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Performance Evaluation of *In Vitro* Screening Kits for Hepatitis B in Resource-Limited Settings

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ABSTRACT

Objective: The aim of this study was to assess the performance of different screening assays for the detection of hepatitis B virus surface antigen (HBsAg).

Methodology: This single-centre, cross-sectional prospective study was conducted at the Peshawar Regional Blood Centre. A total of 210 blood donor samples were tested including 70 positive and 140 negative samples for HBsAg. Six rapid screening devices, two ELISA and one CLIA were assessed in comparison with Polymerase Chain Reaction for the detection of HBsAg. The specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), Positive Likelihood Ratio (PLR), Positive Likelihood Ratio (NLR), and efficiency were calculated using the PCR results as the gold standard.

Results: The results showed that CLIA showed 100% sensitivity and 100% specificity. The ELISA ChiL showed 98.57% sensitivity and 99.29% specificity while the other ELISA AiD had 97.14% sensitivity and 100% specificity. The rapid devices showed variable results.

Conclusion: The chemiluminescence immunoassay (Liaison Murex assay) showed results similar to PCR. Screening in blood banks should be performed by ELISA or other higher technology.

Keywords: Hepatitis B virus, chemiluminescence immunoassay, PCR, antigen.

INTRODUCTION

The hepatitis B virus (HBV) is a significant problem for global public health and a leading cause of cirrhosis, hepatocellular carcinoma, and chronic hepatitis. According to estimates by the World Health Organization (WHO), 820,000 people died from cirrhosis and hepatocellular carcinoma in 2019, while 296 million people worldwide are living with chronic HBV infection. Besides, there were 1.5 million new infections per year.[1] Around 8% of the population carries HBV chronically in regions where it is highly endemic, including portions of South America, East and Southeast Asia, and sub-Saharan Africa.[2]

In recent years, a number of programmes have been launched to control and eradicate hepatitis. Under aim 3.3 of the UN Sustainable Development Goals (SDGs) from 2015, the UN urged all countries to "fight hepatitis"[3] The WHO Global Health Sector Strategy on Viral Hepatitis (WHO-GHSS) was endorsed by the World Health Assembly in 2016 with the aim of eradicating viral hepatitis as a menace to public health.[4] With comparison to the baseline year of 2015, the WHO-GHSS recommended impact objectives of a 30% decrease in new cases and a

10% decrease in HBV-related fatalities by 2020, and a 95% decrease in new cases and a 65% decrease in deaths by 2030.

In over 80% of individuals with persistent HBV infection, there will be no symptoms of infection. HBV testing is essential for public health, especially for blood screening in blood banks, because the illness is frequently asymptomatic. HBV dissemination is facilitated by chronic carriers with low viremia levels and undetected acute infections.

Hepatitis B surface antigen (HBsAg) is the first serological marker to show during the course of HBV infection and is a critical marker for HBV infection screening and laboratory diagnosis. The immunoassays' detection threshold affects HBsAg sensitivity.

Due to the genetic variety of HBV, HBsAg test sensitivity may also depend on HBsAg antigenic variation. In actuality, certain HBsAg mutants that arise as a result of immunological pressure selection may avoid being detected by commercial HBsAg tests.[5-7] Besides, HBV naturally exhibits heterogeneity due to the variety of genotypes and subtypes.

HBsAg is classically identified using sensitive immunoassays with an immunoassay equipment that is either based on ELISA (enzyme linked immunosorbent assay) or CLIA (chemiluminescence immunoassay) principles. Furthermore, WHO advises using affordable, quick, and simple tests that operate similarly to immunoassays, which may be carried out in peripheral health centres' laboratories in countries with limited resources. With a quick turnaround time for the patients to get the test results, it could be able to detect HBsAg. A wide range of rapid immunochromatographic tests (ICTs) are circulating and used for hepatitis B screening in Pakistan during clinical research, for routine diagnosis, and for blood screening mostly without the approval of the Drug Regulatory Authority Pakistan (DRAP) which is the regulatory body of the health ministry of Pakistan. Very little information is available on the evaluation of these rapid tests and this study was conducted to evaluate the performance of different HBsAg screening tests (ICT, ELISA, CLIA) in comparison with Polymerase Chain Reaction (PCR). This will enable national authorities to develop a sustained supply of inexpensive, high-quality screening kits by enabling them to make informed decisions about the assays to be utilized.

