DOI: https://doi.org/10.53350/pjmhs20231769

ORIGINAL ARTICLE

Correlation between Hepatic Enzymes and Viral Load of HBV in Hepatitis B patients

TABINDA IJAZ[1], FAYYAZ AHMAD[2], MUHAMMAD ATIF[3], SAJJAD ULLAH[3], AHMED BILAL WAQAR[4]
[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]PESSI, MNCH, KLP, Lahore, Pakistan
[3]University Institute of Medical Laboratory Technology, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan
[4]The University of Chenab, Gujrat Pakistan
Correspondence to Dr. Ahmed Bilal Waqar, E-mail: drabwaqar@yahoo.com

ABSTRACT

Background: Hepatitis B virus is a causative agent of hepatitis B, and it is a severe global health issue.
Aim: To investigate the increased level of aminotransferases and its association with viral load of HBV infected patients and to find the most sensitive and reliable technique for identification of HBV infection.
Methods: This cross-sectional study included 198 patients of HBV. Blood samples were collected from these patients in serum tubes for detection of HBV by different techniques such as Immunochromatographic (ICT), Enzyme linked immunosorbent assay (ELISA), and whole blood was collected which was further processed for DNA extraction and real time PCR.
Results: There were 64 female and 134 males in this study. All the patients were positive for HBV by the ICT method and also by ELISA method but for the RT-PCR out of these 198 patients 89(45%) were positive and 109 (55%) were negative. This study demonstrates no significant correlation between liver enzymes with viral load. The ICT test method is active and simple but not a precise and unique test also has no statistical correlation with viral load, but ELISA is correlated with viral load recognized by real time PCR. (P value=<0.001, 95% Cl=0.40-0.69, r value =0.56).
Conclusion: Our results concluded that the immunochromatographic technique is fast and easily available but not a precise and definitive test and has no statistical correlation with viral load, but ELISA is associated with viral load proved by RT-PCR. Real time polymerase chain reaction is the gold standard test for the detection of Hepatitis B virus infection. The practical implication of this study is that ICT technique is no longer a reliable technique for the diagnosis of HBV infection and the patients positive on ICT should be confirmed for HBV in PCR for both diagnostic and prognostic purposes.
Keywords: Hepatitis B Virus, Liver enzymes, Immunochromatographic, Viral load, Enzyme linked immunosorbent assay

INTRODUCTION

HBV infection presents a great burden of disease world widely with around 350 million people suffering from this dangerous disease and around one million deaths annually[1]. After infection hepatitis B virus enters the blood circulation and it affects the hepatic cells[2]. HBV has DNA genome and complete sets of antigens and proteins to play with immune system of the host[3]. HBV is more prevalent in underdeveloped countries and is widely distributed in Africa and east Asia region and also in China where its rate is 9.09%[4].

HBV infection is also prevalent in Pakistan with positivity rate of 2.5% which is continuously increasing[5]. Although Pakistan has an extensive vaccine program against HBV infection with 54% of kids receive three doses of hepatitis B vaccine[6]. A study in Lahore showed that 49% of regular athletes and 42% of students and employees in pharmaceutical industry have been vaccinated against HBV infection[7].

Hepatitis B has many transmission routes and is the major contributor to the epidemic of liver infections[8]. The major sources of spread of HBV are through donation of blood transfer, use of contaminated syringes used for drugs, organ transplantation, use of contaminated blades for shaving by street barbers, dental and surgical therapies and unprotected sexual contact[9]. The pathway through HBV genetic material is transmitted in humansis by the entry of viral DNA into covalently closed circular DNA (cccDNA) that is responsible for the spread of the infection in the hepatocytes[10]. Many viral receptors have been responsible for assisting the entry of viral DNA into the host cells and these receptors have been targeted by many studies to find a cure against the spread of HBV infection[11].

Inlaboratories viral infections can be detected through various techniques. Most common method of diagnosis is based on immunochromatographic method, ELISA and PCR[12]. In addition to this other blood markers are also used for screening and prognosis purposes such as bilirubin and liver enzymes ALT, AST, ALP. The ICT method is more popular because it is cheap, and the results are available immediately and can be employed for large scale screening purposes. On the other hand, ELISA and PCR tests are confirmatory tests with greater precision and accuracy but they are much costly and not every laboratory has the facility of ELISA and PCR especially in the underdeveloped countries[13].

