Original Article

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Real Time Polymerase Chain Reaction Assessment of Hepatitis C Infection in a Tertiary Care Centre

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Abstract

Introduction: RNA viruses are very vulnerable for mutations and therefore pose maximum difficulty in diagnosis and treatment. Hepatitis C virus is among one of them. In addition to pose higher mutation rate, its geographic distribution in different countries and even in different states in India is quite remarkable. This study is being undertaken to study its prevalence by real time polymerase chain reaction (RT-PCR) method, which is a gold standard for diagnostic assessment for such type of viral infections.

Materials and Methods: This study was conducted in the blood bank on 820 units of blood at Teerthanker Mahaveer Medical College and Research Centre (TMMCRC) and associated hospital with prior consent of medical superintendent of the hospital. Along with other screening test RT-PCR was applied as gold standard. All conditions for this type of diagnostic tests were maintained and results were obtained and appropriate statistical test applied.

Results: Of the 820 units, 0.51% showed the seropositivity. On statistical analysis, there was statistically significant ($P < 0.05\%$) seropositivity and higher response rate ($P < 0.005$) was observed. The seropositivity was found to be maximum in old age (55-65 years males), while it was found to be least in young 18-25 years males.

Conclusion: As we go through the literature available on the topic we found that there is a major difference in the seropositivity in different continents of the world and even the different states in India. Hence, we conducted the study using RT-PCR method and found the prevalence rate of 0.51% in our hospital, which addresses to us for being more vigilant at the time of receiving blood.

Keywords: Hepatitis, RNA virus, Real time polymerase chain reaction, Seropositivity.

INTRODUCTION

Hepatitis C is a RNA virus that belongs to the Flaviviridae family and genus *Hepacivirus* (Choo *et al.* 1989) saw first it in infected animals.[1] Worldwide prevalence of hepatitis C virus (HCV) is approximately 1.8%.[2] The HCV is single-stranded RNA virus. Being RNA virus it has got maximum genetic variability in terms of mutations. The high mutation rate is associated with high morbidity and mortality. Unsafe blood transfusion and neglect of prescribed guidelines to prevent such type of infections are the leading cause of the emergence of HCV in India. HCV genome differs in different geographical regions.[3] Antiviral therapy is affected by the genome of the virus.

While in India seropositivity rate varies from 0.11% to 3.8%, but globally it varies from 0.36% to 18.6%.[4]

In addition to various tests available for detecting HCV antibodies, polymerase chain reaction (PCR) real time PCR (RT-PCR) for HCV is the test of choice because it easily detects antibodies in serum in 7-12 days after infection.[5] Real-time PCR method has got added advantage of lower detection limits.

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MATERIALS AND METHODS

This study was conducted in the blood bank (pathology department) TMMCRC, Moradabad, India. A total of

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820 units of blood were screened. Persons found reactive for anti-HCV were informed. After information persons who gave the consent to be included in the study were accessed for further detailed history, examination and other blood tests, other blood tests which were performed included liver function test, prothrombin time, activated partial thromboplastin time, and HCV RNA detection and detection of HCV RNA by real-time PCR HCV RNA by RT-PCR.

To guarantee ideal conditions, serum samples were stored at temperature −80°C. The study was approved by the Ethics Committee of Medical Research Division of TMMCRC, Moradabad, India.

RT-PCR technique involved following steps in sequential manner

1. Denaturation at 92°C for 1 min,
2. Annealing at 52°C for 1 min,
3. Extension at 72°C for 1 min, for 30 cycles.

The amplified products were analyzed under ultraviolet illumination.

Statistical Analysis

The data were statistically evaluated using Student's - *t*-test.

RESULTS

Of the 820 units, 4 were found positive for anti-HCV antibodies, showing seropositivity of approximately of 0.51%. When checked for concomitant infections no one found to be positive. Of the four, three responded back to the department (making it to 75%). A significantly lower HCV seropositivity ($P < 0.05$) and a higher response rate ($P < 0.005$) pattern were observed.

The seropositivity was found to be maximum in old age (55-65 years males), while it was found to be least in young 18-25 years males (Table 1, Figure 1).

Samples that were positive showed the band size of 270 base pairs. Following molecular genomic sequencing of different regions were obtained (Table 2, Figure 2).

