Original Research Article

Comparison of diagnostic modalities for diagnosis of hepatitis-C virus infection using Rapid Antigen Diagnostic Kit and ELISA

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Abstract

Introduction: Hepatitis-C virus (HCV) causes liver disease which can cause acute & chronic hepatitis, Illness may range from asymptomatic cases to serious life long illness which may result in chronic hepatitis & hepato-cellular carcinoma. HCV is blood borne virus transmission of which is mainly parental, infection also spread by sexual contact & from vertical transmission from mother to baby. **Material & Method:** Study was conducted for a period of six month. Sample send for routine anti HCV antibodies were subjected to ELISA & rapid card test. Considering ELISA as gold standard, rapid card test was compared with it. **Result:** Out of 2295 blood samples tested on rapid card 56 samples were reactive while 2239 sample were non reactive. On further testing with ELISA 2 samples were false positive and 8 samples were detected false negative using ELISA as gold standard test. Sensitivity of rapid test was 85.7% while specificity of rapid test was found to be 99.91%. Positive predictive value (PPV) was 96% while negative predictive value (NPV) was 99.64% . P value was <0.001 which is statistically significant and in favor of ELISA. **Conclusion:** Present study shows that rapid tests are inferior as compared to ELISA and hence they should not be recommended in screening of blood donor and for treatment initiation.

Keyword: Hepatitis C virus, Chronic hepatitis, Hepato-cellular carcinoma, Enzyme linked immuno sorbent assay (ELISA).

Introduction

Hepatitis C virus (HCV) viral infection is a major cause of healthcare problem alongwith burden of health management care cost of international concern[1]**.** Globally about 120-130 million people infected with HCV which is approximate 3% of the total world 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 coloured etc[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]**.**

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Only 15-40 % of HCV infected person could clear virus spontaneously, the reason for which remains unclear[7]**.** Genotype-1 is most prevalent (40-80%%) globally, that leads to more severe liver diseases with hepatocellular carcinoma[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), immune chromatographic 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 immuno blotting assay (RIBA)[9]. Seropositivity by the test occurs as early as 8-10 weeks post exposure and may shows positivity from 6 months 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

Study Type and Study period

The present study is an prospective observational, cross sectional study done for a period of 6 months from October 2020 to March 2021 in department of Microbiology at tertiary care center, Rama medical college, hospital and research center, Pilkhuwa-Hapur.

Ethical Clearance
Permission for the study was taken from the institutional ethical committee (IEC).

Study Population
During the study period from October 2020 to March 2021, a total of 2295 sample were received for routine anti HCV antibody test.

Method for blood collection
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 microlisa utilises 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 structuralproteins 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 has nitrocellulose 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.

Inclusion criteria
All sample of any age groups for anti HCV antibody testing.

Exclusion criteria
Hemolysed and lipemic sample were exclude from the study..

Statistical analysis
Statistical analysis was done using SPSS software version 25. Difference between proportions were determined using chi-square (χ^2). P value <0.05 was taken to be statistically significant and represent 95% of confidence level.

Result
A total of 2295 blood samples were tested, out of these on rapid card 56 samples were reactive while 2239 sample were non reactive(table-1). On further testing with ELISA, 2 samples were false positive and 8 samples were detected false negative using ELISA as gold standard test. Sensitivity of rapid test was 85.7% while specificity of rapid test was found to be 99.91%.
Positive predictive value (PPV) was 96% while neagtive predictive value(NPV) was 99.64% as shown in table-1. p value was 0.001 which is statistically significant and in favor of ELISA. (table-2).
In our study, HCV acquisition is more in male as compared to female and age wise it will shown that HCV infection positivity is more acquired after age of 40 years (Table 3). P value was <0.001 which is statistically significant and showed relationship of age with sex wise groups of infectivity of HCV.

Table-1 Comparison of Rapid card test with ELISA

Rapid card test	ELISA Reactive	ELISA Non-reactive	TOTAL
Reactive	48	2	50
Non-reactive	8	2237	2245
Total	56	2239	2295

Table-2 Evaluation of Rapid test kit with ELISA

Sensitivity	85.71%
Specificity	99.91%
Positive predictive value (PPV)	96%
Negative predictive value (NPV)	99.64%
Diagnostic accuracy	99.56%
P value	<0.0001

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

Age (years)	Male	Female	Total
0-20	2	2	4
21-30	6	1	7
31-40	7	4	11
41-50	5	6	11
51-60	8	4	12
61-70	7	0	07
>70	4	0	04
Total no.= 56	39 (69.64%)	17 (30.4%)	56

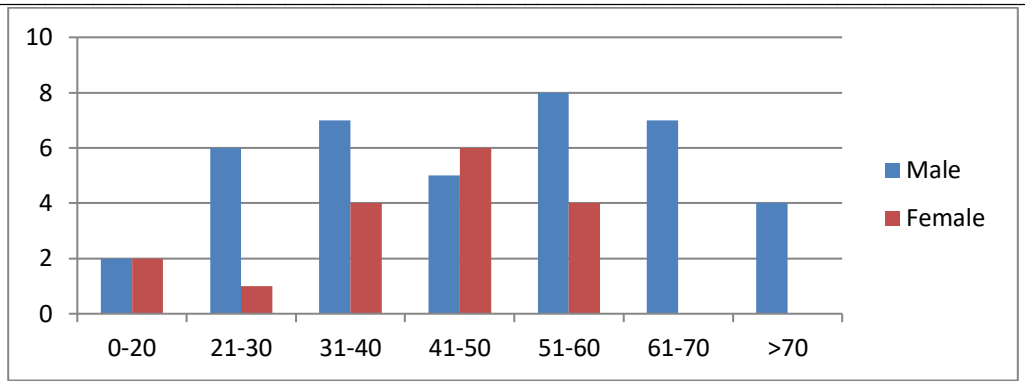

Figure 1: Age and sex groups distribution Of HCV positive

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 card test method (p value <0.0001).Yet rapid card test are cheaper and quicker method for diagnosis of infection with sensitivity of 85.71%, specificity of 99.91%, positive predictive value (PPV) of 96%, Negative predictive value of 99.64% and diagnostic accuracy of 99.56%.

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 of rapid card test sensitivity and specificity are 85.71% and 99.91% respectively which is consistent with study done by Susmita Maity et al[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 India where 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 test card 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, nature of antigen used and the genetic heterogeneity of virus. Most of the rapid test assay use recombinant protein from the prototype virus alone but in case of HCV whose variant shows significant variation in nucleotide 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.

Limitation of the study

We were not able to determine borderline result with further investigation like RIBA and PCR for further confirmation.

Conclusion

A positive result for anti HCV antibodies using a rapid card test does not warrant that the treatment should be intiated positively nor does a negative result of rapid test exclude presence of infection, patient history, and other supplementary laboratory tests should be done before starting treatment of the patient. Present study shows that rapid tests are inferior as compared to ELISA and hence they should not be recommended in screening of blood donor and for treatment initiation.

Authors Contributions

All the authors listed have made a substantial, direct and intellectual contribution to the work, and critically reviewed it and approved for publication.

Data Availability

All datasets generated or analysed during this study are included in the manuscript

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