

Original article:

Comparison of two rapid immunoassays for screening of hcv infection in dialysis patients

¹Swapna S, ²Anant KS, ³Hema Prakash Kumari P, ⁴Vijaya lakshmi P

¹Assistant Professor, Dept. of Microbiology, Gitam institute of medical sciences and research

²Associate professor, Dept. of General surgery, Gitam institute of medical sciences and research

³Professor, Dept. of Microbiology, Gitam institute of medical sciences and research

⁴Tutor, Dept. of Microbiology, Gitam institute of medical sciences and research

Gitam University, Visakhapatnam-530045, India

Corresponding author: Dr K.S Anant , Associate Professor, Department of Surgery, GITAM Inst Medical Sciences & Research , Visakhapatnam – 530045(AP)

ABSTRACT

Introduction: In case of diagnosis of infectious disease, dissonant results may have serious outcomes among the patients as it causes un-necessary mental stress and tension. For appropriate diagnosis of infection as well as disease management and prevention, detection of appropriate test kit is necessary.

Method: ELISA was used as gold standard for comparative evaluation. Ninety two samples were collected and processed using Elisa and two rapid immune assay test kits (HCV Tridot, FLAVI check HCV).

Results: From the results, it was revealed that, out of 92 serum samples collected, male preponderance (69.5%) was seen. Taking HCV Microlisa as a standard reference test, out of 92 samples, 35.8% sero-positivity was observed. In the present study, HCV Tridot sensitivity was 96.9% and FLAVI check sensitivity was 93.9%.

Conclusion: Rapid assays must be used with care and it is also significant to validate these rapid assays by testing them in a given population to evaluate the effectiveness of these assays. Atleast two rapid assays should be carried out for detecting HCV antibodies in dialysis patients as preliminary tests and should further confirmed by gold standard assay method like ELISA for accuracy.

Key words: HCV, Rapid immune assays, ELISA, Dialysis patients

INTRODUCTION:

The prevalence of HCV among the Hemodialysis patients (HD) varies in different countries world-wide from 1% to 85% respectively. The prevalence of HCV is particularly high in developing countries like India and is a major cause of increased morbidity and mortality in patients with end stage renal disease (Wasley and Alter, 2000; Alter, 1997). The hepatitis C virus (HCV) is a prevalent infectious disease generally contracted via HCV infected blood and blood products. HBV and HCV infections are known to cause chronic silent infections which end up in hepatocellular carcinomas, hence accurate detection of the viral marker is essential for controlling the transmission of the virus. In many developing countries, ICA based rapid diagnostic tests are widely used to detect HBsAg and

anti-HCV antibody for both diagnosis and screening of acute and chronic infections, although ideally, screening should be done using more advanced and accurate methods such as EIA, PCR or ELISA (Kayser, 2005). Negative samples from patients referred for screening assays (rapid assays) are seldom re-tested, considering the costs of retesting in resource poor settings. Hence, choosing a test with high sensitivity and NPV is more important than choosing a test with high specificity and PPV for routine use. Although rapid tests are widely used in India, studies on accuracy indices of ICAs in the country are scarce. It is not safe to depend on the studies that have been performed in other countries because of genetic diversities in HBV and HCV can result in differences in accuracy indices (Ansari et al., 2007; Clement, 2002; Gul, 2009). For this reason, it is necessary to validate detection methods prior to allowing their use in diagnostic laboratories. Hence, the current study was planned to compare ELISA and rapid ICA based tests that have been used widely in India for detection of Anti HCV antibody. Due to their easy use and cheaper cost, the rapid tests are being used practically at all primary and most secondary health care facilities in India. Different methods are used for the diagnosis of hepatitis including ICT, ELISA, EIA and PCR. ELISA, EIA and PCR methods are expensive and are used in well equipped labs and major tertiary care hospitals (Forman et al., 2011). Rapid diagnostic ICT kits are a good choice as they are less expensive and do not need high tech manpower or infrastructure. The present study was designed to check the sensitivity and specificity of at least two different rapid kits for Anti HCV and to compare with standard reference test like ELISA. The ultimate goal of this study was to recommend most reliable and cost-effective rapid kits for the diagnosis HCV in areas where advance diagnostic facilities are not available.

MATERIALS AND METHODS:

A total of 92 blood samples from the patients with END stage Renal Disease (ESRD) who were undergoing dialysis in Dialysis Unit of GITAM institute of Medical Sciences and Research from January 2016-December 2016 were included in the study.

Blood sample collection

Blood samples approximately 3 ml were collected from each patient under aseptic precautions into plain sample collection tubes and allowed to stand for 20 minutes at room temperature for clot formation. The serum was separated by centrifuging at 1600 rpm for 10 minutes and stored in separate vials at -20C. Serum samples were tested using 4th generation HCV Tridot and FLAVICHECK HCV kits and confirmed using HCV MICROLISA ELISA Test (Table 1). All the serum samples and the test kits were allowed to come to room temperature and the test were performed according to the manufacturer's instruction.

