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Estimation of Liver Enzymes in Patients Infected with Hepatitis B Virus in Baghdad Hospitals

Akram J. Hammood, Wasan A. Gharbi, Safaa A. Abdul Razzaq

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

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Abstract: Hepatitis is an inflammation in the liver. The hepatitis can be self-limiting or can progress to fibrosis, cirrhosis or liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances (e.g., alcohol, certain drugs), bacteria and autoimmune diseases can also cause hepatitis. Liver function tests are useful in determining the severity and prognosis of some liver illnesses, such as viral hepatitis. HBV infection can alter blood levels of various hepatic enzymes and chemicals, such as ALT, AST, and a rise in these enzymes above normal. It is considered that their top reference limits are unusual. Once there is a hepatitis infection, the enzymes AST and ALT are often released into the circulation. ALT serum level elevation corresponds more with hepatic damage, hence ALT serum level elevation correlates more with hepatic damage. The aim of the research is to know the level of liver enzymes for different stages of the disease in patients in groups with the hepatitis virus Patients with chronic hepatitis B virus group, Advance Chronic hepatitis B Patients with cirrhosis group, Recovered patients with undetected virus group, and apparently healthy subjects group. Levels of serum ALP and TSB were significantly higher among those with chronic hepatitis B compared with healthy individuals. The total number of samples is two hundred (200). The study population consisted of 50 patients with chronic hepatitis B infection (HBsAg seropositive), and 50 apparently healthy (HBsAg seronegative) participants as controls. Fifty patients with cirrhosis (HBsAg seropositive) and 50 patients with undetected virus (HBsAg seronegative). Biochemical markers of liver disease were evaluated by routine methods. Difference at AST, ALT, TSB and ALP levels, compared to healthy controls. The study concluded that deranged AST, ALT, TSB and ALP values correlate with HBV infection and may be a potential tool for disease diagnosis and progression.

Keywords: Total serum bilirubin, alkaline phosphatase, alanine aminotransferase, Aspartate Aminotransferase, Hepatitis B surface antigen.

Corresponding author: (Email: akramjoda641@gmail.com).

Introduction

Hepatitis is inflammation of the liver and can be caused by a variety of different viruses (1). Since the development of jaundice is characteristic feature of many liver diseases, a correct diagnosis of underlying cause of liver diseases can be made by testing patients' sera for the presence of specific anti-viral antigens or antibodies (2, 3, 4). Of the many viral causes of viral hepatitis, few are of great global importance than Hepatitis B virus (HBV). The HBV infection constitutes a serious public health problem, affecting approximately 240 million carriers worldwide (1, 5, 6). Chronic HBV infection had been found to significantly elevate the risk for developing liver cirrhosis and hepatocellular carcinoma (5). HBV is the most common pathogenic infective

cause of hepatitis and affecting millions of people worldwide (2). The virus is endemic throughout the world. It is shed in various body fluids of infected individuals (7, 8).

Infection with HBV leads to a wide spectrum of clinical presentations ranging from an asymptomatic carrier (9, 10). state to self-limited acute or fulminant hepatitis to chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma. Infection with HBV is one of the most common viral diseases affecting man (11). Both viral factors and the host immune response have been implicated in the pathogenesis and clinical outcome of HBV infection (12, 13).

The HBV is an Hepadnavirus with a 42nm partially double stranded DNA composed of a 27nm nucleocapsid core (HBcAg), surrounded by an outer lipoprotein coat (also called envelope) containing the surface antigen (HBsAg) (14). Hepatocytes that are infected in vivo by hepadnaviruses produce an excess of non- infectious viral lipoprotein particles composed of envelope proteins ((15, 16). The virus consists of a nucleocapsid and an outer envelope composed mainly of three Hepatitis B surface antigens (HBsAg) that play a central role in the diagnosis of HBV infection (17). The nucleocapsid contains hepatitis B core antigen (HBcAg), a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (18, 19).

