aim of the study is to determine the seroprevalence of Hepatitis C virus and providing genotyping of the viruses at Thanjavur medical college and hospital. This study aimed at early diagnosis, disease prevention and treatment of HCV. Materials and methods: Observational Study with blood samples collected from 194 patients who registered at OPDs and admitted at IPDs with clinical history of jaundice, abdominal pain, for the period from February 2020 to January 2021, at Thanjavur Medical College and Hospital, Thanjavur. All the samples were screened for anti-HCV antibodies by using ELISA kit. Seropositive samples were further processed for HCV RNA identification by Real time PCR. Positive samples by Real-time PCR were further processed for genotyping analysis. Out of 194 samples, five samples were positive for anti-HCV antibodies by third generation ELISA. Prevalence of Hepatitis C Virus was 2.6%. Serum samples found positive by ELISA were further processed for HCV RNA detection by Real time PCR. Out of five positive samples, 3 samples were positive for HCV RNA by Real time PCR. Serum sample found positive for HCV RNA were further processed for molecular characterization for genotyping. Real time PCR was done for genotyping. Genotyping for Hepatitis C was genotype 1 &3genotype 1 was found in sample one and genotype 3 was found in third and fourth sample. It shows that higher numbers of positive cases were among the age group above 20-30 years (4.44%), and above 60 years (13.33%). Smallest number of positive cases were among the age above 40 years (2.85%). This study estimates the seroprevalence of Hepatitis C for both the sexes in patients attending Thanjavur medical college and hospital. The seroprevalence of Hepatitis C was 2.6%. Seroprevalence study estimates the magnitude and dynamics of disease transmission. Moreover, knowing about the genotypes in the community helps in the development of future vaccine. Further studies of genotype distribution will help in the development, adaptation and prevention strategies. Early diagnosis prevents the disease progression and further complications.

Introduction

Hepatitis means inflammation of the liver. It is caused by different viruses such as Hepatitis A, B, C, D and E. Hepatitis C virus (HCV) are the most common blood borne, hepatotropic viruses (Ansaldi, 2014). HCV are important causes of Liver cirrhosis and hepatocellular carcinoma. Hepatitis C are significant public health problems especially in developing countries like India (Ashis Mukhopadhy, 2008). It is the major cause of chronic liver disease leading to cirrhosis of liver and hepatocellular carcinoma (Bhattacharya, 2003).World health organization (WHO) estimated that 180 million people are affected worldwide (David and Gretch, 1997).

Studies in India stated seroprevalence of HCV is 1.8% among the general population (Eddie Tang, 1991). HCV was the first virus identified using varied molecular biology technique using after extensive testing of serum from experimentally infected animals (WHO, 2003).

Hepatitis C virus (HCV) belonging to genus Hepacivirus in the family Flaviviridae and it is an enveloped positive single stranded RNA virus. Diversity is seen in RNA genome of Hepatitis C because of high rates of mutations. The Hepatitis C virus RNA genome consists of nearly 9500 nucleotides with a single reading frame which is open that encodes a polypeptide precursor of 3000 amino acids.RNA genome is flanked by the non-coding regions at both the 5' and the 3' termini (De Francesco, 1999). The poly protein precursor is cotranslationally processed by host signal peptides to yield the three structural proteins such as Core protein (c) and Envelope Glycoproteins (E1 and E2) and the non-structural proteins (NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B). HCV is divided into six genotypes and 90 subtypes (John D. Scott and David R. Gretch, 2007). Novelty of the study included the seroprevalance of HCV using ELISA Moleculaar biological technique for 194 patients and Genotyping was stuied. Confirmation of genotype grouping may lead to further specifications of medications.

Materials and Methods

The observational study was conducted in Thanjavur Medical College Hospital, Thanjavur. Prior approval attained and studied during the period of February 2020 to January 2021.

Inclusion Criteria

Patients who registered at the OPDs, admitted to the IPDs with clinical history of viral hepatitis, jaundice for evaluation and alcoholic hepatitis were included in the study.

Exclusion Criteria

Patients with documented past history of Hepatitis C viral infections are excluded from this study.

High risk groups such as immuno-compromised, Intravenous Drug Users were also excluded from the study.