Table 1: Commercial Kits, Details of Manufacturer and the principles of the test

Name of the Kit	Manufacturing Company	Principles of the Test
HCV TRIDOT	J. Mitra PVT LTD, New Delhi, India	Cassette ELISA
Flavichck HCV	QUALPRO DIAGNOSTICS, GOA, India	Immunochromatographic test (ICT)
HCV Microlisa	J. MITRA PVT LTD, NEW Delhi, India	Enzyme Linked Immuno Sorbant Assay (ELISA)

HCV TRIDOT:

The 4th generation HCV TRIDOT is a rapid, visual, sensitive and qualitative in vitro diagnostic test, for the detection of antibodies to Hepatitis C virus in human serum or plasma which is based on the principle of cassette ELISA (flow through technology). Highly purified HCV antigens for core NS3, NS4 and NS5 were used. Results were obtained within 3 minutes.

FLAVICHECK HCV:

It is a 4th generation two site sandwich immunoassay for the detection of total antibodies specific to Hepatitis C virus (HCV in human serum / plasma / whole blood). The test employs a genotype cross-reactive recombinant peptide antigen derived from the core, NS3, NS4, and NS5 regions of multiple HCV genotypes. The double antigen sandwich system ensures detection of all anti-HCV-antibody isotypes (viz., IgG, IgM, IgA etc.) to all major HCV genotypes. Results were obtained within 15 minutes.

HCV MICROLISA:

It is a 3rd generation immunoassay for the invitro qualitative detection antibodies against immunosorbent Assay (ELISA) principle. HCV Microlisa utilizes a combination of both structural and non structural antigens i.e. core, E1, E2, NS3, NS4 and NS5.

RESULTS AND DISCUSSION:

Table 2 showed that out of total 92 patients, 64 (69.5%) were males and 28 (30.5%) were females. Male preponderance was seen in the present study which correlates to the studies of Kalantari et al. (2016). The age group ranges between 4 – 83 years with a mean age group of 45 years. All demographic data were collected in a proforma designed by the investigators. Using HCV Microlisa as a standard reference test and out of 92 samples, 33 (35.8%) were seropositive. To evaluate the results of different serological tests, out of 92 samples tested 35 were positive by HCV tridot, 31 by Flavi check and 33 were positive by ELISA (Figure 1). From the Table 3 it was found that, HCV Tridot showed 2 false positives and 1 false negative.. Whereas Table 4 showed that, Flavi check showed 2 false negatives. However 33 serum samples were positive with ELISA. The results were statistically significant ($p < 0.05$). HCV Tridot sensitivity was 96.9% and FLAVI check sensitivity was 93.9%. The results were correlated with the previous studies by Raj et al. (2001) reported, sensitivity was 79% by ICT method. Another study showed 100% sensitivity of ICT method with a specificity of 99.2% by ICT method (Zahoorullah et al. 2001). However Khan et al. (2010) found that sensitivity was only 50% and Kaur et al 2000 reported 100% specific and sensitivity was HCV and use ELISA to pick up all false negative. In contrast to our study Sato et al (1996) and Lin et al (2008) demonstrated an overall sensitivity was almost 100%. Rapid assays 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 the genotypes and subtypes of HCV circulating in the region before using these tests routinely in diagnostic laboratories. There are no approved rapid assays by the food and drug administration (FDA) and CE mark for European Union for HCV detection although several rapid tests for screening for HIV have been approved. Lin et al (2008) In conclusion we reported rapid test are less efficient than ELISA and atleast two rapid assays should be carried out on patients of dialysis patients before going to gold standard assay methods like ELISA, PCR etc. They should be recommended only in poor settings, remote areas and peripheral health facilities. HCV are highly

dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays like ELISA.

Table 2: Distribution of cases based on sex

	Males	Percentage (%)	Females	Percentage (%)
Total No. of Patients (N=92)	64	69.5%	28	30.5%
Total no. of seropositives for HCV antibodies (ELISA) (n=33)	26	78.7%	07	21.3%

Figure 1 shows the Seropositivity obtaining by different tests.

