Original Article

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Microbiology Section

Comparison of Immunochromatographic Test and Electrochemiluminescence Assay with PCR for the Detection of Hepatitis B Virus: A Cross-sectional Study

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ABSTRACT

Introduction: Hepatitis B Virus (HBV) infection remains a significant public health concern globally, necessitating accurate and timely diagnostic methods. Immunochromatographic Tests (ICTs) and Electrochemiluminescence Assays (ECLIAs) are widely used assays for HBV detection due to their rapidity and cost-effectiveness. However, their diagnostic performance should be evaluated to ascertain their reliability.

Aim: To detect the presence of Hepatitis B Surface Antigen (HBsAg) in the selected samples using ICT and ECLIA and to compare it with HBV Deoxyribonucleic Acid (DNA) using a molecular assay.

Materials and Methods: A cross-sectional study was done with serum samples collected from patients visiting the hospital over a period of six months with prior ethical clearance. Serum samples were obtained from 57 patients suspected of HBV infection. The results of ICT, ECLIA, and HBV DNA viral load (by Polymerase Chain Reaction (PCR)) were cross-tabulated and assessed for differences in diagnostic sensitivity. The positivity and correlation of the ICT and ECLIA with PCR were estimated. All statistical analyses were performed using the R programming language.

Results: Out of 57 samples, 53 (92.98%) tested positive in the ICT card test, and 54 (94.74%) were positive in the ECLIA method. McNemar's test showed that the sensitivity of ICT and ECLIA differed significantly compared to HBV DNA PCR. There was a significant positive correlation between ECLIA and HBV-DNA PCR (Spearman correlation, r-value=0.28, p-value=0.035).

Conclusion: The findings suggest that in settings where accurate diagnosis is critical, particularly for screening and monitoring treatment efficacy, molecular assays remain the preferred choice despite their higher cost and complexity. However, in resource-limited settings, ECLIAs can still play a valuable role in HBV screening programs.

Keywords: Chemiluminescence, Immunochromatography, Polymerase chain reaction

INTRODUCTION

India has one of the highest burdens of HBV infection globally. According to estimates from the World Health Organisation (WHO), there are approximately 40 million people living with chronic Hepatitis B infection in India [1]. As per published data, about 200 crores of the world's population have been exposed to HBV, of whom 350 million have a chronic carrier state. India falls in the intermediate endemicity zone with a prevalence rate of 2-7% [2]. Timely and accurate diagnosis of Hepatitis B is crucial for effective management and prevention of complications. Various methods are employed to detect HBV infection, ranging from serological tests to molecular assays.

Chemiluminescence methods such as Chemiluminescent Microparticle Immunoassay (CMIA) and ECLIA have the added advantages of high sensitivity and specificity and are also easily done with quantitative results easily [3]. These results have been used to predict and evaluate the effect of antiviral drug treatment on positive patients. ECLIA is increasingly being considered by labs and tertiary care centres for diagnosing HBsAg due to its better performance in terms of sensitivity, specificity, and result interpretation [4]. In resource-limited settings, ICTs are widely used to detect HBsAg, as they are quick and relatively less costly compared to other diagnostic tests [5]. Hence, the factors determining the choice of a specific serological test in screening or diagnosing symptomatic cases depend on cost-effectiveness, prevalence, and the diagnostic performance of the test. There have been few studies done in India to evaluate the role of ECLIA and ICT in HIV and HBV infections [6-8]. However, studies addressing the comparison of ICT and ECLIA with HBV DNA PCR are limited, which is crucial in resource-limited settings [9,10]. Thus, this study aimed to detect the presence of HBsAg in the selected samples using ICT and ECLIA and to compare it with HBV DNA PCR. The primary objective was to compare and correlate the positivity among different methodological kits for Hepatitis B detection.

MATERIALS AND METHODS

The study was carried out as a cross-sectional study at the Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, a tertiary care hospital present in Melmaruvathur, Tamil Nadu, India. The study involved serum samples obtained from patients over a period of six months, from May 2023 to October 2023. These patients were suspected to have Hepatitis B and were advised by clinicians to undergo HbsAg detection. The study was approved by the Institutional Review Board (Reg No. 206 (5) 2022).

