Original Article

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Microbiology Section

A Comparison of Rapid Screening Test and ELISA for the Diagnosis of Hepatitis B Surface Antigen in Patients Attending a Tertiary Care Hospital, Tamil Nadu, India

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ABSTRACT

Introduction: Hepatitis B virus infection is a major public health problem and leading cause of death worldwide. World Health Organisation (WHO) estimates that in 2015 about 257 million population were living with chronic Hepatitis B virus infection with a global prevalence of 3.5%. Among the several viral antigens of Hepatitis B, Hepatitis B Surface Antigen (HBsAg) is an important viral antigen which is recognised as a superior marker for Hepatitis B virus detection. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary.

Aim: To compare the results of rapid screening tests and ELISA for the diagnosis of HBsAg.

Materials and Methods: A cross-sectional study was conducted from July 2019-August 2019 in a tertiary care hospital. A total of 200 blood samples received for HBsAg testing were centrifuged. Serum of all the 200 blood samples were tested for HBsAg using both rapid Immuno Chromatographic Card (ICT) method (HEPAVIEW - one step test for HBsAg, Viola Diagnostic System, A Division of Tulip Diagnostics Pvt., Ltd.) and Enzyme Linked Immuno Sorbent Assays (ELISA) (Merilisa HBsAg- Meril Diagnostics Pvt., Ltd.) method. Data for rapid card method and ELISA were noted and entered into MS excel spread sheet and analysed.

Results: Among the total 200 blood samples tested by HbsAg rapid card, five samples were positive and the remaining 195 were negative. For rapid card test, the sensitivity was 83.4%, specificity 100%, Positive Predictive Value (PPV) 100% and Negative Predictive Value (NPV) 99.4% and for ELISA the sensitivity, specificity, PPV and NPV were all 100%.

Conclusion: The overall performance of the rapid ICT for HBsAg was less sensitive to ELISA. So, only ELISA can be encouraged in all setups irrespective of their developmental and economical status not only to prevent the complications of Hepatitis B infection but also for early diagnosis and better treatment of patients.

Keywords: Enzyme linked immunosorbent assay, Hepatitis B virus, Immuno chromatographic card test

INTRODUCTION

Hepatitis B virus infection is a major public health threat and a leading cause of mortality [1]. WHO had estimated that in 2015 about 257 million population were living with chronic Hepatitis B virus infection with a global prevalence of about 3.5% [2]. Viral hepatitis is a systemic disease infecting liver [1] and has been one of the leading causes of a range of hepatic complications including chronic hepatitis, cirrhosis of liver, fulminant hepatitis and hepatocellular carcinoma not only globally but in India also [3].

India is considered to have an intermediate level of Hepatitis B virus endemicity (3.7% prevalence) which constitutes 11% of estimated global burden [3]. Hepatitis A, B and C viruses constitute for most of the viral hepatitis cases [4]. Among the hepatitis viruses, Hepatitis B virus is the only Deoxyribonucleic Acid (DNA) virus. Among the several viral antigens of Hepatitis B virus, HBsAg is an important viral antigen which is recognised as a superior marker for Hepatitis B virus detection. HBsAg, earlier known as Australia antigen is the first serological marker to circulate in the blood of infected individuals even 2-4 weeks before the appearance of the clinical symptoms. The levels of HBsAg are particularly elevated during the symptomatic phase and decline thereafter [5]. Hepatitis B virus infection transmits via blood/blood products, needle prick injuries, sexual relationships and vertically from mother to foetus. Screening of HBsAg will expose previously unsuspected chronic HBV infection in young, otherwise healthy individuals. It has the added benefit of making it possible to refer such patients for appropriate antiviral therapy prior to significant liver damage and associated functional insufficiency are developed [6]. Since, Hepatitis B virus is potentially infectious and leading to serious complications, true positives have to be identified earlier for better treatment.

