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Original Article

Microbiology Section

Comparative Study between ELISA and TrueNat for Hepatitis B Virus and Hepatitis C Virus among Antenatal Women Attending Tertiary Care Medical Institute in Manipur, India

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ABSTRACT

Introduction: Viral hepatitis is one of the leading causes of death and disability. Nearly 1.5 million people die every year from Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) related liver diseases. Reported consequences of HBV and HCV infection in pregnancy include an increased likelihood of occurrence of preterm delivery and low birth weight and perinatal transmission.

Aim: To compare Enzyme-Linked Immunosorbent Assay (ELISA) and closed system Polymerase Chain Reaction (PCR) TrueNat for the detection of HBV and HCV among antenatal women attending a tertiary care medical institute.

Materials and Methods: This was a hospital-based cross-sectional study conducted at the Department of Microbiology, JNIMS, Imphal, Manipur, India, during the period from December 2020 to January 2022. Serum samples were tested using ELISA as well as on the TrueNat PCR. Data was analysed using Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: Out of 60 samples, using ELISA (QUALISA), 24 were found to be positive for HBV surface antigen (HBsAg) and 14 were found to be positive for HCV antibodies (HCV Ab). On further testing using TrueNat chip-based closed system Real Time-PCR (RT-PCR) Test, 12 were found to be positive for HBV Deoxyribonucleic Acid (DNA) and five were found to be positive for HCV Ribonucleic Acid (RNA).

Conclusion: TrueNat PCR could eliminate the problem of false positivity in the detection of HBV and HCV. It is useful to make clinical decisions on starting antiviral therapy and also in documenting the efficacy of the antiviral therapy.

Keywords: Enzyme linked immunosorbent assay, Polymerase chain reaction, Viral hepatitis

INTRODUCTION

Viral hepatitis is one of the leading causes of death and disability globally and causes atleast as many deaths annually as tuberculosis, Acquired Immunodeficiency Syndrome (AIDS) or malaria [1]. HBV and HCV are very serious health problems worldwide. It is estimated that approximately one-fifth of the world population i.e., 500 million people are chronically infected with HBV and HCV [2]. HBV and HCV predominantly spread via parenteral route and are notorious to cause chronic hepatitis which in turn can lead to critical complications such as cirrhosis of liver and Hepatocellular Carcinoma (HCC) [3]. The burden of HBV and HCV infection remains disproportionately high in Low- and Middle- Income Countries (LMICs) particularly in Asia and Africa [4]. India is second after China in terms of number of chronic hepatitis infection as the disease has overtaken Human Immunodeficiency Virus (HIV)/AIDS and malaria in terms of death rate [5]. Outcomes of HBV and HCV infection in pregnancy include an increased occurrence of preterm delivery, low-birth weight and perinatal transmissions [6]. Moreover, these infections have been found to be linked to threatened preterm labour, antepartum haemorrhage and gestational diabetes mellitus [7]. Symptoms of such patients are non specific which may include jaundice, nausea, anorexia, abdominal pain or discomfort, fatigue, malaise, myalgia, and dark urine. Clinical symptoms cannot differentiate various viral hepatitis. Pregnant women with chronic HBV and HCV viral infection may progress to decompensated cirrhosis and develop ascites, hepatic encephalopathy, coagulopathy, and oesophageal variceal bleeding [8]. The outcome depends on the severity of the disease and presence of portal hypertension indicating poor prognosis overall [6].

Testing, in conjunction with accurate diagnosis of HBV and HCV infection is the gateway for both prevention as well as care and treatment services [9]. Testing will also provide an opportunity to link to therapeutic interventions and thereby reducing the transmission. Various methods can be used for the diagnosis of hepatitis including Reverse Passive Haemagglutination (RPHA), rapid Immunochromatographic Test (ICT), ELISA, Enzyme Immunoassay (EIA), Chemiluminescent Immunoassay (CLIA), PCR, TrueNat, etc., [10]. Present study aims to compare the diagnostic utility of ELISA which was considered to be the gold standard against the current gold standard PCR. The objective of this study was to compare ELISA and closed system PCR TrueNat for the detection of HBV and HCV among antenatal women attending a tertiary care medical Institute.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study conducted in the Department of Microbiology, JNIMS, Imphal, Manipur, India, during the period from December 2020 to January 2022. Institutional Ethics Committee (IEC) approval (No. Ac/03/IEC/JNIMS/2018) was taken for the study and written informed consent was obtained from all participants.

Inclusion criteria:

1. Blood samples were collected from antenatal women who were 18 years or older attending JNIMS having deranged Liver Function Tests (LFT) and/ or recent development of jaundice.

