MAK HILL Publications International Journal of Tropical Medicine

Research Article
doi: 10.59218/makijtm.2023.2.18.22

Prevalence of Hepatitis C Virus and its Correlation with Genotypes in Chronic Liver Disease Patients Attending a Tertiary Care Center, Coimbatore

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ABSTRACT

Hepatitis C virus is an important cause of chronic hepatitis and primary hepatocellular carcinoma. HCV genotypes can vary in pathogenicity and can have an impact on treatment outcome. The analysis of Hepatitis C virus genotypes has become an important factor for planning treatment. Present study was aimed to study prevalence of HCV and its correlation with genotypes in chronic liver disease patients attending a tertiary care center, Coimbatore. Present study was single-center, prospective, observational study, conducted in patients with chronic liver disease subjected to Anti-HCV ELISA and RT-PCR. Genotyping is done by RT-PCR. Among 200 patients, majority belong to age group between 41-70 years (76.5%). 110 (55%) were males and 90(45%) were females respectively. Out of 200 patients tested for Anti-HCV antibodies by ELISA, 56 (28%) were positive. Out of 61 cases of chronic hepatitis, 14 (22.9%) and from 133 cases of cirrhosis 38 (28.6%) were positive for Anti-HCV antibodies by ELISA. 29 (14.5%) were detected to have HCV RNA. 26 (13%) samples had been found to be positive by both ELISA and PCR. Genotype 3 was most common type observed in 17(58.6%) in this study followed by the genotype 4 which was seen in 6 (20.6%) cases. Four cases (13.7%) showed genotype 1 and genotype other than 1-4 was also observed in 2 (6.9%) cases. The present study highlighted that HCV genotype 3 is the predominant genotype among chronic liver disease in our geographical area.

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Key Words

Hepatitis C virus, chronic liver disease, hepatocellular carcinoma, genotypes

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Received: 7 August 2023
Accepted: 24 August 2023
Published: 25 August 2023

Citation: S. Viji, V.M. Theeba and M. Banumathy 2023. Prevalence of Hepatitis C Virus and its Correlation with Genotypes in Chronic Liver Disease Patients Attending a Tertiary Care Center, Coimbatore Int. J. Trop. Med., 18: 18-22, doi: 10.59218/makijtm.2023.2.18.22

INTRODUCTION

Hepatitis C virus is an important cause of chronic hepatitis and primary hepatocellular carcinoma. HCV genotypes can vary in pathogenicity and can have an impact on treatment outcome[1]. Acute infection becomes persistent with long term viremia in 50-85% of infected individuals. Persistent infection with HCV leads to cirrhosis and hepatocellular carcinoma. The high rate of chronicity and lack of successful vaccine makes Hepatitis C virus a serious threat to public health[2].

The first step used for the detection of Hepatitis C virus is serological tests. Third generation ELISA with increased sensitivity and more specificity has been the standard method for HCV serology. Molecular technique detects HCV RNA and confirms active infection. Quantitative assay is necessary for monitoring prognosis[3,4].

The virus shows considerable genetic diversity. It can be differentiated into 6 major genotypes and nearly 100 subtypes based on RNA sequence analysis. Genetic variabilities are recognized as an important factor in the prognosis and outcome of HCV mediated chronic liver disease. HCV genotypes have distinct geographical distribution. Duration of treatment, cure rates the need for combination therapy with the new direct acting antivirals (DAA) also based on the genotypes and subtypes[5,6]. The formulation of treatment strategies using direct acting antiviral requires knowledge of prevalence of HCV genotype. The analysis of Hepatitis C virus genotypes has become an important factor for planning treatment. Present study was aimed to study prevalence of HCV and its correlation with HCV genotypes in chronic liver disease patients attending a tertiary care center, Coimbatore

MATERIALS AND METHODS

Present study was single-center, prospective, observational study, conducted in Department of Microbiology, Coimbatore Medical College, Coimbatore, India. Study duration was of 12 months from August 2015 to July 2016. The approval for this study was obtained from the ethical committee prior to its conduct. Informed consent was obtained from the patients/guardian of the patients.