The ICT could be a fast, cheap and time saving technique and have high degree of sensitivity and specificity however there are some possibilities to false positive and false negative results. Liver enzymes additionally increase in all liver diseases other than HBV, so these don't seem to be specific for HBV. Anti HBsAg enzyme linked immunosorbent serologic assay shows a sensitivity in the range of 91-97% while PCR is considered to be 100% sensitive and specific for HBV infection[14].

We frequently utilize four diagnostic techniques for the detection of HBV in serum. The ICT fast test kit, Liver enzymes, ELSIA test for the detection of antibodies against HBV and Nucleic acid-based identification, Real Time PCR. In this study we will explore all these techniques and find out the utilities of these techniques in diagnosing HBV patients.

MATERIAL AND METHODS

Study Design: This cross-sectional study was conducted in Department of Chemistry, Government College University, Lahore, Pakistan from October 2018 to September 2019 after IRB permission. A total of 198 patients were included in this study. Blood samples were collected from these patients under aseptic sterile condition in both serum tubes and also whole blood was collected for further DNA extraction and PCR analysis.
Hepatitis B surface Antigen screening through Immunochromatographic technology (ICT): This is achromatographic immunoassay established method. The strip is precoated with anti-HbsAg Abs. HBsAg in the serum makes complex with the with anti HbsAg Abs present in the strips and the positive reaction is denoted by the color line in the control and sample area and negative results is denoted by a single line in the control region only(ABBOT, USA).

Received on 23-12-2022
Accepted on 24-05-2-23

Serum Biomarkers: Serum sample was used for analysis of total bilirubin and liver enzymes such as ALT, AST and ALP (Bio Rad, Pakistan) on Micro lab 300 instrument, which is a semi-automated clinical chemistry analyzer, Merck. The analyzer determines the amount of change in absorbance over an established time interruption. The rate of change of absorbance is directly proportional to the activity of the enzymes in the serum sample.

Enzyme linked immunosorbent assay (ELISA): ELISA for HBsAg was used for the estimation of HbsAg in blood serum of patients. ELISA method is a sandwich immunoassay technology in which Microtiter well plate is pre coated with monovalent antibodies and antigen from serum make an antigen antibody complex. On addition of conjugate which contain the enzyme labelled second antibody, a sandwich is formed. After addition of substrate a color product is formed whose absorbance is taken at 450 nm in ELISA reader and calibration curve is prepared and results are calculated (ABBOT, USA)

DNA extraction: DNA extraction was done by the viral spiral kit, the DNA was extracted with 4 steps. (Lyses, binding, washing and elution) and extracted DNA was further processed by RT-PCR (QIAamp, Germany).

Amplification of DNA through Real time Polymerase chain reaction (RT-PCR): DNA amplification was done by RT-PCR after optimization of the working conditions. Dream Taq was used for amplification with DNA and forward and reverse primers as shown in table 1. Denaturation was done at 94 C, annealing was carried out at 58 C and extension was done at 72C. PCR reaction was run for 35 cycles to obtain the desired results.

Table 1: Primers and probes

Sequences ID	Polarity	Oligonucleotide sequences 5-3	Length	Product Size
HBV Forward	Forward	GTGTCTGCGGCGTTTTATTCA	20	98bp
HBV Reversed	Reversed	GACAMACGGGCAACATACCTT	21	
HBV Probe		/5Cy5/CCTCTKCATCCKGCTGCTATGCCTY MWC/31AbRQSp/	28	

Statistical Analysis: The statistical data was entered and analyzed by using SPSS software version 21. A p value of <0.05 was considered as statistically significant.

RESULTS

Demographic Data: In this study a total of 198 patients were included. The age range of the patients was from 5-60 years. There were 64 female and 134 males in this study. 58 patients were single, and 140 patients were married. All the patients were positive for HBV by the ICT method and also by ELISA method but for the RT-PCR out of these 198 patients 89 (45%) were positive and 109(55%) were negative as shown in table 2.

Table 2: Analytical and scientific aspects of participants admitted in this consideration (n=198)

Characteristics		Prevalence (%)
Age (years)	5-15	6(3)
	16-30	69(35)
	31-45	87(44)
	45-60	36(18)
Sex	Female	64(32)
	Male	134(68)
Marital status	Single	58(29)
	Married	140(71)
ICT HbsAg	Positive	198(100)
	Negative	0(0)
ELISA for HbsAg	Positive	198(100)
	Negative	0(0)
RT PCR for Hepatitis B	Positive	89(45)
	Negative	109(55)

TaqMan Probe HBV real time PCR HBV Viral Load (log IU/ml) was used in RT PCR as shown in Figure 1 and amplification if HBV DNA is shown in figure 2.

