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A Comparison of Rapid Card Test with Enzyme-Linked Immunosorbent Assay for the Detection of Hepatitis B Surface Antigen [HBsAg] in Tertiary Care Hospital

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Abstract

Introduction: *Hepatitis B virus (HBV) infection is one of the leading causes of death worldwide. The most important marker for HBV infection is HBsAg. In case of diagnosis of infectious disease, discordant results may have serious consequences among the patients as it causes unnecessary mental stress and tension. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary.*

Aims and Objective: *The aim of this study was to compare the analytical sensitivity of a rapid card test in comparison to Enzyme-Linked Immunosorbent Assay (ELISA) method in the detection of HBV infection among blood samples of all age group.*

Material and Methods: *A prospective study was conducted from November 2016 to December 2016 in a tertiary care hospital at Jhalawar, Rajasthan, India. A total of 1162 blood samples were received for HBsAg diagnosis in the Department of Microbiology & Immunology, Jhalawar Medical College, Jhalawar, Rajasthan, India. Each blood sample was tested for HBsAg using both rapid card test [HEPACARD-DIAGNOSTIC ENTERPRISES] and ELISA [MERILISA-HBsAg-MERIL DIAGNOSTIC] method.*

Result: *Using ELISA as a confirmatory method, sensitivity of rapid card test was 95.12%, specificity was 99.82%, positive predictive value was 95.12%, negative predictive value was 99.82%, diagnostic accuracy was 99.65%, and kappa statistic value was 0.949.*

Conclusion: *The card test's sensitivity and specificity is comparable with ELISA. These rapid kits are cheaper and easy to perform and their use should be encouraged at rural settings where sophisticated setups are not available, so that the patient should be channelized faster towards specific and accurate diagnosis.*

Keyword: *Hepatitis B virus, Hepatitis B Surface Antigen (HbsAg), Enzyme-Linked Immunosorbent Assay (ELISA)*

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INTRODUCTION

Hepatitis B Virus (HBV) infection is a global public health problem. It is estimated that approximately 360 million people are infected worldwide with this virus [1]. Viral hepatitis is a systemic disease primarily involving the liver. Most of the cases of acute viral hepatitis are caused by Hepatitis A Virus (HAV), Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV). HBV has a double stranded DNA encoding for P, X, core and surface proteins. The complex antigen found on the surface of HBV is called Hepatitis B surface antigen (HBsAg). Antibodies against HBV proteins are other immunological markers of infection, of which Anti-Hepatitis B core antigen, Hepatitis B envelope antigen and Hepatitis B envelope antibody are also identified shortly after HBsAg, and are important markers of past or present HBV infection [2]. HBsAg appears in serum 2–10 weeks after exposure to HBV and before the onset of symptoms or elevation of serum aminotransferase levels. In self-limiting acute HBV infection, HBsAg usually becomes undetectable after 4–6 months. Persistence of HBsAg for more than six months implies progression to chronic HBV infection [3]. Consequently, HBsAg has been found to be a useful viral marker for both population screening and diagnosis of acute HBV infection or Chronic Hepatitis B infection [3].

HBsAg rapid card test is a rapid screening test for the qualitative detection of HBsAg in whole blood, serum or plasma specimen. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, serum or plasma [4]. While ELISA is an enzymatic immunoassay technique of the "sandwich" type for the detection of HBV in human serum or plasma. The test uses monoclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg now recognized by the World Health Organization (WHO) [5] and the most part of variant HBV strains [6].

