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Seroprevalence, Genotypic identification and Comparative evaluation of diagnostic methods for Hepatitis C Virus in our Tertiary Care Hospital, Kanchipuram.

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KEYWORDS

Seroprevalence, Genotypic identification

ABSTRACT:

Introduction: Hepatitis C virus (HCV) has posed a major public health problem globally. Since majority of HCV infected patients are asymptomatic, diagnosis of HCV infection is mainly based on the detection of anti-HCV antibodies by Rapid Diagnostic Tests (RDTs), Enzyme Linked Immunosorbent Assay (ELISA), Chemiluminescent immunoassay (CLIA) and HCV Ribonucleic Acid (RNA) by real time Polymerase Chain Reaction (PCR) of serum samples. The present study was done to know the seroprevalence of HCV by various methodologies such as RDTs, 3rd generation ELISA, CLIA and real time PCR and to analyze the prevalent genotype in our hospital.

Materials and methods: An observational cross-sectional study was carried out in the Virology Section, Department of Microbiology, Meenakshi Medical Collage Hospital and Research institute (MMCHRI), Kanchipuram, India, for a period of 12 months from November 2022 to October 2023. The study includes 700 samples from suspected patients of hepatitis. Sample were processed as per standard protocol and were subjected to Rapid, ELISA, CLIA and Real time PCR. Further the positive samples were subjected to gene detection by PCR.

Results: Out of 700 samples, anti-HCV antibodies was detected by RDTs is 7(1%) samples, ELISA is 7(1%) samples, CLIA is 9(1.28%) samples and HCV RNA by RT-PCR in 9(1.28%) samples. Out of 9 positive samples, the predominant genotype was identified as 3b type followed by 1a type.

Conclusion: This study aim to strengthen the proper screening for HCV infection to reduce the morbidity and mortality. RT PCR test revealed that few samples that were negative by other methods turned out to be positive in PCR. Real time PCR is considered as the gold standard test. It can be reiterated that genotyping is important for diagnosis, treatment planning along with follow up. Early diagnosis and initiation of treatment can prevent complications.

INTRODUCTION

Hepatitis C virus (HCV) is a spherical enveloped, positive-sense, and single-stranded RNA virus that belongs to genus Hepaciviridae, a member of family Flaviviridae. [(1)] In 1989, the HCV virus was first discovered in USA with more than three to four million people getting infected every year due to hepatitis. The World Health Organization has estimated that 3 percent of the 7 billion people worldwide are being chronically infected by hepatitis virus [(2)]. HCV is transmitted through contaminated blood which makes the intravenous drug users, hemodialysis (HD) patients, and recipients of blood products at higher risk. Moreover, it has been

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found that one of the most common modes of HCV transmission is nosocomial. HCV infection leads to 27% of cirrhosis, 25% of hepatocellular carcinoma, and causes more than 350,000 deaths each year [3]. The high rate of chronicity and lack of successful vaccine makes Hepatitis C virus a serious threat to public health. World Health Organization (WHO) has projected that 10-24 million people are living with active HCV infection in India and seroprevalence among healthy population ranged from 0.09-2.02% in India [4]. HCV is known to have marked genetic heterogenicity. Presently HCV can be classified into at least 6 major types (1, 2, 3, 4, 5, 6) and series of subtypes. Genotype 1a is common worldwide. In India the most prevalent genotype is 3[5].

MATERIALS AND METHODS

An observational cross-sectional study was conducted in the Department of Microbiology, Meenakshi Medical College Hospital and Research institute (MMCHRI), Kanchipuram from November 2022 to October 2023. Blood samples were collected from 700 patients who are attending OPDfor Hepatitis investigation at MMCHRI & RI, Kanchipuram. After ethical committee approval (MMCHRI IEC/PG/69/OCT/22), informed consent was obtained from the patients. Samples were collected from patients of all age group, both sex, patient with chronic hepatitis, hemodialysis, history of blood transfusion, needle stick injury, post transplantation, post surgery patients referred from various department.Those who are not willing to participate in the study were excluded. A detailed demographic data and clinical history was taken and patients were explained about the test procedures. A detailed questionnaire was given and asked for history of jaundice, loss of appetite, fever, weight loss, co-morbiditis (Diabetes, Carcinoma, HIV, HBV, HAV). Patients were assessed for the presence of risk factors such as history of blood transfusion, hemodialysis, needle stick injury, post transplantation, post surgery, tattooing, alcohol abuse, IV drug use, sexual promiscuity. After getting informed consent, under aseptic precautions, 5 ml of blood sample collected and processed as per protocol. Samples were subjected to RDTs (HCV Tridot, Diagnostic Enterprises, Pvt Ltd.) and ELISA (QUALPRO Pvt Ltd.), CLIA (Matrix lab Pvt, Ltd). Confirmation done for positive samples with RTPCR (Helini Biomolecules Pvt Ltd.).

