

REVIEW ARTICLE

Hepatitis B surface antigen and liver function tests as prediction markers for patients infected with the hepatitis B virus.

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Abstract:

Background: The infection with the hepatitis B virus represents a significant global health challenge with serious complexities like the failure of the liver and hepatocellular carcinoma. It results in an estimated mortality of 1.12 million cases in 2022.

Aim of Study: This study aimed to use hepatitis B surface antigen and liver function tests as prediction markers for patients infected with the hepatitis B virus and compare them with viral load in Wasit Province, Iraq.

Materials and Methods: This study involved 75 patients, both male and female, who made up the study population. Hepatitis B virus (HBV)-positive patients' samples were gathered from Al-Karama Teaching Hospital and the General Public Health Central Lab in Kut City between December 2023 and May 2024, in addition to 75 apparently healthy volunteers as a control group. The enzymatic liver biomarkers (the ALT, the AST, the ALP, and the TSB) were detected by utilizing the Roche Cobas C111 system, and the hepatitis B surface antigen was detected by utilizing the ELISA.

Results: Both ELISA and viral load results were positive for all patients, and liver enzymes showed a significant and remarkable rise among the group of patients. **Conclusion:** The paper of research gives insight, as the utilization of ELISA is much more effective, and it could be used in monitoring the progress of HBV infection along with liver enzymes.

Keywords: Hepatitis B virus, HBS Ag, prediction.

Introduction

Hepatitis B virus (HBV) infection is a main cause of liver illness worldwide, often progressing to cirrhosis of the liver and hepatocellular carcinoma. Diagnostic and prognostic tools such as enzyme-linked immunosorbent assay (ELISA) and liver function tests are essential for clinical evaluation. Hepatitis B surface antigen (HBsAg) is an established early marker of HBV infection (1). Serum HBsAg quantification in chronic hepatitis B (CHB) patients provides valuable insight into viral replication and complements HBV DNA monitoring (2-4).

Covalently closed circular DNA (cccDNA), the transcriptional template of HBV, plays a central role in virus-related persistence. HBsAg is translated from mRNA transcribed from active cccDNA, reflecting the burden of infected hepatocytes. Therefore, intrahepatic HBsAg levels are proposed as surrogate markers for cccDNA concentration (5). Given this relationship, HBsAg quantification is considered indicative of the host immune response to therapy, independent of the virological response measured by serum HBV DNA (4).

The study by Al-Salih et al. in 2021 on two hundred patients with chronic hepatitis B who were admitted to the Teaching

Hospital of Liver and Gastroenterology in Baghdad concluded that chronic patients had higher stages of ALP, GPT, GOT, and TSB than the control, and elevated liver enzymes strongly indicate that liver cells have been damaged (6).

This study aimed to use hepatitis B surface antigen and liver function tests as prediction markers for patients infected with the hepatitis B virus and compare them with the viral load in Wasit Province, Iraq.

2. Materials and Methods.

2.1. Study Population.

This study involved 75 patients, both male and female, who made up the study population. Hepatitis B virus (HBV)-positive patients' samples were gathered from Al-Karama Teaching Hospital and the General Public Health Central Lab in Kut City between December 2023 and May 2024, in addition to 75 apparently healthy volunteers as a control group.

2.2. Collection of Sample.

Each person (cases and controls) included in the current study samples had five milliliters of venous blood drawn from them via vein puncture; the blood was then distributed nearly evenly (5,0 milliliters). Blood samples were collected into ethylene



diamine tetra acetic acid (EDTA) tubes and (2.5 ml) in serum tubes. Plain plastic containers were then made and labeled.

Every individual provided a 2.5 ml blood sample, which was kept at room temperature (25°C) for one hour. The serum was then separated by centrifugation, which was carried out for 5 minutes at 4000 rpm. The serum was split into five equal fractions using a micropipette and placed into five test tubes. The serum was then used to identify hepatitis B surface antigen (HBS Ag) and measure the following biochemical parameters using commercial kits. Until the assay was completed, the sera were kept at -20°C. Viral DNA was extracted from the blood in EDTA tubes to determine the viral load. Data were statistically analyzed using SPSS v26.

