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Study of Incidence of Hepatitis B and A Comparative Study to Check the Specificity & Sensitivity of Immunochromatographic Technique (ICT with Enzyme Linked Immunosorbant Assay (ELISA) for Hepatitis B Infection

Medical Science

KEYWORDS : Hepatitis B, Immunochromatographic test, ELISA, Incidence, Specificity

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ABSTRACT

2-5% of the adult population in India is estimated to be infected with Hepatitis B (HB), which has been declared endemic in Asia by World Health Organization (WHO). Detection of HB is expensive and time consuming. This study determined the incidence of HB among residents of Navi Mumbai and evaluated the performance of Rapid ICT as against standard ELISA test; to rationalize ICT 's reliability to detect HB. Incidence of HB was calculated from among 5629 patients who visited the hospital during study period and amongst those 135 randomly selected patients, 50 and 85 rapid diagnostic test positive and negative, respectively were subjected to ELISA. It was observed that in comparison with ELISA, rapid ICT test had 94.2% sensitivity and 97.61% specificity. Hence, it may be concluded that ICT can be used as a rapid screening test, however patients exhibiting strong symptoms of HB infection, but detected negative by ICT test should be confirmed by ELISA.

Introduction

Hepatitis B virus (HBV) infection is a global public health problem. Almost two billion people in the world have been infected by HBV and with nearly 350 million people having chronic HBV infection. Around 10% of HBV infected patients develop acute fulminant and since HBV is the most common cause of liver cirrhosis, hepatocellular carcinoma and liver failure HBV infection has high mortality rate.[1],[2],[3] Studies estimate that 15-25% people with chronic HBV infection would die owing to liver failure aggregating to an estimate of one million people each year. [3][4] Hence, as a precautious measure blood banks have made it mandatory to test blood sample for HBV along with other tests like HIV, HCV, VDRL, Malaria parasites and so on.

WHO estimates (August 2008) Hepatitis B as a major health problem in India. Based on the prevalence of Hepatitis B carrier state in the general population, countries are classified as having high (8% or more), intermediate (2-7%), or low (less than 2%) HBV endemicity. India has intermediate endemicity of Hepatitis B, with Hepatitis B surface antigen (HBsAg) prevalence between 2% and 8% and the number of HBsAg carriers in India has been estimated to be over 40 million (4 crores).[5] Quantitative Enzyme Linked Immunosorbant Assay (Q-ELISA) method is considered to be the most specific test for detection of HBV infection and is widely used at well-equipped research centers/laboratories or blood banks. However they have some limitations like, large number of samples is required to perform the test. Minimum six controls (positive, negative and cut-off value controls) must be used with each test run for validity and performance of the test under consideration. Also expensive reagents, instruments and trained staff are a prerequisite.

Hence we studied the reliability of a rapid screening test ICT, which gives results in 15-20 minutes as against standard ELISA which takes 2-3 hours.

Materials and Methods

Time and location: This study was conducted between, January and December, 2010, as a hospital based study at MGM Hospital, Kamothe, Navi Mumbai. The main reason for selecting this area was that no previous studies were conducted to check the incidence as well as to compare the specificity and sensitivity of ICT with respect to standard ELISA method for detection of Hepatitis B viral infection specifically in this region. The population of Navi Mumbai is estimated to be 2 million (Census of India 2001).

Study sample: For calculating incidence of the disease, a total of 5629[1] blood samples were received at microbiology laboratory for testing of HBsAg. The test was carried out using Hepacard (manufactured by "Biomed Industries" plot no. 20-A, Sector-1, Industrial Estate, Parwanoo-173220, Himachal Pradesh) method.

To compare the sensitivity and specificity of ICT and ELISA, 135 patients were selected randomly, out of which, 50 were ICT positive and 85 were ICT negative and all the samples were then subjected to ELISA test.

Sample Collection: 2 ml of blood sample was collected under aseptic conditions in a plain and sterile test tube and serum was separated by centrifugation at 1509 g.

