

Original Article:**Burden of hepatitis C virus infection and its genotypes among the blood donors at Tirupati, Andhra Pradesh**

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ABSTRACT

Background: Safe blood donation remains a challenge in resource limited countries. False positive serological tests lead to wastage of large number of blood units. Nucleic acid amplification test (NAAT) technology has greatly enhanced the accuracy in identification of transfusions transmitted infections.

Methods: The present study was undertaken to study the seroprevalence of hepatitis C virus (HCV) and asses the concordance between seropositivity with the presence of HCV ribonucleic acid (RNA) and to know the distribution pattern of HCV genotypes in healthy blood donors.

Results: Among the 9287 donors screened (88.3% males), 7153 (77%) were voluntary donors while 2134 (23%) were replacement donors. Among blood donors, 27 (0.3%) samples tested HCV seropositive. Among 27 anti-HCV positive samples only 11 (41%) were found positive for HCV RNA. Among the 5 samples subjected to sequencing, three were found to be genotype 1a while two were genotype 3a.

Conclusions: Our observations suggest that implementing NAAT test for HCV screening will be helpful in minimizing false-positive test results in the Indian setting.

Key words: *Hepatitis C virus, Genotype, Seroepidemiologic studies*

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INTRODUCTION

Hepatitis C virus (HCV) has been implicated in the causation of transfusion associated hepatitis (TAH). The most striking feature of HCV is its ability to persist in the host. It is transmitted primarily through parenteral route i.e., transfusion of blood or blood products. But, after the implementation of mandatory HCV antibody screening in 1990, incidence of TAH has decreased in most developed countries to 1 per million transfusions.^{1,2} The few cases that still occur are due to the newly infected individuals donating blood in the window period, as it takes 6-8 weeks to develop antibodies against the virus.

In India, mandatory screening for HCV was introduced as late as 2002. A study³ from Vellore in South India reported that 61% of 90 patients with chronic HCV infection had acquired the infection following blood transfusion.³ Lower prevalence of HCV is being observed more recently.

Six HCV genotypes and 80 subtypes have been identified around the world.⁴ Identification of HCV genotype before prescribing therapy is important because it has significant influence on disease severity, response to interferon therapy and also on duration of therapy. Genotype 1b has been shown to be associated with more severe hepatic disease as compared

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to other genotypes. Interferon therapy is less effective for genotypes 1 and 4 as compared to 2 and 3; also duration of treatment for genotypes 1 and 4 is 48 weeks as compared to 24 weeks for genotypes 2 and 3.⁴

Indian data on the prevalence of HCV infection and its genotype among general population as well as in healthy blood donors are limited. Some studies have reported the seroprevalence of HCV and its genotypes among patients with liver disease and those who have received multiple blood transfusions.^{5,6}

The present study was undertaken to study the burden and HCV seropositivity and evaluated the concordance of HCV seropositivity with the presence of HCV ribonucleic acid (RNA) and to study the distribution pattern of HCV genotypes in healthy blood donors.

MATERIAL AND METHODS

This is a prospective study conducted from June 2013 to May 2014 in the Department of Microbiology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati. The study was approved by the Institutional Ethical Committee. Five mL of peripheral venous blood samples were collected from the donors following standard procedure at the time of blood donation in a single sitting. Informed consent was obtained at the time of blood donation regarding participation in the study. Samples were divided in three aliquots and kept at -20 °C for further processing.

Enzyme linked immunosorbent assay (ELISA) was performed in the blood bank using Erba lisa Mannheim, India kit. All the positive samples were retested using Qualisa HCV ELISA kit (Qualpro diagnostic, Goa, India). Signal to cut-off ratio (S/CO) of the positive samples was calculated. HCV RNA was extracted from plasma using QIAmp viral RNA extraction kit from plasma (Qiagen GmbH, Hilden, Germany. Catalogue No. 52904).

Real-time polymerase chain reaction (RT-PCR) was performed in the Department of

Microbiology using Rotor Gene Q Machine with Artus HCV QS-RGQ Kit (Qiagen GmbH, Hilden, Germany, Catalogue no. 4518363).

The following primers were used: forward primer: ACTTTTCCAATCAACGCCAC; reverse primer: CTGAGCAAGGGCTTACAAGG. Initial denaturation was done at 94 °C for 2 minutes followed by denaturation at 94 °C for 45 seconds, annealing at 53.6 °C for 30 seconds and final extension at 72 °C for 1 minute.

