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Original Research Paper

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PREVALENCE OF HEPATITIS C VIRUS GENOTYPES REPORTED FROM A TERTIARY CARE CENTRE OF KERALA.

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ABSTRACT

Hepatitis C is a global health problem and an estimated 71·1 million individuals are chronically infected with hepatitis C virus. The prevalence of HCV infection in high-risk group of patients like those receiving blood transfusions, subjects with haemodialysis ,reuse of needles are expected to be higher than in general population.

AIM OF STUDY-To study the prevalence of Hepatitis C infections

MATERIALS AND METHODS-The study was conducted at Travancore medical college a teaching hospital. A total of 450 samples were included and tested for hepatitis C virus by RT-PCR.

RESULTS- Of which 41 samples were positive for HCV RNA and HCV genotype 1 (n = 14), include subtype 1a (n = 11), subtype 1b (n = 2), genotype 3 (n = 22) and genotype 4 (n =5).

CONCLUSION- The study discovered the presence of HCV genotypes 1, 3&4. The current study shows a prevalence of 9% HCV infection. It was more likely to occur in older persons than younger ones. Multiple haemodialysis' and post transfusion hepatitis continues to be an important cause of HCV related liver disease in India.

KEYWORDS : Hepatitis C virus, Genotypes, haemodialysis, Polymerised chain reaction Hepatocellular Carcinoma

Hepatitis C is a global health problem and an estimated 71·1 million individuals are chronically infected with hepatitis C virus. In India, it is estimated that around 12.5 million people are suffering from chronic hepatitis [1].Of the exposed have an estimated 75% to 85% likelihood of developing chronic infection, while the remainder experience spontaneous clearance of the virus.[2] Hepatocellular carcinoma is the fifth most common cancer and major cause of death in patients with chronic HCV infection and responsible for approximately one million deaths each year .But now, with the advancement in medical research, newer anti-HCV drugs have been developed which are also useful in the advanced cirrhosis caused by HCV [3].

The prevalence of HCV infection in high-risk group of patients like those receiving blood transfusions, subjects with haemodialysis, reuse of needles are expected to be higher than in general population. The chronicity of hepatitis C virus (HCV) infection is due to the existence of various genotypes and its various subtypes.[4] It is transmitted through blood and blood products. Healthcare providers are at risk through needle-stick injuries. Babies born to mothers with hepatitis C are also at risk. Less commonly, the hepatitis C virus is transmitted through sexual contact with an infected partner. Testing of donated blood for HCV has helped reduce the risk of transfusion associated hepatitis C from 10% to 01%.[5]

It is an enveloped, single stranded, positive sense, RNA virus belonging to the family Flaviviridae and is the only member of the genus Hepacivirus with a genome containing approximately 9500 nucleotides. HCV has a high genetic heterogeneity. The estimated mutation rate is 1.92×10^{-3} nucleotide site[6].The envelope regions, especially HVR1, (hypervariable region 1) have the highest mutation rate.On the basis of genomic variability, HCV is classified into seven major genotypes and 67 subtypes.

In published studies, genotype 3 is reported as the most common genotype in India, accounting for 54–80% of cases.[7] Studies from northern, western and eastern parts of the country have uniformly shown predominance of genotype 3; however, in southern India, both genotypes 1 and 3 HCV are found to be prevalent [7,8]. So the present study was decided to investigate the distribution of HCV genotypes and its risk factors in our area.

MATERIALS AND METHODS

The study was conducted at Travancore medical college a teaching hospital with a bed capacity of 900 located at Kollam District,Kerala, India in the Department of Microbiology from January 2016 to February 2017. Human ethical clearance for this study was obtained from the Institution Ethics Committee. Patients visiting the haemodialysis unit, gastroenterology, surgery departments whose sample was sent to the Microbiology Department, were included in the study. A total of 450 samples were included and tested for hepatitis C virus. All the patients were screened for seropositivity of hepatitis B virus (HBV), HCV and human immunodeficiency virus (HIV). Those seropositive for HCV were further subjected to HCV RNA detection and HCV genotyping.Detailed history of transfusion of blood/blood products, dental extraction, surgeries, previous dialysis and high-risk behaviours like sexual promiscuity, sharing of needles,razors and tattooing were collected from patients.

