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Research Article

A COMPARATIVE EVALUATION OF SCREENING HEPATITIS B SURFACE ANTIGEN BY ENZYME-LINKED IMMUNOSORBENT ASSAY AND RAPID CARD TEST

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A B S T R A C T

Background: Hepatitis B virus (HBV) is one of the major causes of death in developing countries. The most important marker for diagnosis is detection of Hepatitis B surface antigen in blood. Objective: The aim of present study was to compare two different brand rapid card test kits (Brand A and Brand B) for screening of hepatitis B virus infection with gold standard enzyme linked immunosorbent assay method. Method: This study was conducted in Department of Microbiology at government Medical College and associated hospital for a period of 6 months. Result: Out of 4200 blood samples tested for hepatitis B surface B antigen (HBsAg), 89 (2.09%) were positive by enzyme linked immunosorbent assay (ELISA), 87 (2.04%) positive by Brand A rapid card and 86 (2.02%) were positive by Brand B rapid card. The sensitivity of rapid card test Brand A was 97.75%, specificity was 100%, positive predictive value was 97.75%, negative predictive value was 99.95%, and diagnostic accuracy was 99.95%. The sensitivity of rapid card test Brand B was 96.62%, specificity was 100%, positive predictive value was 96.63%, negative predictive value was 99.93%, and diagnostic accuracy was 99.93%.Conclusion: The sensitivity and specificity of both brands rapid card test (Brand A and Brand B) is comparable with ELISA. These rapid kits are easy to perform and less cheap in compare with cost of ELISA. There use should be encourages at rural area where cannotafford the cost of ELISA test so that the patient should be channelized faster towards specific and accurate diagnosis.

INTRODUCTION

Hepatitis B virus which causes hepatocellular carcinoma is one of the important target for global elimination by 2030 [1]. The world health organization estimated that 257 million people were living with HBV infection in 2015 and responsible for 887000 deaths every year [2-3].Based on the prevalence of HBV in different areas of the world are classified as high (≥8%), Intermediate (2-7%), or low (≤2%) HBV endemicity. India has an intermediate prevalence of hepatitis B virus with a 4% to 5.4% infected population [4-5]. HBV has a double-stranded DNA genome of around 3200 base pairs encoding for P, X Core and surface proteins. The envelop proteins are surface glycoprotein and assigned as hepatitis B surface antigen [6-7]. HBsAg appears in serum within 2-10weeks after exposure to HBV and before the onset of symptoms or elevation of serum aminotransferase level. Chronic HBV infection progresses nonlinearly through 3-4 phases, from the immune-tolerance phase to immune clearance or immume-active phase to non-replicative inactive phase and possible HBsAgusually becomes undetectable after 4-6 months [8-9].

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HBsAg has been found to be an important viral marker for population screening as well as diagnosis because it is the primary way to identify persons with chronic HBV infection and several characteristics of this serological marker increase the precision of HBsAg estimates, including high specificity, long serum persistence, low possibility of chronic cases losing HBsAg [10,11,12]. Early and accurate detection of HBV infection using sensitive and specific methods allow investigators to evaluate the status of HBV infection and develop strategies to prevent transmission. There are many methods for diagnosis of hepatitis B surface antigen but rapid card test is a rapid screening test for qualitative detection of HBsAg in whole blood, serum or plasma specimen. The test utilized a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, serum, or plasma [8, 13]. On the other hand ELISA is enzymatic immunoassay technique of the sandwich type for the detection of HBV in human serum or plasma, in which antigens or antibodies are covalently bound with suitable enzymes that can catalyze the change of substrates into dyed products. It is an approved technique to investigate diverse serological markers [14]. The rapid immunochromatography tests are known to have less sensitivity and specificity than enzyme immunoassay [15, 16]. A major concern in utilizing rapid screening test is that these tests should have a high degree of sensitivity and a reasonable

level of specificity to minimize false positive and false negative results. The present study was designed to check the sensitivity and specificity of two different rapid cards of HBsAg which are frequently used in many labs and hospitals of Kannauj district, Uttar Pradesh, India and to compare with already confirmed cases on ELISA. The ultimate goal of this study was to recommend most reliable, specific and sensitive rapid cards for the diagnosis of HBV in areas where advance diagnostic facilities are not available.