Figure 1: TaqMan Probe HBV real time PCR HBV Viral Load (log IU/ml)

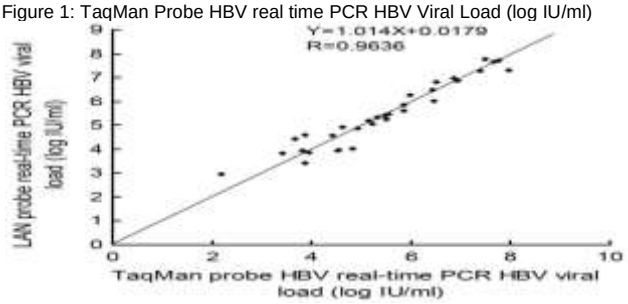
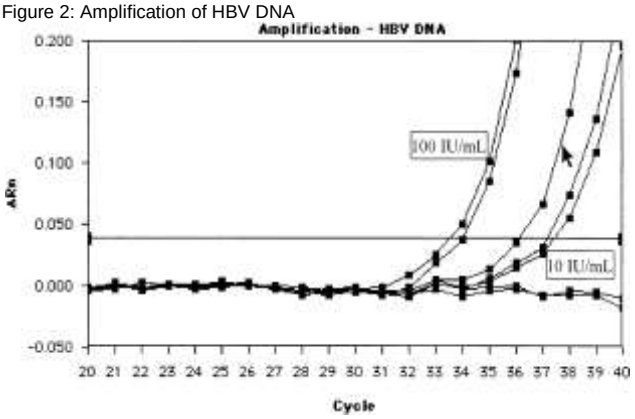


Figure 2: Amplification of HBV DNA



Mean value of ALT was 29.14 ± 10.16IU/L, and AST was 25.36±143.8 IU/L, and ALP was 215.50±50.8 IU/L and bilirubin was 0.533±0.34 IU/L. It was found that liver enzymes and bilirubin in serum were within reference range in most of the HBV patients.

Correlation of hepatic enzymes with viral efficacy: Pearson correlation coefficient was used to find out the correlation of hepatic enzymes with viral load and found no statistically significant results (P≥0.001; Table 3).

Table 3: Correlation of hepatic enzymes with HBV viral load

Parameters Viral Load	ALT IU/l	AST IU/l	ALP IU/l
P-value	0.48	0.36	0.67
R-value	-0.06	-0.08	-0.04
95%CI	-0.26- 0.14	-0.28- 0.13	-0.27- 0.15

Correlation of ELISA against viral load: On a subset of samples (n=89) those were positive on PCR and ELISA to a correlation analysis was done. Results point out there is a wide spread link between ELISA and viral load estimated through RT_PCR(P=<0.001, r=0.57, 95%CI=0.39-0.68).(P<0.001; Table4).

Table 4: Correlation of ELISA with HBV viral load

Parameters Viral Load	ELISA
P-value	<0.001
R-value	0.57
95%CI	0.39-0.68

DISCUSSION

This study was aimed to find the association of liver enzymes, ICT method, ELISA method with viral load in HBV positive subjects. In this study, out of total 198 HBV subjects 64 were female and 134 were male. It has been determined that an Asian male severally affected with hepatitis B virus and has a 20-25% chance of developing HBV infection as compared to females[15]. In all areas of world estimated that male have more chances of hepatocellular carcinoma (HCC)[16]. There can be multiple factors behind this high

prevalence of HBV infection in males as compared to females such as male are more exposed to risk factors such as using injections for drugs, going to barber shops for shaving, sexual intercourse which makes them more vulnerable to HBV infection[17].

In this study the age related with large incident of hepatitis B contamination is 31 to 45 years (44%), followed by 16 to 30 years (35%), and age between 46 to 60 years (18%) and minimum occurrence rate used to be discovered in age group 5 to 15 years (3%). Keeping in mind the routes of HBV transmission, people with ages between 16 and 45 are more exposed to the risk factors involved in HBV infection[17]. Our finding is in agreement with Yewande et al., 2018 who also report similar age group having high frequency of HBV infection and mention that children less than 15 years and adults more than 45 years are less involved in behaviors putting them at risk of HBV infection[18].

