Journal of MedVerse Research & Practice
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Hepatitis C virus- seroprevalence, genotypic identification, and comparative assessment of diagnostic techniques in a tertiary care hospital in Kanchipuram.

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Submission Date: 19.05.2024	Accepted Date: 16.06.2024	Published Date: 30.06.2024
DOI: 10.65188/nurexus.1005		

Abstract

Introduction: Hepatitis C virus (HCV) infection constitutes a significant global public health concern. Given that numerous infected individuals remain asymptomatic, primary diagnostic modalities encompass Rapid Diagnostic Tests, Enzyme-Linked Immunosorbent Assay, Chemiluminescent Immunoassay, and real-time Polymerase Chain Reaction for HCV RNA detection. This investigation sought to evaluate HCV seroprevalence utilizing diverse techniques, including real-time PCR, CLIA, third-generation ELISA, and RDTs, in conjunction with identifying prevalent genotypes.

Materials and methods: The Virology Section of the Department of Microbiology at the Mahatma Gandhi Medical College and Research Institute, Pondicherry, India, conducted an observational cross-sectional study from September 2022 to November 2023, involving 700 samples from individuals suspected of HCV. After processing, samples were tested via RDTs, ELISA, CLIA, and real-time PCR according to established protocols, with PCR employed to identify genetic material in positive cases.

Results: Among the 700 samples, anti-HCV antibodies were detected in 9 (1.28%) via RDTs, 9 (1.28%) via ELISA, and 11 (1.5%) via CLIA, and HCV RNA was identified in 11 (1.5%) by RT-PCR, with genotype 3b constituting 55% of positive results, followed by 1a type at 36%.

Conclusion: Some samples initially deemed negative by other assays testedpositive by RT-PCR, reinforcing its status as the gold standard diagnostic method. The significance of genotyping in diagnosis, treatment planning, and monitoring is underscored by timely interventions capable of preventing complications.

Keywords: Hepatitis C, seroprevalence, genotype, diagnosis.

Introduction

The Hepatitis C virus (HCV) belongs to the Flaviviridae family and is characterized by its single-stranded RNA structure [1]. The HCV virus was first identified in the United States in 1989 and causes hepatitis in over three to four million people annually. According to estimates from the World Health Organization (WHO), approximately 3% of the global population, or around 210 million people, are chronically infected with HCV [2]. HCV transmission primarily occurs among intravenous drug users, hemodialysis patients, and individuals receiving contaminated blood products. In addition, nosocomial transmission plays a significant role in the spread of the virus. Each year, more than 350,000 deaths are

attributed to HCV infection, with 25% resulting from hepatocellular carcinoma and 27% from cirrhosis [3].

HCV remains a major global health concern due to its high chronicity rate and the absence of an effective vaccine. In India, WHO estimates suggest that 10–24 million individuals are actively infected with HCV, with a seroprevalence ranging from 0.09% to 2.02% among healthy populations [4]. The virus exhibits remarkable genetic heterogeneity, comprising multiple subtypes and at least six major genotypes, designated 1 through 6. Genotype 1a is distributed worldwide, while genotype 3 predominates in India [5].

With advancements in molecular diagnostics, the use of techniques such as real-time polymerase chain reaction (PCR), chemiluminescent immunoassay (CLIA), third-generation enzyme-linked immunosorbent assay (ELISA), and rapid diagnostic tests (RDTs) has become essential for detecting, quantifying, and characterizing HCV infections. The present study aims to determine the most prevalent HCV genotype and to estimate the seroprevalence of HCV within the target population using these advanced diagnostic methods.

Materials & Methods

The Microbiology Department at Mahatma Gandhi Medical College and Research Institute, located in Pondicherry, India, conducted an observational cross-sectional study from September 2022 to November 2023. Blood specimens were obtained from 700 patients who presented to the outpatient department for hepatitis evaluation within the research protocol.

Informed consent wasprocured from all participants following ethical committee approval. Individuals of all ages and sexes, including those diagnosed with chronic hepatitis, patients undergoing hemodialysis, and those with previous blood transfusions, needle stick injuries, or referrals from various departments, were deemed eligible for sample collection. Those who declined to participate in thestudy were excluded. Participants were thoroughly informed about the study procedures, and their demographic and medical information was documented in detail.

A comprehensive survey gathered data on various symptoms, including jaundice, decreased appetite, elevated body temperature, weight loss, and coexisting health conditions (such as diabetes, cancer, HIV, HBV, and HAV). The study also evaluated potential risk factors among the participants, including previous blood transfusions, kidney dialysis, accidental needle injuries, organ transplantation, surgical procedures, tattoos, alcohol consumption, intravenous drug use, and multiple sexual partners.

After obtaining informed consent and following sterile techniques, 5 mL of blood was collected and processed according to established protocols. The blood samples underwent analysisusing CLIA (Matrix Lab Pvt, Ltd.), ELISA (QUALPRO Pvt Ltd.), and RDTs (HCV Tridot, Diagnostic Enterprises, Pvt Ltd.). Samples that tested positive were further verified using RT-PCR(Helini Biomolecules Pvt. Ltd).