Specimen collection

A 5-ml venous blood samples were taken aseptically from all eligible patients in sterile vacutainers, after obtaining informed oral consent. The blood was allowed to clot for 45 minutes at room temperature and serum was separated after centrifugation. Serum sample was stored at -20°C until further testing. Serum is tested for antibodies of Hepatitis C by ELISA Method. The HCV screening was done using commercially available enzyme linked immunosorbent assay kits (ErbaSure HCV Gen3) for detection of anti-HCV

antibody according to manufacturer's instructions. Serum sample found positive for anti-HCV by ELISA were further processed for real time PCR assay (PureFast® Total RNA Minispin purification kit), for HCV RNA detection.

HCV RNA was extracted using One Step RT-PCR Probe Master Mix which contains Reverse transcriptase, RNase Inhibitors, Taq DNA polymerase, Taq reaction buffer, 3.5mM MgCl2, 1µl of 10mM dNTPs mix and PCR additives. Serum samples positive for HCV RNA were subjected to genotyping by HELINI HCV genotyping Real-time PCR kit.

Statistical Analysis

All statistical analysis were performed using SPSS version 22 with $p < 0.05$ accepted as statistically significant. The Chi-square test was used for categorical variables.

Results and Discussion

During the study, a total of 194 samples were collected and tested for anti-HCV Ab at Central service diagnostic laboratory, Department of Microbiology, Thanjavur medical college and hospital, Thanjavur.

(Table.7 & Fig.1) shows the total number of anti- HCV Ab positive and anti HCV Ab negative cases. Out of 194 samples tested, only 5(2.6(%)) out of 194 showed higher OD value in anti HCV Ab ELISA test. Remaining 189 (97.4(%)) showed negative results.

(Table/Fig-2) shows the age and sex distribution of the total number of cases with anti-HCVAb seroprevalence.

The seroprevalence of anti –HCV Ab was found to be 2.6(%).

The seroprevalence of anti-HCV Ab among males were 2.79(%) and females were 1.96(%).The highest seroprevalence of anti HCV Ab was found in males above the age of 60 years.

(Table/Fig-3) shows the age wise distribution of anti HCV Ab positive cases. It shows that higher numbers of positive cases were among the age group above 20-30 years (4.44(%)), and above 60 years (13.33(%)).

Minimum no of positive cases was among the age above 40 years (2.85(%)). Significance was calculated with chi square test. P value was $0.118 > 0.05$.

HCV RNA detection by Real-time PCR

Result of Hepatitis C Real-time PCR assay

Hepatitis C Real-time PCR assay was done for 8 samples, including positive & negative samples by ELISA from 194 study groups.

Hepatitis C Real-time PCR

From (Table/Fig-4) out of 8 samples 3 sample (38%) were positive for HCV RNA and 5 samples were negative (62%) for HCV RNA by Real- time PCR assay.

Out of 8 samples 3 (38%) sample were positive both by ELISA and Real- time PCR assay and 3 (38%) samples were negative by both ELISA and Real time PCR assay. Two (24%) samples which were initially positive for anti-HCV antibodies by ELISA were negative by Real-time PCR for HCV RNA analysis.

(Table/Fig-5) shows amplification plots of RT PCR for 8 samples and one standard. Cycle threshold for standard was 26.75, and for 3 samples were 29.58, 31.90, and 33.24 respectively.

Quantitative analysis by Real time PCR

HCV = 2.7 copies load.

Result of Molecular characterization by HCV Genotyping

Three positive samples which were positive for HCV RNA by Real-time PCR assay was further processed for Molecular characterization by Genotyping. Real-time PCR was done for Genotyping. From (Table/Fig-6) its evident that Genotype 1 was detected in sample 1 and genotype 3 was found in sample 3 and 4.

Table.7 and Fig.7) shows amplification plot of RT PCR genotyping of HCV. Cycle threshold for 3 samples were 16.58, 26.96 &27.89 respectively. From (Table/Fig-7) it is confirmed that Sample-1 belongs to HCV genotype – 1 and samples 3 & 4 belongs to HCV genotype – 3.

Interpretation by RT-PCR genotyping Amplification Plot

Sample-1 = HCV genotype - 1 detected in FAM channel

Samples 3 &4 are HCV genotype - 3 detected in FAM channel.

Hepatitis C infections are a serious global and public health problem. HCV infections are transmitted mainly by blood and blood products. Worldwide two billion people are infected with HCV, and 350 million are chronic carriers. Based on the prevalence of HCV infections, HCV cause asymptomatic infection to chronic infection. It is one of the major causes of liver cirrhosis and hepatocellular carcinoma (Kamili, 2012). WHO estimates that there are 180 million people affected worldwide. According to WHO global prevalence of HCV is 3%. The seroprevalence of HCV among general population in India is1.8% (Murphy, 2007).