Early and accurate detection of Hepatitis B virus infection using sensitive and specific methods allow investigators to evaluate the status of Hepatitis B virus infection and develop strategies to prevent transmission. HBV is a highly infectious virus which causes silent infection. Therefore, accurate detection of the viral markers is important for controlling the transmission. Hence, it is essential to validate the detection methods prior to allowing their use in laboratories [7]. So identification of appropriate test kit is necessary to avoid false positive and false negative results.

Now-a-days many techniques are available to detect HBsAg in patients sample such as rapid ICT, ELISA, Enzyme Immunoassay (EIA), Nucleic Acid Amplification Test (NAT) and Polymerase Chain Reaction (PCR). Among these ELISA, EIA, NAT, PCR methods are costly and used in advanced laboratories. Rapid kits are cost effective and do not need technical human support [8]. HBsAg card test is a rapid screening test for the qualitative detection of HBsAg in whole blood, serum and plasma [1]. This test utilises both mono and polyclonal antibodies to selectively detect elevated levels of HBsAg.

Whereas, HBsAg ELISA is a direct solid phase enzyme linked immuno assay based on “sandwich” type of detection of Hepatitis B virus in human samples [7]. This uses monoclonal antibodies which has the ability to bind with various subtypes and strains of Hepatitis B virus as now recognised by WHO [9,10].

Hepatitis B rapid card test methods are popular and most commonly used methods in developing countries such as India even though

ELISA is considered as more reliable method, since rapid screening tests are rapid, easy to perform, user friendly and the manufacturers recommend their use [10]. Apart from rapid results and ease of use, ICT can be used with whole blood from finger prick instead of serum or plasma with no significant difference in accuracy [11]. Ideal rapid test should have good sensitivity and reasonable specificity to reduce false positive and false negative results [7]. This study was conducted to evaluate the sensitivity and specificity of rapid screening tests with ELISA for the better diagnosis and the early treatment of patients.

MATERIALS AND METHODS

A cross-sectional study was carried out from July 2019-August 2019 in a tertiary care centre at Coimbatore, Tamil Nadu, India. A total of 200 blood samples were received for HBsAg detection in the Microbiology Department, Government Medical College and ESI hospital, Coimbatore, Tamil Nadu, India. Ethical Approval was obtained from Institutional Ethics committee (Approval No: 19054). Informed consent was not necessary for this study as only samples were included.

Inclusion criteria: Samples received for HBsAg testing from various departments (General Medicine, General Surgery, Obstetrics and Gynaecology, Orthopaedics, Otorhinolaryngology) of the hospital.

Exclusion criteria: Stored (>3 days)/contaminated samples, heat inactivated samples, samples which uses sodium azide as preservative.

Sample processing: All 200 blood samples were centrifuged and serum was separated. Serum of all the 200 received blood samples were tested for HBsAg using both rapid ICT method (HEPAVIEW – one step test for HBsAg, Viola Diagnostic System, A Division of Tulip Diagnostics pvt., Ltd.,) and ELISA (MerilisaHBsAg – Meril Diagnostics pvt., Ltd.,) method.

Procedure

Rapid Card Test process: HEPAVIEW- one step test for HBsAg is a single-step immunoassay based on the antigen capture or sandwich principle. In this method, monoclonal antibodies are conjugated to colloidal gold and polyclonal antibodies are immobilised on a strip made of nitrocellulose, in a lean line. The sample in the given well flows sideways through an absorbent pad where it mixes with the single reagent. If the sample is HBsAg positive, the colloidal gold-antibody conjugate binds to the antigen and the antigen-antibody-colloidal gold complex is formed. This complex then moves through the nitrocellulose strip by capillary action. When the complex meets the line of immobilised antibody (test line) 'T', complex is trapped which forms a band which is pink in colour indicating the sample is reactive for HBsAg. For control, an additional line of antimouse antibody (control line) 'C', has been immobilised at a distance from the test line on the strip. If the sample is positive, test will result in the formation of a pink band upon contact with the conjugate [12].