METHODOLOGY

The current study was executed at the Regional Blood Centre (RBC), Peshawar, from March 2019 to February 2020, which is the only modern centralized blood bank in the region. The RBC is equipped with advanced technology for all blood banking procedures adopting and implementing standards of national and international level. Screening for HBsAg was performed utilizing different commercially available rapid ICT, ELISA, CLIA and confirmed by PCR as a reference gold standard. The molecular analyses (PCR) were carried out at the Department of Blood Transfusion Services, Pakistan Institute of Medical Sciences (PIMS), Islamabad. The sample size was calculated according to WHO sample size calculator with confidence level 95%, and absolute precision of 5%. A total of 210 blood donors were screened for HBsAg on six types of rapid ICT devices, two types of ELISA, one CLIA and confirmed by PCR. These included 70 positive and 140 negative samples for HBV DNA as confirmed by PCR.

The Ethical Committee of the Khyber Medical University, Peshawar endorsed the study vide letter No DIR/KMU-EB/ES/000609. Standard ethical principles were compiled throughout the study. The privacy and confidentiality of study participants were guaranteed at all levels during the study including the non-disclosure of the identity of the donors duly accompanied with a distinctive ID number. The donors were selected after a detailed screening examination in the donor management department of the RBC. Informed consents were taken from them. As per standard guidelines, donors between the age of 18-60 years with weight > 50 kg, a haemoglobin level of more than 12.5 g/dl, and blood pressure (systolic not more than 160 diastolic not more than 100 mm of Hg without medication), and pulse rate (60 to 100 beats per minute) were selected.[8] Given below is the number of tests performed on different techniques/kits.

The six rapid testing commercially available kits for HBsAg were CHiL Rapid Test (CHIL Tibbi Mal., San, Tic. Ltd. Stl, Izmir, Turkey, Advance Quality TM One-Step Test (InTec Products, Inc.), ACCU-TELL ® Rapid Test Cassette (AccuBioTech Co., Ltd.), SD Bioline Rapid Test (Standard Diagnostics, Inc. Kyonggi-do, Korea), ACON Rapid Test Cassette (ACON Laboratories, Inc. San Diego, CA, USA), Toyo

Cassette Test (TurkLab Tibbi Malzemeler San. Ve TIC. AS Izmir.

Two commercially available kits for HBsAg ELISA kits are CHiL ELISA Test (CHIL Tibbi Mal., San, Tic. Ltd.Stl, Izmir, Turkey) and AiD™ ELISA PLUS (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. China). All samples were tested by the Chemiluminescence immunoassay using a fully automated Diasorin Liaison Murex assay system and then confirmed by PCR (Bioneer Corporation, Korea). To maintain the efficacy of kits, good storage practices (GSP) and standards were observed as per the manufacturer's manuals for storage. The specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), Positive Likelihood Ratio (PLR), Positive Likelihood Ratio (NLR), and efficiency were calculated using the PCR results as the gold standard.

RESULTS

A total of 210 blood donors were enrolled and blood from each participant was collected and further processed accordingly. The majority of participants were males, i.e. 98.2% (n=275) while females were only 1.7% (n=5). 38 years was the mean age of the participants. Given below is the tabulated description in table **I** of the tests performed on different techniques for HBsAg with their specificity, sensitivity, PPV, NPV, PLR, NLR, and efficiency stated.

DISCUSSION

Worldwide, a large number of HBsAg rapid tests utilising immunochromatographic assays have been developed. The primary difficulty with these tests is identifying the low concentrations of the target antigen that are present in a sizable fraction of asymptomatic carriers.[9] Due to the cheaper cost of testing and easier logistics, a quick diagnostic test is a viable substitute for epidemiologic surveys in a situation with limited resources when ELISA is not accessible. This is not true in the instance of blood transfusion services, where using poor ICT devices can be fatal.