Different methods are used for the diagnosis of hepatitis including Rapid card test, ELISA, Enzyme Immunoassay (EIA) and Polymerase Chain Reaction (PCR). ELISA, EIA and PCR methods are expensive and are used in well-equipped labs and major tertiary care hospitals. Rapid diagnostic kits are a good choice as they are less expensive and do not need high technical manpower or infrastructure [7]. Since 1990s, rapid tests are available for detection of HIV infection. They were intended for field survey diagnosis, emergency and home testing. In addition to rapid test for Anti-HIV, HBsAg and Anti HCV have been used for blood screening in many resource poor areas to save resources and overcome lack of funding, equipment and electrical supply. The rapid card test is known to have less sensitivity and specificity than EIA but some have sensitivity and specificity comparable to EIA [8]. A major concern in utilizing rapid screening tests 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 rapid card test of HBsAg which are frequently used in different laboratories and hospitals and to compare with already confirmed cases on ELISA. The ultimate goal of this study was 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.

MATERIAL AND METHOD

Collection of Specimen

A prospective study was conducted from November 2016 to December 2016 in a tertiary care hospital at Jhalawar, Rajasthan, India. A total of 1162 blood samples were collected and tested for HBsAg in the Department of Microbiology & Immunology, Jhalawar Medical College, Jhalawar, Rajasthan, India.

Sample Processing

Each blood sample was tested for HBsAg using both rapid card test [HEPACARD-DIAGNOSTIC ENTERPRISES] and ELISA [MERILISA HBsAg-MERIL DIAGNOSTIC] method.

Rapid Card Test Process

HEPACARD 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 flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg, 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 antibody-antigen-antibody colloidal gold complex. This forms a pink band indicating the sample is reactive for HBsAg. To serve as a procedural 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 test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate.

ELISA Method

MERILISA HBsAg is based on microwells coated with monoclonal anti-HBsAg antibody. The conjugate is polyclonal anti-HBsAg antibody labelled with horseredish peroxidase. Samples and controls are incubated in the wells and HBsAg if present bind to monoclonal anti-HBsAg antibody on the microwell. In a subsequent step conjugate is added which in turn binds to any specific antigen already bound to the antibody on the well. Unbound conjugate is washed away and a solution containing 3,3',5,5'-

tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound conjugate develop a blue to bluish green colour which is converted to a yellow to orange colour when reaction is stopped with sulphuric acid. After incubation the reactions are stopped with sulphuric acid and colour is read spectrophotometrically. The intensity of colour produced in the wells is directly proportional to the concentration of HBsAg in the sample.

RESULT

- Out of 1162 blood samples tested on rapid card test, 41 samples were positive and 1121 samples were negative for HBsAg. On further testing with ELISA, 2 false positive and 2 false negative samples were detected (Table 1).
- Using ELISA as a gold standard confirmatory method, sensitivity of rapid card test was 95.12%, specificity was 99.82%, positive predictive value was 95.12%, negative predictive value was 99.82%, diagnostic accuracy was 99.65% and kappa statistic value was 0.949 (Table 2).

Table 1: *Comparison of Rapid Card Test with ELISA.*

Rapid card test	ELISA positive	ELISA negative	Total
Positive	39	2	41
Negative	2	1119	1121
Total	41	1121	1162

Table 2: *Evaluation of Rapid Test Kits with ELISA.*

HBsAg by Rapid card test	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy	Kappa statistic value
	95.12%	99.82%	95.12%	99.82%	99.65%	0.949

DISCUSSION

In the present study ELISA was compared with the rapid kits for the screening of HBsAg. For HBsAg screening, rapid tests are equally sensitive to ELISA and yet they are cheaper and quicker. Within the rapid tests, the sensitivity and specificity was same but there were variations in the cost. ELISA, EIA, PCR 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. 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 a reasonable specificity so as to minimize false positive and false negative results.