Our results are in agreement with previous studies who also report that there is no correlation between liver enzymes and hepatitis B virus[19]. ALT, AST, ALPcan increase in many other liver ailments such as HCV, liver fibrosis, hepatocellular carcinoma[20]. Chen *et al*., in 2010 also reported that there is no correlation between the stages of hepatitis and enzymes of liver (P = 0.811 and 0.603). However, it was also noted that high levels of antigens detected by ELISA are statistically significantly correlated with RT-PCR[21].

Simpler and rapid test methods such as ICT are in common use in laboratories within limited facilities, less trained workers and in remote areas. Additionally, ICT methods are cheaper and easily available. Excessive and unregulated use of ICT methods can lead to severe concerns and sometimes patients' diagnosis is compromised. Specialized diagnostic techniques such as EIA, ELISA and PCR etc are highly sensitive and specific for HBV infections but they are only available in major labs with specialized workforce and advanced equipment[22].

This study has also done the correlation of ELISA with viral load of HBV and found a positive statistically significant correlation between ELISA and RT-PCR[23]. The present study demonstrates that ELISA is a more sensitive approach rather than ICT and ELISA is well correlated with viral load[24]. ICT technique is no longer a reliable technique for the diagnosis of HBV infection and the patients positive on ICT should be confirmed for HBV in PCR[25]. There are chances of false positive results on ICT because many of those tests which were initially positive for ICT device later found negative for HBV on PCR. Though all ICT tests were also positive for ELISA and only 89 were further positive on PCR. A large number of samples that were negative on PCR suggest that in ELISA it was mishandling, contamination in wells, improper washing or errors in results interpretations[26].

CONCLUSION

The Hepatitis B testing by less sensitive ICT device should not be used as a definite testing for the diagnosis of HBV infection. The HBVDNA viral load has no relation with hepatic enzymes; however, a positive correlation was found between ELISA and hepatitis B viral load. Other investigations with large sample size and patients follow up are required to evaluate comparison of enzymes liver with viral load in detail.

