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TO STUDY THE SEROPREVALENCE OF HEPATITIS-C VIRUS INFECTION AMONG PATIENTS UNDERGOING SURGERY AT A TERTIARY CARE HOSPITAL IN KANPUR

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Abstract

Background: Hepatitis C virus (HCV) is among the major causes of acute and chronic hepatitis that leads to hepatic cirrhosis and carcinoma. According to global estimates, the prevalence rate of HCV in India is continuously increased in past decades and contributed around more than 11 million patients. Routine sensitive and specific methods are needed so that precise diagnosis can be made especially in patients undergoing surgical interventions. The present work compared the sensitivity and specificity of the rapid card test versus real time PCR (RT-PCR) for HCV confirmation in patients undergoing surgical procedure in several departments.

Aim: The aims of the present study are to compare the Sensitivity and Specificity of the Rapid card test versus RT-PCR in detection of HCV and also determine the sero-prevalence of HCV in the study region**.**

Methods: A cross-sectional study was conducted in the Department of Microbiology and Department of Medicine RMCH&RC UP from April 2023 to May 2024.All patients undergoing surgery were included in this study. Rapid HCV card test along with RT-PCR were performed to screen patients prior to surgery, and for each patient who tested positive using the rapid HCV, qualitative RT-PCR testing was done to confirm active viral load. Comparison of sensitivity and specificity were made between RAPID card test and RT-PCR.

Results: The sero-prevalence of HCV in this study was 2%, among 2400 patients screened for HCV infection by using Rapid card & RT-PCR tests. 45 samples were found positive with Rapid Card test. While 48 sample were found positive by RT-PCR. Males were more affected than females. Sensitivity and specificity observed for rapid card was 85.29% and 100%, and RT-PCR showed100% sensitivity and specificity.

Conclusion: The study results concluded that RT-PCR method is more sensitive and specific compared to rapid card test for the detection of HCV and should be performed in all positive cases to confirm and to know the viral load of HCV.

Keywords: Sero-prevalence, Hepatitis C Virus, Surgery, Rapid Card, RT-PCR.

INTRODUCTION

Hepatitis c virus is a spherical single stranded RNA virus belonging to flaviviridae family. It can cause both acute and chronic hepatitis. It is a major cause for liver cancer. Hepatitis c is a blood borne infection. Globally it is estimated that about 71 million people are suffering from chronic hepatitis C infection, belonging to a family named Hepadnaviridae.[1,2,3] it is capable of causing active and can cause severe infection. HCV can lead to life threatening liver diseases across the world. That is why hepatitis C viral infection, has become one among the other important global ill health problems. Near about 1.2 million people die due to chronic hepatitis per annum and also die due to cirrhosis and hepatocellular carcinoma [4]. And approximately 350 million remain as severely infected with hepatitis C virus worldwide [5, 6]. They continue to be under the threat of developing chronic hepatitis, cirrhosis and hepatocellular carcinoma[2,3].India reported over 40 million of hepatitis C virus carriers which is equivalent to 10- 15% of the total of HCV carriers in the world. Also India accounts for deaths of around 1.15 lakh people as a result of hepatitis C related complications. Spread of hepatitis C viral infection happens through parenteral, sexual also as vertical transmission. Burden of hepatitis C infection in India is unknown. It is estimated about 6 million people suffer from HCV in India. Other risk of hepatitis C infections are intravenous drug abusers, spouses of infected partner, homosexual men, health care personnel and frequent recipients of blood and blood products, hemodialysis, organ transplantation etc.[7] Around 50 million new cases occur annually as a result of perinatal transmission infection[8].Diagnosis of HCV infection is confirmed by demonstrating specific antibodies or antigen in serum of patients. And RT-PCR the foremost important laboratory test for diagnosis of HCV infection is HCV RNA which is the first antigen to appear [9]. During convalescence it falls to undetectable levels, if it persists for quite six months than it indicate the carrier phase and a hazard for chronic hepatitis and hepatocellular carcinoma.[10]The major consequence of HCV infection is hepatic fibrosis which may progress to life threatening cirrhosis and an increased risk of hepatocellular carcinoma. Anti HCV antibodies appear in 8–9 weeks after exposure and are detectable in more than 95% of chronic cases however in acute hepatitis antibodies are variably present detectable in 50–70% patients. Detection of HCV RNA remains the gold standard for the diagnosis of hepatitis C. Therefore patients showing presence of Anti HCV should be confirmed by nucleic acid amplification.[11]Contaminated surgical equipment,surgical disposables, needles, self-pricks during procedures andblood transfusion can be the causes of transmission of viruses from patient to patient and even to the doctors, paramedicalstaff and nurses[12]. Surgical procedures and dental treatment procedures in the health care setting are the important Transmission mode of hepatitis C viruses. [13] HBVand HCV co infection has been reported in high risk patients screened before surgery.Hence this study is undertaken to find out, the sero-prevalence of hepatitis-c virus infection among patients undergoing surgery at a tertiary care hospital in Kanpur.

