



## Receiver Operating Characteristic Analysis of Liver Function Tests in Patients with Chronic Viral Hepatitis B in Baghdad, Iraq

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### Abstract

Hepatitis B virus (HBV) infection is a global health challenge, and clearance or persistence of HBV is mostly determined by host immune responses. Therefore, this study aimed to determine HBV effects in chronic HBV patients living in Baghdad receiver operating characteristic (ROC) analysis. The impact of HBV genotypes on liver-function parameters was explored. A case-control study was conducted during June-October 2018 on 80 chronic HBV patients and 96 matched control. The results revealed that most patients were males 50 (62.5%), while female patients accounted for 30 (37.5%). The sera of patients were positive for anti-HBc (hepatitis B core antigen) IgG and -HbsAg (hepatitis B surface antigen) antibodies, while they were negative for anti-Hbc IgM antibody. Such profile is consistent with the interpretation of chronic HBV infection. Four liver-function tests (LFTs); total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assessed. Significantly increased levels of ALP, ALT and AST were observed in HBV patients compared to normal healthy control, while TSB showed no significant variation. However, in receiver operating characteristic (ROC) analysis, only ALP and AST occupied an excellent area under curve (AUC > 0.90).

### 1. Introduction

Hepatitis B virus (HBV) infection is a global health challenge. It has been estimated that 257 millions of humans are infected with the virus, resulting in an estimated annual mortality of 887,000 due to cirrhosis and/or hepatocellular carcinoma [1, 2]. A community-based study in Iraq reported a prevalence of 1.6% for hepatitis B surface antigen (HBsAg). In addition, the anti-hepatitis B core antigen (HBc) and anti-HBs antibodies were reported at prevalence of 9.7 and 17%, respectively [3]. Accordingly, the endemicity of HBV in Iraq was considered to be low / intermediate level. Sample surveys are critical in providing information in a broad range of areas, serving as a valuable resource for guiding actions and policies. In the United States, the National Center for Health Statistics (NCHS) is the principal health statistics agency under the Center for Disease Control (CDC). It conducts several population surveys, such as the National Health and Nutrition Examination Survey (NHANES), the National Health Interview Survey (NHIS), and the National Survey of Family Growth (NSFG). These large-scale surveys use complex sampling designs, including stratification, multistage cluster sampling, and unequal selection probabilities to obtain a representative sample more efficiently in terms of time and cost [4]. Failure to

account for the complex survey design may result in biased parameter estimators, underestimated standard errors, and possibly misleading conclusions [5]. A further cytokine (IL-35) has been suggested to play a role in maintaining viral persistence. IL-35 is the latest member of IL-12 family, which is recognized to have anti-inflammatory and immunosuppressive properties recent studies suggest a critical immune-pathogenic role for IL-35 in potentiating a chronic infection [6, 7] Liver-infecting viruses (A, B, C, D, and E) have a substantial influence on humanity, generating high rates of morbidity and mortality in infected persons with acute and chronic infections. Therefore, mankind has spent decades attempting to develop diagnostic and preventive techniques, such as vaccine, pharmaceuticals, and liver transplantation [8]. Infections with Hepatitis B are comparable to HIV, malaria, and tuberculosis as the four most lethal infectious diseases globally [9]. The World Health Organization (WHO) has a strategy for 2030 with the participation of 114 member states to manage and neutralize both hepatitis B and C [10]. The aim of this study Receiver operating characteristic analysis (area under curve) of total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) among hepatitis B virus infection patients.

## 2. Materials and Methods

### 2.1. Patients and Control

- related serum status was negative, the statistical work, the mean age  $\pm$  standard deviation of the patients and the control group was  $40.7 \pm 13.8$  and  $43.6 \pm 11.8$  years, respectively.

### 2.2. Laboratory Methods

#### 2.2.1. Liver Function Tests

The sera of patients and control were quantitatively assessed for total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by using ready-to-use kits (Linear Chemicals, Spain), and instructions of manufacturer were followed. The four kits were based on a similar procedure (colorimetric method). The TSB was given in units of  $\mu\text{mol/L}$ , while APL, ALT and AST were expressed as U/L.

