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A Comparative Study on Detection of Hepatitis B Surface Antigen and Anti HCV Antibodies by Rapid Diagnostic Tests and ELISA Method at Tertiary Care Hospital in South India

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Abstract

Hepatitis B virus and Hepatitis C virus have been causing significant morbidity and mortality throughout the world including India. The most important markers for HBV and HCV infection are HBsAg and Anti HCV antibodies respectively. For diagnosis of infection as well as disease management and prevention, selection of appropriate test kit is necessary. The aims of this study were to compare Rapid tests with ELISA for Hepatitis B and Hepatitis C virus infection and to recommend most reliable and cost-effective rapid card test for the diagnosis of HBV and HCV in areas where advance diagnostic facilities are not available. The present study was conducted in the central microbiology laboratory at a Tertiary care Hospital, Devanahalli from a period of sep 2019 to March 2020. In our study, total no. of 1500 samples were tested for HBsAg and 150 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. On testing for 1500 samples of HBsAg, 70 samples were positive by ELISA but only 65 were positive by Rapid tests and Out of 150 samples for HCV, 10 were positive for Anti HCV antibodies by ELISA but only 9 samples were positive by Rapid tests.. These rapid kits are cheaper and easy to perform and their use should be encouraged at rural settings. ELISA is much more sensitive than rapid tests for screening of infections like HBsAg and HCV.

Keywords: HBsAg, Anti HCV antibodies, Rapid test, ELISA, Sensitivity, Specificity.

INTRODUCTION

The Viral Hepatitis is also known as "Serum Hepatitis". 1-5% infected people turn out to be chronic carriers of HBV Virus. Most of the chronic carriers secrete hepatitis B surface antigen (HBsAg) into blood and other secretions of the body like saliva and vaginal fluid. These chronic carriers are potentially infectious to other seronegative people. HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection due to its appearance in almost 2-4 weeks. HBsAg particles contain common "a" antigen, linked to two sets of mutually exclusive determinants, "d" or "y" and "w" or "r" giving the four main types-adw, adr, ayw and ayr. (19, 20, 22) Hepatitis C Virus was identified in 1989 as the main aetiological agent of non-A, non-B hepatitis (NANBH) leading to more than 90% of post-transfusion hepatitis cases. It can cause many condition like acute chronic hepatitis, liver cirrhosis and hepatocellular carcinoma throughout the world.so proper diagnosis is very important. The antiHCV was proved to be highly valuable, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily done by detecting anti-HCV in serum/plasma. (3, 5, 16,) Conventional ELISA is the most commonly used screening method for general population in Tertiary Care Hospitals of India

. Due to short comings of ELISA tests like high costs, nonavailability in many laboratory and testing sites, costly equipments, time consuming, highly skilled personnel for interpretation, rapid tests are gaining more importance and comparison of performance is needed. (11) Rapid diagnostic kits are better alternatives as they are less expensive and do not need high technical manpower or infrastructure. (14). They were meant for field survey diagnosis, emergency and home testing. The rapid card test is known to have less sensitivity and specificity than EIA but some have sensitivity and specificity comparable to EIA (2). This study was conducted to aid in early detection and treatment of HBV and HCV infections and its prevention in community.

Aims of study:

1] To compare the efficacy of ELISA test kits and Rapid test kits for testing Hepatitis B and Hepatitis C infection.

2] To determine the sensitivity and specificity of rapid card test of HBsAg and HCV

3] To recommend most reliable and cost-effective rapid card test for the diagnosis of HBV and HCV in areas where advanced diagnostic facilities are not available.

MATERIALS AND METHODS:

The present study was conducted in the central microbiology laboratory at Tertiary care hospital, Devanahalli from period Sep 2019 to March 2020. The study was conducted after taking clearance from Institutional Ethical Committee. In our study, total no. of 1500 samples were tested for HBsAg and 150 samples for HCV by ELISA method. A total of 1650 blood samples were collected from the outpatient and inpatients. Out of these, total no. of 1500 samples were tested for HBsAg and 150 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. The results of the reactive sample by ELISA were compared with the rapid tests. The collected blood was allowed to clot & serum was separated. The sample were stored at 2-80c & tested within 7 days of collection. Patients' serum samples were subjected to following tests for detection of Anti-HCV antibodies

Hepalisa Ultra Test:

This is a 4 th generation micro well ELISA test for the detection of hepatitis B surface antigen (HBsAg) in Human Serum / Plasma. [12, 14], manufactured by J. Mitra& Co. Pvt. Ltd. New Delhi, India. It is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. Samples were processed according to kit insert. Sample found to be reactive by HEPALISA ULTRAtest were again tested by rapid test i.e HEPACARD test.