Inclusion criteria:

- Patients with chronic hepatitis, fulminant hepatitis, hepatic failure, cirrhosis of liver, hepatocellular carcinoma, persistently elevated liver enzymes, serum bilirubin> 6 months, willing to participate in present study

Exclusion criteria:

- Patients with acute hepatitis
- Auto immune diseases of liver
- Inborn errors of metabolism
- Systemic diseases affecting liver function
- Hepatic congestion due to cardiac failure
- Drug induced hepatic dysfunction

Study was explained to patients in local language and written consent was taken for participation in the study. Blood samples were collected from 200 patients admitted with chronic liver disease for detecting Hepatitis C virus infection in various clinical departments of coimbatore medical college hospital.

The patient's name, age, sex, address, Inpatient/outpatient number and detailed clinical history were noted. General and systemic examination was carried out. 10 mL of blood was withdrawn aseptically by venepuncture. The 5mL blood sample for serum was transferred to plain vacutainer tube for anti HCV ELISA. The 5mL blood sample for plasma was collected in a K_2EDTA vacutainer tube and the tube was centrifuged at 3000 rpm for 10 min for separation of plasma. This was stored at-20°C for PCR. ELISA test for HCV antibody is performed using hepascan kit as per manufacturer instruction and plasma samples were tested for detection of HCV-RNA by reverse transcription and real time polymerase chain reaction (RT-PCR). Genotyping of the positive samples were done out by real time RT-PCR. Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Statistical analysis was done using descriptive statistics.

RESULTS

200 patients with clinical, biochemical and ultrasonographic evidence of chronic liver disease enrolled in the present study. The age-wise distribution of the patients in the study group ranges from 21-70 years, with a mean of 45. Most of the cases belong to age group between 41-70 years (76.5%). 110 (55%) were males and 90 (45%) were females respectively. Age-wise analysis in the present study showed high sero-positivity among individuals in the age group of 31-40 years (31.8%) followed by age group 41-50 years (30.1%) and 51-70 years (27%). Among the 56 Anti-HCV positive patients, 33 (30.8%) and 23(24.7%) were males and females, respectively (Table 1).

Table 1: General characteristics

Age groups (years)	No.	Percentage	ELISA positive cases (%)
21-30	12	6.0	2 (16.67)
31-40	22	11.0	7 (31.8)
41-50	53	26.5	16 (30.1)
51-60	48	24.0	13 (27)
61-70	52	26.0	14 (26.9)
>70	13	6.5	2 (15.3)
Gender			
Male	110	55.0	33 (30)
Female	90	45.0	23 (25.5)

Table 2: Distribution of Anti-HCV and PCR positive cases

Clinical presentation	No of cases n = (200)	ELISA positive cases (%)	PCR positive cases (%)
Chronic hepatitis	61	14 (22.9)	8 (13)
Cirrhosis	133	38 (28.6)	18 (13.5)
Hepatocellular carcinoma	6	4 (66.7)	3 (50)
	200	56 (28)	

Table 3: Biochemical profiles in anti-HCV positive cases (n = 56)

Liver enzyme	Normal (%)	Elevated (%)
Alanine aminotransferase (ALT)	36 (64.2)	20 (35.7)
Aspartate aminotransferase (AST)	39 (69.65)	17 (30.35)
Serum alkaline phosphatise	37 (66.1)	19 (33.9)
Serum bilirubin	32 (57.14)	24 (42.8)

Table4: Distribution based on probable history of exposure to HCV infection

Risk factor	No. of cases (n = 200)	Anti HCV positive cases (%)	HCV RAN positive cases
Blood transfusion due to non-surgical cause	37	11 (29.7)	4 (10.8)
Haemodialysis	21	5 (23.8)	4 (19)
Surgery and Blood transfusion	42	8 (19)	5 (12)
Unsafe injection	32	6 (19)	1 (3.1)
IV drug abuse	3	1 (33)	-
Unknown	65	26 (40)	15 (23.1)