#### 2.2.2. Statistical Analysis

Receiver operating characteristic (ROC) curve was constructed for each parameter, and the area under curve (AUC), sensitivity and specificity were consequently estimated. Spearman bivariate correlation was employed to understand the correlation between certain parameters. The statistical package SPSS version 19.0 was employed to carry out these analysis.

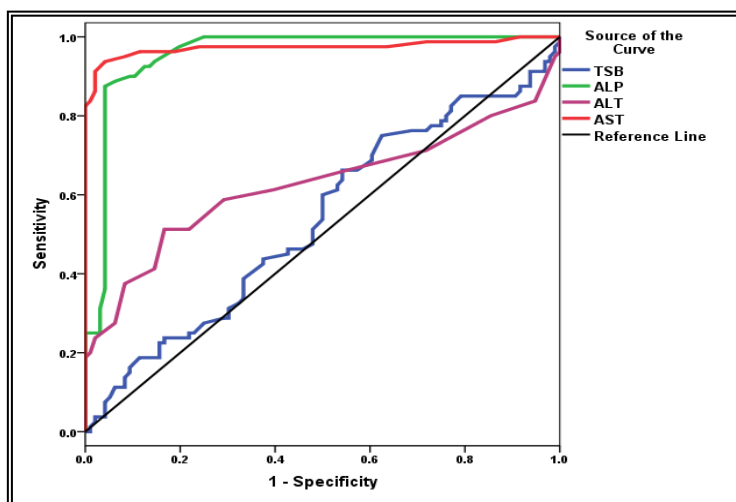
## 3. Results and Discussion

Receiver operating characteristic (ROC) is a statistical analysis used to describe the discrimination accuracy of a diagnostic test or prediction model. It constructs an area under curve (AUC) and through which the sensitivity and specificity of a test is estimated at a specific cut-off value. Thus, the accuracy of a test is measured by the AUC, which has a range from 0.5 to 1.0, and accordingly the accuracy of a test is determined. For instance, an AUC range of 0.60-0.70 makes the test poor from the diagnostic point of view, while it is excellent if the range is 0.9 – 1.0 [11]. The obtained results declared that ALP and AST were excellent diagnostic tests in patients with chronic HBV infection. The AUCs of both tests were 0.957 and 0.973, respectively, and the associated sensitivities and specificities were 90.0% and 95.0%, and 89.6% and 91.7%, respectively. The recorded p-value was less than 0.001 (highly significant). For ALT, although the AUC was significant ( $p = 0.004$ ), its AUC was 0.626. Such value limits its diagnostic significance and it is considered as poor. In the case of TSB, no diagnostic significance was estimated in chronic HBV infection, and the estimated AUC was 0.536 ( $p = 0.146$ ) (Table 1 and Figure 1).

**Table (1).** Receiver operating characteristic analysis (area under curve) of total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) among hepatitis B virus infection patients.

Liver Function Test	AUC (95% C.I.)	Sensitivity (%)	Specificity (%)	Cut-off Value	P value
TSB ( $\mu\text{mol/L}$ )	0.536 (0.449 – 0.622)	53.8	50.0	6.6	0.416
ALP (U/L)	0.957 (0.925 – 0.988)	90.0	89.6	72.0	< <b>0.001</b>
ALT (U/L)	0.626 (0.536 – 0.175)	61.3	60.4	16.5	<b>0.004</b>
AST (U/L)	0.973 (0.945 – 1.000)	95.0	21.5	< <b>0.001</b>	

AUC: Area under curve; CI: Confidence interval; p: Probability.



**Figure (1).** Receiver operating characteristic analysis (area under curve) of total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) among hepatitis B virus infection patients (details of the figure are given in table -1).