Conventional PCR product was confirmed for the presence of HCV RNA by running on 1% agarose gel. Figure 1 shows the 250bp band corresponding to HCV in gel documentation of PCR product. The PCR product was sent for nucleotide sequencing to the Helini Biomolecules commercial lab, Chennai.

After receiving the sequence report, genotype was determined by using Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) [Available at URL: [http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn & PAGE_TYPE = Blast Search & LINK_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Accessed on November 10, 2014]

Statistical analysis

Data were recorded on a predesigned proforma and managed using Microsoft Excel 2007 (Microsoft Corp, Redmond, WA). Statistical software IBM SPSS, Version 20 (IBM SPSS Statistic, Somers NY, USA) was used for statistical analysis. Chi-square test was used to assess the statistical significance between categorical variables. A p-value less than 0.05 was considered statistically significant.

RESULTS

During the period June 2013 - May 2014, 9287 donors comprising 9126 (98.3%) males were screened. Of these 7153 (77%) were voluntary and 2134 (23%) were replacement donors. Of the 9287 screened donors, 27 (0.3%) were reactive for HCV antibodies. Of the 27

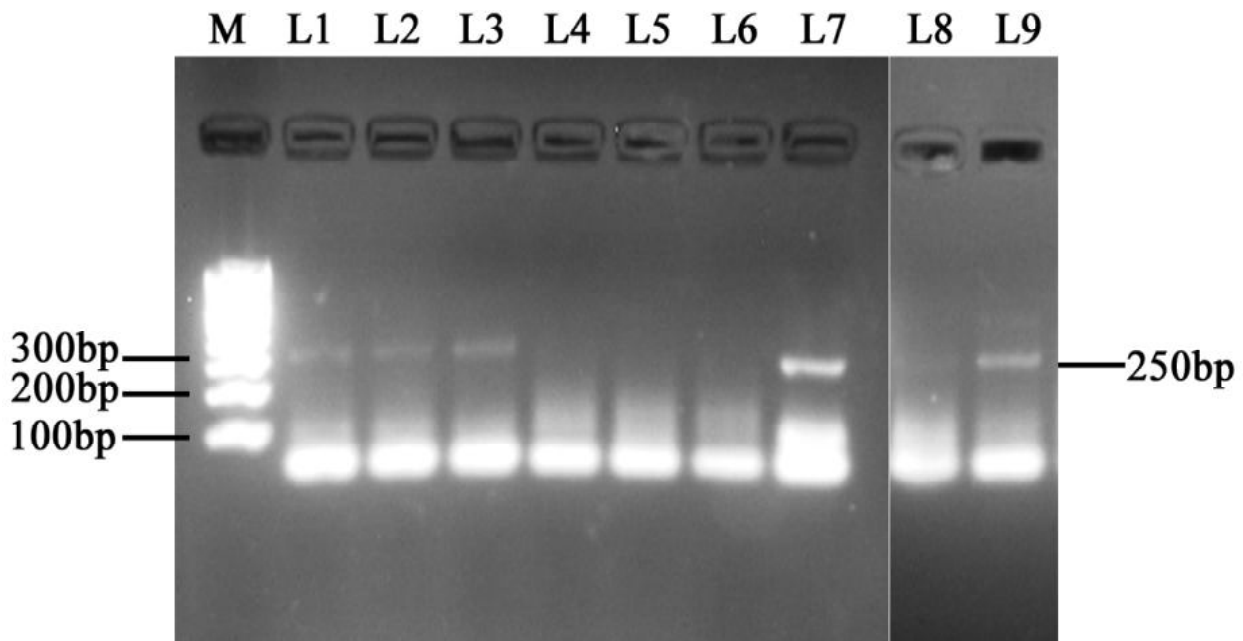


Figure 1: Ethidium bromide stained 1% agarose gel electrophoresis from 5'UTR-Core region: 250 bp product, Lane M = DNA ladder 100 bp, Lanes L1, L2, L3, L7 and L9-donor samples; 5' UTR = 5' untranslated region; bp = base pair

seroreactive samples, the S/CO of 5 samples was greater than 3.8. Eighteen (0.25%) of the voluntary donors and 9(0.42%) of the replacement donors, were seroreactive for anti-HCV antibodies. There was no statistically significant difference observed in the seroreactivity between voluntary and replacement blood donors ($p=0.147$).

Twenty four of the 9126 male donors (0.26%) and 3 of the 161 female donors (1.86%) were reactive for anti-HCV antibodies. A significantly higher proportion of female donors tested HCV seroreactive compared to male donors [3/161(1.9%) vs 24/9126 (0.3%); $p = 0.011$]. Majority of donors ($n=5162$; 55.5%) were in the age group of 21-30 years and few ($n=113$, 1.21%) were aged above 50 years. The occurrence of anti-HCV positivity was highest in the age group of 41-50 years (0.45%) and none tested positive in the age group of 51-60 years (Table 1).

