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Study the Prevalence of Hepatitis B and Evaluation of Two Rapid Immunochromatographic Tests for the Detection of Human Hepatitis B Surface Antigen in a Tertiary Care Hospital in New Delhi, India

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Hepatitis B viral (HBV) infection is a leading cause of liver disease worldwide. This study was done to see the prevalence of hepatitis B in patients reporting at our hospital and evaluation of two rapid Immunochromatographic testing (ICT) kits for HBs Ag detection. A total no. of 5024 serum samples were collected wef Jan 2011 to July 2011 from patients and were tested for HBs Ag by ELISA. The two ICT tests- *Instachk™* by InTec Products INC and *HEPA™CARD* by Reckon Diagnostcs Pvt. Lt. were performed on Elisa tested samples for HBV- Reactive (n = 137) and HBV- Non reactive (n = 150) samples. Prevalence of Hepatitis B in our study was found to be 2.73 %, 137 being reactive out of 5024 samples by ELISA. The I.C.T. results showed a sensitivity of 94.16 % and 51.09%, specificity of 100% and 96.67 %, positive predictive values of 100% and 93.33 % while the negative predictive values of 94.93 % and 68.39 % for *Instachk™* and *HEPA™CARD* kits respectively. These results suggest that the rapid ICT kits for HBsAg have only limited efficacy and should be backed by superior methods like ELISA and PCR where possible.

Key words: Prevalence, HBV, hepatitis, ELISA, Immunochromatographic tests, HBs Ag.

Hepatitis B is a major public health problem worldwide. Approximately 30 percent of the world's population, i.e. more than 2 billion persons, have serological evidence of current or past HBV infection. Of these, an estimated 360 million have chronic HBV infection and at least 600,000 persons die annually from HBV-related chronic liver disease, including cirrhosis and liver cancer[1,2].

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India has intermediate endemicity of Hepatitis B, with Hepatitis B surface antigen (HBsAg) prevalence of 2% to7% among populations studied. The prevalence does not vary significantly by region in the country. The number of HBsAg carriers in India has been estimated to be over 40 million (*4 crore*). It has been estimated that, in India of the 25 million infants born every year, over one million run the lifetime risk of developing chronic HBV infection. Every year over 100,000 Indians die due to illnesses related to HBV infection[3].

The prevalence of HBV infection within dialysis units reported in developing countries varies from 2–20%[4,5]. Prevalence of HBV Infections in Indian Patients with Chronic Renal Failure has been reported to be 7% to 7.45%[4,6].

The prevalence of HBsAg positivity among asymptomatic pregnant women in North India has been reported to be 1.1% with 71% having high HBV DNA levels. These women may have a high risk of transmitting infection to their newborns[7].

The considerable morbidity and mortality associated with serum hepatitis has initiated vigorous efforts to identify the causative agent(s)[8].Confirmation of diagnosis in HBV infection and assessment of prognosis is based on wide array of advanced immunological, molecular and histological assays. HBsAg is an HBV serologic marker that plays a major role in the diagnosis of HBV infection and can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious[9].

The most widely used HBsAg screening tests are Enzyme immunoassays (ELISAs) which are considered to be quite sensitive tests and are widely used at well-equipped reference centers or laboratories[10]. Elisa tests are generally costly so far the instruments and chemicals are concerned so they are the most appropriate for screening large numbers of specimens on a daily basis[8].

However, many laboratories in resource limited countries only process limited numbers of specimens. Hence, individual tests would be more appropriate. Several simple, instrument and electricity-free screening tests have been developed including agglutination, immunofiltration (flow through) and immunochromatographic (lateral flow) membrane tests[9].

The majority of rapid tests are based on immunochromatographic principle. A positive result is indicated by the appearance of a coloured dot or line, or shows an agglutination pattern. While most of these tests can be performed in less than 10 minutes, other simple tests are less rapid and their performance requires 30 minutes to 2 hours. The results are read visually[10].

Objectives

This study was aimed to find the prevalence of hepatitis B in patients reporting at our hospital and to evaluate the performance of two commercially available rapid ICT kits for HBsAg detection.

MATERIALS AND METHODS

A total no. of 5024 serum samples were collected w.e.f Jan 2011 to July 2011 from patients reporting at ESI-PGIMSR and Hospital, Basaidarapur, New Delhi. These included patients clinically suspected for hepatitis (Subgroup A1), patients reporting to ICTC (Integrated Counselling and Testing Centre) for HIV testing including patients reporting to antenatal clinic (Subgroup A2) and patients on dialysis (Subgroup A3).