2.3. Biochemical analysis.

The enzymatic liver biomarkers (the ALT, the AST, the ALP, and the TSB) were detected by utilizing the Roche Cobas C111 system. The serum-filled gel tube was placed into the device so that it could automatically analyze the data.

2.4. Serological Test.

In clinical laboratories, the enzyme-linked immunosorbent assay test was utilized as the primary screening method for blood to identify the hepatitis B surface antigen (HBsAg) in human serum.

An effective way to diagnose and prevent HBV infection is through serological detection of HBsAg, and ELISA is now a widely used analytical tool for both clinical diagnosis of HBV in infected persons and blood donor screening.

This kit applies the principle of double antibody sandwich ELISA. On the microplate, pure hepatitis B surface antibody (HBsAb) is pre-coated; in a sample, HBsAg will initially interact with HBsAb, after which the enzyme-labeled HBsAb complex is combined with it, and the microplate turns blue. The purpose of this kit is to specifically identify the presence of the hepatitis B surface antigen (HBsAg) in human plasma or serum samples.

2.5. Real-Time PCR Test for the Quantitative Detection of Hepatitis B Virus

The HBV Real-TM Kit Quant Dx is a hepatitis B virus quantitative detection assay that uses real-time polymerase chain reaction. The process begins with plasma DNA extraction, continues with real-time amplification, and culminates in detection by the use of fluorescent reporter dye probes that are specific to HBV. Simultaneously, an HBV-specific Internal Control (IC) is recognized through dual color detection. Amplification products undergo strand separation at high temperature during each thermal cycling cycle, which paves the way for primer annealing and extension at low temperature (Table 1). Target sequences are amplified by a factor of a billion or more by exponential amplification, which is accomplished by repeatedly cycling between high and low temperatures. The same reaction amplifies both the HBV and IC targets at the same time. Without reopening the reaction tube after real-time amplification, the accumulating product can be detected and quantified by monitoring the fluorescence intensities in real time. The HBV Real-TM Quant Dx assay seeks out a specific sequence within the 5' gene, which codes for HBsAg. The area in question is both highly conserved and unique to HBV.

Table (1): Show the Real-Time PCR instrument program For HBV.

Stage	Temp, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	-	1
	95	5s	-	
	60	20s	-	
Cycling	72	15s	-	5
	95	5s	-	
	60	30s	FAM/Green, JOE/Yellow/HEX	
Cycling2	72	15s	-	40
	95	5s	-	
	60	30s	FAM/Green, JOE/Yellow/HEX	

3. Results

3.1. ELISA Detection of HBsAg

To confirm the hepatitis B virus infection, the suspected patients and healthy control subjects submitted to HBS-Ag detection by ELISA technique, and the results are demonstrated in table (2). The results show 75 (100.0%) of patients have active hepatitis B virus infection by finding positive results for HBS-Ag. While all 75 healthy control subjects (100.0%) had negative results for HBS-Ag, the variation was very significant (P > 0.001).

Table (2): Detection of HBS Ag by ELISA technique in studied groups.

Technique	Patients with HBV =75	control n = 75	P value
ELISA technique (HBS-Ag)			
Positive, n (%)	75 (100%)	0	0.001< ¥ HS
Negative, n (%)	0	75 (100.0%)	

¥: Chi-square test; HS: Highly significant at P ≤ 0.001.

3.2. Viral load diagnosis for HBV.

The present results show the mean levels of viral load were 3728812.04 ± 303791.88 in patients with HBV infection. The frequency distribution of patients according to viral load was as follows: 61 (81.3%) of HBV patients with low viremia, 9 (12.0%) of HBV patients with moderate viremia, and only 5 (6.7%) of HBV patients with high viremia in table (3).

Table (3): mean levels of viral Load in patient.