SEROLOGICAL ASSAY

Blood samples were collected, serum and plasma was separated and dispensed into screw capped vials and stored at –80°C. This sample was utilized for detection of anti-HCV antibodies, HCV-RNA detection and subsequent genotyping analysis.

The statistical analyses were performed in SPSS 20.0(Chicago, IL, USA). The categorical variables were tabulated as counts and percentages, along with 95%confidence intervals (Cis). Samples were subjected to HCV Microlisafourth-generation ELISA (by J. Mitra and Co., Pvt., Ltd.) for the detection of HCV antibodies. Samples in which HCV antibodies were detected were subjected to HCV-PCR for confirmation. (QI Aamp Viral RNA Mini Kit (Qiagen Inc., Germany) was used for the detection. Confirmed positives in PCR were subjected for sequencing (SciGenom Labs Pvt. Ltd., Kerala). To simplify the genotype and subtype, a phylogenetic tree was constructed using MEGA 5.05software.[8]

HCV-RNA detection.

This method is based on real-time PCR for the amplification and uses specific fluorescent probes for the detection of the HCV genome.Following manufacturer's manual, HCV RNA was automatically extractedfrom 650 μL samples, reverse transcribed into complementary DNA (cDNA), and amplified and amplicons were detected using CobasAmpliPrep /CobasTaqMan HCV machine, version 2. HCV genotyping (nested reverse transcriptasepolymerase chain reaction) were carried out by sequencing of highly conserved regions such as NS5, core, E1 and 5'UTR using HCV genotype kit cobas® HCV GT, manufactured by Roche used for identification of HCV genotypes 1-6 and can discriminate between subtype a and b

of genotype-1.RT-PCR was performed on a Z480 thermocycler from the same automated platform . Detection of both the HCV genome and its genotype is achieved using fluorescent hydrolysis probes. A positive control containing extracts from genotypes 1, 3 and 4 allow monitoring of the three RT-PCR reactions. A negative control is also included in the kit.

RESULTS

The study has been designated to test 450 samples for HCV. Of the 450 samples screened, 46 samples were positive for HCV antibodies by ELISA test. These 46 samples were subjected to PCR, of which 41 were positive for HCV RNA. These 41 cases had active HCV infection. The study discovered the presence of HCV genotypes 1, 3&4. The HCV sequences showed three distinct clusters corresponding to HCV genotype 1 (n = 14), include subtype 1a (n = 11), subtype 1b (n = 2), genotype 3 (n = 22) and genotype 4 (n =5). Fig 1

The risk factors commonly associated with each genotype were different. Genotypes 1 and 3 were predominantly seen transmitted by blood transfusions, multiple haemodialysis, Chronic liver disease(CLD), Injectable drug users.(Table-1).In 6 patients (out of 41), the mode of transmission could not be ascertained. The age of the patients varied from 21 to 65 years and majority of cases were within the age group of 35 to 55 years.