In our study, sensitivity of rapid test kit was 95.12% and specificity was 99.82%. Raj *et al.* [9] reported that sensitivity was 79% and specificity was 98.9%. Another study showed 100% sensitivity of rapid test kit with a specificity of 91.7% for HBsAg [10]. Kaur *et al.* [11] reported 100% specificity and 93.4% sensitivity of ELISA to pick up all false negative. A study reported by Ansari *et al.* [12] showed that rapid assays with strip or device had sensitivity between 97.5% and 99.2% and specificity between 97.5% and 99.2%. In a different study using two ICAs, the sensitivity and specificity were 100%. Lin *et al.* [13] demonstrated an overall specificity of 98.7% and its sensitivity was almost 100%. A study from India by Kaur *et al.* has observed that ICAs has a specificity of 100% but the sensitivity was 93.4% [11]. Study from Seoul showed 97% sensitivity and 100% specificity for detecting HBsAg [14]. Another study among healthy individuals from Karachi, Pakistan showed comparable sensitivity and specificity of ICT kits with ELISA technique [15].

In contrast to our study, Khan *et al*. [16] found sensitivity 53% (HBsAg) although the specificity was 100%. Ideally rapid devices should have a high degree of sensitivity and a reasonable specificity to minimize false positive and false negative results. False positive in our study was 4.88% and false negative was 0.18%. False positivity was high in our study similar to Gul *et al*. [17]. Although in many instances false positive results are preferable to false negative results when screening large groups, as positive serology triggers repeat testing with alternative method for case confirmations but false

negative results may jeopardize human safety. Different ICA-based rapid assays used for HBsAg detection in the serum may not have the same accuracy index in every region since there can be differences in the prevalence of HBV infection in a given population. Most of these rapid assay use recombinant proteins from the prototype virus alone, specifically for HBV. Eight type of genotype of HBV are prevalent in different regions of the world. Moreover, the circulating subtype/s and genotypes of HBV shows varied geographical and epidemiological distribution [5]. In such cases ICA that does not cover this particular subtype/s will not detect this type when testing. This may be the reason why one serum sample that was nonreactive for one step test was reactive using the ELISA [18].

Further work is needed as data on the circulating genotypes and mutants of HBV are widely available in India. Failure of rapid test kit to detect HBV reactive samples may be due to inadequate coating of the antigen, different nature of antigen used and genetic heterogeneity of the virus prevalent in that area [19].

In our study positive predictive value (PPV) was 95.12%, negative predictive value (NPV) was 99.82%. Another study by the WHO showed a better performance (higher PPV, higher NPV and fewer cases of false negatives) value. PPV is the ability of an assay to identify actual infected individuals among all persons giving a positive result with the kit being used. NPV is the ability of an assay to identify correctly the real non-infected individuals among persons giving a negative result with the kit being used. A good assay for an infectious agent like HBV from a diagnostic point of view is one with a high PPV and less cases of false negatives [5].

Advantages and Disadvantages of Rapid Card Test Over Conventional ELISA Kits

- Compared with conventional ELISA which needs 4 h, Rapid card test results are available within 20 min. This will be very helpful in initiating immediate treatment and minimizing the serious complications and mortality of HBsAg. Conventional ELISA cannot be performed for single or small number of samples, since it would be quite uneconomical. Hence, they are used for testing of large samples and single plate can be used once with 92 samples or twice with 44 samples each time.
- Rapid card test are quite susceptible to unfavourable storage conditions, so this is essential to do periodic quality control checks to avoid false positive or false negative results.

CONCLUSION

In conclusion we reported that rapid test is less efficient than ELISA. These rapid card tests should be recommended only in resource limited poor settings, remote areas and peripheral health facilities for screening purpose. HBV are highly dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays such as ELISA. This study had some limitations. One was the relatively small sample size. Another was the lack of confirmatory testing for HBsAg. PCR is the gold standard for HBsAg detection. A major concern in utilizing rapid screening tests 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 rapid kits of HBsAg which are frequently used in different labs and hospitals and to compare with already confirmed cases on ELISA. The ultimate goal of this study was to recommend most reliable and cost-effective rapid kits for the diagnosis of HBV in areas where advance diagnostic facilities are not available.