Table 5: Comparison of results: ELISA and PCR

	ELISA +ve Cases		PCR +ve cases		Both +ve cases	
Result	No	Parentage	No	Parentage	No	Parentage
Positive	56	28	29	14.5	26	13.0
Negative	144	72	171	85.5	141	70.5

Table 6: Prevalence of HCV genotypes among chronic liver disease patients

Genotype	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype other than 1,2,3,4
No of cases (n = 29)	4 (13.7%)	0 (0%)	17 (58.6%)	6 (20.6%)	2 (6.9%)

Out of 200 patients tested for anti-HCV antibodies by ELISA, 56(28%) were positive. Out of 61 cases of chronic Hepatitis, 14 (22.9%) and from 133 cases of cirrhosis 38 (28.6%) were positive for Anti-HCV antibodies by ELISA (Table 2).

Liver enzymes in HCV related chronic liver disease are may be fluctuating or normal. In the present study showed ALT was elevated in 35.7%, AST in 30.35% and alkaline phosphatase in 33.9% of anti-HCV positives. The present study also showed that serum bilirubin was elevated only in 2.8% of anti-HCV positives (Table 3).

In the present study the probable risk factor for HCV transmission was observed as blood transfusion due to non-surgical causes in 11 cases (29.7%), surgery and blood transfusion in 8 cases (19%), haemodialysis in 5 cases (23.8%), unsafe injection in 6 cases (19%) and IV drug abuse in only one case (33%). No risk factors were identified in other anti-HCV positive cases (40%) (Table 4).

Out of 200 samples tested for the presence of HCV RNA by real time only RT-PCR, 29 (14.5%) were detected to have HCV RNA. 26(13%) samples had been found to be positive by both ELISA and PCR. Among 144 ELISA negative samples, 3 (2.1%) were PCR positive. Thirty ELISA positive samples were tested negative by PCR (Table 5).

Genotype 3 was most common type observed in 17 (58.6%) in this study followed by the genotype 4 which was seen in 6 (20.6%) cases. Four cases (13.7%) showed genotype 1 and genotype other than 1,2,3,4 was so served 2 (6.9%) cases (Table 6).

Genotype 3 was the commonest type observed in 58.6% patients which shows good response to therapy. The genotype 4 was seen in 20.6% patients showing rising trend and associated with poor prognosis. Genotype 1 was seen in 13.7% which respond poorly to treatment. Genotype other than 1,2,3,4 was observed in 2 (6.9%) cases. Genotype 3 was a predominant genotype among both genders and in all age groups.

DISCUSSIONS

Hepatitis C virus infection is a serious threat to health care system. It can cause varying clinical conditions ranging from acute infection to chronic hepatitis and hepatocellular carcinoma. The seroprevalence of HCV varies in different parts of the country. The complex and uncertain nature of HCV infection and its chronicity emphasises the difficulties in prevention and control of HCV. The information about seroprevalence of HCV among chronic liver disease cases and relative distribution of each genotype are essential tool to formulate effective treatment strategies[7,8].

In the present study, 200 CLD patients were screened and 56 (28%) were tested positive for anti-HCV antibodies. The prevalence among chronic liver disease patients has been high when compared with general population (0.87%)[9]. This sero-revalence rate is comparable with the sero-prevalence of anti-HCV 18% by Kundu *et al.*[10] and 22.5% by Ayele *et al.*[11]. This implies that there has been no appreciable change in the sero-prevalence of HCV over the last 10-years period.

The majority of the CLD cases belong to age group 31-70 (76.5%). Sero-prevalence was highest among persons in the age group 31-50 years (30.6%) and a substantial prevalence till 70 years of age. This finding goes in correlation with study done by Sandhu and Dahiya[12] and Anbazhagan *et al.*[13]. This sero-prevalence pattern of high age distribution i.e., after 3rd decade of life indicates that the HCV transmission would have either occurred in their late childhood or during the adulthood period. The chronic HCV infection may lead to progression of liver diseases. Now, it may end up with cirrhosis, CLD and HCC over a period of 20-40 years.