Exclusion criteria:

1. Patients with history of other disease conditions such as gallstones, tumours, haemolytic anaemia, etc., that causes jaundice.
2. Patients who refused to take part in the study.
3. Patients who were <18 years of age.

Sample size: Sample size was calculated by taking the prevalence from another study by Roy A et al., [11]

Formula: $n=\frac{z^2pq}{d^2}$

Where, n=sample size

z=1.96 for 95% confidence interval

p=prevalence from previous study

=2.4

q=100-p

d=allowable error

=4%

$$n=\frac{(1.96^2\times2.4\times(100-2.4)}{4^2}$$

$$\therefore n=56.23$$

Now, keeping room for some adjustment, a sample size of 60 was taken for the study.

Laboratory Methodology

Five mL of blood was collected aseptically from each patient. The blood samples were centrifuged at 3000 rpm for 10 minutes and the separated serum was aliquoted in pre labelled, screw-capped sterile scintillation vials. Each sample was tested using ELISA and TrueNat PCR as follows:

ELISA: 3rd generation ELISA (QUALISA, Tulip Diagnostics) kits were used for detection of both HBsAg and HCV Ab. The tests were performed following the manufacturer's guidelines.

TrueNat: In our set-up, ICMR approved TRUELAB Uno Dx PCR ANALYSER machine was used for performing HBV and HCV assays. A 0.5mL of the serum sample was transferred into the lysis buffer bottle using 1 mL transfer pipette. Then, the entire content of the lysis buffer tube were transferred to the sample chamber (black cap) of the cartridge using a 3 mL transfer pipette. The cartridge was inserted into the extraction machine and the completion of extraction was indicated by a beep sound after 20 minutes. The elute was collected in Elute Collection Tube (ECT), then 6 μL of it was dispensed into microtube containing freeze dried RT-PCR reagents. It was incubated for 30 seconds at room temperature to obtain a clear solution. Using the same pipette and tip the clear solution was dispensed into the white reaction well of the microchip and the PCR amplification machine is started. Single assay had a turnaround time of 60 minutes [12].

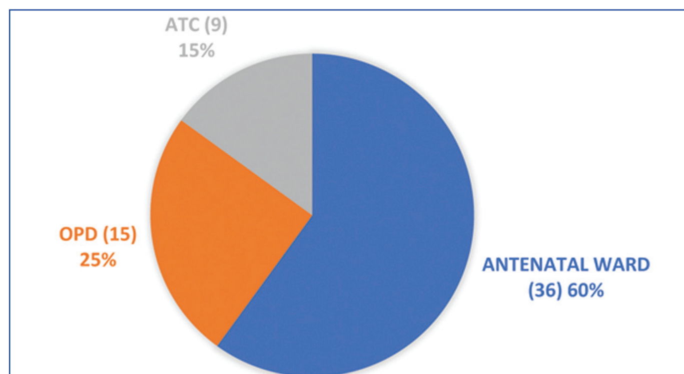
Amplification test and Interpretation: At the end of 60 minutes, a graphical representation of the data with three amplification curves is displayed on the RT-PCR analyser screen to indicate the progress of the test. Both the target and the Internal Positive Control (IPC) curves will take a sheer, expanding path when the fluorescence will cross the threshold value in case of positive samples. The Cycle threshold (Ct) of the specimen will depend on the number of virus copies in the sample. Throughout the test duration, only the IPC curve will take an augmented path in case of negative samples. In case the IPC curve remains horizontal, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for positive result or "NOT DETECTED" for negative result [12].

STATISTICAL ANALYSIS

Analysis of the data was done using Microsoft Excel Sheet and SPSS 23.0. Chi-square test was used to calculate p-value for the comparison. A p-value <0.05 is considered to be statistically significant.

RESULTS

During the study period, 60 samples were collected from patients who fulfilled the study criteria. Of these, 36 were from the Antenatal ward (IPD), 15 were from OPD, and nine were from Accident and Trauma Centre (ATC) [Table/Fig-1]. Maximum number i.e., 48.3% (29/60) of the participants belonged to the age group 21-30 years with the mean age being 29.87±3.3 years.



[Table/Fig-1]: Distribution of samples from various wards.

Of the 60 samples, eight were found to be positive on both ELISA and TrueNat PCR. However, four samples that were negative on ELISA were detected with HBV DNA on TrueNat PCR. The difference between the two tests was found to be statistically significant [Table/Fig-2]. The sensitivity of ELISA with respect to TrueNat is 67%, and the specificity is 67%. Positive Predictive Value (PPV) was found to be 33.4% and Negative Predictive Value (NPV) is 88.9%.