Laboratory Techniques: ICT, as a preliminary test, and ELISA as a confirmatory test were performed on the selected samples.

ICT for detection of HbsAg- HEPACARD test strips were used. It is a one step immunoassay, based on the principle of antigen capture, used to diagnose acute and chronic carriers of HBV. Before proceeding with the test procedure, the reagents and specimens were brought to room temperature (RT). Upon removing the test strips from the foil pouch and placing them on a clean dry surface, 2 drops (~70µl) of test sample were added to the card strips. The reaction was incubated for 20 minutes at RT and the results were then interpreted in accordance with the appearance of the band.

ELISA for detection of HBsAg- HEPALISA (Manufactured by J. Mitra & Co. Pvt. Ltd., New Delhi) and QUALISA (manufactured by QUALPRO Diagnostics, Goa) test kits were used for in-vitro detection of HBsAg including subclasses of HBsAg (ad, ay) in human plasma or serum. Kit reagents and samples were thawed to room temperature (20ºC-25ºC) before beginning the procedure. 2 negative and 3 positive controls were included in each run. The reagents were prepared as per the protocol provided with the kit. The O.D. (Optical Density) was read at 450nm in ELISA reader (manufactured by Biotek Instruments, Inc.) after rectifying A-1 well as blank. Cut off value was calculated as per kit protocol. Standard O.D. values for positive and negative controls were checked.

Interpretations of results:

Positive- Specimens with absorbance value equal to or greater than the cut-off value were considered HBsAg positive (reactive).

Negative- Specimens with absorbance value less than the cut-off value were considered HBsAg negative (non-reactive).

Calculations

The total numbers of the positive cases were 133[2]. The rate of incidence is calculated as per the formula:

Rate of Incidence =	Number of Newly detected cases		X 100
	Total population studied		
=	133	X100	
	5629		
=	2.36%		

The rate of incidence of hepatitis B was determined to be 2.36%.

Sensitivity and Specificity were calculated by comparing the results of ICT against the results of ELISA, while considering the later as standard. Please refer Table No 1 for more details.

Table No. 1

ICT positive and ELISA positive	48[a]	True positive
ICT positive and ELISA negative	02[b]	False positive by ICT
ICT negative and ELISA positive	03[c]	False negative with ICT
ICT negative and ELISA negative	82[d]	True negative

Sensitivity = true positive/ (true positive + false negative) X100 [a/a+c X 100]

Specificity = true negative/ (true negative+false positive) X 100 [d/d+b X 100]

Performance characters of ICT device are as follows (Table 2)- ICT can detect hepatitis B surface antigen in serum or plasma at a concentration of, or more than, 0.5ng/ml

Table No. 2

Sensitivity	94.12%
Specificity	97.61%
Positive predictive value	96%
Negative Predictive value	88%

Statistical significance of ICT
Table No 3

	Positive	Negative	
ICT	50 [a]	85 [b]	135 [a+b]
ELISA	51 [c]	84 [d]	135 [c+d]
Total	101 [a+c]	169 [b+d]	270 [a+b+c+d]

Chi square test $\Sigma [(O-E)2 / E]$
Where, O= observed frequency and
E= expected frequency.

Interpretation of Chi Square test

$\chi 2$ CAL < $\chi 2$TAB
There is no significant difference between the two tests (at 95% C.I. or 5% level of significance and P>0.05)

$\chi 2$ CAL > $\chi 2$TAB
There is significant difference between the two tests. (at 95% C.I. or 5% level of significance and P<0.05)

So expected frequency calculated by the standard formulas are obtained are presented in Table No 4.