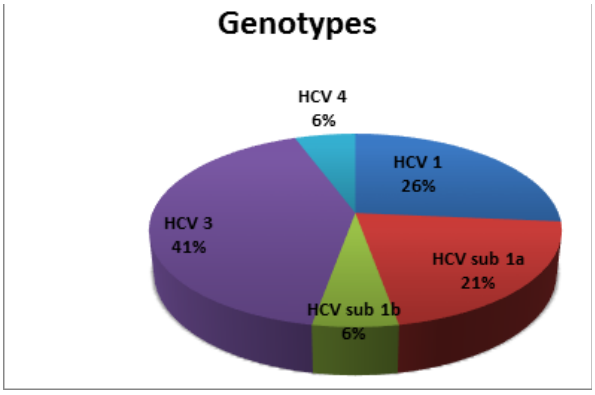

Fig: 1 HCV-Genotypes Pattern of Study Population

Table-1 Mode Of Transmission And Distribution Of Hepatitis C Virus Genotypes

Mode of transmission	HCV -1 (14)	HCV -3 (22)	HCV -4 (5)
Blood transfusions	1	2	0
Multiple hemodialysis	7	9	1
Liver cancer	0	3	0
Chronic liver disease	0	1	3
IV drug abusers	2	0	1
Health care associated	1	0	0
TOTAL 41	14	22	5

DISCUSSION

The prevalence of HCV in India has been higher in comparison to Western Europe and USA[9] The HCV prevalence reported by various groups in Indian population is 1 in 4138[10] 1 in 1997[11] and 1 in 2536[12]. HCV genotype 3 is more prevalent in the northern, eastern and western parts of India and genotype 1 is more prevalent in the southern India [13]. Despite, the widespread prevalence of genotypes 3 and 1, there also exists increased prevalence of genotypes 4 and 6 in certain regions of the country. Genotype 4 is found mostly in south Indian patients from the States of Andhra Pradesh and Tamil Nadu. Genotype 6 is found to be prevalent in patients belonging to northeastern parts of India [14].

HCV infection is a major health problem among hemodialysis patients in developing countries. This could either be due to the non- adherence to strict infection control measures or the unavailability of vaccine to prevent hepatitis C infection. It depends on sterilisation of equipments, hygiene of hospital, patient, rotation of dialysis machine and the undertaking of rigorous universal precaution rules in the dialysis unit. In India, HCV is responsible for acute viral hepatitis in up to 21 per cent[15] and chronic liver disease in 14-26 per cent cases of HCV infections [16]. Longer duration of HD and getting HD at more than one centre are deemed important in acquiring HCV infection.[17] Genotype 1 is the most common genotype worldwide; 2% of the world is infected with this genotype. It is also the most predominant genotype in Central Asia.[18] Genotype 1 is further subtyped into 1a and 1b; Genotype 1b is predominantly associated with hepatocellular carcinoma.[18]. The risk factors most commonly associated with genotype 1 in the study were a history of blood transfusions and multiple haemodialysis. Genotype 2 accounts for 9.1% of the cases worldwide. This genotype is most predominant in West Africa. Subtypes 2aand 2b are commonly found in North America, Europe and Japan.[18,19] In the present study genotype 2 is not isolated. Genotype type 3 commonly occurs in Indian subcontinent and Southeast Asia[13]. Genotype 3 is less virulent and requires treatment with pegylated interferonand ribavirin[18]. The risk factor commonly associated with genotype 3 in our study was multiple haemodialysis, blood transfusion and history of chronic liver disease.The risk factors associated with genotype 4 in our study were a history of chronic liver disease, history of multiple haemodialysis and IV drug abusers. Genotype 4 is prevalent in North Africa and Middle Eastern countries mainly associated with intermediate drug resistance to pegylated interferon and ribavirin .[18,19] Genotype 4 has not been reported as yet from Kerala, and the presence of genotype 4 is significant in terms of disease management and have a close association with migration to the middle east countries.

CONCLUSION

Hepatitis C is a pathogen responsible for a significant proportion of liver disease in various regions of India. The current study shows a prevalence of 9% HCV infection. It was more likely to occur in older persons than younger ones. Multiple haemodialysis and post transfusion hepatitis continues to be an important cause of HCV related liver disease in India. This could either be due to the non-adherence to strict infection control measures or the unavailability of vaccine to prevent hepatitis C infection. Screening of blood for anti HCV antibody needs to be widely practised to control the frequency of transfusion related liver disease in India. The occurrence of genotype 4 shows a new genotype spread in Kerala. Genetic diversity can results in emergence of mixed genotypes recombination and evolution of new drug resistant genotype. So the study reinforces the need for continuous monitoring to analysis divergence and heterogeneity of HCV viruses.