Table 1: Age wise distribution of seropositivity

Age in years	Rate of seropositivity (%)
16-25	0.26
26-35	0.32
36-45	0.41
46-55	0.42
56-65	0.51

DISCUSSION

HCV RNA is one of the most important parameters for diagnostic and prognostic significance of hepatitis C.

Real-time PCR technology has now become the technique of choice for highly sensitive assessment of RNA targets.[6-8]

Chances of transmission of HCV through contaminated transfusion are approximately 88-90%. Safe transfusion does not only involve only screening of blood, but other factors like age, area of study, incidence and prevalence, and proper history taking. In the present study, anti-HCV seropositivity was 0.51% among healthy donors. Studies from Northern India found HCV seroprevalence ranging from 0.53% to 5.1% in their blood donors.[9-11] This well correlates with our study.

A study conducted in other part of western India showed seropositivity between 0.33% and 2.6%.[12,13] This difference from our study may be due to different

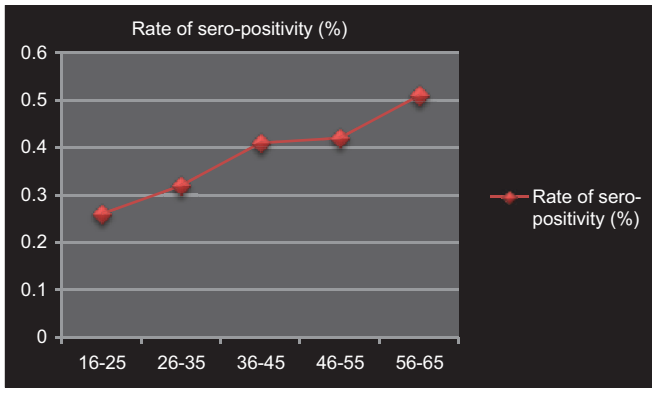

Figure 1: Graphic representation of age wise distribution of seropositivity

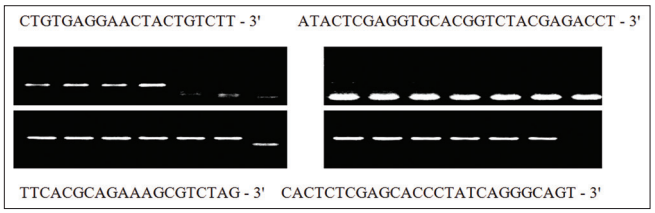

Figure 2: Base pairing sequence of hepatitis C virus - RNA by real time polymerase chain reaction

Table 2: Sowing base pairing sequence of HCV - RNA

Region	Base pairing sequence
Outer sense: 5'	CTGTGAGGAACTACTGTCTT – 3'
Outer antisense: 5'	ATACTCGAGGTGCACGGTCTACGAGACCT – 3'
Inner sense: 5'	TTCACGCAGAAAGCGTCTAG – 3'
Inner antisense: 5'	CACTCTCGAGCACCCTATCAGGGCAGT – 3'

HCV: Hepatitis C virus

methods of testing, strict regulations whether followed or not like in our study and high-risk donors did not return back for confirmation.

In our hospital, we take blood of those only who well know about safe sexual practices, relatives of patients, and who full fill our criteria of safe blood donation. Even after that 0.51% seropositivity reflects very strict testing of blood.

When we compared and analyzed the seropositivity between relative donors and voluntary donors, we found seropositivity was less in blood units of relative to some extent, but not statistically significant ($P > 0.5$) from voluntary donors.

Our study is well in accordance with the study conducted by Schreiber *et al.* 2001,[14] who also did not find statistically significant difference ($P > 0.05$) between donors of different socio-economic class, and other concurrent factors like safe sexual practices, and relatives of patients.

When we track the record, we found that seropositivity was maximum in age group between 55 and 65 years of age group individuals, which is similar in results shown by Okayama *et al.*[15,16]

CONCLUSION

Spread of HCV, through blood and blood products is an important mode of transmission because even the very meager amount of blood transfers a large amount of the infective agent into potential recipient. There have been various methods used to combat such type of blood borne infections, which effectively reduced the prevalence of such type of infections in developing countries. In India, various studies showed varied results, so taking their reference is not adequate to implement in our hospital and to avoid spread of hepatitis C we employed the ultimate test RT-PCR for confirming the susceptibility of hepatitis C infection and we were successful in achieving the results. Still we feel that a large sample than this should be taken to validate the study.

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