Table No. 4

	Positive	Negative
ICT	50.5 [E1]	84.5 [E2]
ELISA	50.5 [E3]	84.5 [E4]

Observation Table
Table No. 5

O	E	O-E	(O-E)2	(O-E)2/E
50	50.5	-0.5	0.25	0.005
85	84.5	1.5	2.25	0.002
51	50.5	1.5	2.25	0.04
84	84.5	-0.5	0.25	0.002
TOTAL				0.049

$\chi 2 = \Sigma [(O-E)2 / E]$

$\chi 2$ CAL = 0.049

$\chi 2$ CAL < $\chi 2$TAB

0.049 < 3.84 ($\chi 2$ 1, 0.05)

Hence, there is no significant difference between the ICT and ELISA methods for screening of HBsAg (at 95% C.I. or 5% level of significance and P>0.05)

Discussion

ELISA test is considered as standard diagnostic tool for many serological tests. However, ELISA requires expensive instruments, reagents and trained staff. Hence there was a need for simpler technology and ICT were developed. The advantage with ICT is that the test can be performed on a single blood sample with a built in control. Moreover, it does not require trained staff and produces quick results (20 minutes). However there was a need to compare the performance of ICT with ELISA.

The Rapid tests that are currently available for the detection of HBsAg range in sensitivity from 50-94% relative to ELISA results depending on the technology applied.[6]

The ICT test was carried out on 5629 patients during study period as (a) surgical (pre-operative check-up), (b) clinical (exhibiting clinical suspicion) or (c) routine check-up. The incidence of HBsAg infection was calculated from the total samples with the help ICT positive results. From total 5629 patients, 133 were positive on whom HBsAg test was carried by ICT. The incidence of Hepatitis B was found to be 2.36% in the population of Navi Mumbai. For the comparative study, 135 patients were selected from the total 5629 patients, out of which 50 were rapid diagnostic test positive and 85 were rapid diagnostic test negative samples.

Out of 135 patients, 51 were positive and 84 were negative by ELISA. There were 03 false negative and 02 false positive by ICT. The minimum antibody concentration of detection for HBsAg of ICT is 0.5 ng/ml and for ELISA is 0.2 ng/ml. The three false negative cases of ICT could have been between 0.3-0.5 ng/ml which is below the detection limit of ICT and so were not detected by ICT but were detected by ELISA. Considering quantitative ELISA results, as gold standard method, our rapid tests with HEPACARD showed sensitivity of 94.12% and specificity of 97.61%, which is comparable with the results obtained by D.T.Y. Lau, et al. In their study, a commercially developed, rapid Immunochromatographic test for detection of HBsAg was evaluated using a total of 2463 selected samples (827 frozen sera, 1011 fresh sera, and 625 whole blood samples). Results of the rapid test were compared with standard enzyme immunoassay (EIA) methods for HBsAg detection. The overall sensitivity and specificity for the detection of HBsAg were 95% and 100%.[7] In a similar study carried out by Abdelbagi M. Nagi et al., the

results obtained for HBsAg showed that no significant difference (P>0.05) was observed between the two techniques.[8] In an experiment carried out in Seoul, Irwig L et.al., (2002) documented that Immunochromatographic technique has sensitivity of 97% and specificity of 100% for detecting surface HBsAg. [9] The performance of rapid tests varies widely but some have sensitivity and specificity levels that meet standards established by ELISA for HBsAg[9,10] However, it is known to be less sensitive than ELISA.[11]

Conclusion

The difference between ICT device and ELISA test as gold standard is statistically not significant (at P>0.05). These findings correlate well with the previous studies.

Detection value of ELISA is 0.2ng/ml while that of ICT is 0.5ng/ml. Hence, there is a need for improving the detection limit of ICT.

It is concluded that the rapid/simple tests were easy to perform and interpret, required no (or minimal) laboratory equipment, and could be taught easily to local laboratory personnel. The cost of these tests is less than that of routine ELISA methods [12]

Also, we conclude that the ICT test could be used as a rapid screening test to detect presence of HBsAg in patients but the positive results should be confirmed by performing ELISA test for the same sample.

However, if ICT test result is negative and there is suspicion of HBV infection considering the previous history and clinical symptoms, it will be necessary to repeat the ICT and ELISA be used as confirmatory.

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