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ORIGINAL ARTICLE

To compare the diagnostic accuracy of the Rapid Antigen Diagnostic Kit with ELISA for detecting hepatitis-C virus infection

Neha Jain

Assistant Professor, Department of Microbiology, Saraswathi Institute of Medical Sciences, India

ABSTRACT:

Aim: To compare the diagnostic accuracy of the Rapid Antigen Diagnostic Kit with ELISA for detecting hepatitis-C virus infection. **Material and method:** The present study is a prospective observational, cross-sectional study done in department of Microbiology. During the study period a total of 1990 sample were received for routine anti HCV antibody test. All sample of any age groups for anti HCV antibody testing were included in this study. Patients with hemolysed and lipemic sample were exclude from the study. **Result:** A total of 1990 blood samples were examined, with 50 samples showing reactivity on the quick card test and 1940 samples showing non-reactivity. Upon doing further ELISA testing, it was found that 2 samples had false positive results, while 7 samples were falsely identified as negative when compared to the gold standard test. The fast test demonstrated a sensitivity of 86% and a specificity of 99%. A positive predictive value (PPV) of 97% and a negative predictive value (NPV) of 99%. The p-value of 0.001 indicates statistical significance, supporting the use of ELISA. **Conclusion:** A positive outcome from a rapid card test for anti HCV antibodies does not necessarily indicate that treatment should be immediately initiated. Similarly, a negative result from the rapid test does not rule out the possibility of infection. Prior to initiating treatment, it is important to consider the patient's medical history and conduct additional laboratory tests.

Keywords: Rapid Antigen Diagnostic Kit, ELISA, Hepatitis-C virus, Infection.

Corresponding author: Neha Jain, Assistant Professor, Department of Microbiology, Saraswathi Institute of Medical Sciences, India

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INTRODUCTION

Hepatitis C virus (HCV) infection is a significant healthcare issue that has a substantial burden in terms of health management costs and is of worldwide concern[1]. Approximately 120-130 million individuals worldwide are infected with HCV, accounting for approximately 3% of the global population[2]. Majority with HCV infectivity, develop chronic manifestations such as liver cirrhosis or hepatocellular carcinoma later in life[2]. As per WHO concensus in the year 2016 approximately 399000 people died from liver cirrhosis and hepatocellular carcinoma that was a sequalae of HCV infection[3].HCV is a positive sense ssRNA of the size of 40-80 nm, belongs to family flaviviridae genus hepacivirus ®. The most common mode of transmission of HCV infection is through exposure to small quantity of infected blood which can occur by transfusion of infected blood or blood products or by re-use of syringes, sexual or vertical transmission. HCV transmission is also seen in intravenous drug abuser[4]. Infectivity period for HCV infection ranges from 2 weeks to 6 months[5]. Patient may be asymptomatic in 80% of cases. Symptomatic patient may exhibit symptom like fever, fatigue,decrease appetite, nausea, vomiting abdominal pain, dark colored urine, yellowish discoloration of skin and eyes (jaundice) grey colouredetc[6].HCV accounts to approximately 15-20% cases of acute cases of hepatitis. Of these 50-80% will develop chronic disease which will eventually lead to liver cirrhosis & hepatocellular carcinoma[7].Only15-40% of HCV infected person could clear virus spontaneously, the reason for which remains unclear[7]**.** Genotype-1is most prevalent (40-80%%) globally, that leads to more severe liver diseases with hepatocellularcarcinoma[8]. Quantification & genotyping of HCV is of great importance to decide the duration of antiviral therapy along with the prognosis of the patient. HCV infection is diagnosed in two steps[2] initial diagnosis of HCV infection is mainly with screening methods like HCV antibodies using Enzyme linked immunosorbent assay(ELISA),immunechromatographic rapid card test &Chemiluminescence immunoassay(CLIA),the positive results from the screening test are confirmed with supplementary assay which are more specific like polymerase chain reaction(PCR) & recombinant immunoblottingassay(RIBA)[9]. Seropositivity by the test occurs as early as 8-10 weeks post exposure and may shows positivity from 6months to life-long[10].Though HCV is considered curable disease in early stage and accurate diagnosis plays key role for initiation of treatment and prognosis. The present study is undertaken to compare rapid card test based on immunochromatography principle with gold standard ELISA for detection of anti HCV antibodies. ELISA is given more specific results as compared to rapid card test and prevent the detection of false

positive tests that comes out frequently with rapid card test.

MATERIAL AND METHOD

The present study is a prospective observational, cross-sectional study done in department of Microbiology. During the study period a total of 1990 sample were received for routine anti HCV antibody test.All sample of any age groups for anti HCV antibody testing were included in this study. Patients with hemolysed and lipemic sample were exclude from the study.