MATERIAL AND METHODS: A cross-sectional study was conducted in the Department of Microbiology and Medicine RMCH&RC UP from April 2023 to March 2024.All patients undergoing surgery were included in this study. Rapid HCV card test along with RT-PCR were performed to screen patients prior to surgery, and for each patient who tested positive using the rapid HCV, qualitative RT-PCR testing was used to confirm active viral load. Comparison of sensitivity and specificity were made between RAPID card test and RT-PCR.

Sample collection: After informed consent of patients, 2-3ml of peripheral venous blood samples were collected in Plane and EDTA vial from the patients.Blood samples (3 mL) from patients were taken via vein puncture and transferred to EDTA tubes. After that, the collected blood samples with tubes were packaged in a cool container and the processed then transferred to the Department of Microbiology for further processing. After centrifugation (3000 RPM for 5 minutes), the serum was collected from the blood samples and stored at -80 °C for further testing. Furthermore, prior to collecting blood samples, we conducted a case study on a patient using the following methods: After screening a total of 2400 patients and data from records based on treatment duration and samples were collected for this research study. The following information was collected before the blood sample collection.

Sample processing: Plane vial sample and EDTA vial samples were centrifuged first then Serum from plane vial was processed for Rapid detection of HCV infection by Rapid card (SD Biosensor) and positive samples were repeated by HCV Tri-dot for confirmation and Plasma from EDTA vial were processed for molecular detection of HCV infection by RT-PCR.

Test procedure:

HCV from rapid card test: [SD-BIOSENSOR]

Lateral flow Immuno-chromatographic assay used for HCV testing, Qualitatively it gives result after adding 10 µl serum and 3 drops workingbuffer of HCV.

Positive result- after 15-20 min double band appears (One is test line band second is control band)

Negative result- single control band appear only.

Invalid – if control band not appeared.

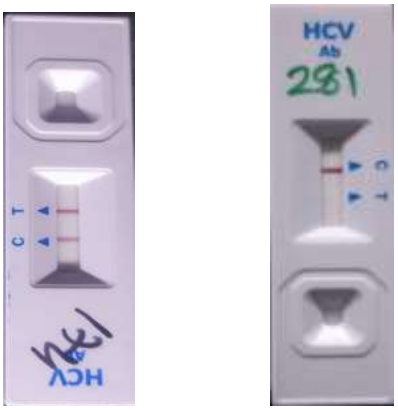

Figure 1: Rapid Card (Standard Q HCV Ab Kit) shows reactive and non -reactive results.

HCV Tri-dot (For confirmation of Positive results by SD card)

Test Procedure

Step No. 1

Add 3 drops of Buffer Solution to the center of the device.

Step No. 2

Hold the dropper vertically downwards and add 1 drop of patient's sample (50 µl serum or plasma) using the

Sample dropper provided. (Use a separate sample dropper for each specimen to be tested).

Step No. 3

Add 5 drops of Buffer Solution.

Step No. 4

Add 2drops of Protein- A Conjugate.

Step No. 5

Add 5drops of Buffer Solution.

Step No. 6

Read result immediately and discard the device considering it to be potentially infectious.