MATERIAL AND METHOD

This prospective study was conducted in the government medical college and associated hospital at Tirwa, Kannauj, India, from November 2018- April 2019.Two most common used brands of rapid cards for HBsAg in many laboratories and hospitals were selected for the study. ELISA was used as gold standard for comparative evaluation. Prior to selection of rapid cards, a verbal survey was done in the major laboratories and hospitals to find out which brands rapid cards are being used by these outlets. For study Reckon diagnostic and SD bioline were selected.

A total of 4250HBsAg samples included 89 positive by ELISA and 4161 negative by ELISA. All samples were selected and tested on two different immunochromatography cards. As this is prospective study all samples during study period were included.

Sample collection-2-3 ml whole blood Samples were collected from patients in plain vial with clot activator and left them for 10-15 minutes at room temperature. Blood samples were then centrifuged at 1500 round per minutes for 5-10 minutes to collect serum. We used serum for performing ELISA and rapid card tests for all patients.

Sample processing- Each blood sample was tested for HBsAg using two different brands rapid card (Brand A= Reckon diagnostics pvt. LTD, Brand B= SDbioline, standard diagnostic, INC.) and ELISA by Hepalisa- J.Mitra& Co. Pvt. Ltd

Before performing the test all the samples and reagents were brought to room temperature as per kit manual.

Determination of Hepatitis B surface antigen

By Enzyme linked Immuno-sorbent assay

HEPALISA assay test kit by J. MITRA Diagnostic manufacture in India is used for ELISA technique. HEPALISA is a solid phase enzyme linked immunosorbent assay based on the 'Direct Sandwich' principle. The microwells are coated with monoclonal antibodies with high reactivity for hepatitis B surface antigen. The samples are added in the wells followed by addition of enzyme conjugate (polyclonal antibodies linked to horseradish peroxidase). A sandwiched complex is formed in the well wherein hepatitis B surface antigen (from serum samples) is trapped or "sandwiched" between the antibody and antibody horseradish peroxidase conjugate. Unbound conjugate is then washed off with wash buffer. The amount of unbound peroxidase is proportional to the concentration of HBsAg present in the sample. Upon addition of the substrate buffer &chromogen, a blue colour develops. The intensity of developed blue colour is proportional to concentration of HBsAgin sample. To limit the enzyme-substrate reaction, stop solution is added & a yellow colour develops which is finally read at 450nm spectrophotometrically.

Test Procedure: All the samples were run along with negative control (NC) and positive control (PC) according to test procedure given by manufacturer (J. Mitra Diagnostic).

Calculation of Result: Compute mean of NC and PC absorbance.

Test validity:

Positive control acceptance criteria

PC or PC$\bar{x}$ must be >0.5, if it is so, then run is invalid

Negative control acceptance criteria

NC or NC$\bar{x}$ must be <0.150

Cut off value

Cut off value is determined by using the given formula below

Cut off value = NC$\bar{x}$+0.1

Where NC$\bar{x}$ is the mean absorbance of negative control
All samples with absorbance value more than cut off value were taken as positive for hepatitis B surface antigen. The minimum detectable concentration of HBsAgby this assay is estimated to be 0.1ng/ml as per the kit used.

Determination of Hepatitis B surface antigen by Rapid card test

Rapid card (A= Reckon diagnostics pvt. LTD, B= SD diagnostic pvt. LTD)is a one -step immunoassay based on the antigen capture or sandwich principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and to flow laterally through an absorbent pad where it mixed with the signal reagents. If the sample contains hepatitis B surface antigen, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen-antibody-colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (test line) 'T', complex is trapped forming an antigen-antibody colloidal gold complex. This forms a pink band indicating the sample is reactive forHBsAg.

Test procedure for both Brand's rapid card (Brand A& Brand B) as per kits

- Using the dropper provided put 2-3 drops (25µl) of serum into the sample well.
- Let the reaction to proceed until the appearance of positive line and control line or upto 20 minutes.
- Read result after 20 minutes. Strong positive reaction may visible within 5 minutes.

Interpretation of Result:		
Interpretation	Control Line	Test Line
Negative Test	Pink Line	No pink Line
Positive Test	Pink Line	Pink Line
Invalid Test	No pink Line	No pink Line/ Pink Line

Test card was stored at 4^{O}C as advised by manufacturer. The test kit was kept away from direct sunlight, moisture and heat.

RESULT

The results of different rapid cards on the basis of sensitivity, specificity, negative predictive value, positive predictive value, disease prevalence and diagnostic accuracy of Immunochromatography technique with that of ELISA which is considered as gold standard technique for the detection of HBsAg.