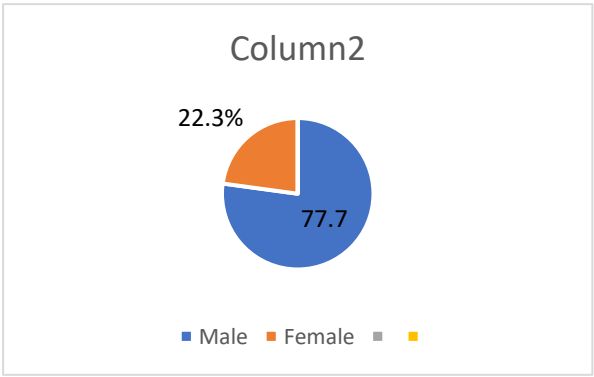
RESULTS

A total of 700 samples were subjected to RDT, CLIA, ELISA. Out of which 7 (1%) samples were positive for RDT, ELISA, and 9 (1.28%) samples were positive for CLIA. These 9 samples were subjected to HCV RNA RT-PCR for gene detection. Significantly more predominant was seen in males (77.7%) followed by females (22.3%) was shown in Table-1. The maximum age group was seen between 30-40 years are shown in Table-2. Of 9 seropositive cases, 4 (57%.1) were from dialysis department followed by surgery and obstetrics departments was described in Table -3. Tattooing was the major risk factor among the 9 samples followed by dialysis was defined in Table- 4. All the 9 positive cases were subjected to genotypic identification by RT-PCR. The prevalent genotype was identified in our study was 3b (56%) followed by 1a (33%) genotype was shown in Table- 5.

Table 1: SHOWS THE POSITIVE CASES OF HCV

TOTAL POSITIVE=9

MALE =7(77.7%) FEMALE =2(22.3%)



Male is more predominant than female for HCV infection

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Table 2: Age wise distribution among positive samples

AGE	MALE	FEMALE
<30	-	-
30-40	3(33.3%)	-
40-50	2(22.2%)	1(11.1%)
50-60	1(11.1%)	1(11.1%)
>60	1(11.1%)	-

Maximum number of positive cases was in the age group of 30 - 40

Table 3 : Seropositivity of the test done

STUDY POPULATION	TOTAL SAMPLE TESTED	RDT(%)	THIRD GENERATION ELISA(%)	CLIA(%)	PCR
DIALYSIS	470	4(57.1%)	4(57.1%)	5(55.5%)	5(55.5%)
MEDICINE	19				
SURGERY	100	1(14.2%)	1(14.2%)	1(11.1%)	1(11.1%)
ORTHOPEDICS	27			1(11.1%)	1(11.1%)
OBESTRICS AND GYNECOLOGY	22	1(14.2%)	1(14.2%)	1(11.1%)	1(11.1%)
BLOOD BANK	62	1(14.2%)	1(14.2%)	1(11.1%)	1(11.1%)

Maximum number of samples collected from dialysis department

Table 4 :SHOWS THE RISKFACTORS OF HCV

RISK FACTORS	N(%)
TATOO	3(33%)
SURGERY	1(11.1%)
BLOOD TRANSFUSION	1(11.1%)
DIALYSIS	2(22.2%)
INJECTING DRUG USERS	1(11.1%)

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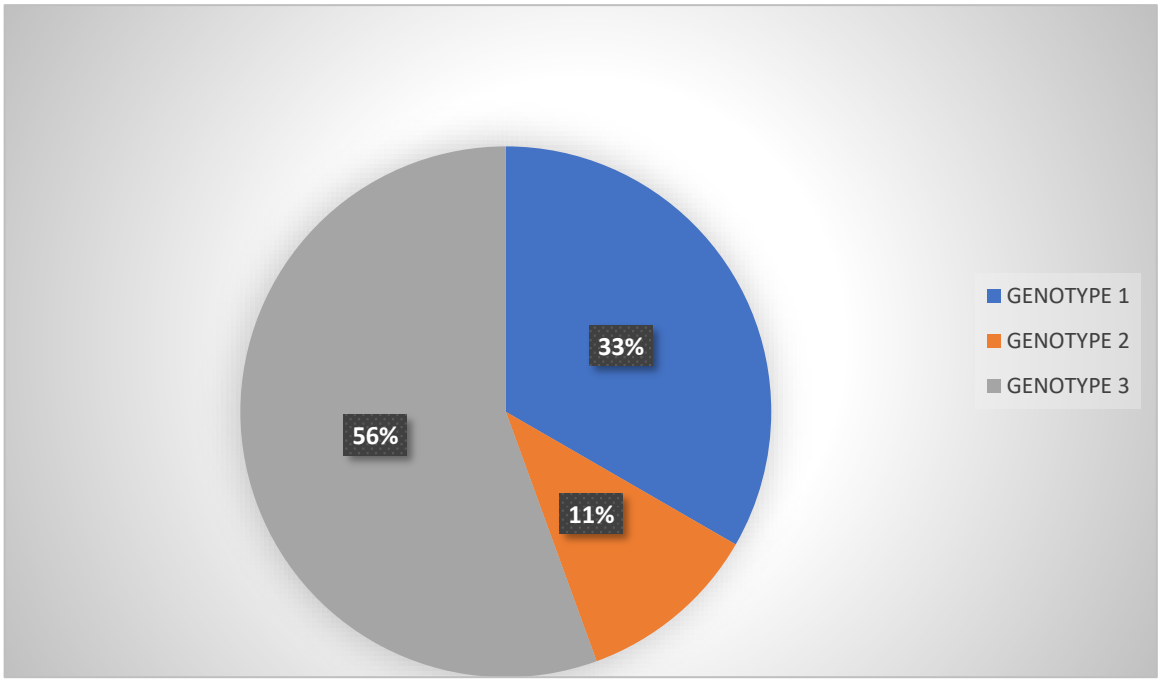
SEXUAL EXPOSURE	1(11.1%)