The study obtained approval from the Institutional Ethics Committee of Mahatma Gandhi Medical College and Research Institute, Pondicherry, India (Ref No: MGMCRI/IEC/2022/91837). A detailed Participant Information Sheet was provided to all participants, and written informed consent was obtained prior to their participation in the study.

Results

Table 1: Sero-positivity shown in different diagnostic methods and the sample used

Study subjects	No of the samples tested	RDT(%)	3rd generation ELISA (%)	CLIA(%)	PCR
Dialysis	469	5(55.5%)	5(55.5%)	6(54.5%)	6(54.5%)
Medicine	20	0	0	0	0
Surgery	101	2(22.3%)	2(22.3%)	2(18.2%)	2(18.2%)
Orthopedics	26	0	0	1(9.1%)	1(9.1%)
Obstetrics & gynecology	21	1(11.1)	1(11.1%)	1(9.1%)	1(9.1%)
Blood bank	63	1(11,1%)	1(11.1%)	1(9.1%)	1(9.1%)

All 700 samples included in this study underwent ELISA, CLIA, and RDT testing. Eleven (1.5%) samples tested positive for CLIA, and nine (1.2%) tested positive for RDT and ELISA. These 11 samples were used to detect the genes using HCV RNA RT-PCR. Six (54.5%) of the 11 seropositive cases came from the dialysis department, followed by the surgery, orthopedics, and obstetrics and gynecology departments.

Table 2: Age distribution of HCV-positive subjects (N=11)

Age group	Frequency (%)
30-40	4(36.4%)
40-50	3(27.2%)
50-60	3(27.2%)
>60	1(9.2%)

Table 2 : Shows that the maximum age group was between 30 and 40 years (36.4%). This was followed by 40-50 years (27.2%), 50-60 years (27.2%), and > 60 years of age (9.2%). None of the patients aged < 30 yearsshowed HCV seropositivity.

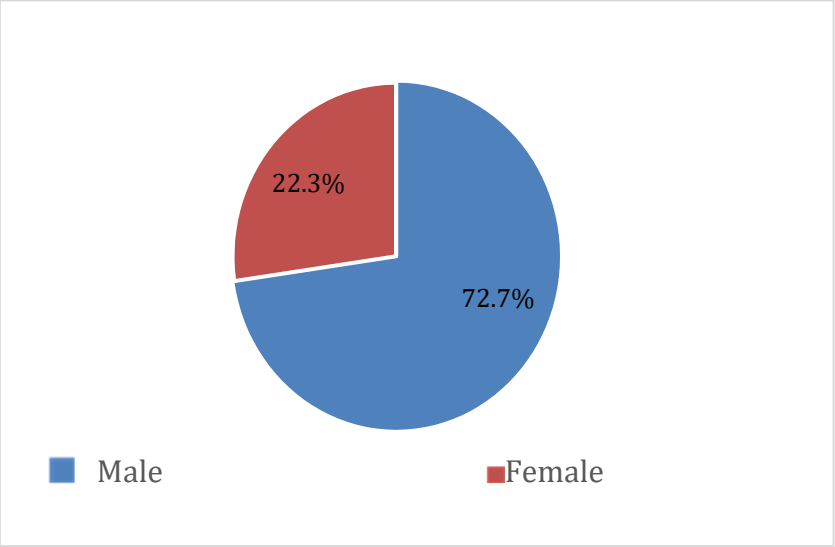

Figure 1: Gender distribution

The above pie chart shows that HCV was positive in more males, with a proportion of 72.7%, which is more than two-thirds of the cases that have shown seropositivity for HCV.

Table 3:Risk factors among HCV-positive study subjects (N=11)

Risk factors	Frequency (%)
Tattoo	4(36.4%)
Surgery	2(18.1%)
Blood transfusion	2(18.1%)
Dialysis	3(27.2%)
Injecting drug users	2(18.1%)
Sexual exposure	2(18.1%)

As indicated in Table 4, tattooing (36.4%) was the primary risk factor among HCV-positive subjects. This was followed by dialysis (27.2%).