Hepacard :

The 3rd Generation HEPACARD is a rapid and qualitative in vitro diagnostic test for the detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma, manufactured by J. Mitra & Co. Pvt. Ltd. New Delhi, India.Test procedure followed and results were interpreted according to manufacturer instructions. Appearance of pink coloured line, one each in test region "T" and control region "C" indicates that the sample is REACTIVE for HBsAg. .

HCV Micro ELISA test:

The 3rd generation HCV Micro Elisa is an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies against HCV (anti-HCVs) in human serum or plasma, manufactured by J. Mitra& co. Pvt. Ltd. New Delhi, India.The results were read on Microplate spectrophotometer at 450 nm. Cut off value was calculated as per the manufacturer's guidance and the results were interpreted accordingly. Cut off value = 0.1xPCx+0.1, PCx = Mean absorbance of positive control

Interpretation: - According to their absorbance values, samples were interpreted as either reactive for HCV antibody (HCV positive) or non-reactive for HCV antibody (HCV negative) if test specimens with absorbance value is within 10% below the cutoff, it should be suspected for the presence of antibodies and should be retested in duplicate. Sample found to be reactive initially by HCV Microlisa test were again tested by visual rapid test.

HCV TRI-DOT:

The 4th Generation HCV TRI-DOT is a rapid and qualitative in vitro diagnostic test for the detection of antibodies to Hepatitis C Virus in human serum or plasma.

Interpretation: - Results are noted as per manufacturer guidelines and results were interpreted accordingly. If

Volume 4, Issue 5; September-October 2021; Page No 331-337

test dots T1, & T2, appear pink, result should be considered reactive for antibody to HCV. If only control dot appear it indicates that the sample is nonreactive for anti-body to HCV. Sample found to be positive for HCV antibodies by both HCV Microlisa test & HCV TRI-DOT method would be further tested for hepatitis B Surface antigen by ELISA.

RESULTS:

In our study, A total of 1650 blood samples were collected from the outpatients and in patients. Out of these, total no. of 1500 samples were tested for HBsAg and 150 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. On testing for 1500 samples of HBsAg, 70 samples were reactive by ELISA but only 65 were reactive in Rapid tests and Out of 150 samples for HCV, 10 were reactive for Anti HCV antibodies by ELISA but only 9 samples were reactive by Rapid tests. [Table 1] Table 4. shows that using ELISA as a gold standard confirmatory method, sensitivity of rapid card test for HBsAg was 92.85%, specificity was 100%, PPV (positive predictive value) was 100%, NPV (negative predictive value) was 99.67%, whereas sensitivity of rapid card test for Anti HCV Antibodies was 90%, specificity was 100%, positive predictive value was 100%, negative predictive value was 99.34%, . In the present study ELISA was compared with the rapid kits for the screening of HBsAg and Anti HCV Antibodies. For HBsAg and Anti HCV Antibodies screening, rapid tests are equally specific compared to ELISA. But ELISA is more sensitive compared to rapid tests. ELISA and other advanced methods are laboratory based, time consuming and require trained personnel. Rapid test enables early detection at sites where laboratory facilities or trained manpower are not available or there is issue of accessibility. (18, 19) The rapid tests reduce the potential for loss of follow up of a case when results are not given straight away. The high laboratory cost is another factor that reduces the willingness to screen the general population. Ideally rapid devices should have a high degree of sensitivity and specificity. This study agrees with previous studies in other countries, which have stated that rapid test kits are not sensitive enough to be used solely for the detection of HBsAg and HCV. (9, 18) Our study also found ELISA to be much more sensitive than Rapid Test. Our results were correlated with the Previous studies by Ijaj H *et al.,* 2012 (Pakistan)[6], Adeyemi AA *et al.,* 2013 (Nigeria)[1] and Parth R *et al.,* 2016(India)[13]. Comparison of studies conducted by other researchers showed slight variations in results. Our study has provided authentic scientific data based on the affected population. We conclude that HBV and HCV directly affects epidemiology, morbidity, mortality, socioeconomic and preventive aspects, So particularly in developing countries like India, the present study and other similar studies by early detection of viral prevalence for in assessment of disease burden in community and in controlling the complications of HBV and HCV viral infections.