13. Interpretation of results:

Non-reactive result:

1. Appearance of only one dot at the control region"C" indicates that the sample is non-reactive for antibodies to HCV.

Reactive result:

1. Appearance of two dots, one at the control region"C" & other at the test region "T1" indicates that the sample is reactive for antibodies to HCV.

2. Appearance of two dots, one at the control region"C" & other at the test region "T2" indicates that the sample is REACTIVE for antibodies to HCV.

3. Appearance of all the three dots, one each at "C" "T1"& "T2" region indicates that the specimen is reactive for antibodies to HCV.

Invalid result:

If no dot appears after the completion of test, either with clear background or with complete pinkish/purplish background the test indicates error.

This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. The specimen should be retested on a fresh device (Refer sample / specimen processing).

Figure 2:HCV Tri-Dot shows reactive and non -reactive results

HCV RNA by RT-PCR: [TRU PCR KIT]

HCV RNA is extracted from Plasma using TRU-PCR RNA extraction kit, RT-PCR was performed in central molecular lab in Department of Microbiology by using BIO-RAD C1000 Touch Thermal cycler Cfx-96^{TM}Real time System with TRU-PCR kit**.**

Figure 3: RT-PCR testing BIO-RAD (C1000 Touch Thermal cycler)Cfx-96^{TM} Real time System to confirm active viral**.**

1. Test Procedure:

The TRUPCR HCV Viral Load kit is designed for the quantitation of hepatitis C viral RNA in human serum or plasma EDTA from HCV infection individual specimens using Real Time PCR System. Endogenous control gene is included in the kit to monitor the quality of extracted RNA from clinical samples. The result from the TRUPCR HCV Viral Load Kit must be interpreted within the context of all relevant clinical and laboratory finding.

2. Reagent included in the kit:

Reagent	Description	Volume 24 reactions	Volume 48reactions	Volume 96reactions
Master Mix	PCR buffer including Taq polymerase	240 µL×1	240 µL×2	240 µL×4
Enzyme Mix	Enzyme Mix for RT	24 µL×1	24 µL×2	24 µL×4
HCV Primer Probe Mix	Primer probe mix for HCV detection and Endogenous internal control detection	96 µL×1	96 µL×2	96 µL×4
HCV Standard	HCV STD 1(6×10^4IU/µL) HCV STD 2(6×10^3IU/µL) HCV STD 3(6×10^2IU/µL) HCV STD 4(60IU/µL)	20 µL×1 each	50 µL×1 each	50 µL×2 each
Negative Control	Sterilized water	1000 µL×1 each	1000 µL×2 each	1000 µL×4 each

3. Real Time PCR Protocol:

1. Prepare the PCR Mix as follows
2. Transfer 15µL of above prepared Reaction mix in 0.2 ml PCR Tubes and closethe tubes.
3. For 15µL of above reaction mix, add up to 10 µL of RNA or standard or negative control and make up the final volume 25 µL.

Name of the Reagent	For 1rxn
Maxter Mix	10.0µl
HCV Prime Probe mix	4.0µl
Enzyme Mix	1.0µl
Total reaction volume	15µl

4. Program setup:

Define the following setting for temperature Profile and Dye Acquisition.

Step	Temperature°C	Time	Dye Acquisition	Cycles
1	55	10 min	-	1
2	94	10 min	-	1
3	94	15 sec	-	40
	56	45 sec	Yes	
	72	15 sec	-	

5. Channel selection:

Detection	Detector name	Reporter	Quencher	Gain setup
HCV	HCV	FAM/Green	None	Auto
Endogenous IC	IC	HEX/Yellow/VIC	None	Auto

6. Result Analysis:

Qualitative Result analysis: TRUPCR HCV Viral load kit can be used for quantitative detection of HCV RNA by using standard 2 as positive control with expected Ct values 22±2.