Seroprevalence study is important to assess the magnitude and disease transmission in the community (Sharma, 2010). The present study has been taken to assess the seroprevalence of HCV antibodies in both the sexes and different age groups in patients attending Thanjavur Medical college and hospital.

Quantitative analysis by Real time PCR

Viral load is determined by total number of viral copies per ml of blood sample (Kaushal B. Shah; Mondelli and Silini, 1999). Viral load depends upon stage of illness. Since it is a real time PCR, quantification of the viral load can also be measured (Saha, 2014; Sayeedul Hasan Arif *et al.,* 2015; Shagufta Hussain *et al.,* 2010). The viral load for the one positive sample is 300 copies/ml that is equal to 57 IU/ml.

Prevalence of Hepatitis C

The prevalence of Hepatitis C was 2.6% from this present study.

This seroprevalence is almost similar to the study conducted at Andhrapradesh which was 2.02% (Mondelli and Silini, 1999).

In India seroprevalence of HCV varies from 1.5% from Cuttack to 4.8% from Pondicherry (12). Study conducted by Sharma *et al.,* in 2010 from Jaipur stated that Seroprevalence for Hepatitis C as 1.7% (Jeulin *et al.,* 2013; Miriam J. Alter, 2007). This study does not support my present study.

Table.1 Anti-HCVAb Positive by ELISA

Result	n=194	Percentage (%)
Positive	5	2.6(%)
Negative	189	97.4(%)

Table.2 Age and Sex Wise Anti-HCVAb Seropositivity

Age(yrs)	Male n=143	Positive	%	Female n=51	Positive	%	Total Positive (%)
<20	7	0	0%	6	0	0%	0(0%)
21-30	29	1	3.44%	16	1	6.25%	2(9.69%)
31-40	42	0	0%	6	0	0%	0(0%)
41-50	26	1	3.84%	9	0	0%	1(3.84%)
51-60	28	0	0%	8	0	0%	0(0%)
61-70	10	2	20%	5	0	0%	2(20%)
70&above	1	0	0%	1	0	0%	0(0%)
TOTAL	143	4	2.79%	51	1	1.96	5(2.6%)

Table.3 Age Wise Anti-HCVAb Positive

Age (Years)	No. of Cases Tested	Positive	Percentage (%)
≤20	13	0	0(%)
21-30	45	2	4.44(%)
31-40	48	0	0(%)
41-50	35	1	2.85(%)
51-60	36	0	0(%)
61-70	15	2	13.33(%)
71&above	2	0	0(%)