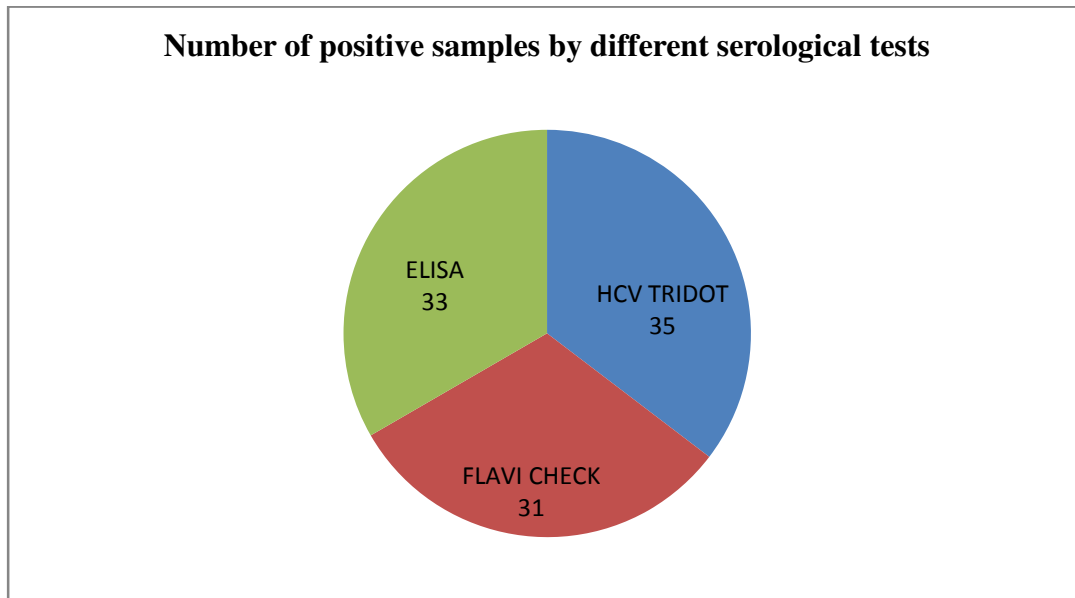


Table 3: Evaluation of HCV Tridot with HCV Elisa test

	ELIS A Positi ve	ELISA Negative	Total	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
HCV Tridot Reactive	32	3	35	96.9%	94.9%	91.4 %	98.2%	95.6%	p<0.05
HCV Tridot Non -reactive	01	56	57						
Total	33	59	92						

Table 4: Evaluation of HCV FLAVI CHECK with HCV Elisa test

	ELISA Positive	ELISA Negative	Total	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Flavi check Reactive	31	0	31	93.9%	100%	100%	96.75	97.8%	p<0.05
Flavi check Non reactive	02	59	61						
Total	33	59	92						

REFERENCES:

1. Hamid Kalantari, Shahram Ebadi1 , Majid Yaran2 , Mohammad R. Maracy3 , Zahra Shahshahan. Prevalence and risk factors of hepatitis B and C viruses among hemodialysis patients in Isfahan, Iran. *Advanced Biomedical Research*. 18: IP: 117.211.83.18.
2. Raj AA, Subramaniam T,Raghuraman S, Abraham P.2001. Evaluation of an indigenously manufactured rapid immunochromatographic test for detection of HBsAg. *Indian Journal of Pathology and Microbiology* 44:413- 4.
3. Zahoorullah, Akhtar T, Najib ul Haq, Shah MZ 2001. Latex agglutination and immuno-chromatographic screening tests verses reverse passive hem-agglutination for B surface antigen in serum. *Pakistan Journal of Medical Research* 2001; 40: 69-71.
4. Khan JK, Lone DS, HameedA, Munim MR, Bhatti M, Khattak AA 2010. Evaluation of the performance of two rapid immunochromatographic tests for detection of HBsAg and anti HCV antibody using ELISA tested samples. *Annals of King Edward Medical University* 16(S1):84-7
5. Kaur H, Dhanao J, Oberoi A 2000. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. *Indian Journal of Medical Science* 54:432-4
6. Lin Y, Wang Y, Loua A, Day G, Qiu Y, Nadala Jr 2008. Evaluation of a new hepatitis B virus surface antigen rapid test with improved sensitivity. *Journal of Clinical Microbiology*; 46:3319-24
7. Sato K, Ichiyama S, Iinuma Y, Nada T, Shimokata K, Nakashima N.1996 Evaluation of Immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody, Dainascreen HBsAg and Daina screen Ausab. *Journal of Clinical Microbiology* 34:1420-22.
8. Wasley A, Alter M.J. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis*. 2000 ;20(1):1-16
9. Alter M.J. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis*. 1997 ;1(3):559-68
10. Kayser FH, Bien KA, Eckert J, Zinkernagel RM. *Medical Microbiology* 2005. 10th ed. New York.

11. Ansari MHK, Omrani MD, Movahedi V 2007. Comparative evaluation of immuno-chromatographic rapid diagnostic tests and PCR methods for detection of human hepatitis B surface antigen. *Hepatitis monthly* 7:87-91
12. Clement F, Dewint P, leroux 2002.evaluation of a new rapid test for the combined detection of Hepatitis surface B antigen and Hepatitis B e antigen *journal of clinical microbiology* 40:4603-06
13. Gul N, Sarwar J, Idris Muhammad, Jamila Farid, Farhat R 2009. Seroprevalence of hepatitis C in pregnant females of hazara division. *Journal Ayub Medical College Abbottabad* 21: 83-6.
14. Forman MS, Valsamakis A. Hepatitis C virus. In: Versalovic J, Carrol KC, Funke G, Jorgensen JH, Landry ML, Warrock DW, editors. *Murray's Manual of Clinical Microbiology*. 10th ed. Washington: American Society of Microbiology Press; 2011. pp. 1437–55.