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Original Research Paper

Microbiology

EFFICACY OF RAPID IMMUNOCHROMATOGRAPHIC TEST IN THE DIAGNOSIS OF HEPATITIS B.

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ABSTRACT

Introduction: Hepatitis B (HBV) is a hepatotropic viral disease causing complications like fulminant hepatitis, cirrhosis of liver and liver cancer. The standard method for diagnosis of Hepatitis B is detection of HBsAg by ELISA. But in emergency conditions and in primary healthcare centers an efficient rapid test is required for Hepatitis B diagnosis.

Aims & Objectives:

To assess efficiency of rapid test to ELISA in detection of HBsAg in the diagnosis of Hepatitis B in general population at a tertiary health care center.

Method: A prospective, cross sectional study was conducted for a period of one year at a tertiary care hospital and included 600 subjects of all age groups. Five milliliter of blood was collected for testing Hepatitis B by both rapid (HBsAg Trustline, Chennai, India) & ELISA (HBsAg MERILISA, Gujarat, India) tests. Results were analyzed statistically.

Result:

HBsAg Rapid test gave a sensitivity of 94% & specificity of 100% with a PPV of 100% & NPV of 99.83%, taking ELISA as the gold standard.

Conclusion:

The Rapid test(HBsAg Trustline) was found to be an efficient point of care test in the diagnosis of Hepatitis B and could be used in healthcare setup where rapid and accurate results are desired.

KEYWORDS : Hepatitis B, Rapid HBsAg test, HBsAg ELISA

INTRODUCTION:

Hepatitis B (HBV) is a hepatotropic viral diseases and can cause acute as well as chronic infection. It can cause fulminant hepatitis leading to hepatocellular carcinoma. As little as 0.00001ml of blood is sufficient to cause Hepatitis B infection [1]. PCR is the gold standard used in the diagnosis of Hepatitis B but the most common method is detection of HBsAg by ELISA. ELISA requires infrastructure, time and skilled personnel. In cases like needle prick injury, enucleation cases, emergency surgical procedures or whenever there is an urgent need to know patient's serostatus, a rapid, accurate test becomes necessary for optimum diagnosis & management of Hepatitis. Thus, the present study was carried out to find out efficacy of rapid test for HBsAg in the diagnosis of hepatitis B.

AIMS & OBJECTIVES:

To assess efficacy of HBsAg rapid test to HBsAg ELISA in the general population for the diagnosis of Hepatitis B at a tertiary health care center.

MATERIALS AND METHODS

The present study is a prospective, cross sectional study and conducted at Department of Microbiology of a tertiary care hospital in Mumbai for a period of one year (1st May 2017-30th April 2018) and included 600 subjects. Sample size was calculated by following formula

$$\text{Sample size}(n) = Z_{1-\alpha/2}^{2} \frac{P(1-P)}{d^2}$$

Where,

n = sample size

P = anticipated population proportion

d = absolute precision required on either side of the proportion

α = error

Z = constant

Inclusion Criteria:

The inclusion criteria of the study were to include patients of all age groups and both sexes coming with clinical suspicion of hepatitis, namely complaints of fever with nausea, vomiting, joint pain, loss of appetite, jaundice and pain in abdomen. These patients were either registered at the outpatient department (OPD) or were patients admitted in wards and had been advised to undergo hepatitis B screening.

Exclusion Criteria:

Patients who had previously tested positive for hepatitis B & also patients who refused to give consent were excluded from the study.

Ethics Permission:

The study was initiated after approval was given by Institutional Ethics Committee.

METHODOLOGY:

Study Group:

The study group included different segments of the general population with varied risk factors and co morbidities and all who satisfied the inclusion criteria.

Study Procedure:

All patients (n= 600) satisfying the inclusion criteria were included in the study. A detailed clinical history as per case record form was taken & patients were explained about the test procedure. Written consent was taken.

Collection Of Specimen:
Five milliliter of fasting blood sample from each patient was collected in a plain vaccutainer. The serum was separated in sterile vials and then tested by a rapid HBsAg immunochromatographic test and HBsAg ELISA for hepatitis B, as per kit literature. ELISA was taken as the gold standard. The details of rapid test and ELISA test kits used in the present study are as follows:

- HBsAg Trustline, Athenese Dx Pvt. Ltd. (Chennai, India), lot no. F0505171, expiry date 04/05/2019.
- HBsAg MERILISA kit of Meriline diagnostic Pvt. Ltd. (Gujarat, India), lot no. MI011844, expiry date 2019/03.

Known serum specimens who had tested positive & negative were included as external positive & negative quality controls respectively.

The rapid test used in the present study is a qualitative test based on the principle of lateral flow immunochromatographic assay for the qualitative detection of hepatitis B surface antigen (HBsAg). The rapid test detects HBsAg at a level equal to or higher than 0.5ng/ml as claimed by the manufacturer. 60-90 μl of sample of serum or plasma is to be used for the test. The results are available in 15 minutes. As per the manufacturers kit insert the relative sensitivity is of 99.21% and relative specificity 99.78%.