Inclusion criteria: Serum samples collected from both genders were included, irrespective of their age.

Exclusion criteria: Samples obtained from patients who underwent repeated testing for HbsAg detection were excluded from the study. **Sample size:** The calculated sample size for this study was 73 samples, with an estimated precision of ±0.2, a 95% confidence interval, and an HbsAg positivity rate of 12.2% [11,12]. However, only 57 samples were included in this study due to financial and time constraints in obtaining and processing the samples.

Study Procedure

The detection of HBsAg was carried out by two methods: rapid antigen testing by immunochromatography test (ICT, HEPA card) and electrochemiluminescence immunoassay (ECLIA, ElecSys HBsAg, Roche Diagnostics). Both techniques detected HBsAg as per the manufacturer's instructions, using appropriate calibrators and controls. ECLIA results were reported based on the Cut-Off Index (COI), where COI <0.9 represented a negative result, COI between 0.9 and <1.0 represented a borderline result, and COI ≥1.0 represented a reactive result [13]. The ICT was a qualitative test, and it was done as per the manufacturer's instructions, with results reported as either positive or negative [14]. Serum samples were kept frozen at -70°C until further analysis. Viral load, i.e., Hepatitis B Virus DNA testing, was done by Quantitative Polymerase Chain Reaction (Q-PCR) (Artus HBV PCR Kits CE, Qiagen) according to the manufacturer's instructions. HBV DNA PCR results with <10.21 IU/L or <83.74 copies/mL were reported as "undetected" (1 IU/L equals 8.21 copies/mL, hence the detection limit of 10.21 IU/L corresponds to 83.74 copies/mL) [15].

STATISTICAL ANALYSIS

The data were entered into a Microsoft Excel sheet and analysed using R version 4.1.1. McNemar's test was done to compare the sensitivity of ELCIA and ICT with HBV DNA PCR. Spearman's correlation was done to determine the relationship between HBsAg levels detected by ECLIA and HBV DNA levels detected by PCR. A p-value of <0.05 was considered statistically significant.

RESULTS

The total number of subjects recruited for the study was 57, with 20 females (35.09%) and 37 males (64.91%). The comparison of the results of ICT versus HBV DNA PCR is shown in [Table/Fig-1]. Among the samples that were reported positive by ICT, the HBV DNA PCR detected the virus in 32 samples (60.38%) [Table/Fig-1]. ICT and HBV DNA PCR showed a statistically significant difference in sensitivity (McNemar's test, p-value=0.0002). The comparison of the results of ECLIA versus HBV DNA PCR is shown in [Table/Fig-2]. Among the samples that reported positive by ECLIA, the HBV DNA PCR detected the virus in 34 samples (62.96%) [Table/Fig-2]. ECLIA and HBV DNA PCR showed a statistically significant difference in sensitivity (McNemar's test, p-value=0.00003).

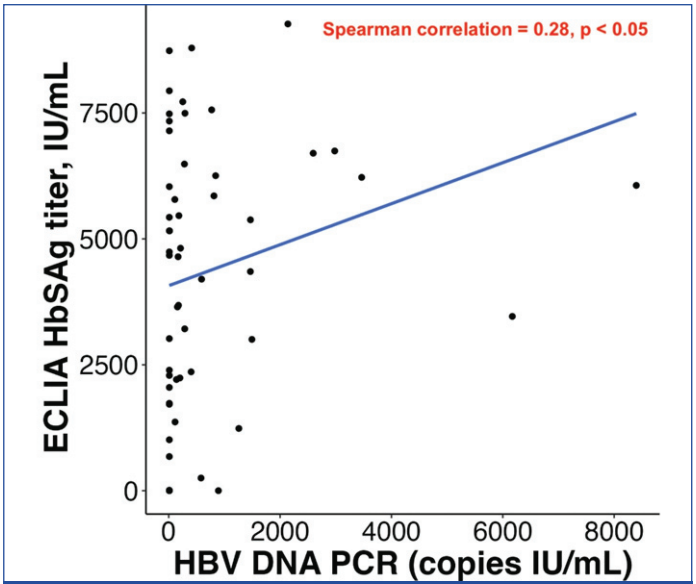
		HBV DNA PCR			p-value
		Positive	Negative	Total	
Immunochromatography (ICT)	Positive	32 (60.38)	21 (39.62)	53	0.0002
	Negative	3 (75)	1 (25)	4	
	Total	35	22	57	