Among 27 anti HCV seroreactive donor samples, 11 (41%) were positive for HCV RNA by real time PCR. The HCV positivity by ELISA and PCR is shown in Table 2.

Samples with S/CO greater than 2 were positive by RT-PCR while samples with S/CO less than 2 values tested negative by real time PCR.

Table 1: Anti-HCV seroreactivity in various age groups

Age (years)	Anti-HCV seroreactivity (n/N)	% positive
18-20	4 (1402)	0.28
21-30	10 (5162)	0.19
31-40	9 (1960)	0.45
41-50	4 (650)	0.61
51-60	0 (113)	0
Total	27 (9287)	0.29

n = number positive; N = number tested; HCV = hepatitis C virus

Table 2: HCV Seropositivity by ELISA and real time PCR

RT-PCR	ELISA positive (n = 27)
Positive	11
Negative	14

Note: RT-PCR was done only for ELISA positive samples

ELISA=enzyme linked immunosorbent assay;
RT-PCR = real time polymerase chain reaction

Among the five samples processed for sequencing three samples (3, 4 and 5) were found to be genotype 1a while two samples (1 and 2) were genotype 3a.

Figure 2 shows the nucleotide sequence reports of the five samples.

DISCUSSION

Transmission of HCV through blood transfusion has become a rarity presently because of mandatory HCV antibody screening before transfusion. Prevalence of HCV infection in general population in India ranges from 0.1%-7.9% while among blood donors it ranges from 0.29%-1.85%.^{7,8} Highest prevalence rate is reported from Egypt (3.5%), which is attributed to the mass treatment campaign of parenteral Schistosomiasis therapy. Lower rates of anti-HCV antibodies have been reported from Turkey (0.17%) and USA (0.3%). Neighbouring countries Pakistan and China have reported seroprevalence of 2% and 0.86% respectively.^{7,8}

The prevalence of HCV infection among apparently healthy screened donors in the present study was 0.3% which is comparable to studies from Delhi and Pune.^{9,10} Low prevalence has been reported from Karnataka (0.05%-0.23%) and Gujarat (0.07%-0.09%).^{11,12} Highest prevalence of 1.62% was estimated from Kolkata.¹³ Several reasons have been cited for differences in prevalence rates in various studies, such as, differences in the donor population groups and social practices between different regions of the country, literacy rates, use of different generation of ELISA kits, effectiveness of donor screening to exclude donors with the history of high risk behaviour and pre-donation counselling. The difference in HCV infection among voluntary (0.25%) and replacement donors (0.42%) was not statistically significant ($p = 0.147$) as reported in other studies.^{14,15}

Prevalence of HCV infection among female donors (1.9%) was significantly higher as compared to males (0.26%) ($p = 0.011$) which is in contrast to the observation reported in other studies.^{9,12} This observation merits future study.

Some studies^{16,17} which reported increasing trend with age attributed this to infection acquired in the past due to the use of unsafe needles and contaminated equipments used in the health care related procedures. On the contrary, some studies¹⁶⁻¹⁸ have reported decreasing trend in the anti-HCV positivity with the increasing age, possibly implying a higher exposure rate of HCV among younger population group.¹⁶⁻¹⁸ Neither an increasing nor a decreasing trend was observed in our study ($p = 0.17$).

Of the 27 anti-HCV reactive samples, 11 (41%) have shown the presence of HCV RNA by RT-PCR. The prevalence of HCV among blood donors in our study based on ELISA was 0.3%, while based on RT-PCR it was 0.11%. Several studies^{19,20} have reported that the prevalence of HCV decreases when NAT is used for testing as compared to ELISA.

Recent studies have reported that higher the anti-HCV antibody titre in patient's serum; more are the chances of it being true positive. This was the basis of inclusion of measurement of anti-HCV S/CO ratio that indirectly represents higher antibody levels in patient's sample. Centers for Disease and Control (CDC) in 2003 recommended the inclusion of S/CO ratio to determine the need for supplementary testing.^{21,22} It was estimated that for ELISA, a S/CO of 3.8, and for Chemiluminescence immunoassay (CLIA) a S/CO of 8, predicted true viraemia in 95%-98% cases.^{21,22}

It was observed that the samples with high viral load and S/CO greater than 3.8 (5 samples) showed a clear band when the samples were subjected for RT-PCR. Real Time PCR is more sensitive as compared to reverse transcriptase

