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A Comparative Study of Elisa, ICT & CMIA in the Detection of Hepatitis B Surface Antigen among Patients Attending a Tertiary Care Hospital in South India

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Abstract

Background: Hepatitis B virus (HBV) is the second most common cause of acute viral hepatitis after hepatitis E virus (HEV) in India. The complex antigen found on the surface of HBV is called Hepatitis B surface antigen (HBsAg). Both serological and molecular screening tests are being employed for the diagnosis of HBV infection. Among all HBSAg assays, ELISA techniques are the most frequent used because of their effectiveness. Rapid test (Immunochromatographic tests- ICT) enables early detection at places where laboratory facilities or trained manpower are not available or there is issue of accessibility. Chemiluminescent microparticle immunoassays (CMIA) use signal amplification to give quantitative measurements.

Aims & Objectives: To compare the results of all three diagnostic tests(ICT, ELISA, CMIA) in the screening of HBsAg in blood samples.

Material And Methods: 100 blood samples collected aseptically for Hepatitis B surface antigen screening were centrifuged and serum was subjected to all three tests(Immunochromatographic Test, ELISA and CMIA) as per manufacturer's instructions and results were compared.

Results: Among 100 serum samples tested, 1(1%) was reactive by ICT, 4(4%) were reactive by ELISA and 5 (5%)were reactive by CMIA.

Conclusion: The present study inferred that both ELISA and CMIA had comparable results, but the sensitivity of ICT was very less.

Keywords: CMIA, ELISA, ICT, Hepatitis B virus, HBsAg.

Introduction

The enigma of hepatitis began in 3rd millennium B.C. in Sumeria with the first description of jaundice. Epidemic icterus was reported by Hippocrates (460 to 375 B.C.) followed by numerous vague descriptions by Greeks and Romans.[1]

Hepatitis B Virus infection is a global public health problem. It is estimated that approximately 360 million people are infected worldwide with this virus [2].

World Hepatitis Day is observed on 28th July, the birth anniversary of Nobel Laureate Baruch Samuel Blumberg.

Hepatitis B virus (HBV) is the second most common cause of acute viral hepatitis after hepatitis E virus (HEV) in India.[3] Population prevalence of chronic HBV infection in India is around 3–4%. There is a wide variation in hepatitis B surface antigen (HBsAg) prevalence in different geographical regions in India.[4] Genotypes 1 and 4 of HBV and genotypes 1 and 3 of HCV are more prevalent in India.[3]

Infection with HBV results in a wide spectrum disease from subclinical to fulminant hepatitis leading to death. Since these virus are mostly transmitted by transfusion of contaminated blood and blood product, however, other subjected modes of transmission like intravenous drug abuse, close personal contact, use of shared needle, razor etc cannot be ignored. Approximately, 75% acutely infected patients develop chronic hepatitis infection that commonly progress to liver cirrhosis and hepatic malignancy.[5]

The complex antigen found on the surface of HBV is called Hepatitis B surface antigen (HBsAg). In a typical Hepatitis B infection, Hepatitis B surface antigen (HBSAg) will be detected within 2 to 5 weeks before symptoms or jaundice develop. [6]

Both serological and molecular screening tests are being employed for the diagnosis and patient monitoring of HBV infection. Among all HBSAg assays, ELISA techniques are the most frequent used because of their effectiveness.

Enzyme-linked Immunosorbent Assay (ELISA) is an enzymatic immuno-assay technique of the “sandwich” type for the detection of Hepatitis B virus in human serum or plasma. The test uses monoclonal antibodies selected for their ability to bind themselves to the various sub-types of HBSAg .[7]

Rapid test (Immunochromatographic tests- ICT) enables early detection at places where laboratory facilities or trained manpower are not available or there is issue of accessibility. Most rapid tests are based on immunochromatographic principles. [8]

Chemiluminescent microparticle immunoassays (CMIA) use signal amplification to give quantitative measurements.

This study aimed to compare all three diagnostic tests(ICT, ELISA, CMIA) in the diagnosis of HBsAg . There are very few such studies reported from India and thus this study would enhance our knowledge regarding these tests.

Material and Methods

Study Design: Cross-sectional Hospital based study

Study Duration: 2 months (1st June- 31st July 2019)

Study Population: 100 patient blood samples sent for screening from various clinical departments

Inclusion criteria

- Samples from patients of all age groups and both the sexes.
- Blood samples sent for screening of Hepatitis B Surface Antigen from various clinical departments.
- Both in-patients & out-patients.