Groups	Mean	SD	SE
HBV	3728812.0395	303791.88	3531.12
Classification			
Low viremia, n (%)	61 (81.3%)		
Moderate viremia, n (%)	9 (12.0%)		
High viremia, n (%)	5 (6.7%)		

For sake of understanding, these various levels of viral loads in this study are classified as low (below 10000 copies\ ml), medium (10000–100,000 copies\ ml), high (above 10,0000 copies\ ml) Figure (5).

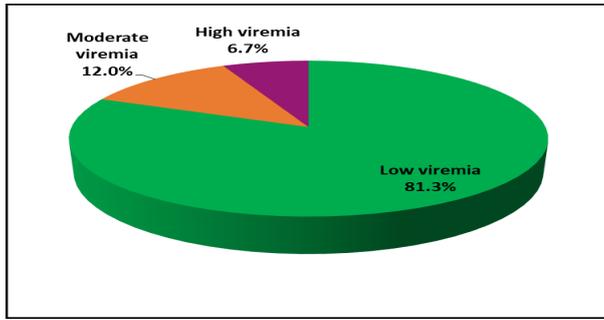


Figure (5): Distribution of patients according to Viral load.

3.3. Liver Function Tests:

3.3.1. Results of liver function tests (ALT, AST, ALP, and TSB) in patients and healthy controls.

The comparison of liver function exams (ALT, AST, ALP, and TSB) among patients and the control group has been carried out, and the results were established in table (4) and figures (1-4). The results were as follows:

1. ALT: Patients showed a mean level of 77.10 ± 11.72 IU/L compared to 20.53 ± 4.80 IU/L in controls ($p < 0.001$).
2. AST: Patients averaged 58.75 ± 7.35 IU/L vs. 17.70 ± 5.81 IU/L in controls ($p < 0.001$).
3. ALP: Elevated in patients (216.35 ± 28.2 IU/L) compared to controls (75.04 ± 9.08 IU/L, $p < 0.001$).
4. TSB: 2.02 ± 0.22 mg/dL in patients vs. 0.95 ± 0.21 mg/dL in controls ($p < 0.001$).

Table (4): Comparison of liver function tests (ALT, AST, ALP, and TSB) in patients and controls.

Groups		ALT	AST	ALP	TSB
HBV Patients	Mean \pm SD	77.10 \pm 11.72	58.75 \pm 7.35	216.35 \pm 28.2	2.02 \pm 0.22
	Range	46.00-99.60	41.00-89.00	157.80-297.00	1.60-2.70
Control	Mean \pm SD	20.53 \pm 4.80	17.70 \pm 5.81	75.04 \pm 9.08	0.95 \pm 0.21
	Range	8.90-29.00	8.20-33.10	54.00-99.00	0.33-1.70
p-value		< 0.001 † HS	< 0.001 † HS	< 0.001 † HS	< 0.001 † HS

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

TSB Total serum bilirubin, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase

Figure (1): Mean levels of ALT of patients and control subject.

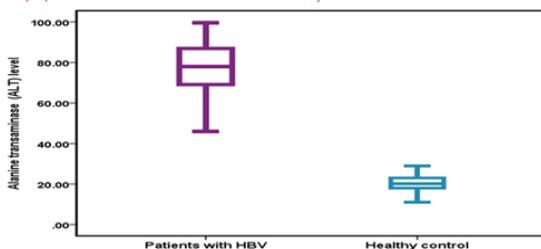


Figure (2): Mean levels of AST of patients and control subject.