Sample type	**Case**	**Amplification Signal in**		**Interpretation**
Control		**FAM**	**HEX**	
	Standard 2	Present (22±2)	Present(25±3)	Standard is working properly
	NTC	Absent	Absent	NTC is working properly
Sample	1	Present	Present/ Absent	HCV specific RNA detected
	2	Absent	Present	HCV specific RNA not detected. sample does not contain detectable amount of HCV specific RNA
	3	Absent	Absent	PCR inhibition ,retest the sample from extraction

Detection of the internal control is not required for positive results in the FAM. high HCV load in the sample can lead to reduced or absence of internal control signal.

Cut-off-This assay runs for 40 cycles, however no amplification beyond 38 cycles should be considered for any interpretation, hence cut off is 38 Ct.

Quantitative Result analysis:

The standard curve is plotted using the data obtained from the defined standards with the Ct Threshold Cycle and Log Starting Quantity.

Interpret the values for unknown samples only if the slope of standards is between 3.1 to 3.9 and PCR efficiency is between 90%-110% (0.9-1.1)and there should be no amplification in negative control.

Inclusion criteria:

Patient screened for Hepatitis-C infection among pre-operative patients of all age groups.

Exclusion criteria:

- Patients already diagnosed and are on Treatment of HCV infection
- Samples received without clinical history.
- Patients not giving informed consent.

Statistical analysis:

Data recorded on the case report form and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

RESULT

The sero-prevalence of HCV in this study was 2%, among 2400 patients screened for HCV infection by using Rapid card & RT-PCR tests. 45 samples were found positive with Rapid Card test. While 48 sample were found positive by RT-PCR. Males were more affected than females. In our study it was found that 48% study population were females and 52% were males. Also among the study participants, 28% belongs to urban areas while 72 % hailed from rural.

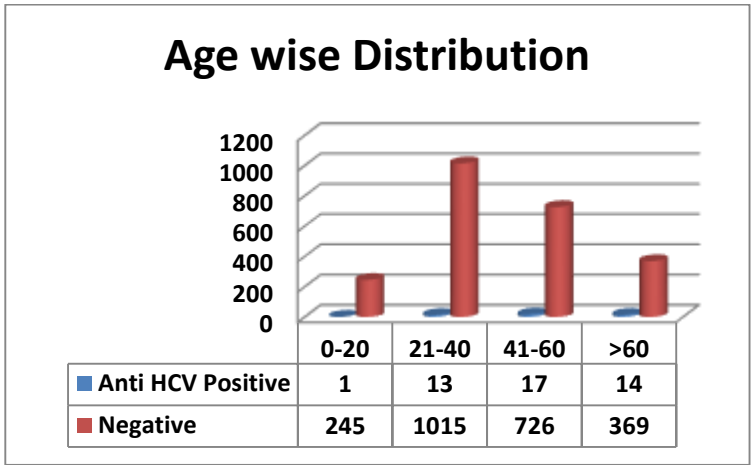

Figure 4: Age wise Distribution of Hepatitis C Virus patients

Age wise distribution of study participants were shown in figure 4 and observed that maximum positive HCV participants were in the age group of 41-60 years followed by more than 60 years and 21-40 years. The least number of HCV participants belongs to the age range of 0-20 years.

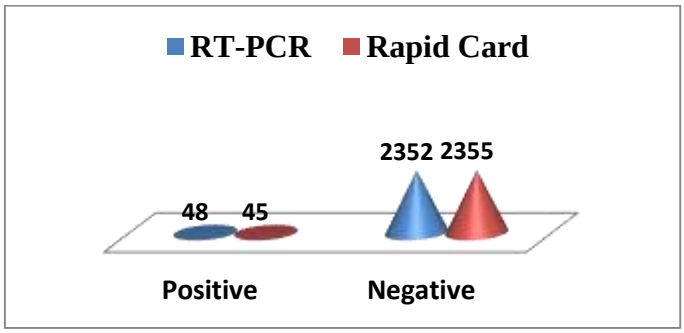

Figure 5: Shows comparisons of RT-PCR Test V/S Rapid Card Test.

In our study, the positive HCV patients were recorded highest with the RTPCR, compared to Rapid card test as shown in figure 5.