Out of 4250 samples, 89 (2.09%) were HBsAg positive by ELISA.
The age range of the HBsAg positive patients (n=89) was between 5-80 years with mean of 36.34±17.44 years. (Table No. 1)
Out of 89 ELISA positive samples tested on rapid card test Brand A, 87 samples were positive and 02 samples were negative for HBsAg.

On further testing on rapid card test Brand B, 86 samples were positive and 03 samples were negative for HBsAg.

The reason for false negative is unclear; this may be due to low viremia or less than 0.5ng/ml. The rapid card test (Both brands) used in this study can detect hepatitis B surface antigen in serum or plasma as low as 0.5ng/ml while HEPALISA kit has a sensitivity of 0.1ng/ml as per kit manual. For further satisfactory statement titre of hepatitis surface antigen, viral load, and other quantitative immunological markers should be perform.

On comparison with ELISA, two false negative were detected for brand A, and three false negative were detcted for brand B.

Table No. 1 Age distribution of subject (n=89) of HBsAg positive

Age Group	Subject Tested
<10	02 (2.25%)
11-20	04 4.5%)
21-30	47 (52.80%)
31-40	07 (7.87%)
41-50	11 (12.36%)
>50	18 (20.22%)
Total (n)	89 (100%)

Table No 2 Comparison of ELISA, Rapid card Brand A and Brand B

Total No of subjects	4250
Positive by ELISA	89 (2.09%)
Positive by Brand A	87 (2.04%)
Positive by Brand B	86 (2.02%)
False negative by Brand A	02 (2.24%)
False negative by Brand A	03(3.37%)

Using ELISA as a gold standard confirmatory method, comparison between ELISA and Brand A

The sensitivity of rapid card test Brand A was 97.75%, specificity was 100%, positive predictive value was 97.75%, negative predictive value was 99.95%, diagnostic accuracy was 99.95%, and disease prevalence was 2.09%. (Table No 3)

Using ELISA as a gold standard confirmatory method, comparison between ELISA and Brand B

The sensitivity of rapid card test Brand B was 96.62%, specificity was 100%, positive predictive value was 96.63%, negative predictive value was 99.93%, diagnostic accuracy was 99.99%, and disease prevalence was 2.02%. (Table No. 3)

DISCUSSION

Serological assays detect the host immune response (antibodies to HCV) or a viral antigen (HBsAg, HCVcAg). They are based on the immunoassay principle, and are available in the form of rapid diagnostic tests (RDTs) or laboratory-based enzyme immunoassays (EIAs), chemoluminescence immunoassays (CLIAs) and electrochemoluminescence immunoassays (ECLs).

In contrast, NAT technologies are typically used to detect the presence of the virus, determine if the infection is active and if the individual would benefit from antiviral treatment. NAT technologies are also used to determine when antiviral treatment should be discontinued (due to non-response or resistance) or to confirm virological cure (HCV) or effective suppression (HBV).

Most laboratory-based serological immunoassays (EIAs, CLIAs and ECLs) detect antibodies, antigens or a combination of both and differ only in the mode of detection of immune complexes formed. A cut-off value, usually determined by the manufacturer of the assay, specifies the point at which the results are considered to be reactive, and therefore, EIA results are generally reported as optical density divided by the assay cut-off (OD/CO) values.

Rapid diagnostic tests (RDTs) are single-use disposable assays that are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and can give a simple qualitative result in under 30 minutes. Quality-assured RDTs are therefore particularly useful in settings where conventional laboratory-based testing services are not available or accessible.

Table No 3 Evaluation of Rapid Card Test Brand A and Brand B with ELISA

Rapid card Brands For HBsAg		ELISA (Gold Standard)			Results for screening test									
		Reactive (n=89)	Non-reactive (n=4161)	Total	True Positive	True Negative	False Positive	False Negative	Sensitivity	Specificity	Positive Predictive Valve	Negative Predictive Value	Diagnostic accuracy	Kappa Value
Brand A (SD Bioline)	Reactive	87	-	87	87	4163	-	02	97.75%	100%	100%	99.95%	0.975	0.975
	Non-Reactive	02	4161	4163										
Brand B (Reckone)	Reactive	86	-	86	86	4164	-	03	96.62%	100%	100%	99.93%	0.975	0.975
	Non-reactive	03	4161	4164										

The choice of assay format will depend on a variety of factors, most importantly, performance characteristics (sensitivity and specificity), cost, ease of use and the characteristics of the testing site, such as storage facilities, infrastructure, and level of staff skills. WHO recommends the use of standardized testing strategies to both maximize the accuracy of HBsAg or HCV antibody testing while simplifying the process through streamlining procurement and training [17].