REFERENCES

1. Chen DS. Toward Elimination and Eradication of Hepatitis B. *J Gastroenterol Hepatol*. 2010; 25: 19–22p.
2. Courouce AM, Lee H, Drouet J, *et al*. Monoclonal Antibodies to HBSAg: A Study of their Specificities for eight different Sub-types. *Developments in Biological Standardization*. 1983; 54: 527–34p.
3. Perrillo R. Hepatitis B and D. Liver, In: Feldman M, Friedman LS, Brandt LJ (Ed.). *Sleisenger and Fordtran's*

gastrointestinal and liver disease: Pathophysiology, diagnosis, management, Vol. II, 9th Edn. Philadelphia: Saunders; 2010. 1287–311.
4. Nanu A, Sharma SP, ChatterJee K, *et al.* Markers for Transfusion Transmissible diseases in Northern Indian Voluntary and Replacement Blood Donors: Prevalence and Trends. *Vox Sang*. 1997; 73: 70–3p.
5. World Health Organization. *Status of blood safety in the WHO African Region. Report of the 2004 Survey WHO Regional Office for Africa, Brazzaville*. Geneva: WHO; 2007.
6. Torane VP, Shastri JS. Comparison of ELISA HBSAg and HCV among healthy blood donors in a tertiary Care Hospital in Mumbai. *Indian J Med. Microbiol.* 2008;2: 284–5p.
7. Rahman M, Khan SA, Lodhi Y. Unconfirmed reactive screening tests and their impact on donor management. *Pak J Med Sci*. 2008; 24: 517–19p.
8. Allain JP, Lee H. Rapid tests for detection of viral markers in blood transfusion. *Expert Rev Mol Diagn*. 2005; 5: 31–41p.
9. Raj AA, Subramaniam T, Raghuraman S, *et al*. Evaluation of an indigenously manufactured rapid immunochromatographic test for detection of HBsAg. *Ind J Pathol Microbiol*. 2001; 44: 413–14p.
10. Zahoorullah, Akhtar T, Najib ul Haq, *et al*. Latex agglutination and immuno-chromatographic screening tests verses reverse passive hem-agglutination for B surface antigen in serum. *Pakistan Journal of Medical Research.* 2001; 40: 69–71p.
11. Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. *Ind J Med Sci.* 2000; 54: 432–4p.
12. Ansari MHK, Omrani MD, Movahedi V. Comparative evaluation of immuno-chromatographic rapid diagnostic tests and PCR methods for detection of human hepatitis B surface antigen. *Hepatitis Monthly.* 2007; 7: 87–91p.
13. Lin Y, Wang Y, Loua A, *et al*. Evaluation of a new hepatitis B virus surface antigen rapid test with improved sensitivity. *J Clin Microbiol.* 2008; 46: 3319–24p.
14. Irwig L, Bossuyt P, Glasziou P, *et al*. Designing studies to ensure that estimates of test accuracy are transferable. *BMJ*. 2002; 324: 669–71p.
15. Qasmi SA, Aqeel S, Ahmed M, *et al*. Detection of Hepatitis B Viruses in Normal Individuals of Karachi. *J Coll Physicians Surg Pak*. 2000; 10: 467–9p.
16. Khan JK, Lone DS, Hameed A, *et al.* Evaluation of the performance of two rapid immunochromatographic tests for detection of HBsAg and anti HCV antibody using ELISA tested samples. *Annals of King Edward Medical University*. 2010; 16(S1): 84–7p.
17. Gul N, Sarwar J, Idris Muhammad, *et al*. Seroprevalence of hepatitis C in pregnant females of hazara division. *Journal Ayub Medical College Abbottabad*. 2009; 21: 83–6p.
18. Purdy MA, Talekar G, Swenson P, *et al*. A new algorithm for deduction of hepatitis B surface antigen subtype determinants from the amino acid sequence. *Intervirology*. 2006; 50: 45–51p.
19. Torane VP, Shastri JS. Comparision of ELISA and Rapid screening test for the diagnosis of HIV, HBV and HCV among healthy blood donor in a tertiary care hospital in Mumbai. *Ind J Med Microbiol*. 2008; 2: 284–5p.

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