Distributing the serum level of LFTs according to their relation to the medians (> median and  $\leq$  median) confirmed the results of ROC analysis. The highest odds ratio (OR) was associated with ALP and AST (78.74 and 198.33, respectively), and the two associations were highly significant ( $p < 0.001$ ). A significant OR was also recorded for ALT, but it was much lower than that of ALP and AST (OR = 2.41;  $p = 0.006$ ). For TSB, no significant association was observed (Table 2).

**Table (2).** Distribution of liver-function test levels (> median and  $\leq$  median) in hepatitis B virus infection patients and control.

LFT	HBV Patients (N = 80)				Control (N = 96)				OR (95% CI)	P Value
	> Median		$\leq$ Median		> Median		$\leq$ Median			
	N	%	N	%	N	%	N	%		
TSB	41	51.2	39	48.8	46	47.9	50	52.1	1.14 (0.63-2.06)	0.762
ALP	74	92.5	6	7.5	13	13.5	83	86.5	78.74 (28.66-216.37)	< 0.001
ALT	49	61.2	31	38.8	38	39.6	58	60.4	2.41 (1.32-4.42)	0.006
AST	77	96.2	3	3.8	11	11.5	85	88.5	198.33 (53.76-731.68)	< 0.001

LFT: Liver-function test; TSB: Total serum bilirubin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; OR: Odds ratio; CI: Confidence interval; p: Probability.

The obtained results highlighted the diagnostic potential of LFTs in chronic HBV infection and their order of significance was AST, ALP and ALT. Whereas, TSB failed to show any related significance. Therefore, the biomarker expectation of AST, ALP and ALT is suggested in the present study. Different biochemical markers of diverse clinical and epidemiological potential are augmented when HBV infection is established. These markers can occur independently or in different combinations; this is dependent on the disease stage and its natural history [12]. Among these biomarkers are LFTs, which are important in the evaluation of hepatic function and disease severity in HBV infection patients [13]. As in present study, it has been depicted that HBV infection may alter serum levels of certain hepatic enzymes (i.e. ALP, AST and ALT) [14, 15]. In this context, marked elevation of ALT in sera of chronic HBV patients with acute flare-up has been suggested. The release of AST and ALT into the bloodstream is a consequence of hepatocellular damage due to HBV infection; so an elevation of both enzymes is more frequently correlated with hepatic injury. With respect to ALP, its level has been significantly associated with HBsAg seropositivity (17); an observation that is supported by the present findings, because all HBV patients were seropositive for HBsAg and marked with a significant increased ALP level. A similar conclusion can be drawn for AST and ALT, and an increase in the levels of both enzymes has been reported in HBsAg-positive patients [15]. Although, AST, ALP and ALT were associated with HBV risk and their diagnostic values were suggested, they may represent an initial non-specific testing of HBV infection and specific diagnosis of HBV infection must involve the evaluation of further specific HBV serological markers that include certain HBV antigens and antibodies [16]. For TSB, its serum level showed no significant variation between HBV patients and control; therefore such serum protein might not be related to HBV chronicity. Such findings might be expected, because increased levels of TSB are generally associated with acute HBV infection rather than chronic infection [17]. It has been suggested that bilirubin might be implicated in the protection of specific kinds of diseases resulting from oxidative damage, and appeared to have the innate capacity to resist oxidative damage that occurs during acute HBV infection [17, 18]. Serum level of liver function enzyme and bilirubin are also subjected to alteration due to other conditions; for instance, medications used or occupational exposure to toxins by subjects prior to this study. Additionally, serum alcohol and HBV DNA serum level might also have an effect. As these conditions were not considered, they represent important limitations of present study results.

#### 4. Conclusions

The four parameters of liver functions were influenced by the HBV infection, but from the diagnostic view points, alkaline phosphatase (ALP) and aspartate aminotransferase (AST) recorded an excellent area under curve in the receiver operating characteristic analysis; therefore, their diagnostic significance is enhanced.

**Conflict of Interest:** The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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