[Table/Fig-1]: Cross tabulation of ICT results versus HBV DNA PCR results. The data is represented as absolute number (percentage). The row percentage is calculated. McNemar's test was done to compare the diagnostic sensitivity between the two tests. The p<0.05 is considered as statistical significance

		HBV DNA PCR			p-value
		Positive	Negative	Total	
Electrochemiluminescence immunoassay (ECLIA)	Positive	34 (62.96)	20 (37.04)	54	0.00003
	Negative	1 (33.3)	2 (66.7)	3	
	Total	35	22	57	

[Table/Fig-2]: Cross tabulation of ECLIA results versus HBV DNA PCR results. The data is represented as absolute number (percentage). The row percentage is calculated. McNemar's test was done to compare the diagnostic sensitivity between the two tests. The p<0.05 is considered as statistical significance.

The correlation of ECLIA with HBV DNA PCR was plotted and found to have a significant positive correlation between ECLIA and HBV DNA PCR (Spearman correlation, r-value=0.28, p-value=0.035) [Table/Fig-3].



[Table/Fig-3]: Correlation of ECLIA and HBV DNA PCR.

DISCUSSION

The results of this study provide valuable insights into the performance and correlation of the ICT and ECLIA methods with the gold standard molecular assay, HBV DNA PCR, for the detection of HBV infection. The high positivity rate of 92.98% observed with the ICT card test proves that it is the best available screening method and strengthens its utility as a rapid and convenient diagnostic tool for HBV screening. However, it is essential to note that among the samples identified as positive by ICT, only 60.38% were confirmed positive by HBV DNA PCR This indicates a considerable rate of false positives with the ICT method, which may lead to misdiagnosis and unnecessary concern among patients, leading to the initiation of treatment or a delay in surgical procedures. Therefore, while ICTs offer quick results and ease of use, their lower specificity compared to molecular assays highlights the importance of confirmatory testing, especially in clinical settings where accuracy is most needed.

Conversely, the ECLIA method showed a positive correlation with HBV DNA PCR, as evidenced by a significant Spearman correlation coefficient of 0.28 (p-value<0.05). This suggests that ECLIA may offer better sensitivity and specificity compared to ICTs, making it a more reliable option for HBV detection, particularly in settings where molecular assays are not readily available or feasible. The correlation observed between ECLIA and HBV DNA PCR indicates that ECLIA results can serve as a useful indicator of HBV infection status, although confirmatory testing may still be warranted in certain cases, especially when clinical suspicion is high or when discordant results are obtained.

The high cost of diagnostic tests, the need for lifelong monitoring, and the stigma attached to accessing healthcare are the various barriers preventing patients from getting guideline-based treatment [16]. Furthermore, a mutation in the ORF region that codes for HBsAg can cause a conformational change and result in HBsAg mutants, leading to the risk of a false negative HBsAg result with certain test kits [17]. Many studies have evaluated the performance of various methods in different study settings [6,17-20]. Most of the studies compare Rapid Diagnostic Tests (RDTs) with Enzyme Immunoassays (EIAs) [20]. There are only a few studies available that compare RDTs with Nucleic Acid Amplification Tests (NAAT) as reference standards [9,10]. The sensitivity and specificity varied according to the study settings and the kits used. Molecular assays directly detect HBV DNA in serum or plasma, providing quantitative measurement of viral load and assessing viral replication, including PCR. In a meta-analysis conducted by Amini A et al., it was found that the pooled sensitivity and specificity of HBsAg detection by ICT were 90.0% (95% CI: 89.1, 90.8) and 99.5% (95% CI: 99.4, 99.5), respectively, with EIA as the reference standard. The estimates