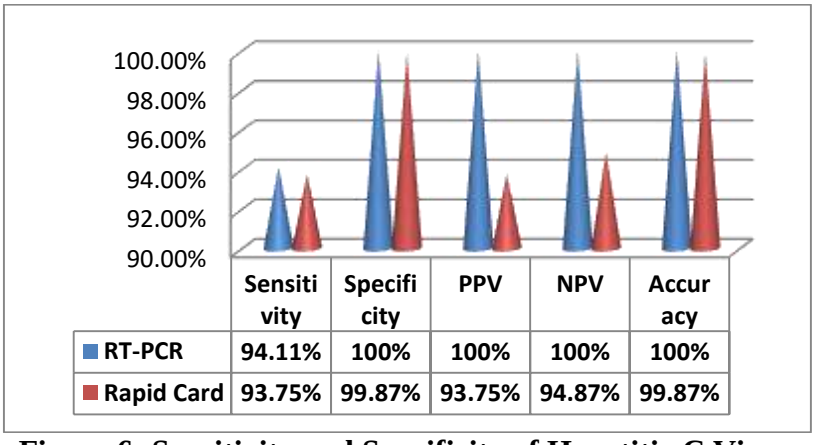

Figure 6: Sensitivity and Specificity of Hepatitis-C Virus.

Hepatitis C virus infection confirmation in study participants were shown in figure 6 and found that RTPCR method showed sensitivity of 94.11% compared to rapid card illustrating sensitivity of 93.75%. Specificity of RTPCR was highest compared to rapid card (100 % v/s 99.87%).

DISCUSSION

Blood samples possess utility for screening of HCV infection especially in resources deprived areas or in areas where population finds difficulty to reach healthcare settings.

In the present study the prevalence rate of HCV was 2%. In another study was conducted by Ibrahim AM et al. [12] in which the prevalence of HCV was 0.4% .The reason for low prevalence of HCV infections in surgical patients could be due screening for hepatitis increase prevalence rate of HCV in our region.

In our study, among the recruited participants, the 1248 (52%) were male and 1152 (48%) were female participant. Similar pattern was observed in previously published study in which the authors found that among 3254 samples collected, 3034 (93.24%) were males and the remaining 220 (6.76%) were females [16].

In the present study patient of all age groups were included but 41-60 year age group was found to be more affected. Similar pattern was observed in previously published study in which the authors Agrawal S et al. In their study, conducted at Manipur observed maximum hepatitis C cases in 41-50 year age group [14]

In our study, high prevalence of HCV was found in rural population compared to urban population. There was more number of participants from rural population, compared to urban population. Our results are in complete agreement with previously published study in which authors showed HCV prevalence was higher among rural residents (4.7% [3.8%, 5.7%]) than urban residents (1.6% [1.1%, 2.2%])[17]. In our study, RTPCR showed 100% specificity and 94.11% sensitivity in screening of HCV compared to the rapid card tests which have credential of 99.87% specificity and 93.75% sensitivity. Our results are in range of previously published study in which authors have performed screening of HCV using anti-HCV antibodies and found sensitivity (92.6%) and NPV (79.5%)[17].

The authors of previously published study also showed results in similar pattern they found sensitivity of 97.55% and specificity of 96.71% with PPV of 79.03% by HCV-RDT [16]. Similar results have been shown by Maity S et al., (sensitivity of 95.5% and specificity of 100%, J Mitra and Co, PVT Ltd), Mane A et al., (sensitivity of 99.4% and specificity of 99.7%, SD Bioline)and Chevalier S et al., (sensitivity of 97% and specificity of 100%, SD Bio line) [24-26].

Limitations of the study-The major limitations associated with RT-PCR installation at primary health care facilities include high cost of operation and non availability of skilled persons to handle such sophisticated technique.

Conclusion:: The study results concluded that RT-PCR method is more sensitive and specific compared to rapid card test for the detection of HCV and should be performed in all positive cases to confirm and to know the viral load of HCV. RT-PCR possesses better sensitivity and specificity compared to Rapid card test for screening of viral disease. Government should focus on fabrication of such sophisticated at health centers.

Ethical clearance:

The ethical committee clearance certificate was taken before starting of study by institutional medical ethical committee.

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