Exclusion criteria

- Clotted blood samples
- Hemolysed blood samples
- Patients who don’t give consent.

Methodology: This study was conducted in the diagnostic laboratory of Department of Microbiology, Mandya Institute of Medical Sciences after obtaining approval from Institutional Ethical Committee. 100

blood samples collected aseptically for Hepatitis B surface antigen screening were centrifuged and serum was subjected to all three tests(Immunochromatographic Tests, ELISA and CMIA) as per manufacturer's instructions and results were compared.

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.Cut-off value was calculated by adding 0.100 to the mean of the Negative Control replicates.

Virucheck Immunochromatographic test card(Tulip Diagnostics) was used for testing the serum for HBsAg. Coloured bands at Control region and Test Region was considered Positive. Virucheck detects Hbsag at concentrations as low as 0.5ng/ml.

The Architect i1000SR HBsAg Qualitative II 4TH Gen(Abbott Laboratories, Abbott Park, Illinois, USA) is a chemiluminescence microparticle immunoassay (CMIA) for the qualitative detection of HBsAg in human serum and plasma. Values higher than 1.00 (≥1.00) indicated the reactive result, values lower than 1.00 indicated the nonreactive result. All tests including calibrations and control were performed and interpreted in accordance with the manufacturers' recommendations.

Results

Among 100 serum samples tested, 1(1%) was reactive by ICT, 4(4%) were reactive by ELISA and 5 (5%)were reactive by CMIA.

Table 1: Results of all 3 diagnostic tests

TESTS Results	ELISA N (%)	ICT N (%)	CMIA N (%)
Reactive	4 (4%)	1 (1%)	5 (5%)
Nonreactive	96 (96%)	99 (99%)	95 (95%)
Total	100	100	100

Table 2: Comparison of ICT with ELISA

ICT	Elisa Positive	Negative	Total
Positive	1	0	1
Negative	3	96	99
Total	4	96	100

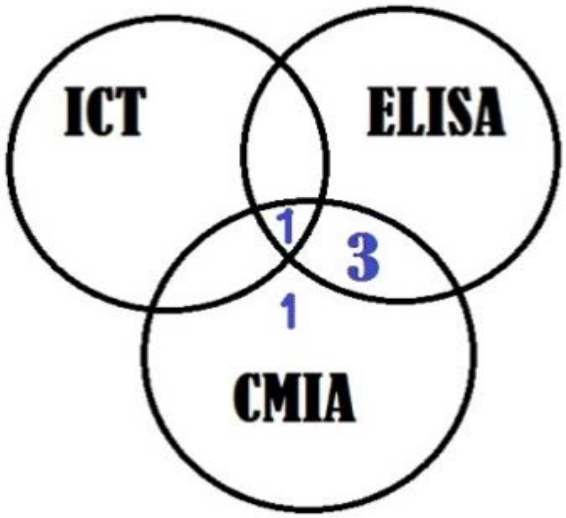
Sensitivity of ICT =25%, Specificity of ICT= 100%, PPV=100%, NPV= 96.9%

Table 3: Comparison of CMIA with ELISA

CMIA	Elisa Positive	Negative	Total
Positive	4	1	5
Negative	0	95	95
Total	4	96	100

Sensitivity of CMIA=100%, Specificity= 98.9%, PPV=80%, NPV=100%

Fig 1: Comparison of results of all 3 tests



Among 5 Reactive samples by CMIA, 3 were males and 2 were females. 1 female among 2 was pregnant. The Instrument value of all of them were above 2000(>cut off value of 1) except 1 pregnant female whose Instrument value was 1.41. This was subjected to repeat CMIA which showed the Instrument value as 1.38. She was suggested PCR & PCR was negative. This suggests the possibility of CMIA being false positive inspite of repeat tests.

Discussion

Hepatitis B virus (HBV) is a hepadnavirus which can cause either acute or chronic infection, and the associated illness ranges in severity from asymptomatic to symptomatic, progressive disease.[9]

Among 100 serum samples tested, 1 was reactive by ICT, 4 were reactive by ELISA and 5 were reactive by CMIA. All 4 samples reactive by ELISA were reactive by CMIA, in addition, 1 more sample was reactive by CMIA.

We observed discordant results between the ICT and ELISA with 3 samples(negative with ICT but positive with ELISA).