Statistical Analysis:
The results of the study were analyzed statistically by Fischer's exact test and odds ratio.

RESULT AND DISCUSSION:
Hepatitis B is widely distributed in the world. An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive) [2].

In the present study, a total of 600 serum specimens of patients satisfying the inclusion criteria were subjected to both HBsAg ELISA and HBsAg rapid tests. Of 600, seventeen were HBsAg reactive and 583 did not show HBsAg in ELISA. The mean age of the patients tested positive by ELISA was 38.12 years and consisted predominantly of males.

Table–1 Evaluation Of HBsAg Rapid To HBsAg ELISA Tests

HBsAgRapid	HBsAgELISA		Total
	Positive	Negative	
Positive	16(2.7%)	0(0%)	16(2.7%)
Negative	1(0.2%)	583(97.2%)	584(97.3%)
Total	17(2.8%)	583(97.2%)	600(100%)

Table- 2 Statistical Parameters Of HBsAg Rapid Test For Hepatitis B

sensitivity	a/(a+c)	0.9412
specificity	d/(b+d)	1.0000
positive likelyhood ratio	sen/(100-sp)	0.0095
negative likelyhood ratio	100-sen/sp	99.0588
disease prevalence	(a+c)/(a+b+c+d)	0.0283
diagnostic accuracy	(a+d)/(a+b+c+d)	0.9983
Positive Predictive Value (PPV)	a/(a+b)	1.0000
Negative Predictive Value (NPV)	d/(c+d)	0.9983

In the present study, it was observed that of the 17 positive cases, sixteen (2.7%) cases were positive by both rapid test and ELISA (true positive), While 1 (0.2%) of 17 cases was negative by rapid test but positive by ELISA (false negative). None of the sample showed false positive test by rapid test. Hence, the sensitivity of rapid card test used in the present study was 94.12% & specificity was found to be 100% using ELISA as the gold standard test. The positive predictive value was found to be 100% & negative predictive value was found to be 99.83% (**Table 2**). The time taken for performing the rapid test and ELISA was 15 minutes and approximately 2 hours respectively. No special infrastructure was required for the rapid test as compared to ELISA which requires ELISA reader, ELISA washer, etc for performing ELISA. Performing and then reading and interpreting the results of ELISA required skilled personnel compared to that for the rapid test.

Hepatitis B is a hepatotropic virus affecting humans all over the globe. The viruses may have a deleterious effect on liver if undiagnosed or untreated. The number of HBV related deaths due to liver cirrhosis and/or hepatocellular carcinoma (HCC) increased between 1990 and 2013 by 33%, relating to 686,000 cases in 2013 worldwide [3]. Hence, there is a need to diagnose the disease at the earliest by having a point of care test which is rapid, accurate, does not require skilled personnel and also does not require an elaborate infrastructure. This would thus, enable the test to be performed both in field or tertiary health care settings.

Table 3 – Comparative Studies Of HBsAg Rapid Tests In The Diagnosis Of Hepatitis B

Study by	Year	Kit Name / Manufacturer	Sensitivity	Specificity
Ansumana R. *et al.* [4]	2013	SD Bioline (Standard Diagnostics, Republic of Korea)	98%	99%
Khuroo M. *et al.* [5]	2014	Vikia Hbs Ag (Biomerieux, Brazil)	99 %	99%
Khuroo M. *et al.* [5]	2014	Determine HBsAg (Abbott, Japan)	98%	99%
Tiwari Y. *et al.* [6]	2016	Hepacard (J. Mitra & Co. Pvt. Ltd. New Delhi, India)	95.12%	99.82%
Present study	2018	HBsAg Trustline (Athenese Dx Pvt. Ltd. Chennai, India)	94.12%	100%

Table 3 shows various studies comparing sensitivity and specificity of Rapid HBsAg test of different manufacturers. Different immunochromatography assays (ICA) based rapid tests used for HBsAg detection in the serum may not have the same accuracy index and varies with the different manufacturers as seen in the table. The rapid test used in the present study has sensitivity lower than other test kits but it has the highest specificity thus making it an accurate point of care test which could be used in health care settings which do not have the infrastructure nor the skilled personnel to perform ELISA or PCR for diagnosing Hepatitis B.

Self evaluation of the test kits also helps in assessing the actual performance of the test kits with reference to its sensitivity, specificity, PPV & NPV. Hence, choosing the rapid test with the highest sensitivity and specificity is essential to help detect cases of Hepatitis B more accurately for optimum management.

CONCLUSION:
In the present study, the Rapid test (HBsAg Trustline) for diagnosis of Hepatitis B was found to have a sensitivity of 94% and a specificity of 100% making it an efficient point of care test in the diagnosis of Hepatitis B, especially in healthcare setup where quick results are desired. Rapid antigen detection test have maximum utility in casualty, emergency laboratories and in intensive care setups not only in tertiary care hospital but also in primary health care centers.

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