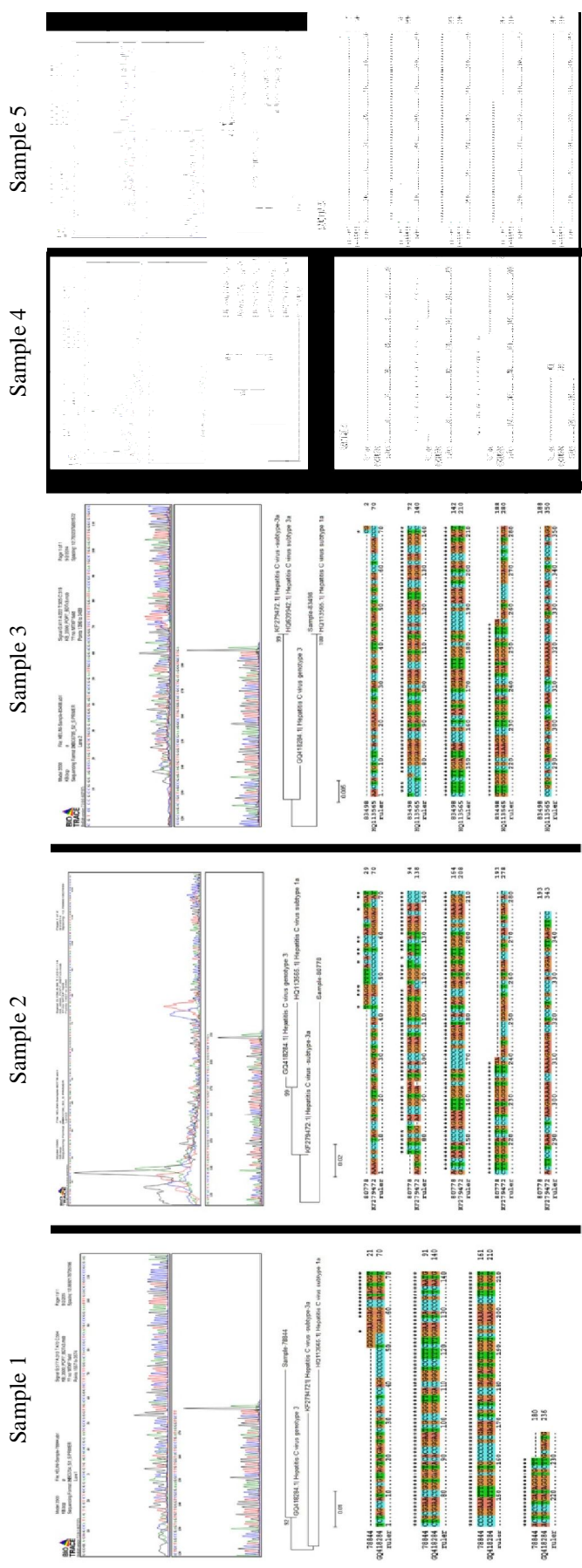


Figure 2: Sequence report of the five PCR positive samples. Samples 3, 4 and 5 belong to 1a and samples 1 and 2 belong to 3a
 PCR= polymerase chain reaction

PCR; therefore samples with low viral load may be negative in conventional method.

Distribution of HCV genotypes varies throughout the world in different geographical regions. Subtype 1a is common in Europe, Australia and North and South America where as subtype 1b is common in North America and Europe. The distribution of HCV genotypes varies in different geographical regions of India. Genotype 3 is the predominant genotype in North India whereas genotype 1 in South India. Among the five samples, three were genotype 1a, while two were genotype 3a which is in concordance with the previously published studies from south India.^{3,5,6}

As only five genotypes were identified in our study, it is difficult to comment on the age and sex distribution of different genotypes but all these five donors were males. All the three 1a genotypes were donors less than 35 years of age whereas both the genotypes 3a were from donors aged more than 45 years of age. In literature no significant differences have been found in genotype distribution with respect to age and sex. But a study²³ from Pakistan reported that subtype 1a/1b were more common in younger patients and subtypes 2a/2b and 3a/3b were common in older patients. More extensive work needs to be done before conclusively commenting on the various genotypes prevalent in this region. Knowing the predominant genotypes is important to plan future prevention and treatment strategies as the treatment of different genotypes and prognosis differs considerably. To conclude, safe blood donation remains a challenge in resource limited countries. Though NAT is costly, it has definite advantage over serological tests for early diagnosis of HCV infection. Given the high false positive serological results we recommend implementation of NAT in blood banks so as to avoid unnecessary wastage of precious blood.

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