



ORIGINAL RESEARCH PAPER

Microbiology

COMPARISON OF FOURTH GENERATION ELISA AND RAPID DIAGNOSTIC TEST FOR DIAGNOSIS OF HEPATITIS C VIRUS(HCV) INFECTION IN A TERTIARY CARE HOSPITAL.

KEY WORDS: Fourth Generation ELISA, Rapid Diagnostic Test, sensitivity and specificity

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ABSTRACT

Introduction: Hepatitis C virus (HCV) infection has emerged as one of the major global health challenge affecting about 2 - 3% of the world population. Epidemiological studies have shown that HCV infection is a major risk factor for development of Acute hepatitis, chronic liver disease, cirrhosis and Hepatocellular carcinoma (HCC). Early diagnosis of HCV is important to link hepatitis testing to care and treatment initiation. **Aim:** To compare sensitivity and specificity of rapid diagnostic test (RDT) with fourth generation ELISA **Material and Method:** This study was conducted in the Department of Microbiology at Atal Bihari Vajpayee Institute of Medical Sciences (formerly Post Graduate Institute of Medical Education and Research) and Dr Ram Manohar Lohia Hospital from January 2018 to December 2018. Blood samples of patients suspected with hepatitis were tested using ELISA and rapid diagnostic test **Results:** In our study 26378 blood samples were tested for HCV, using fourth generation ELISA. Of these, 581 (2.20%) samples were found to be positive by ELISA. These HCV positive samples along with equal number of ELISA negative samples were tested by rapid diagnostic test. Sensitivity and specificity of the rapid diagnostic test was found to be 72.98 % and 100% respectively. **Discussion:** Rapid diagnostic test can be used during emergency hours but their results must be followed by ELISA test results in a tertiary care hospital. Reporting of false negative results should be minimized for rapid linkage to treatment initiation and to avoid silent transmission of infection.

Hepatitis C virus (HCV) infection has emerged as one of the major global health challenge affecting about 2 - 3% of the world population and is responsible for around 700,000 deaths per year globally¹. The overall prevalence of HCV in India is estimated to be 1-1.9% and due to large population size this is a public health concern^{2,3}. In India, HCV is responsible for acute viral hepatitis in up to 1.16-10.6%⁴. While chronic hepatitis due to HCV infection accounts for upto 10.8% to 48.5%⁵. Epidemiological studies have shown that the persistence of HCV infection is a major risk factor for development of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). According to a study by Gupta et al, HCV was found to be positive in 27% of cirrhosis and 25% of patients with HCC².

HCV is a RNA virus and its genetic diversity is constantly evolving due to rapid globalization. On the basis of genomic variability, HCV is classified into six genotypes and many subtypes². HCV is generally transmitted through transfusion of infected blood and blood products, unprotected high-risk sexual activity, unsafe injection practices, organ transplantation from infected donor and mother to fetus. After transmission, the virus may remain in latent phase due to suppression by host immune system. However, during its infectious phase, the replication of HCV is very fast, and around 10¹² viral particles can be produced per day^{2,6}. In a population, infected with HCV, it has been observed that only 10-15% individuals are symptomatic. Spontaneous clearance is seen in 25-50% of symptomatic and 10-15% of asymptomatic individuals. 10-20% of chronically infected individual's progress to cirrhosis over a period of 20 years which may further lead to decompensated cirrhosis and HCC later in life in some individuals².

The characteristics of persistent HCV infection and development of the chronic infection depends on various factors such as age, immune response, degree of inflammation, HIV/HBV co-infection or lifestyle habits such as consumption of alcohol².

Laboratory plays an important role in screening of HCV infection as HCV reactive persons can be linked to care and early treatment initiation.

HCV can be detected either by molecular or serological assays^{2,6}. As per WHO, serological testing strategy, in adults and children older than 18 months of age, a single serological assay can be used for serological evidence of past or present infection and nucleic acid testing is recommended for evidence of viraemia. Serological assay can either be based on detection of antibody or antibody/antigen using either laboratory based immunoassay (Enzyme linked immunosorbent assay-ELISA) or rapid diagnostic test (RDT) which meets the minimum safety, quality and performance standards⁷. Serological assays used for the detection of the HCV infection have evolved to minimize the number of false positives with improvement in their specificity. Successive generations of the HCV ELISA have not only improved the sensitivity and specificity but have also reduced the time for the detection of infection. Fourth-generation assays are also known as 'antigen-antibody combo' tests because the principle of these assays is based on the detection of both anti HCV antibody and HCV antigen at the same time. These assays are highly sensitive and are responsible for high reduction in detection time. The average detection period is 26.8 days for these assays^{2,6}. RDT are based on the principle of detection of antibodies and are configured to generate results within half an hour so that they can be used for point-of-care

testing and are easy to perform. RDT assays do not require sophisticated instrument and highly trained staff. The main disadvantage with RDT is long detection period and their inability to discriminate between active and non-active infection. RDT gives positive results due to the presence of anti-HCV IgG even after the clearance of viraemia by immune system². ELISA would be preferred in area where access to laboratory is easy while RDT would be preferred in remote and periphery areas where access to laboratory is difficult⁷. The molecular assays not only help in the early diagnosis but also confirm the viremia. The diagnosis and quantification of HCV RNA with highly sensitive and specific method plays a crucial role in the management of HCV infection^{2,6}. However, limitations of molecular assay are that they test negative during resolution of HCV infection and during low viremia in acute HCV infection, where ELISA gives positive results⁸.