Author's name and year	Place of study	Number of subjects	Methods compared	Parameters assessed	Conclusion
Roy S et al., 2018 [6]	West Bengal	198	Rapid Diagnostic Test (RDT), ECLIA and ELISA	HbsAg, anti-HCV, anti-HIV 1 and 2	RDT and ECLIA performed better in screening HIV and HBV.
Amini A et al., 2017 [20]	United Kingdom	23,716	Meta-analysis RDT, EIA, PCR	HbsAg, HBV DNA viral load	RDTs performed good sensitivity and excellent specificity compared to EIA accuracy of RDTs was lower compared to nucleic acid testing.
Chen CH et al., 2004 [23]	Taiwan	67	ECLIA and PCR	HbsAg and HBV DNA	HbSAg showed a better correlation with HBV replication in careers.
Dembele B, et al, 2020 [5]	Ivory coast	699	RDTs of four different kits vs EIA	HbsAg	RDTs from Alere and Vikia and Biomerieux performed better.
Present study, 2024	Tamil Nadu, India	57	ECLIA and ICT with PCR	HBsAg	ICT and ECLIA differed significantly compared to HBV DNA PCR.

[Table/Fig-4]: Comparison of the present study with similar studies [5,6,20,23].
ECLIA:Electrochemiluminescence assay; ELISA:Emzyme linked immunosorbemt assay;PCR:Polymerase chain reaction

varied among different kits [20]. The same study also showed that the pooled sensitivity and specificity of EIA were 88.9% (95% CI: 87.0, 90.6) and 98.4% (95% CI: 97.8, 98.8), respectively, with CLIA as the reference standard [20].

A study conducted by Dembele B et al., compared four different RDTs and reported that the sensitivity varied from 97 to 100%, and the specificity varied between 99 and 100% [5]. The clinical significance and correlation of quantitative assays qHBsAg with HBV DNA was first reported by Park et al., [21]. A number of studies have documented the positive correlation between qHBsAg and the viral DNA load, indicating that quantification of HBsAg can be used as an inexpensive alternative for monitoring the treatment response [22,23]. But, there are also contraindications in some studies that have reported a lack of correlation between the two, as shown by Mathai F et al., published that there is a weak but significant correlation between HBsAg and HBV DNA (p-value=0.024, r-value=0.171) [24]. A comparison of the present study with similar studies has been done in [Table/Fig-4] [5,6, 20, 23].

Overall, the findings highlight the importance of choosing appropriate diagnostic methods based on their performance characteristics and the clinical context. While rapid tests like ICTs offer quick results and may be suitable for screening purposes, they should be used judiciously, with confirmatory testing performed whenever possible to minimise the risk of false positives. On the other hand, ECLIA remains a reliable and promising diagnostic tool for HBV detection, particularly when access to molecular assays is not available in those areas. Further research is warranted to explore ways to enhance the performance of RDTs while maintaining their ease of use and affordability, ultimately improving the diagnosis and management of HBV infection.

Limitation(s)

The sample size of the study was limited due to financial and time constraints. The sensitivity and specificity could not be calculated due to the smaller sample size, and the study participants included those who had a high index of suspicion for Hepatitis B.

CONCLUSION(S)

The findings suggest that while ICTs and ECLIAs offer rapid and convenient options for HBV detection, they may exhibit lower sensitivity and specificity compared to molecular assays. Therefore, in settings where accurate diagnosis is critical, particularly for screening purposes and monitoring treatment efficacy, molecular assays remain the preferred choice despite their higher cost and complexity. However, in resource-limited settings where access to molecular assays is limited, ICTs and ECLIAs can still play a valuable role in HBV screening and surveillance programs. Further studies are warranted to explore strategies for improving the performance of RDTs while maintaining their affordability and accessibility.

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