The samples were tested for HBs Ag by ELISA using Monolisa^{TM} HBsAg ULTRA kit from BioRad (France). The two ICT tests- *Instachk*^{TM} by InTec Products Inc.(Germany) and *HEPA*^{TM}*CARD* by Reckon Diagnostcs Pvt. Lt.(India) were performed on Elisa tested samples stored at -20 ºC for HBV- Reactive (n = 137) and HBV- Non reactive (n = 150) by allotting random numbers in a double blind control manner. A repeat ELISA was done to confirm the results.

All the conditions for the storage of the kits were strictly followed. The sera were thawed on the day of doing the tests. The tests were performed as per manufacturer's instructions. The results were read and recorded by two independent medical technologists and supervised by the consultant.

Monolisa HBsAg Ultra is a fourth generation one-step sandwich enzyme immunoassay using a solid phase coated with monoclonal antibodies (three). It is capable of detecting variant strains and has a detection limit of less than 50 picogram/ ml. The cutoff value is calculated as the mean negative control value plus 0.050. Samples with a signal/cutoff ratio of ≥1.0 are reactive, and those with a signal/cutoff ratio of <0.9 are nonreactive[11].

The ICT tests are rapid, qualitative, one-step two-side sandwich immunoassay based on the immunochromatographic sandwich principle. The method employs monoclonal antibody-dye conjugate (colloidal gold) alone in case of *Instachk*^{TM} or monoclonal antibody-dye conjugate in combination with polyclonal solid phase antibodies in case of *HEPA*^{TM}*CARD* to selectively identify HBs Ag with a high degree of sensitivity (claimed to be 0.5ng/mL for *HEPA*^{TM}*CARD* and 1.0 ng/ ml for Instachk™). The result was taken as positive if two coloured lines (test and control)

were seen, negative if only the control line was seen and invalid if no control line was seen, which was repeated[12,13].

Sensitivity, specificity, positive predictive value and negative predictive value of these tests were calculated using ELISA as the gold standard[14].

RESULTS

Out of 5024 samples tested, 137 were found to be reactive for HBs Ag by ELISA. Prevalence of Hepatitis B in our study was found to be 2.73 % being highest in subgroup A1 i.e. 8.02 % in pts. clinically suspected for Hepatitis, 7.19% in dialysis pts. and 1.25 % in pts. reporting to ICTC including ANC (Table 1).

Table 1. Prevalence of Hepatitis B in various subgroups tested

TEST	No. of Samples tested for HBs Ag			TOTAL
	SubGroup A1 (Suspected for Hepatitis)	SubGroup A2 (ANC & ICTC)	Sub Group A3 (Dialysis pts.)	
ELISA Reactive	78	49	10	137
TOTAL samples tested	972	3913	139	5024
Prevalence	8.02 %	1.25 %	7.19%	2.73%

Table 2. Comparison of ICTs with ELISA for HBs Ag detection

ICT(*Instachk*)™	ELISA Reactive	Non Reactive
Reactive	129	0
Nonreactive	8	150
Total	**137**	**150**
ICT (***HEPA™*** card)	Reactive	Non Reactive
Reactive	70	5
Nonreactive	67	145
Total	**137**	**150**

The I.C.T. *Instachk™* was found to be 94.16 % sensitive and 100% specific. The *HEPA™CARD* was found to be 51.09 % sensitive and 96.67 % specific. The negative predictive value was 94.93 % and 68.39 % for. *Instachk™* and *HEPA™CARD* kits respectively. The positive predictive values were 100% and 93.33 % with *Instachk™* and *HEPA™CARD* kits respectively. The *HEPA™CARD* test yielded more false-negative results when compared with the *Instachk™* diagnostic test kit (Table 2).

Various other criteria for the two diagnostic tests were evaluated and are listed in Table 3.