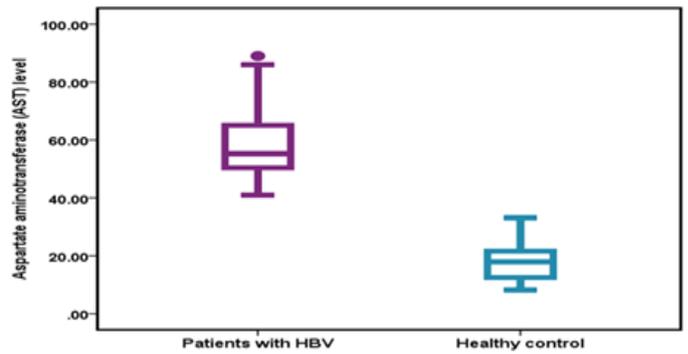
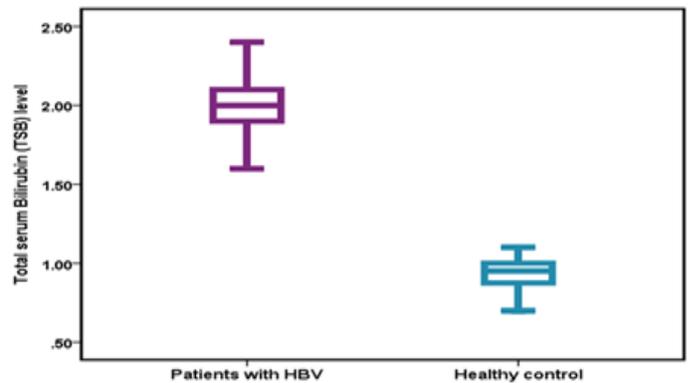


Figure (3): Mean levels of ALP of patients and control subject.



high viremia, respectively; the mean level was significantly higher in patients with high viremia compared to other groups ($P < 0.001$). Furthermore, the present results show there was a significant difference between patients with low viremia, patients with moderate viremia, and patients with high viremia according to both serum ALP and TSB ($P > 0.05$).

Table (5): Frequency distribution of liver function tests according to viral load

Groups		ALT	AST	ALP	TSB
Low viremia	Mean \pm SD	77.65 \pm 6.41 ^A	56.87 \pm 5.8 ^A	211.13 \pm 21.3 ^A	2.00 \pm 0.09 ^A
	Range	67.80-89.20	49.00-71.00	185.70-254.00	1.90-2.20
Moderate viremia	Mean \pm SD	90.62 \pm 1.40 ^B	74.11 \pm 2.18 ^B	270.2 \pm 7.72 ^B	2.31 \pm 0.07 ^B
	Range	89.50-92.00	71.40-78.00	258.00-279.50	2.20-2.40
High viremia	Mean \pm SD	95.60 \pm 2.33 ^B	83.62 \pm 3.78 ^C	292.0 \pm 4.03 ^B	2.24 \pm 0.21 ^B
	Range	93.90-99.60	80.0-89.0	288.70-297.00	2.00-2.40
p-value		< 0.001 † HS	< 0.001 † HS	< 0.001 † HS	< 0.001 † HS
Different letters denote to the significant differences at $p < 0.05$					

SD: standard deviation; †: Kruskal wallis test; **: significant at $P > 0.05$

4. Discussion

According to present findings, there is a significant rise in ALT, AST, ALP, and TSB in HBV patients, which underscores liver dys-

function associated with viral replication and immune response. ELISA proved highly sensitive in detecting HBsAg, supporting its utility in early diagnosis.

The findings align with those reported by Abbood and Atiyah Al-Mhanah (2019), who found that 80% of HBV-infected patients of both sexes were HBsAg-positive using the ELISA technique. Similarly, Davoodian and Sadeghifard (2011) reported that 55 out of 58 chronic hepatitis B (CHB) patients (94.8%) tested positive for HBsAg via ELISA (7&8).

Asaad et al., 2015, in Saudi Arabia and Khaled et al., 2013, in Egypt reported similar results since all their 160 and 140 HBV patients were HBsAg-positive (9&10).

Another set of studies, that of Al-Joudi et al. (2014) and SB KV et al. (2015), reported significantly lower detection rates, as they found only 3.5% (7 out of 200) positive for HBsAg. Trépo et al. (2014) indicated that in the very early phase of infection, HBsAg is usually not detectable; however, it is very persistently positive in CHB patients. Reduced sensitivity of the diagnostic test may be due to mutations either in the antigen or antibody structures. More precisely, mutations at the S gene, those that are responsible for antigen expression, can lead to a situation where ELISA gives a false-negative result despite active infection being present within a patient. On the other side, however, ELISA has been recommended as a highly sensitive method for HBsAg identification and is widely used (11-14).