METHODOLOGY

Obtain 3.5 mL of blood in a plain red top tube by venepuncture after cleaning and disinfecting selected vein area. The patient does not need special preparation. Blood sample were centrifuged at 3000 rpm and serum was tested for performing ELISA and rapid test.These samples were subjected to ELISA (considered as gold standard) and rapid card test based on immuno chromatography lateral flow principle for comparison purpose to evaluate the performance efficacy of rapid card test in comparison to ELISA for screening purpose.The 3rd generation HCV microlisa (J. Mitra & Co. Pvt. LTD) which detects antibodies against HCV in human serum & plasma. The third generation HCV microlisautilises combination of antigen with sequence of both HCV structural and non-structural antigens like core E1, E2, NS3, NS4, NS5. The available antigen markers for structural and non structural proteins that are coated on to the micro wells, diluted sample and control are then incubated as per manufacture instruction. Antibody to HCV if present will bind with antigen in the wells, then the wells will be washed using buffer to remove unbound anti HCV or other human IgG. An enzyme conjugate anti human IgG conjugated with HRP is added and later washed to remove excess enzyme conjugate complex. In next step finally prepared substrate is added and incubated. Enzyme-substrate complex will lead to development of colour in micro wells and finally stop solution 1N sulphuric acid is added and optical density of developed colour is read spectro photometrically in ELISA reader.

Rapid test (IS IT HCV ONE PLUS, Medsource Ozone Biomedicals Pvt. Ltd.) is a rapid in vitro antigen test for qualitative detection of antibody specific to HCV. It is a double antigen lateral flow chromatography immunoassay. The test cassette contain conjugate pad containing recombinant HCV fusion antigen (core, NS3, NS4 and NS5) conjugated with colloidal gold (HCV antigen conjugate) and control antibody conjugated with colloidal gold. The cassette hasnitrocellulose membrane strip containing test line (T line) and a control line C line. The test line is precoated recombinant HCV antigen (core, NS3, NS4 & NS5) and control line is precoated with anti human antibody. Sufficient volume of specimen is added in sample well,this specimen migrate laterally because of capillary action. Antibody to HCV if present in the specimen will bind to HCV antigen conjugate and the immuno complex will be captured on the nitrocellular membrane by precoated non conjugated HCV fusion antigen forming colour T-line suggestive of positive result. The test contain control 'C' line which should also form colored line for positive test to be considered valid or else test has to be repeated again using new test kit.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software version 23. Difference between proportions were determined using chi-square ($\chi 2$). P value <0.05 was taken to be statistically significant and represent 95% of confidence level.

RESULT

A total of 1990 blood samples were examined, with 50 samples showing reactivity on the quick card test and 1940 samples showing non-reactivity (as shown in Table 1). Upon doing further ELISA testing, it was found that 2 samples had false positive results, while 7 samples were falsely identified as negative when compared to the gold standard test. The fast test demonstrated a sensitivity of 86% and a specificity of 99%.

The table-1 has a positive predictive value (PPV) of 97% and a negative predictive value (NPV) of 99%. The p-value of 0.001 indicates statistical significance, supporting the use of ELISA. Table 2. Our research found that the acquisition of HCV is higher in males compared to females. Additionally, the data showed that HCV infection is more likely to be acquired beyond the age of 40 years (Table 3). The p-value was less than 0.001, indicating a statistically significant association between age and the infectivity of HCV in different sex groups.

Table-1: Comparison of Rapid card test with ELISA

Rapid card test	ELISA Reactive	ELISA Non-reactive	TOTAL
Reactive	43	2	45
Non-reactive	7	1938	1945
Total	50	1940	1990

Table-2: Evaluation of Rapid test kit with ELISA

Sensitivity	86
Specificity	99
Positive predictive value(PPV)	97

Negative predictive value(NPV)	99
Diagnostic accuracy	99
P value	<0.0001

Table-3: Age and sex groups distribution of HCV positive cases

Age(years)	Male	Female	Total
0-20	2	1	3
21-30	4	2	6
31-40	7	3	10
41-50	5	5	10
51-60	7	5	12
61-70	6	0	6
>70	3	0	3
Totalno.=56	34	16	50

DISCUSSION

In present study, ELISA is considered as gold standard test and compared it with rapid kit for screening of HCV antibody. Present study finding suggests that for screening of anti HCV antibody ELISA is a superior method for diagnosis as compared to rapid cardtest method (p value <0.0001). Yet rapid card test are cheaper andquicker method for diagnosis of infection with sensitivity of 86%, specificity of 99%, positive predictive value(PPV) of 97%, Negative predictive value of 99% and diagnostic accuracy of 99%.