In our hospital, which is a tertiary care hospital, HCV diagnosis is carried out by serological assays (4th generation ELISA and RDT) and facility for viral load is also available. As per WHO recommendation, single assay is recommended for diagnosis of chronic HCV infection in adults and children older than 18 months age⁷. The ELISA is routinely being performed for screening of HCV and RDT is being used only during emergency hours. This study was conducted with the aim to compare the sensitivity and specificity of the rapid diagnostic test with ELISA.

Material and methods

This study was conducted in the Department of Microbiology at Atal Bihari Vajpayee Institute of Medical Sciences (formerly Post Graduate Institute of Medical Education and Research) and Dr Ram Manohar Lohia Hospital from January 2018 to December 2019. Blood samples of patients suspected with hepatitis were received from various departments of our hospital in a plain vial for testing for HCV. Serum was separated and tested for HCV infection using fourth generation ELISA which was considered as the confirmatory serological assay. Samples tested positive by ELISA were also tested by RDT. Equal number of ELISA negative samples were also randomly selected and tested by RDT. Results were analyzed. All the results were entered in Microsoft excel sheet and correlation tables were made. The sensitivity and specificity was calculated by 2*2 tables⁹ taking fourth generation ELISA as the gold standard against RDT.

Results

Out of 47297 blood samples tested for HCV, using fourth generation ELISA, 1038(2.19%) samples were found to be positive. Of these 1038 (2.19%) ELISA positive samples, 249(23.98%) samples were found to be negative with RDT and 789(76.01%) samples were found to be positive with RDT. Five hundred and eighty one (1038) randomly selected ELISA negative samples were tested by RDT, all were found to be negative. Thus, none of the randomly selected ELISA negative samples (n=1038), tested positive with RDT. Using fourth generation ELISA as serological confirmatory method, sensitivity of the rapid card test was found to be 76.01 % and specificity 100% (Table1). Positive predictive value and negative predictive value was found to be 100% and 80.65% respectively.

Table 1: Evaluation of ELISA and RDT in diagnosis of HCV

Rapid Diagnostic Test	ELISA Positive	ELISA Negative	Total
Positive	789	0	789
Negative	249	1038	1287
Total	1038	1038	2076

Discussion

In our study fourth generation ELISA was taken as confirmatory serological assay for detection of HCV and the results of RDT were compared with it. In our study, HCV positivity was found to be 2.19% which is slightly more than

the reported prevalence in India (1-1.9%)^{2,3}. Prevalence rate in India varies from region to region, according to a study by Sood et al prevalence in Punjab is 3.6%¹⁰ while Goel et al¹¹ reported pooled seroprevalence of 0.44% to 0.88%. Higher positivity rate in our hospital could be due to the fact that it is a tertiary care hospital with advanced facilities and patients from neighboring states are referred for confirmation of diagnosis and treatment.

HCV screening using RDT showed sensitivity of 76.01% and specificity of 100%. A study done by Farooqui et al found sensitivity to be 70.58% and specificity to be 93.61%¹². Our results are also in concordance with the previous study by Raj et al. who reported, sensitivity of 79% by RDT method¹³. However, Khan et al. reported the sensitivity of RDT to be only 50%¹⁴. In contrast to our study, Sato et al¹⁵ and Lin et al¹⁶ demonstrated an overall sensitivity of RDT to be 100%. Another study done by Zahoorullah et al. also showed 100% sensitivity and specificity of 99.2% with RDT¹⁷.

According to WHO report, based on five studies sensitivity of individual RDT ranged from 83% to 100% while specificity ranged from 99% to 100%. There is variable performance standard across different brands and within the same brand of RDT⁷. Thus RDT must be used with caution and it is also important to validate these rapid assays by testing them in a given population to assess the effectiveness of these assays in detecting all the genotypes and subtypes of HCV circulating in the region before using these tests routinely in diagnostic laboratories. Most of these rapid assays use recombinant proteins from the prototype virus alone. In such cases RDT that does not cover a particular subtype will not detect the type when testing. Another reason for false negative results can be due to inadequate coating of the antigen, different nature of antigen used and genetic heterogeneity of the virus prevalent in that area^{10,12}. Fourth generation ELISA detects antigens along with antibody thus increasing the sensitivity². These may be the reasons behind samples being reactive using the ELISA and non-reactive by RDT. Ideally RDT should have a high degree of sensitivity and a reasonable specificity to minimize false positive and false negative results. However false positive results are preferable to false negative results during screening population groups, as positive test results trigger repeat testing with alternative method for confirmation of diagnosis. Whereas with false negative results there is likelihood of missing the diagnosis^{18,19}.

Conclusion

In conclusion we report RDT are less sensitive (72.98%) than fourth generation ELISA. RDT should be recommended only in resource constraint or peripheral health facilities. In a tertiary care hospital RDT should be used during emergency hours; however their results should be followed by ELISA test results. It is important to diagnose HCV infection at the earliest as it has to be linked to care and early initiation of treatment. Timely initiation of treatment also decreases chances of chronicity and development of complications. As HCV is a highly dangerous infection for community; false negative results leave a threat of silent transmission and spread of disease.

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