Test		TrueNat		Total	p-value
		Positive	Negative		
ELISA	Positive	8	16	24	0.03*
	Negative	4	32	36	
Total		12	48	60	

[Table/Fig-2]: Comparison of ELISA with TrueNat PCR for HBV detection. (Chi-square test).
*=statistically significant; p<0.05

Of the 60 samples, three were found to be positive on both ELISA and TrueNat PCR. However, two samples that were negative on ELISA were detected with HBV DNA on TrueNat PCR. The difference between the two tests was found to be statistically significant [Table/Fig-3]. The sensitivity of ELISA with respect to TrueNat is 60%, and the specificity is 80%. PPV was found to be 21.4% and NPV is 95.6%.

Test		TrueNat		Total	p-value
		Positive	Negative		
ELISA	Positive	3	11	14	0.04*
	Negative	2	44	46	
Total		5	55	60	

[Table/Fig-3]: Comparison of ELISA with TrueNat PCR for HCV detection. (Chi-square test)
*=statistically significant; p<0.05

Among the 60 cases, two were found to have HBV and HCV co-infection with the presentation of acute jaundice. Furthermore, two of the patients were found to have HIV and HBV co-infection among the study population.

DISCUSSION

Viral hepatitis has a high burden of disease globally, where more than one-fifth of the world's population is estimated to be living with chronic infection of HBV and HCV [13]. The common cause of jaundice in pregnancy is acute viral hepatitis which if left untreated could lead to cirrhosis in approximately 40% of individuals due to progressive liver fibrosis, a clinical condition that confers increased risk of hepatocellular cancer, hepatic failure and other complications of advanced liver disease [14].

In the present study, out of the total 60 samples that were collected, 29 (48.3%) were from the age group 21-30 years, followed by the

age group of 31-40 years accounting for 38.3% of the samples. Similar findings were observed in studies conducted by Bayo P et al., and Kumari K et al., [15,16]. The mean age of the study population was 29.87±3.3 years. Maximum samples were collected from the Antenatal ward of the Obstetrics and Gynaecology department (60%), followed by out-patient department (25%) and ATC (15%).

In present study, there were 16 samples for HBV which was positive in ELISA but were found to be below quantifiable range or not detectable by TrueNat PCR, whereas in the case of HCV, 11 such samples were analysed. These findings can be correlated with similar findings obtained by Mehta V et al., with 69.7% positivity rate [17]. Present study was also in tandem with different studies done in India by Barman B et al., which showed 39.3% and 21.9%, for HBV and HCV, respectively by PCR, Safi MAA and Ansari K and Omrani in which positivity rate was found to be 38% by EIA and Mohan KV et al., showed 24% by PCR [18-21]. Several studies done in different parts of the world by Bahadar N et al., [22], Kwenti TE et al., [23], Poel CL van der et al., also showed similar result with present study where Nucleic Acid Amplification (NAAT) testing are recommended over other assays [24]. A study by Shenge JA et al., highlights the low sensitivity of non NAAT tests like Rapid Diagnostic Test (RDT) assays which is most commonly used in the healthcare infrastructure like district hospitals of Manipur [25]. However, in a study by Vandana S and Sameer RV the result of immunochromatographic card test was found to be concordant with hepatitis B ELISA [26]. Tang W et al., conducted a multisystemic review and meta-analysis which validated point of care viral load assays demonstrate excellent diagnostic performance when used in various settings and populations [27]. An observation in a control group study by Kwenti TE et al., HCV RNA could be detected in only in few samples that were positive with the ELISA which can either signify a false positive result, or the phenomenon known as spontaneous viral clearance, which was seen in 10-60% of individuals that have been infected with the virus who have the ability to clear the virus from their system even without treatment [23]. This phenomenon further emphasises the importance of investigating further before beginning treatment [28].

In present study, 16 samples that were positive on ELISA were not detected with HBV DNA on TrueNat PCR. The key factors that affect the accuracy and reproducibility of ELISA could be during each assay step, any substances may get adsorbed to the solid phase due to Non Specific Binding (NSB), which can cause a high background reading or false immunosignal [29]. Cross-reaction resulting from enzyme-labelled secondary antibody against detection antibody can lower the assay selectivity thus causing inaccurate and irreproducible findings as in the case of studies conducted by Mendel CM and Mendel DB; and Dietzgen RG and Francki RI [30,31]. Contamination during sample processing could be another reason for this incongruence.

The high positivity rate in this study for both HBV and HCV may be due to sampling from patients who presented with deranged LFT and/or recent development of jaundice. Similar findings were observed in a study by Desikan P et al., were LFTs are potentially important predictors for HBV and HCV infection [32].