Several studies have reported increased levels in the serum of both liver enzymes and bilirubin in chronic hepatitis B (CHB) patients since these indicators are consistent with hepatocellular injury. Gharbi and Razzaq (2022), along with Fazaa et al. (2022a), noted significantly higher values for ALP, TSB, ALT, AST, and bilirubin fractions in HBV patients as compared to healthy controls. This happens due to damage to the liver cells; enzymes then find their way into the circulation (15-18).

The current findings align with previous reports (19; 20), indicating that ALT levels may rise during the natural course of CHB or antiviral therapy and are linked to both disease progression and risk of hepatocellular carcinoma (HCC). Liu et al. (2022) also found a correlation between elevated ALT and hepatic inflammation across different fibrosis stages in CHB patients (21).

Multiple studies (22; 23; 24; 25) support the association between HBV infection and elevated ALT, AST, ALP, and TSB levels, reflecting hepatocellular damage. Ghasemi & Zahediasl (2012), Liang (2009), and Mbaawuaga et al. (2014) further reported a correlation between elevated ALP and HBsAg seropositivity. Similarly, Abulude et al. (2017) highlighted increased AST and ALT in HBsAg-positive individuals, suggesting these enzymes as initial, though non-specific, diagnostic indicators for HBV (26-29).

Consistent with these findings, our study also revealed significantly higher ALP levels in CHB patients compared to controls, in agreement with (30) and (31). ALP is present in the membranes of biliary canaliculi and hepatic sinusoids; thus, its elevation may result from intrahepatic or extrahepatic biliary obstruction or sinusoidal damage, as described by (32) and (33).

Our current study found that elevated HBV DNA levels were as-

sociated with increased ALT and AST, consistent with findings from Turkey (34), which reported a significant correlation between serum ALT/AST and HBV DNA levels. Similarly, Madan et al. (2010) observed that ALT levels and liver histology correlated with HBV DNA levels. Peng et al. (2003) also reported that high HBV DNA levels were linked to elevated ALT and enhanced necroinflammatory activity, regardless of HBeAg status (35 & 36).

Witjes et al. (2011) supported these associations, noting that patients with high viral loads showed increased AST, ALT, and total bilirubin, along with decreased albumin (37). X. Wang et al. (2013) confirmed higher ALT, AST, and HBV viral load in CHB patients (38). Esmaeelzadeh et al. (2017) found a correlation between HBV DNA and ALT, but not AST, echoing prior reports (40; 41; 42) that established a significant association between ALT and viral load (39).

Hasanjani Roushan et al. (2005) observed no correlation between AST and HBV DNA in HBeAg-negative patients, though ALT remained associated with viral load (43). This pattern was reaffirmed by Kim HyeonChang et al. (2004b), who reported a positive correlation between HBV DNA and ALT in 82 HBeAg-negative CHB patients (44).

These findings suggest ALT may better reflect viral replication, while AST may indicate liver necroinflammation. Although both are associated with hepatitis, some studies propose AST as a more reliable marker of liver damage severity (43). Finally, Fazaa et al. (2022b) found high HBV DNA levels to be significantly associated with elevated ALT, AST, ALP, and TSB, aligning with our results (45).

5. Conclusion

This study gives insight, as the utilization of ELISA is much more effective, and it could be used in monitoring the progress of HBV infection along with liver enzymes. It also concluded that there is a higher correlation between HBV-DAN level and elevated levels of enzymes like the ALT, the AST, the ALP, and the TSB. These increased levels of enzymes may be playing a potential diagnostic and prognostic value in HBV-related liver dysfunction.

Acknowledgement:

I extend my sincere thanks to my supervisor, Dr. Wisam S. Abood Alrubaye, for their valuable supervision and guidance throughout this work. Their support and constructive inputs were instrumental in shaping the direction of the study and producing it in this manner.

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