Table 3. A Comparative Evaluation of the ICTs done

Company	*Instachk™*	*HEPA™CARD*
Assay type	immunochromatographic	immunochromatographic
Antibody type	monoclonal	Monoclonal and polyclonal
Solid phase	Nitrocellulose membrane	Nitrocellulose membrane
Specimen type	serum/plasma/ whole blood	serum/plasma/whole blood
Number of tests per kit	40	10/ 50/ 100
Shelf life	18 months (2ºC - 30ºC)	18 months (4ºC - 30ºC)
Volume of serum needed (µl)	100	200
Total time to perform the assay	15 min/ test	20 min/ test
Sensitivity	94.16%	51.09 %
Specificity	100%	96.67 %
Positive predictive value	100%	93.33 %
Negative predictive value	94.93%	68.39 %

DISCUSSION

Prevalence of Hepatitis B in our study was found to be 2.73 % which correlates with reported prevalence of 2-7% in India[3,15]. The prevalence of HBV infection within dialysis units in our study was 8.02% which is in accordance with prevalence reported in developing countries (2–20%)[4]. Introduction of HBV vaccination, isolation of HBV positive patients, use of dedicated dialysis machines and regular surveillance for HBV infection can help reduce the spread of HBV in this setting[4]. Prevalence of 1.25 % in antenatal group is similar to that in other studies[7].

The most widely used HBsAg screening tests are ELISAs as they are the most appropriate for screening large numbers of specimens on a daily basis. However, they are costly and less frequently available in economically deprived countries. Also many laboratories in resource limited countries only process limited numbers of specimens and prefer individual tests[10].

Cheaper, rapid, simple, instrument and electricity-free ICTs for qualitative detection of HBsAg in human serum, plasma, or whole blood developed by different diagnostic firms are available with claims of high sensitivity and specificity. The advantage of ICT method is that it can be completed in 10-20 minutes and performed by nurses or technicians with a minimum of training. It is practical for use at the provincial or peripheral health care level, since the test strips are stable for one to two years at ambient temperatures, if packaged appropriately. Also nowadays ICTs have been developed to detect HBsAg from whole blood[9,14].

The claims as per literature of the company for ICT devices by *Instachk™* and *HEPA™CARD* used in our study fall in the range of 98.8%-100% and 97-100% respectively. (Both for specificity and sensitivity)[12,13].

Results of the present study showed that the sensitivity and negative predictive values of rapid ICT kits used for HBsAg screening were significantly low and sensitivity claims of companies are mere boast. This is in accordance with other studies which have reported similar findings[3]. These claims are usually made on the basis of studies carried by workers observing ideal conditions of manufacture, transport and storage. However these conditions may not be observed for in actual laboratory settings especially in resource limited countries. Under such conditions it becomes pertinent to test the claims of the companies under the prevalent conditions of manufacture, storage and transport before the purchase[8,15].

Failure of these kits to detect Hbs Ag reactive samples maybe due to: 1. Inadequate coating of antigens, 2. Nature of antigens used and 3. Genetic heterogenecity of the virus[2]. It could be due to short incubation period of the ICT employed which do not detect low concentration of antigens as compared to the classic type of immunoassays which employ longer incubation times allowing reaction to proceed to completion[8].

On the other hand in our study specificity and positive predictive values of the rapid testing kits was fairly high which was also seen in other studies[14, 16]. It should be noted that the ICT may identify HBsAg negative samples reasonably well, but, because of their short incubation times the assays do not always identify low concentration of antigens[8,17].

However, an earlier study found that the rapid tests showed sensitivity between 97.5-99.2% and specificity of 97.5-99.2%. with quantitative PCR results as a gold standard method, that were quite acceptable and suggested that they can be easily used in small, rural laboratories for serologic screening of high-risk clients and positive serology should be followed by repeat testing with alternative methods[9].

But still the studies relating to this comparison are much scarce as the trend is more towards the superior assays. Further in-depth studies, using large sample size at different hospitals and health centres, and employing advanced confirmatory techniques (e.g. RIBA, PCR) are needed to explore more information about these tests[12,18].

Finally, the flexibility derived from being able to use whole blood for diagnostic evaluation purposes has significant clinical implications, especially for use in field-based laboratories[12].

CONCLUSION

These results suggest that the rapid ICT kits for HBsAg testing were easy to perform and

interpret, required minimal laboratory equipment, and could be taught easily to local laboratory personnel .However these ICTs have only limited efficacy and fall short of being the ideal screening tests and they should be backed by superior methods like ELISA and PCR where possible. In general, these tests are most suitable for use in laboratories or in a small hospital setting that have limited facilities and/or process low numbers of specimens daily. It becomes pertinent to test the claims of the companies under the prevalent conditions of manufacture, storage and transport before the purchase of these tests. Other factors like price, availability, delivery time should also be taken into account by the laboratory before recommending a particular test.

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