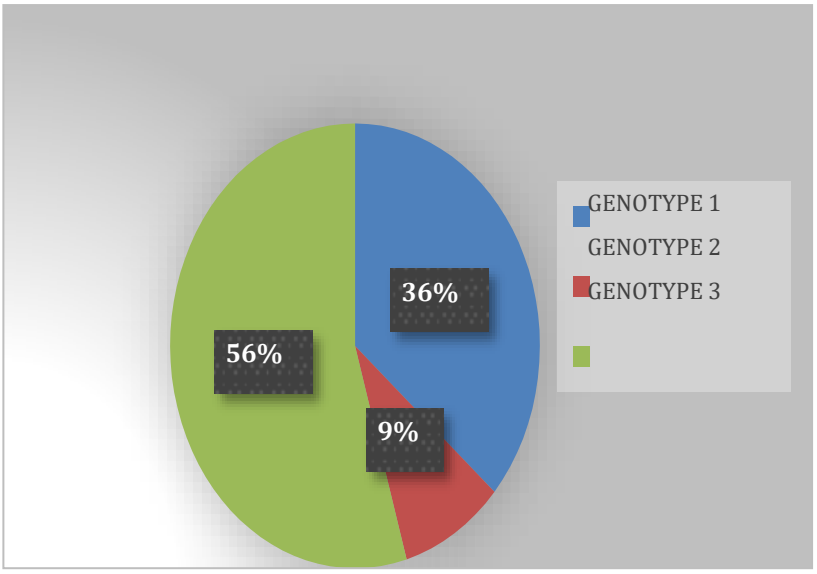

Fig -2: Distribution of different HCV genotypes among study subjects

RT-PCR was used to identify the genotypes of all the 11 positive cases. Fig -2 displays the

genotypes that were found to be most common in our study: 3b (56%) and 1a (33%).In this study,the prevalent genotype was 3b (55%), followed by 1a (36%), and 2 (9%).

Discussion

Studies conducted across India have demonstrated considerable regional variation in Hepatitis C Virus (HCV) seroprevalence, with reported rates of 0.22% in South India, 0.3% in Western India, 1.8% in Central India, and 1.9% in Northern India. In developing countries, unsafe therapeutic injections and blood transfusions are considered the major routes of HCV transmission, as reported by Rajkumar et al., Ramya et al., and Badge et al. [4–6]. In the present study, the observed HCV seroprevalence was 1.5%, which lies between the higher prevalence reported by Sivagamasundari et al. and the lower prevalence documented by Mathur et al. [7,8]. A higher prevalence was noted among males, which is consistent with findings reported by Verma et al., Rajasekaran et al., and Mahajan et al. [9,10,3]. However, this contrasts with observations by Roy et al., who reported a higher prevalence among females. The most affected age group in the present study was 30–40 years, whereas Roy et al. reported the highest prevalence in the 50–60-year age group [11].

During the study period, the majority of samples were obtained from patients attending the dialysis unit. Tattooing, which is a common cultural practice, emerged as a significant risk factor among the study population. In contrast, Rajkumar et al. identified injecting drug users as the predominant high-risk group [4]. Polymerase chain reaction (PCR) detected HCV RNA in eleven samples that were negative by rapid diagnostic tests (RDTs), supporting previous reports that PCR can detect HCV RNA during the early acute phase of infection, even before hepatic enzyme elevation occurs, as noted by Ramya et al. [5]. Additionally, PCR identified two positive cases among seronegative samples, which may be explained by the limited sensitivity of ELISA during the first three weeks of infection, potentially missing early acute HCV cases.

The widespread availability and ease of use of anti-HCV antibody tests in resource-limited settings such as India have led to the acceptance of RDTs as practical screening tools for HCV infection. However, molecular diagnostic techniques remain essential for confirmatory diagnosis and for monitoring treatment outcomes. These techniques were instrumental in the initial identification of HCV, as the virus could not be cultivated easily in laboratory settings, as described by Ramya et al. and Sivagamasundari et al. [6,7]. Nucleic acid testing (NAT), including CLIA-based assays, is considered the gold standard for detecting active HCV replication. NAT is particularly valuable in diagnosing acute HCV infection, as it can detect viral RNA as early as one week following exposure and several weeks before seroconversion, as reported by Mathur et al. [8].

Genotypic analysis in the present study revealed genotype 3b (56%) and genotype 1a (33%) as the predominant circulating genotypes. Similar genotype distribution patterns have been reported by Thanjavur et al. [1]. In contrast, Appalaraju et al. reported genotype 4 as the most prevalent genotype in South India, followed by genotype 3 [12]. Overall, genotype 3 remains the most common genotype in India, accounting for approximately 63.85% of cases, while genotype 1 accounts for 25.72% [12]. Given that the incidence of HCV infection is nearly four times higher than that of HIV infection, there is an urgent need for enhanced public awareness and preventive strategies. Although HCV genotype influences therapeutic decision-making, recent advances have led to the development of direct-acting antiviral agents targeting the NS5B region, which are effective against multiple genotypes, as reported by Appalaraju et al. and Preethi et al. [12,13]. While genotyping is not currently mandated in treatment guidelines, it remains crucial for epidemiological surveillance and

vaccine development, as emphasized by Gowri et al. and Anoop Kumar et al. [14,15].

Conclusion

Tests for detecting HCV infection using serology are simple, reliable, widely available, and cost-effective. However, these methods are not effective in identifying early-stage HCV infections. The most accurate technique for diagnosing HCV infection remains HCV RNA RT-PCR. Additionally, genotyping plays a vital role in managing HCV treatment and monitoring disease progression. Some research has identified genetic variations that may be attributed to population movement or travel. As a result, it is essential to implement rigorous infection control measures in both critical and non-critical settings.

Conflict of Interest: Nil

Reference

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