Tattooing was the major risk factor among 9 samples followed by dialysis

Table 5: PREVALENCE OF HCV GENOTYPES



In this study, prevalent genotype is 3 followed by 1.

Discussion :

HCV seroprevalence reported in South India is 0.22%, 0.3% in Western India, 1.8% in Central India and 1.9% in North India. In the developing world, unsafe therapeutic injections and transfusions are likely to be the major modes of transmission [5]. In our study the sero prevalence of hepatitis c virus is 1.28% which is slightly lower than sivagamasundari et al[6]., who have reported sero positivity of 2.6% and similar study conducted by AjayMathur et al[7]., reported 0.05% prevalence in his study and 0.22% by gowri et al[9]. In our study, Males were more preponderant than females, similar findings were reported by Verma et al[11],Rajasekaran et al[13] and Rajkumar et al [4].this finding was consistent with Col partha roy et al[10] in which female is more predominant. The maximum age group affected is 30-40 years, similar study is conducted by Partha Roy, Col, Retd,[a,] et al[10] and reported 50 – 60 years is mostly affected age group in his study. During this study period maximum number of samples collected from Dialysis department. Majority of the participants had a risk factor of tattooing since it is one of the traditional aspects.,this study was conflict with Rajkumar et al[3], injecting drug users are mostly affected.. PCR was able to detect 9 samples which were shown negative by RDTs. This might be due to the fact that PCR can determine minute amounts of HCV-RNA in serum or plasma and it will be positive in all acute infections with or without elevation of hepatic enzymes, as previously reported[4] . On the other hand, PCR picked up 2 positive samples from those seronegative samples. This is possible during early acute phase of HCV infection as ELISA can be best employed after first three weeks of infection. RDT method is consider one of the screening methods for HCV infection in detecting anti HCV antibodies due to faster, easy availability of antibodies and easy resource availability in developing countries like India. Molecular virological techniques play a key role in diagnosis and monitoring of treatment for HCV. Because it is difficult to cultivate the virus in cell culture, molecular techniques were instrumental in first identifying HCV, making it one

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of the first pathogens to be identified by purely molecular methods · CLIA (NAT) is considered the 'gold standard' for detecting active HCV replication [8] . HCV NAT is extremely useful in establishing the diagnosis of acute HCV infection, since RNA is detectable as early as 1 week after exposure via needle-stick or blood transfusion and at least 4-6 weeks prior to seroconversion as demonstrated in a number of transmission settings . Our results revealed that predominant genotype is 3b(56%) followed by 12(33%). Similar findings seen with Naveen at al[(1)]. A study conducted in south india by Appalaraju et al. showed Genotype 4 is the dominant type followed by genotype 3[(12).] In India, genotype 3 is the predominant genotype (~63.85%) followed by genotype 1 (25.72%) [(14)]. As the HCV infection is 4 times higher than the HIV infection rate, it is very essential to provide awareness programs to the public. The clinical use of antiviral drugs could be driven by the HCV genotype [1], but now there are a few days of single drugs specifically targeted to the non-structural NS5B region, so that almost all types of genotypes can be affectively inhibited by a single drug, so that the method of genotyping detection is not used in the therapeutic guidelines but necessary for successful vaccine development.

Conclusion: Serological assays were simple, reliable, easily available and cost effective for the detection of HCV Infection. But these will not be helpful for the early identification of HCV infection, whereas the HCV RNA RT-PCR is the gold standard method for the detection of HCV infection. Genotyping is also very important for treating the HCV infection & to know the progression of the disease. Genetic variation was observed in few studies, may be due to the migration or travel. Hence, strict infection control practices should be implemented in all critical & non-critical areas.

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