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REFERENCE

1. European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2012; *56* : 908-43.
2. Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. *J Clin Microbiol* 2002; *40* : 4407-12.
3. Bennett JE, Dolin R, Blaser ME, Mandell MD. Hepatitis C. Douglas and Bennett's Principles and Practices of Infectious Diseases. 8th ed., Vol. 156. Philadelphia, USA: Elsiever Saunders; 2010. p. 1904-27
4. John M, Oommen S, Jagan OA, George S, Pillai S. A study on the circulating genotypes of hepatitis C virus in a tertiary care hospital in Central Kerala. Indian J Med Microbiol 2018;36:532-6.
5. Degenhardt L, Charlson F, Stanaway J, Larney S, Alexander LT, Hickman M, et al. Estimating the burden of disease attributable to injecting drug use as a risk

factor for HIV, hepatitis C, and hepatitis B: findings from the Global Burden of Disease Study 2013. Lancet Infect Dis.2016 Dec;16(12):1385–98. doi:10.1016/S1473-3099(16)30325-5 pmid:27665254
6. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. Hepatology. 2014;59:318–27
7. Verma V., Chakravarti A., Kar P. Genotypic characterization of Hepatitis C virus and its significance in patients with chronic liver disease from Northern India. Diagn Microbiol Infect Dis. 2008;61(4):408–414.
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M,Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance,and maximum parsimony methods. Mol Biol Evol 2011;28:2731-9.
9. Singh B. Bernex: The International Hospital Federation (IHF); 2009. Nucleic Acid Testing (NAT) screening of blood donors in India A Project Report, International Hospital Federation Reference Book, 2008/2009; pp. 66–8
10. Mangwana S, Bedi N. Significance of nucleic acid testing in window period donations: Revisiting transfusion safety in high prevalence-low resource settings. J Pathol Nepal. 2016;6:3.
11. Agarwal N, Chatterjee K, Coshic P, Borgohain M. Nucleic acid testing for blood banks: An experience from a tertiary care centre in New Delhi, India. Transfus Apher Sci. 2013;49:482–4.
12. Kumar R, Gupta S, Kaur A, Gupta M. Individual donor-nucleic acid testing for human immunodeficiency virus-1, hepatitis C virus and hepatitis B virus and its role in blood safety. Asian J Transfus Sci. 2015;9:199–202.
13. Bhattacharya PK, Roy A. Management of hepatitis C in Indian context: An update. *J Liver* 2015; *4* : 187.
14. Christdas J, Sivakumar J, David J, Daniel HD, Raghuraman S,Abraham P, *et al*. Genotypes of hepatitis C virus in the Indian sub-continent: A decade-long experience from a tertiary care hospital in South India. *Indian J Med Microbiol* 2013; *31* :349-53.
15. Kar P. World Hepatitis Day-new challenges. Indian J Med Res. 2011;134:1–3.
16. Morishima C, Gretch DR. Clinical use of hepatitis C virus tests for diagnosis and monitoring during therapy. Clin Liver Dis. 1999;3:717–40.
17. Jasuja S., Gupta A.K., Choudhry R. Prevalence and associations of Hepatitis C viremia in hemodialysis patients at a tertiary care hospital. Indian J Nephrol. 2009;19(2):62–67.
18. Pybus O, Pybus OG, Charleston MA, Gupta S, Rambaut A, Holmes EC, *et al*. The Epidemic Behavior of the Hepatitis C Virus. Science 2015;292:2-5.
19. Puri P., Anand A.C., Saraswat V.A. Consensus statement of HCV task force of the Indian National Association for Study of the Liver (INASL). Part I. Status report of HCV infection in India. J Clin Exp Hepatol. 2014;4(2):106–116