Conflict of interest: Nil

REFERNCES

1. Hu KQ, Pan CQ, Goodwin D. Barriers to screening for hepatitis B virus infection in Asian Americans. Digestive diseases and sciences. 2011 Nov;56:3163-71.
2. Pan XB, Lu YQ, Lin SZ, Ye J, Wu N, Lou YY, Ni L, Han JC, Zheng XQ, Wei L. An assessment of upper limits of normal for ALT and the impact on evaluating natural course of chronic hepatitis B virus infection in Chinese children. Official journal of the American College of Gastroenterology| ACG. 2018 Nov 1;113(11):1660-8.
3. Allain JP. Transfusion risks of yesterday and of today. Transfusion clinique et biologique. 2003 Feb 1;10(1):1-5.
4. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet. 2015 Oct 17;386(10003):1546-55.
5. Waqar M, Khan MA, Rehman Z, Idrees M, Wasim M, Asghar A, Rukh QS. Hepatitis B Virus (HBV) a Severe Health Problem in Mardan, Khyber Pakhtunkhwa. BioScientific Review. 2019 Feb 14;1(1):25-32.
6. Shagufta H, Niveen Asher P, Rabia S. Hepatitis B and C prevalence and prevention awareness among health care workers in a tertiary care hospital.2010
7. Karim SM, Rahman MR, Shermin S, Sultana R. Correlation between aminotransferase ratio (AST/ALT) and other biochemical parameters in chronic live
8. r disease of viral origin. Delta Medical College Journal. 2015 Feb 14;3(1):13-7.
9. Lee DH, Cho Y, Seo JY, Kwon JH, Cho EJ, Jang ES, Kwak MS, Cheong JY, Cho SW, Lee JH, Yu SJ. Polymorphisms near interleukin 28B gene are not associated with hepatitis B virus clearance, hepatitis B e antigen clearance and hepatocellular carcinoma occurrence. Intervirology. 2013;56(2):84-90.
10. Asif N, Arif N, Habib SM, Taimur Z, Hussain A. A Descriptive Study on the Prevalence and Risk Factors of two Killing Diseases: Hepatitis B (HBV), Hepatitis C (HCV). Pakistan Journal of Medical & Health Sciences. 2022 Nov 16;16(09):755-.
11. Albayrak A, Uyanik MH, Cerrah S, Altas S, Dursun H, Demir M, Uslu H. Is HMGB1 a new indirect marker for revealing fibrosis in chronic hepatitis and a new therapeutic target in treatment?. Viral immunology. 2010 Dec 1;23(6):633-8.
12. Chisari FV, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. Pathologie Biologie. 2010 Aug 1;58(4):258-66.
13. Fatima K, Ahmad S, Bukhari H, Ejaz A, Ch S, Nasir M. Diagnosis of Hepatitis C Virus Infection in human serum using ELISA and Raman Spectroscopy. Pakistan Journal of Medical & Health Sciences. 2022 Mar 23;16(02):69-.
14. Fazaa AH, Atya AK, Kredy HM. Evaluation of Liver Function Tests and Their Correlation with HBV Viral Load in Patients with Hepatitis B Virus, Thi-Qar, Iraq. HIV Nursing. 2022 Oct 14;22(2):1112-6.
15. Adeyemi AA, Omolade O, Raheem-Ademola R. Immunochromatographic testing method for hepatitis B, C in blood donors. J. Antivir. Antiretrovir. S. 2013;3(2).
16. Mayaphi SH, Rossouw TM, Martin DJ, Masemola DP, Olorunju SA, Mphahlele MJ. HBV/HIV co-infection: the dynamics of HBV in South African patients with AIDS. South African Medical Journal. 2012 Mar 1;102(3):157-62.
17. Anjali H, Issac A, Anjali MR, Anish TS. Transfusion-transmissible infections among voluntary blood donors at Government Medical College Thiruvananthapuram, Kerala, India. Asian Journal of Transfusion Science. 2012 Jan;6(1):55.
18. Obiomah C, Amilo G, Ndulue I. Evaluation of HBsAg Quantification as Surrogate to HBV DNA Viral Load in Hepatitis B Infected Patients in Anambra State, Nigeria. American Journal of Molecular Biology. 2020 Jun 1;10(03):129.
19. Yewande N, Omotayo FA, Babatunde O, Solomon B, Adebowale O, Babatunde M. Hepatitis B virus infection among sexually active individuals in Nigeria: a cross-sectional study. The Pan African Medical Journal. 2018;30.
20. Alam S, Ahamd N, Alam K, Mostafa G, Khan M. Correlation between hepatitis B viral DNA load and extent of liver pathology in patients with chronic hepatitis B. Hepatitis Monthly. 2008 Sep 30;8(3):185-9.
21. Martin P, Nguyen MH, Dieterich DT, Lau DT, Janssen HL, Peters MG, Jacobson IM. Treatment algorithm for managing chronic hepatitis B virus infection in the United States: 2021 update. Clinical gastroenterology and hepatology. 2022 Aug 1;20(8):1766-75.
22. Chen EQ, Huang FJ, He LL, Bai L, Wang LC, Zhou TY, Lei XZ, Liu C, Tang H. Histological changes in Chinese chronic hepatitis B patients with ALT lower than two times upper limits of normal. Digestive diseases and sciences. 2010 Feb;55:432-7.
23. Al-Matary AM, Al Gashaa FA. Comparison of different rapid screening tests and ELISA for HBV, HCV, and HIV among healthy blood donors and recipients at Jibla University Hospital Yemen. Journal of Medicine and Life. 2022 Nov 1;15(11):1403-8.
24. Zeng LY, Lian JS, Chen JY, Jia HY, Zhang YM, Xiang DR, Yu L, Hu JH, Lu YF, Zheng L, Li LJ. Hepatitis B surface antigen levels during natural history of chronic hepatitis B: a Chinese perspective study. World Journal of Gastroenterology: WJG. 2014 Jul 7;20(27):9178.
25. Trivedi M, Patil S, Shettigar H, Mondal SC, Jana S. Evaluation of biofield modality on viral load of Hepatitis B and C viruses. Journal of Antivirals & Antiretrovirals. 2015;3(7):083-8.
26. Fung J, Lai CL, Fong DY, Yuen JC, Wong DK, Yuen MF. Correlation of liver biochemistry with liver stiffness in chronic hepatitis B and development of a predictive model for liver fibrosis. Liver International. 2008 Nov;28(10):1408-16.
27. Lai CL, Yuen MF. Management of chronic hepatitis B. InGut and Liver 2011 (pp. 95-101). Karger Publishers.