ELISA Method: MERILISA– HBsAg is based on microwells coated with monoclonal anti-HBsAg antibody. Polyclonal anti-HBsAg antibody labelled with horseradish peroxidase is used as a conjugate. Samples and the controls are added in the microwells and incubated. HBsAg if present will attach to monoclonal anti-HBsAg antibody in the microwell. Conjugate was added in the next step which binds to the antigen antibody complex if formed in the well. Any unbound conjugate is washed away and a solution containing 3,3', 5,5'-Tetramethylbenzidine (TMB) and hydrogen peroxide was added to the wells as a substrate. Wells with bound conjugate develop blue colour which get converted to yellow colour when reaction is stopped with sulphuric acid and the colour is read spectrophotometrically. The intensity of colour produced is directly proportional to the concentration of HBsAg in the sample [4].

Sensitivity, Specificity, PPV, NPV: Sensitivity is the ability of the screening test to give a positive finding when the person tested is having the disease. It is expressed as percentage.

$$\text{Sensitivity} = \frac{\text{Persons with the disease detected by the screening test}}{\text{Total number of person tested with the disease}} \times 100$$

Specificity is the ability of the screening test to give a negative finding when the person tested is free of the disease. It is also expressed as percentage.

$$\text{Specificity} = \frac{\text{Persons without the disease detected negative by the screening test}}{\text{Total number of person tested without the disease}} \times 100$$

PPV is the percentage of true positives among total positives. NPV is the percentage of true negatives among the total negatives [8].

STATISTICAL ANALYSIS

A total number of 200 serum samples were tested by using rapid card method and by ELISA method simultaneously. Data's such as Optical Density of the samples/Positive Control/Negative Control/ Cut-off value and reactivity for rapid card method and ELISA were noted and entered into MS excel spread sheet and analysed.

RESULTS

Among the total 200 blood samples tested by HbsAg rapid card, five samples were positive and the remaining 195 were negative. Then the same samples when tested with ELISA, six samples were found to be positive and remaining 194 were negative. Observed data's were made as 2×2 table in [Table/Fig-1] and then sensitivity, specificity, PPV and NPV were calculated using pre-formulated formulas.

Test	ELISA (+ ve)	ELISA (- ve)	Total
Rapid Test (+ ve)	05	0	05
Rapid Test (- ve)	01	194	195
	06	194	200

[Table/Fig-1]: Showing ELISA and rapid test.

[Table/Fig-2] shows the results of ELISA and rapid cards along with the cut-off value and optical density of positive control and test sample for HBsAg positive samples.

[Table/Fig-3] shows the evaluation of Rapid ICT test with ELISA (sensitivity, specificity, PPV and NPV).

Sample number	Cut-off value	Optical Density (OD)		Results	
		Positive control	Test	ELISA	Rapid card
1	0.1	3.115	2.176	Positive	Positive
2	0.1	3.115	2.535	Positive	Positive
3	0.1	3.115	3.023	Positive	Positive
4	0.1	3.115	2.725	Positive	Positive
5	0.128	2.744	0.382	Positive	Negative
6	0.128	2.744	1.765	Positive	Positive

[Table/Fig-2]: Cut-off and OD values of HBsAg positive samples in ELISA.

Test	Sensitivity	Specificity	PPV	NPV
Rapid card	83.4%	100%	100%	99.4%
ELISA	100%	100%	100%	100%

[Table/Fig-3]: Evaluation of rapid card test with ELISA.

DISCUSSION

In the present study, a comparison was done between ELISA and rapid ICTs for the screening of HBsAg. This study aims at comparing

the analytical measures between ELISA and rapid cards for better diagnosis of HBsAg in forthcoming years. From this study results, it was found that for HBsAg screening, ELISA was more sensitive than the rapid card tests and equally specific to the rapid card tests. The following laboratory based test methods such as EIA, ELISA and PCR are time consuming and requires skilled manpower [4]. The high laboratory cost for screening of HBsAg among the poor population is also sought as the rapid card tests are cheaper in diagnosis of HBsAg [7].