Table.4 ELISA and RT-PCR studies

Method	Positive	Negative	Total
ELISA	5	3	8
Real-time PCR	3	5	8

Fig.1 RT-PCR studies

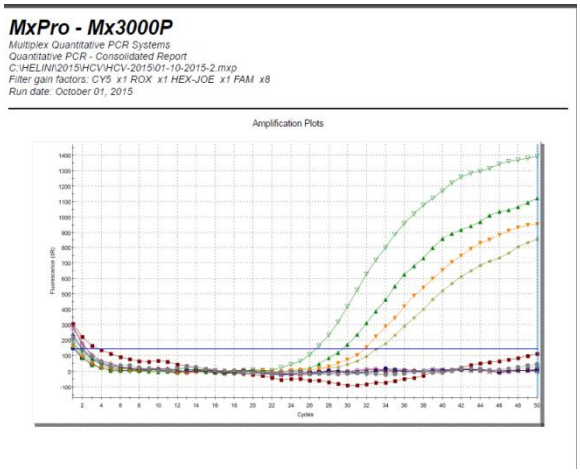


Fig.2 HCV Genotyping

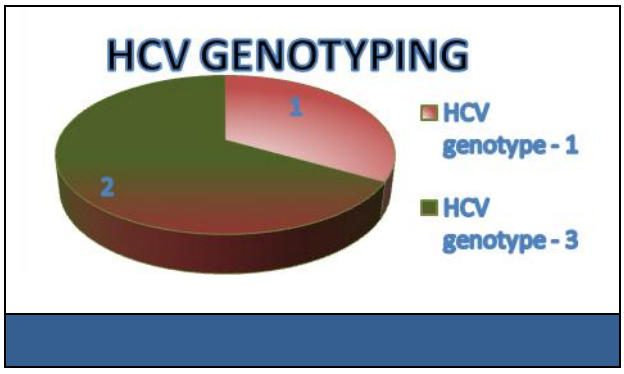
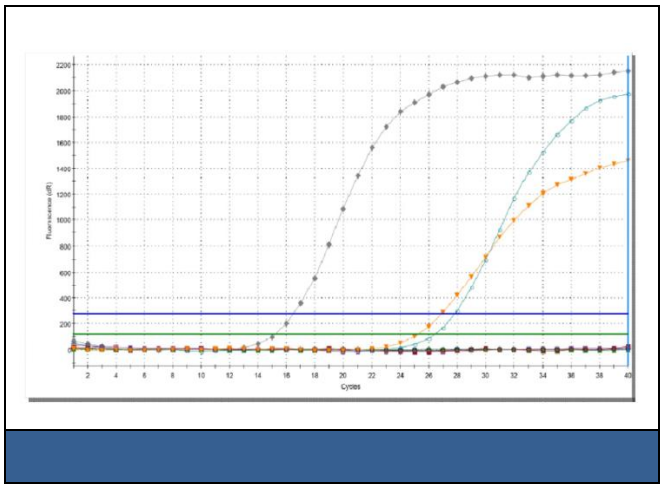


Fig.3 RT-PCR Genotyping of HCV – Amplification plot



Age Prevalence for Hepatitis C

The age prevalence was high among the age groups 20-30 years (4.4%) and >61 years (13.13%). It is almost similar to the study conducted by Smita Sood *et al.,* in 2015 (Younossi and Mchutchison, 1996). Ramarokoto *et al.,* in their study stated that prevalence did not differ significantly based on sex but it increased with age.

Sex Prevalence for Hepatitis C

The seroprevalence of HCV was higher among males than in females. There was no statistically significant difference in the prevalence rates for males and females. Bhattacharya *et al.,* (Swain, 2010) in their study stated that seroprevalence was higher among males than females. Another study reported by Nafees Met (Toshikuni, 2014). Sayeedul Hasan Arif *et al.,*; Ansaldi, 2014; Manjunath P. Salmani stated that the seroprevalence was high among males than females (Yasui, 1998).

Hepatitis C Real-time PCR Assay

Out of 8 samples 3 sample (38%) were positive for HCV RNA and 5 samples were negative (62%) for HCV RNA by Real- time PCR assay. Out of 8 samples 3 (38%) sample. were positive both by ELISA and Real- time PCR assay and 3 (38%) samples were negative by both ELISA and real time PCR assay (Swain, 2010). Two (24%) samples which were initially positive for anti-HCV antibodies by ELISA were negative by Real-time PCR for HCV RNA analysis. Reddy *et al.,* states that antibody tests fail to detect HCV infected patients before seroconversion or during the window period.

But the viral RNA can be detected in the serum. Window period for immunocompetent subjects may extend up to two months whereas for immunosuppressed individuals, it may extend up to 12 months (Swain, 2010). Direct detection of HCV RNA in the serum remains the gold standard test to diagnose HCV infection (Kaushal B. Shah)**.**

Molecular characterization by Genotyping

Three positive samples which were positive for HCV RNA by Real-time PCR assay was further processed for Molecular characterization by Genotyping. Vipoporin p7 is necessary for the production of stable viral particles coated with E1 and E2.

They are glycosylated in ER and new virions are released (Manjunath P Salmani, 2014; Mclauchlan, 2000). Real-time PCR was done for Genotyping (Stephen, 2006). Genotype 1 was detected in sample 1 and genotype 3 was found in sample 3 and 4.

In the present study Genotype 3 was present in two samples and Genotype 1 was present in one sample. Chakravarti *et al.,*; Choo *et al.,* (1989) in their study states that genotype 3 is common in north India (Eddie Tang, 1991). In another study conducted by Christdas *et al.,* states that genotype 3 is predominant in south India. Saha *et al.,* in their states that Genotype 3(34.09%) is common followed by Genotype 1(7.95%) (Chevaliez, 2010).

Strength of the study

Genotyping of HCV was done to know about the Genotype Prevailing in Thanjavur district.

This study estimates the seroprevalence of Hepatitis C for both the sexes in patients attending Thanjavur Medical college and hospital. The seroprevalence of Hepatitis C was 2.6%.The prevalence Hepatitis C was high among males when compare to females. Seroprevalence study estimates the magnitude and dynamics of disease transmission.

Real –time PCR is the gold standard method, because it estimates the viral load and genotyping, because both viral load and genotyping plays an important role in treatment strategy. Moreover, knowing about the genotypes in the community helps in the development of future vaccine. Further studies of genotype distribution will help in the development, adaptation and prevention strategies. Early diagnosis prevents the disease progression and further complications.

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