Transmission through contamination of infectious diseases of the blood and blood-related products remains one of the major route causes of HBV in our society. According to blood safety legislation of Pakistan, screening for five TTIs markers (Hepatitis B, C, HIV, Syphilis, and Malaria) is mandatory on every blood unit collected.[10] In the absence of a stringent regulatory mechanism, the blood transfusion services in Pakistan are weakly monitored. The screening techniques being employed in the blood banks are diverse, ranging from rapid ICT devices to modern CLIA assays.[11]

Table I. Comparative Assessment of Different HBsAg Screening Assays

S.No	Parameters	PCR Bioneer	CLIA DiaSorin	ELISA CHiL	ELISA AiD	Rapid Toyo TurkLab	Rapid SD Bioline	Rapid ACON	Rapid ACCU-TELL	Rapid Advance Quality	Rapid CHiL
1	Total Sample	70	70	70	70	70	70	70	70	70	70
2	True Positive	70	70	69	68	66	68	67	66	65	65
3	False Negative	0	0	1	2	4	2	3	4	5	5
4	False Positive	0	0	1	0	1	0	0	1	2	1
5	True Negative	140	140	140	140	139	140	140	139	138	139
6	Sensitivity (%)	100	100	98.57	97.14	94.28	97.14	95.71	94.28	92.85	92.85
7	Specificity (%)	100	100	99.29	100	99.28	100	100	99.28	98.57	99.28
8	PPV (%)	100	100	98.57	100	98.50	100	100	98.50	97.01	98.48
9	NPV (%)	100	100	99.29	98.59	97.20	98.59	97.90	97.20	96.50	96.52
10	PLR	0	0	-0.00	-0.02	-0.05	-0.02	-0.04	-0.05	-0.05	-0.06
11	NLR	0	0	0.00	0.02	0.05	0.028	0.04	0.05	0.057	0.064
12	Efficiency	136.66	136.66	135.35	134.6	132.1	134.66	133.6	132.1	130.71	131.19

The screening tests for HBV are primarily dependent on immunological screening techniques, most common examples are rapid kits and ELISA**.** The discordance in the results of ICT and ELISA techniques is the main problem and requires to be fixed by the introduction of more significantly sensitive and specific kits. It is an admitted fact that the sensitivity and specificity of PCR, CLIA, and ELISA techniques are far better than rapid ICT kits, but are expensive, time-consuming, and require more specialized technical skills. The occurrence of mutant viruses with altered surface antigens (HBsAg), which prevent their detection by standard immunological procedures, may account for the discrepancy in results about the sensitivity and specificity of rapid testing.[12]

Due to the limitation of resources, in underdeveloped countries, mostly cheap rapid assay techniques are used for blood screening and diagnostic testing. Rapid ICT are cheaper than ELISA, however, they have compromised the level of efficiency.[13] The performance evaluation results of six rapid ICT kits and two ELISA kits showed a great degree of variability and unsustainability. The performance of the CLIA assay was also compared with the PCR which showed equal reliability and accuracy for all samples including the controls.

Earlier studies have reported that SD Bioline kits sensitivities and specificities were 94.1 to 100% and 99.3 to 100% for the HBsAg kit,[14,15] which is in line with our study findings. According to the national data,[16] many blood banks are using rapid ICT kits for screening and diagnostics considering cost as a major concern but ultimately compromising the quality of results. The results of this study are in accordance with the studies conducted earlier in different parts of the globe, including India, Iran, and Egypt stating that the sensitivity and specificity of rapid ICT tests are less than ELISA, CLIA, and PCR individually.[17-19]

CONCLUSION

The sensitivity and specificity of CLIA (DiaSorin) are equivalent to the gold standard PCR for HBsAg in our study. It is the inference that the CLIA technique is as reliable as the PCR for screening of these diseases in blood banks to achieve value for money at large, time effectiveness, and less chance of error due to the availability of fully automated machines and minimum manual interventions which ultimately effects the deliverance of results.

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