According to European union standards anti HCV assay required to have 100% and 99.5% sensitivity & specificity respectively for market approach[11]. In the present study performance evaluation ofrapidcard testsensitivity and specificity are 86% and 99% respectively which is consistent with study done by Susmita Maity etal[12]. Comparative studies on ELISA for rapid card test in of diagnosis of anti HCV antibodies as compared to rapid diagnostic card test with P value <0.0001, which is statistically significant and recommended ELISA specifity. In developing countries like Indiawhere resources are scare & supplement test like RIBA and PCR are not available in all laboratories, ELISA is considered as gold standard screening test but it also requires sophisticated instrument and trained staff, therefore rapid testcard testing can be considered for diagnosis, although they are inferior to ELISA in diagnostic accuracy. Failurity of screening kits in detecting HCV reactive specimens may be attributed to inadequate coating of antigen, natureof antigen used and the genetic heterogeneity of virus. Most oftherapid test assay use recombinantprotein from the prototype virusalone but in case of HCV whose variant shows significant variation innucleotide sequence these rapid may not show praising result[12,13].If rapid card test are done they should be confirmed with ELISA and other supplemental tests like RIBA and PCR. We were not able to determine borderline result with further investigation like RIBA and PCR for further confirmation.

CONCLUSION

A positive outcome from a rapid card test for anti HCV antibodies does not necessarily indicate that treatment should be immediately initiated. Similarly, a negative result from the rapid test does not rule out the possibility of infection. Prior to initiating treatment, it is important to consider the patient's medical history and conduct additional laboratory tests. The current research demonstrates that fast tests are less effective when compared to ELISA, so they should not be suggested for screening blood donors or initiating therapy.

REFERENCES

1. Rock stroh, J.K. and Spengler,U, 2004, HIV & Hepatitis Cviruscoinfection, Lancetofinfectiondisease,4,437-44.
2. Li Cavoli G., Zagarrigo C., Schillaci O., et al. Hepatitis C virus core antigen test in monitoring of dialysis patients. Hepat Res Treat. 2012;83;20-21
3. Freiman J.M., Tran T.M., Schumacher S.G., et al. Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: a systematic review and meta-analysis. Ann Intern Med. 2016;165:345–355.
4. Alberti A, chemello L, BenvagnuL.natural history of hepatitisC.JHepatal.1999;31suppl1:17-24
5. Hepatitis-c. clinical review AA modi, TJ Liang seldio; 2008;14 (1):10-14
6. RavM,BaurK,GeierA.Hostgeneticsvariantsinthepathogenesis of hepatitis C viruses. 2012; 4:3281-3302.
7. SaitoT,Uenoy.TransmissionofhepatitisCviruses:selflimiting hepatitis or chronic hepatitis: World J.Gasteroenterol.2013;19:6961
8. Messina JP, Humphyseys I, Flaxman A, Brown A, cooke GS,Pybus04BarnesE.Globaldistributionandprevalenceofhepatitis c virus genotype, Hepatology. 2015; 61:77-87
9. CheValiezs,parolotskyJM.Hepatitiscvirus:virology,diagnosis&managementofantiviraltherapy.WorldJGastroentrol2007;13:2461-2466
10. Duong C.M., Olszyna D.P., McLaws M.L. Hepatitis B and C virus infections among patients with end-stage renal disease in a low resourced hemodialysis center in Vietnam: a cross-sectional study. BMC Publ Health. 2015;15:192
11. SinyoungKim,Jeong-HOKim,etal.ClinicalPerformanceEvaluationOfFourAut

omatedChemiluminesenceImmunoassay for Hepatitis C virus Antibody detection. J ClinMicrobial.2008,46(12):3919
12. Maity S, Nandi S,Biswas S,etal.Performanceanddiagnosticusefulnessofcommercially available enzyme linked immunosorbent assay and rapid kits fordetection of HIV,HBV and HCV in India.VirolJ.2012;9:290
13. NavA.sharmaSP,Chalterjeek,jyotiP.Markersfreetransfusion transmissible in North Indian voluntary replacementblooddonors:prevalenceandtrends1989-1996voxsang1997;73:70-3
14. KaurH,shanaoJ,oberoiA,Evaluationofrapidkitsfordetection of HIV; HBsAg and HCV infection. Indian J Medicalsciences2000:54:432-4.
15. Vidales-Braz B.M., da Silva N.M.O., Lobato R., et al. Detection of hepatitis C virus in patients with terminal renal disease undergoing dialysis in southern Brazil: prevalence, risk factors, genotypes, and viral load dynamics in hemodialysis patients. Virol J. 2015;12:8
16. Dufour DR. Talastas M. Fernandez MDA, et al.low positive antihepatitis C virus immunoassay result: an important predictor oflow likelihood of hepatitis C virus infection.clinchem.2003; 49:479-486.