Manipur with hardly 0.2% of the total population of India contributes nearly 8% of the country's total HIV positive cases [33]. The high prevalence of HIV infection may be due to high prevalence of Intravenous Drug Users (IVDUs) in the state which is also an international border region with Mynamar. Due to this, the prevalence rate of co-infection of HBV, HCV with HIV are high in this small state. In present study, also 3.3% of the patients had HIV and HBV co-infection. The co-infection would have been much higher if the study was done in the high-risk population as in the case of a study by Goswami P et al., where the prevalence rate was 11% [34].

In a study by Parthiban R et al., the rate of HCV RNA transmission from mother to infant was 25% if the denominator is HCV RNA viremia which is quite high [35]. Moreover, chronic HBV infection which is acquired for the age of five years from vertical transmission is a major cause of HCC [36,37]. This can be prevented by timely testing and diagnosis of HBV and HCV infection. It is the gateway for access to both prevention as well as care and treatment services. Timely testing of the mothers will aid in early detection, treatment and prevention from maternal transmission to new-borns in order to achieve hepatitis-free India in the future. Present study focuses on the maternal testing of HBV and HCV which will impact not just one life but even the future generations that follow a HBV or HCV positive woman.

The Global Hepatitis Health Sector goals of HBV and HCV elimination require knowledge of the regional prevalence and epidemiology of both viruses to implement strategies to mitigate risk factors and reduce transmission [38]. The WHO also recommends the use of nucleic acid based diagnosis prior to therapy [28].

TrueNat works on the principle of quantitative RT-PCR based on Taqman chemistry. TrueNat is an ICMR approved chip-based RT-PCR. It is a test for the quantitative detection of HBV-DNA and HCV-RNA in human plasma, serum and whole blood samples. TrueNat aids in the estimation of viral load. It has the following advantages, such as point of care, portable, wireless data transfer, battery operated, automated and it functions in a wide range of environmental condition with minimal user input, making them suitable for use even in primary healthcare settings that typically have fewer facilities and report PCR results in less than an hour. The Truelab Real Time micro PCR System enables decentralisation and near patient diagnosis [12]. This is an excellent Point-of-Care (PoC) assay for testing of HBV and HCV. With other testing assays which have a turnaround time of 30-45 days depending on the number of patient load, TrueNat will shorten it to a few hours. This will give a high impact on the "same-day test and treat" strategy.

The optimal settings for the establishment of HBV and HCV PoC platforms are likely to be where there are populations at high-risk of loss to follow-up, such as among Persons Who Inject Drugs (PWID), harm reduction centres, prisons and rural settings with no laboratories or no hospitals. These populations would most benefit from a rapid test-and-treat approach. The availability of multi disease testing devices and PoC molecular platforms will bring new integration prospects and provide substantial system efficiencies and cost savings. Moreover, WHO recommends PoC assays for other infectious diseases where their use has been established for a longer period of time, such as HIV [39] and Tuberculosis [40], and they are available in LMICs. Moreover, TrueNat PCR testing platform has been utilised in rural India during the COVID-19 pandemic [41].

Limitation(s)

Limitation of the study was that the study could not be conducted on a larger number of participants. Although the study demonstrated the diagnostic accuracy of PoC HBV and HCV viral load tests, their performance, cost, and accessibility need to be considered when scaling up this service in different settings across India. However, in a resource-poor setting like our state and many parts of India, instead of utilising low sensitive and low specific RDT as screening tests, it is more judicious to use NAAT test like TrueNat which will detect the viruses which cannot be detected by the RDTs. It would be rational for us to use single NAAT test and give early diagnosis and treatment instead of a missed diagnosis which will lead to disease progression and life-threatening complications. For such a fatal disease, cost of a diagnostically accurate test should not be the main concern. The present study was confined to only one institute, and only to the antenatal population with presentations of deranged LFT and/or clinical jaundice, there might be Berkson's bias which draw forth a need to carry out this study in the general population with larger sample size.

CONCLUSION(S)

On comparison, TrueNat PCR was found to be more sensitive and specific than ELISA for detection of HBV and HCV among anenatal women attending our institute. The TrueNat PCR is useful to make clinical decisions on starting antiviral therapy and also in documenting

the efficacy of the antiviral therapy. Testing and diagnosis of HBV and HCV infection is the gateway for access to both prevention as well as care and treatment services. Testing will also provide an opportunity to link to interventions and to reduce transmission and vaccination of hepatitis B as well. TrueNat is a cost-effective, highly sensitive, PoC testing system with high turn over time which can be utilised in the low resource peripheral areas.

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