CONCLUSION:

Rapid test is less efficient than ELISA compared with conventional ELISA which needs long time. Rapid card test results are available within minutes, which is helpful in starting immediate treatment and minimizing the serious complications and mortality. Conventional ELISA cannot be carried out for single or small number of samples, since it would be quite uneconomical. Rapid card test is susceptible to unfavourable storage conditions, so its mandatory to do periodic quality control checks to avoid false positive or false negative results.

These rapid card tests should be recommended only in resource limited poor settings, remote areas and peripheral health facilities for screening purpose. False negative results can lead to silent transmission and spreading of diseases among people and hence more sensitive assays such as ELISA is needed. Hence, it is recommended that, rapid test kit should be used in conjunction with other immunoassay particularly ELISA technique.

Volume 4, Issue 5; September-October 2021; Page No 331-337

TESTS	Reactive by ELISA	Percentage Reactivity	Reactive by RAPID TESTS	Percentage Reactivity	False Negative by RAPID TESTS
HBsAg	70	**4.66**	65	4.33	**0.33**
Anti HCV Antibodies	**10**	**6.66**	**9**	**4.66**	**0.66**

Table.1 Comparative Evaluation of Rapid Test Kits with ELISA forHBsAg and Anti HCV Antibodies Tests

Rapid card test	**HBsAg**	
	ELISA Reactive	**ELISA Non Reactive**
Rapid Reactive	**65**	**0**
Rapid Non-Reactive	**5**	**1430**
Total cases	**70**	**1430**

Table.2 Comparative Evaluation of HBsAg CARD Rapid Test Kit with HBsAg ELISA Test

Rapid card test	**Anti HCV Antibodies**	
	ELISA Reactive	**ELISA Non Reactive**
Rapid Reactive	**9**	**0**
Rapid Non Reactive	**1**	**140**
Total cases	**10**	**140**

Table.3 Comparative Evaluation of HCV DOT Rapid Test Kit with Anti HCV Antibodies ELISA Test

Tests	**Sensitivity**	**Specificity**	**PPV**	**NPV**
HBsAg by Rapid card test	**92.85%**	**100%**	**100%**	**99.67%**
Anti HCV Antibodies byRapid card test	**90%**	**100%**	**100%**	**99.34%**

Table.4 Comparative evaluation of rapid test kits with ELISA as a gold standard confirmatory method