In this study, the sensitivity of ICT was 83.4% and specificity was 100% and the sensitivity and specificity of ELISA was 100%. Similar to present study, Rahman M et al., has reported 53% sensitivity and 100% specificity of ICT [13]. In another study by Irwig L et al., has showed 97% sensitivity and 100% specificity [14]. One more study done by Erhabor O et al., has reported that rapid test kits has 76.9% sensitivity and specificity of 100% [1]. Kaur H et al., reported 93.4% sensitivity and 100% specificity for ICT [15]. Also, Hayder I et al., reported decreased sensitivity and equal specificity of ICT with ELISA [8]. Another study from Iran by Ansari MHK et al., shows comparable results of ICT with ELISA [16]. In contrast to present study, few other studies showed 100% sensitivity with decreased specificity [Table/Fig-4].

Studies	Publication year	Place of study	ICT		ELISA	
			Sensitivity	Specificity	Sensitivity	Specificity
Present study	2022	India	83.4%	100%	100%	100%
Erhabor O et al [1]	2014	North western nigeria	76.9%	100%	100%	100%
Mishra RK et al., [4]	2017	India	95.12%	99.82%	100%	100%
Sharma M et al., [7]	2019	India	100%	99.59%	100%	100%
Hayder I et al., [8]	2012	Pakistan	95-98%	100%	100%	100%
Raj AA et al., [12]	2001	India	95.12%	98.9%	100%	100%
Rahman M et al., [13]	2008	Pakistan	53%	100%	100%	100%
Irwig L et al., [14]	2002	Seoul	97%	100%	100%	100%
Kaur H et al., [15]	2000	India	93.4%	100%	100%	100%
Ansari MHK et al., [16]	2007	Worldwide	97.5-99.2%	97.5-99.2%	100%	100%
Zahoorullah et al., [17]	2001	Pakistan	100%	91.7%	100%	100%
Lin Y et al., [18]	2008	Ghana	100%	98.7%	100%	100%

[Table/Fig-4]: Comparison of sensitivity and specificity of ICT of various studies [1,4,7,8,12-18].

In present study, PPV of rapid card test is 100% and the NPV was 99.4% and for ELISA both were 100%. Rapid test cards fails to pick up sample with low optical density as also proved by Agrawal PC et al., [19]. One more study by the WHO showed better results with higher PPV and NPV and few false negative cases [29]. A good assay for HBV, from a diagnostic point of view is one with high PPV and less number of false negatives. Rapid kits giving more false positive results are better for the diagnosis than those giving more false negative results [7]. A false positive can be followed by more accurate and advanced method to confirm the infection (presence) unlike the false negative results which may jeopardise the human safety [4]. Hence, choosing the rapid kits with good sensitivity and NPV is essential than choosing tests with high specificity and PPV [7]. So, even before using rapid ICT cards one should be aware that different strains of antibodies used may make difference in results.

Immunochromatographic based rapid cards used for HBsAg detection might not have the same accuracy in all regions since there can be difference in the prevalence of HBV infection in a given population [7]. Most of these rapid assays use recombinant proteins from the prototype virus only, especially for HBV. Eight types of genotype of HBV are present in different parts of the world. Furthermore, the circulating subtypes and genotypes of HBV shows different geographical and epidemiological distribution [20]. In such cases, ICT that does not cover this particular subtype and will not detect this type when tested. This might be the reason why one serum sample that was negative for card was positive for ELISA. Failure of the rapid kits to identify HBV reactive samples may be due to: Insufficient coating of the HBsAg specific antibodies; Nature of antibody; Genetic heterogenicity of the virus in that area [6].

So, further studies are required regarding the circulating genotypes and mutants of HBV to prepare specific antibody coated methods to increase the sensitivity.

Limitation(s)

One was the lack of confirmatory testing for HBsAg with gold standard and another was relatively small sample size.

CONCLUSION(S)

This study demonstrates that in detecting HBsAg, ELISA was more sensitive than ICT. These rapid test devices can be used as a screening test for HBsAg only when the resources are limited, remote regions and peripheral health centres for screening purposes. Hepatitis B virus can pose a serious threat of silent transmission and spread among people and also create an urge for more sensitive assays as ELISA. The ultimate goal of this study was to recommend ELISA kits for the diagnosis of HBV irrespective of developmental and economical status of the area which detects positive cases correctly.

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