Our study showed 100% specificity and 25% sensitivity of ICT compared to ELISA. The study conducted in Lahore, Pakistan by Khan et al (2010) demonstrated 93% to 100% specificity for HBsAg by ICT method but the sensitivity was 50%.[10] They observed that ICT fell short of being the ideal screening test for the detection HBsAg as there are a large number of false negative results resulting in low sensitivity and negative predictive values.They concluded that the rapid immuno-chromatographic kits for HBsAg limited efficacy and should be backed by superior methods like ELISA and PCR where possible.

An Indian study reported 100% specificity and 93.4% sensitivity of rapid kits when detecting HBsAg.[11] In another study from Seoul for detecting HBsAg, rapid technique showed 97% sensitivity and 100% specificity.[12]

Very early stages of infection and patients in the recovering stages usually have low viral titers, reflected by low optical density in the test results. These low positive cases may not be detected by rapid screening test like ICT; these can be detected by ELISA and CMIA.[13] Also, failure of the rapid kits to detect the presence of markers of infectious viral diseases may be due to inadequate coating of the antigen, nature of the antigen used and genetic heterogeneity of the virus .[7]

Our study showed Specificity and NPV of CMIA as 98.9% and 100% compared to ELISA. This is in agreement with a study by Borhan etal where Specificity and negative predictive value (NPV) of CMIA in analysis of HBsAg was 98.7% and 100% respectively when compared with ELISA.[13]

1 sample of a pregnant woman which was positive by CMIA(low instrument value) was negative by ELISA & rapid test. False positive results may be expected by CMIA in pregnancy.

The major challenges with ELISA and CMIA is that they are more expensive, require use of instrumentation and requires trained personnel.

Conclusion

The present study was carried out as a pilot basis to compare two methods ICT and CMIA with ELISA for detection of HBV seromarker HBsAg. Although the number of samples tested was limited, we could infer that the two methods(ELISA and CMIA) had performed equally well in limited resource settings.

Limitation of the study: Sample size was small, PCR could not be done for all CMIA positive samples .

References

1. Trepo C. A brief history of hepatitis milestones. Liver Int. 2014;34 Suppl 1:29–37.
2. Chen DS (2010) Toward Elimination and Eradication of Hepatitis B. J Gastroenterol Hepatol 25: 19-22.
3. Aakash S, *et al.* Hepatitis in India: Burden, strategies and plans. NCDC Newsl 2014;3:1-3.
4. Viral Hepatitis – The Silent Disease Facts and Treatment Guidelines by National Centre for Disease Control, Directorate General of Health Service, Ministry of Health and Family Welfare, Government of India; 2015.
5. Ahmed Q, Chowdhury SG, Islam MN, Khan FD, Alam MR, Miah AH. HBsAg amongst unscreened operated patients. Bangladesh Med Red Counc Bull 1991;17:11-6.
6. Courouce AM, Lee H, Drouet J, Canavaggio M, Soulier JP (1983) Monoclonal Antibodies to HBSAg: A Study of their Specificities for eight different Sub-types. Developments in Biological Standardization 54: 527-534
7. Torane VP, Shastri JS (2008) Comparison of ELISA HBSAg and HCV among healthy blood donors in a tertiary Care Hospital in Mumbai. Indian J Med. Microbiol 284-285.
8. Plitt SS, Somily AM, Singh AE. Outcomes from a Canadian public health Prenatal Screening program for hepatitis B: 1997-2004. Can J Public Health 2007; 98: 194-7
9. C.-L.Lai, R.-N.Chien, N.W.Y. Leung et al., “A one-year trial of lamivudine for chronic hepatitis B,” The New England Journal of Medicine, 1998 ;vol.339(2):61–68
10. Khan JK, Lone DS, Hameed A, Munim M, khattak AA, Usman M, et al. Evaluation of the performance of two rapid immunochromatographic tests for detection of hepatits B surface antigen and anti HCV antibodies using ELISA tested samples: Special Edition Annals 2010; 16:84-7.
11. Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. Indian J Med Sci 2000; 54: 432-4.
12. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J.Designing studies to ensure that Cestimates of test accuracy are transferable. BMJ 2002; 324: 669-71.
13. Borhan Uddin Sheik, Dr. Rosy Sultana, Dr. Manjuma Rahman, Mohammad Moniruzzaman. Evaluation of hepatitis b and c virus seromarkers of donor's blood using three different methods. International journal of scientific research. 2017; 6 (7) : 40-42