Volume 4, Issue 5; September-October 2021; Page No 331-337

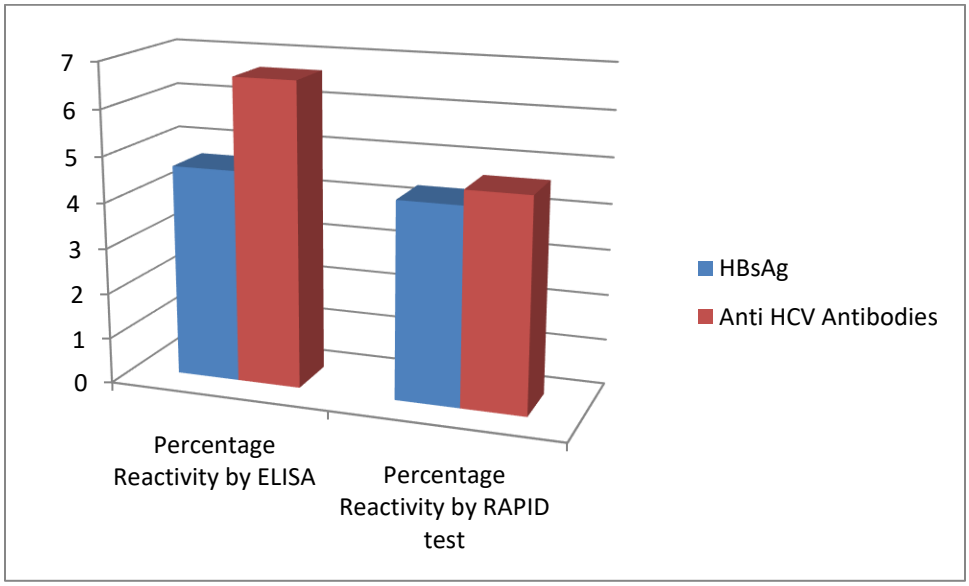

Fig 1: Percentage reactivity by RAPID test and by ELISA

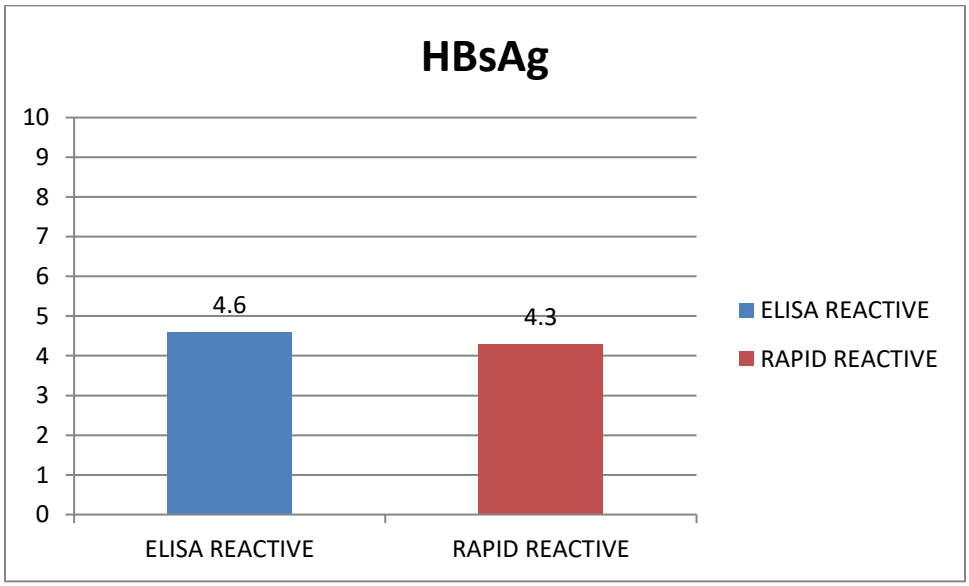

Fig 2: Percentage reactivity of HbsAg by ELISA and Rapid test

Volume 4, Issue 5; September-October 2021; Page No 331-337

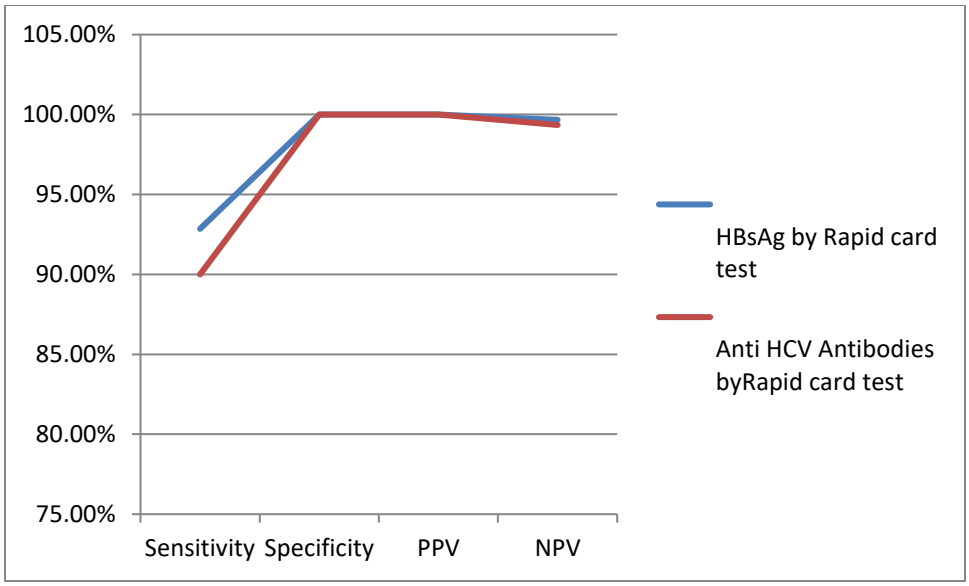

Fig 3: Sensitivity and specificity of HBsAg and Anti HCV by Rapid card test

*Sensitivity (also called the true positive rate, the recall, power, hit rate or probability of detection)-refers to a test's ability to designate an individual with disease as positive. Specificity (also called the true negative rate or selectivity)-refers to a test's ability to designate an individual who does not have a disease as negative. Positive predictive value (PPV also called the precision)-is the ability of an assay to identify actual infected individuals among all persons. Negative predictive value (NPV)-is the ability of an assay to identify correctly the real non-infected individuals among persons.

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Volume 4, Issue 5; September-October 2021; Page No 331-337

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Volume 4, Issue 5; September-October 2021; Page No 331-337