The choice between a one-assay versus two-assay serological testing strategy will depend on the seroprevalence in the population to be tested and diagnostic accuracy (sensitivity and specificity) of the assays used.

In many developing countries, rapid card test is widely used to detect hepatitis B surface antigen for diagnosis as well as screening for hepatitis B virus infections [18] as these are cheap and does not need expertise.

In present study, the infection rate of hepatitis B virus found to be 2.09% by ELISA test, 2.04% and 2.02% by Brand A and Brand B. A prospective study conducted in Kannauj, Uttar Pradesh, India supports our findings with infection rate of 2.28%. [19]

From the systemic review & meta-analysis on the HBsAg diagnostic accuracy of rapid cards Vs enzyme immunoassay and nucleic-acid test (NAT), three studies [20,21, 22] evaluated 7 rapid cards in samples from 510 patients against a NAT reference standard. One study [21] used plasma from Nigerian repeat blood donors. Sensitivities ranged from 38% to 99% and specificities ranged from 94% to 99%. Over all pooled sensitivity and specificity were 93.3% and 98.1% respectively.

Five studies [22,23,24,25, 26] evaluated enzyme immunoassays based on a NAT reference, using samples from 1194 patients. Pooled sensitivity and specificity were 75.7% and 86.1% respectively.

In present study we tested the serum of patientswith two differentbrands (Rapid card Brand A& Brand B) of immunochromatographic methods and subjected to compare same sere to Elisa methods.

The sensitivity of rapid card Brand A and Brand B was 100% for both with reference to ELISA and specificity was 99.95% and 99.93% respectively. Sharma M *et al* [10] reported sensitivity of rapid card was 100% and specificity was 99.56%. Another study by Akhtar *et al* [27] showed 100% sensitivity of rapid card test kit with specificity of 91.7% for hepatitis B surface antigen. A study conducted by Lin *et al* [28] by using ICTs the sensitivity and specificity was 100% respectively. A study conducted by Maity *et al* [29] a comparative study, 3 different HBsAg ICT kits Hepacard, Crystal NS SD bioline, were evaluated, all of them showing 100% sensitivity and 100% specificity. Another study shows the sensitivity of ICT can vary from 50-94% [30].Immunochromatography based assay used for HBsAg detection may not have the same accuracy indices in every region due to differences in a given population. The prevalent subtype of HBV infecting population can be different. ICT for HBsAg detection must be validated before being used in resource limited settings.

The ELISA kit that was used in this study showed to have analytical sensitivity of 0.1ng/ml and detects all the known 11 subtypes of HBV.A similar study shows that ELISA is known to detect the antigen concentration of less than 0.4 ng/ml of HBsAg while as rapid card tests based on lateral-flow technology, which appears to be most sensitive format do not achieve sensitivity of 1 IU/ml for HBsAg [15, 31]. Another study by Mubashirnazir *et al* [32] the ELISA kit that was used shows sensitivity of 0.1ng/ml.

Some studies suggest that the diagnostic performance of RDT is comparable to ELISA [33]. A study by Mizuochi. *et al* [34] shows that newly developed HBsAg rapid test had an analytical detection limit between 0.2 and 0.8 IU/ml values are similar to those of HBsAg EIAs detection.

A positive result can be followed by more accurate and advance method to confirm the infection presence unlike a negative result. In present study negative predictive value for rapid card Brand A and Brand B was 100% for both. Sensitivity and Negative predictive value are two most important parameters for choosing a test rather than specificity and positive predictive value for routine use [35].

Further work is needed as data on the circulating serotype and mutants of hepatitis B virus are widely available in India. Failure to detection hepatitis surface antigen by rapid card may be due to not proper and inadequate antigen coating, genetic heterogeneity of the virus prevalent in that area.

CONCLUSION

Results from this study indicate thatimmunochromatography based rapid card test is a simple, rapid and highly sensitive for screening for hepatitis B surface antigen. Overall performance of these rapid tests was not only compatible with currently established and advanced diagnostic methods but also cheaper. The rapid card test can be used bed site and do not need any expertise to perform and are easy to use. The ultimate goal of this study was to recommend ELISA comparable rapid device for initially screening of hepatitis B, in remote areas or where cost is an issue.

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