Regarding gender of Hepatitis C positive patients, this study has shown that there were 33 (30.8%) males and 23 (24.7%) females showing predominance of male gender. Similar findings were reported by Atreyi Chakraborty *et al.*[14] study. It showed significantly higher prevalence in male (71.6%) patients in comparison with the females (28.4%). Kundu *et al.*[10] study showed 18.92% of males were anti HCV antibody positive and 15.39% of females were anti HCV Ab positive.

Singh *et al.*[15], Grover *et al.*[16] and Anbazhagan *et al*.[13] also have shown male predominance in their studies, which indicates a reasonably higher proportion in males than females in HCV infection could reflect more males coming for treatment in our setting. Besides it could be due to more social mobility in males than females and thus greater vulnerability to be infected.

The variation in the HCV RNA detection rate among sero-positive cases could be due to intermittent viremia or spontaneous resolution of infection. Out of 56 cases positive by ELISA, only 26 (46.42%) were confirmed by real time RT-PCR indicating the HCV infection is active. Sero-positivity among PCR negative patients may be due to cross reaction with non-specific antibody. Hence, all the anti-HCV positive results should be confirmed by testing for HCV RNA. Alternatively, true antibody-positive participants did not have positive RT-PCR results because they had intermittent viremia or spontaneously cleared HCV infection.

Analysis of the HCV genome has shown extreme variability in both structural and non-structural coding regions. This analysis has identified at least six different genotypes which are divided into several subtypes. HCV genotype is the strongest predictor of response to treatment. Different genotype will respond in a different way to treatment. The distribution of HCV genotypes varies according to geographical region. Genotypes 1,2 and 3 are widely distributed throughout the world but the other genotypes are common in particular geographic region. Study of HCV genotypes within a population is a useful for the study of the evolution of HCV infection in different regions.

The present study showed genotype 3 (57.6%) was the major genotype which is like studies by Chakravarti *et al.*[17] and Chistdas*et al.*[7]. The presumption of an asian origin of HCV genotype 3 depends on many subtypes of HCV genotype 3 that were isolated in this area. This suggests that genotype has been present in asian population for many centuries. Genotype 4 is found in 6 (23%) patients in our study. This goes like findings by the study of Raghuraman *et al.*[18]. Genotype 4 is mainly seen in Egypt and Middle East, now shows a rise in trend in South India.

Genotype other than 1,2,3,4 is seen in 2 (7.6%) in this study. Genotype 6 is being increasingly reported in India and appears to be somewhat geographically restricted in its distribution. Genotype 5 is confined to South Africa and Central parts of France and has not yet been reported from this region. Genotype 2 was not detected in our study.

As per Gower *et al.*[6] genotype 1 was the predominant genotype (46%) worldwide followed by genotype 3 (22%), genotype 2 (13%) and genotype 4 in 13%. Genotype 3 was common among older age group 60-70 years whereas genotype 4 was common among 20-50 years. Therefore, we can infer that infection due to genotype 3 in this region occurred much earlier in the past compared to other genotypes.

Third generation ELISA is a useful cost-effective screening test for serological diagnosis of HCV. It has many advantages like they are cost effective and easily available. RT-PCR is recommended in an addition to screening by ELISA to confirm active HCV infection as ELISA may produce false positive results due to non-specific binding antibodies. Hence all the Anti-HCV positive results should be confirmed by RT-PCR testing for HCV RNA.

CONCLUSION

HCV RT-PCR is a highly sensitive and specific method for detecting active infection. Detection of HCV RNA usually precedes the antibody reactivity in serum. It helps to rule out false negative and weakly positive ELISA with clinical signs and symptoms of HCV. The present study highlighted that HCV genotype 3 is the predominant genotype among chronic liver disease in our geographical area. It is expected that distribution of other genotype may be due migration of people, changes in high-risk behaviour and lifestyle. Knowledge of distribution of genotypes helps in predicting therapeutic response and the duration of treatment.

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