HBV DNA can be detected in circulation (using PCR) within 1 month of infection (20), but it remains at the relatively low level of 102–104 genome equivalents per ml for about 6 weeks before the HBV DNA and the secreted HBV e Antigen (HBeAg) and HBsAg increase to their peak titres. HBV core antigen (HBcAg)- specific IgM appears early, and HBcAg- specific IgG persists for life, irrespective of the outcome of infection. Approximately 10–15 weeks after infection, serum Alanine Aminotransferase (ALT) levels begin to rise, which indicates T-cell-mediated liver injury (17, 21, 22).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), a rise in these enzymes above normal (3). Levels of serum alkaline phosphatase (ALP) and total serum bilirubin (TSB) were significantly higher among those with chronic hepatitis B compared with healthy individuals (23, 24).

Materials and methods

The total number of samples is two hundred (200), divided as follows (Patients with chronic hepatitis B virus group, Advance Chronic hepatitis B Patients with cirrhosis group, Recovered patients with undetected virus group, and Apparently healthy subjects group), study population consisted of 50 patients with chronic hepatitis B infection (HBsAg seropositive), and 50 apparently healthy (HBsAg seronegative) participants as controls. 50 Patients with cirrhosis (HBsAg seropositive) and 50 patients with undetected virus (HBsAg seronegative). Samples were taken from both sexes, Ages range from 30 years to 65 years from the Hospital of Gastroenterology and Liver Diseases. individuals that were screened and confirmed using ELISA method participated in this study. And liver enzymes are examined in a machine on Roche/Hitachi cobas c systems.

Sample collection and storage

10 mls of venous blood was carefully drawn into appropriate sample bottles, spun to separate the serum. The serum was separated into a plain sterile sample bottles and stored at -25o C for analysis.

Data management and analysis

The Statistical Analysis System-SAS (25) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability. Estimate of correlation coefficient between variables in this study (25).

Laboratory procedure

In vitro test for the quantitative determination of alanine aminotransferase (ALT). aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Total Serum Bilirubin (TSB), Determination in human serum. The blood was drawn through a syringe, then added to a gel tube, and then transferred to a centrifuge to separate the blood components from the serum and examined in a machine on Roche/Hitachi cobas c systems.

Results

The total number of samples is two hundred (200) samples, this study Patients' groups were included the following (Patients with chronic hepatitis B virus group, Advance Chronic hepatitis B Patients with cirrhosis group, Recovered patients with undetected virus group, and apparently healthy subjects group):

1-Distribution of sample study according to TSB in difference groups:

Table (1) describes the analysis of mean between cases and control of the Total serum bilirubin concentration (mean ± SD). Total serum bilirubin concentration as affected by study groups are presented in table 1. Total serum bilirubin levels were in patients with chronic hepatitis B virus significantly ($P \leq 0.01$) higher than those of apparently healthy subjects (20.96 ± 0.59 versus 10.20 ± 0.74 µmol/L, respectively).

Also, Total serum bilirubin levels were in patients with cirrhosis significantly ($P \leq 0.01$) higher than those of apparently healthy subjects (24.36 ± 0.62 versus 10.20 ± 0.74 µmol/L, respectively). Patients with undetected virus also have significantly ($P \leq 0.01$) high levels of TSB in serum when compared with those of apparently healthy subjects (14.61 ± 0.69 versus 10.20 ± 0.74 µmol/L, respectively).

Serum levels of TSB were in patients with cirrhosis significantly ($P \leq 0.01$) higher than those of patients with chronic hepatitis B virus and patients with undetected virus (24.36 ± 0.62 versus 20.96 ± 0.59 and 14.61 ± 0.69 µmol/L, respectively). While, serum TSB levels in patients with undetected virus were significantly ($P \leq 0.01$) lower than those of patients with chronic hepatitis B virus (14.61 ±

0.69 versus 20.96 ± 0.59 µmol/L, respectively).

Table (1): Comparison of patient status groups in TSB

Study groups	Total serum bilirubin (male up to 24 µmol/L Female up to 15 µmol/L)
Apparently healthy subjects (n=50)	10.20 ± 0.74 d
Patients with chronic hepatitis B virus (n=50)	20.96 ± 0.59 b
Patients with cirrhosis (n=50)	24.36 ± 0.62 a
patients with undetected virus (n=50)	14.61 ± 0.69 c
LSD value	1.855 **
***p*-value**	0.0001

Means with different letters in the same column differed significantly. Mean significant. **(P≤0.01) level.

2- Distribution of sample study according to serum ALP in difference groups:

Table (2) describes the analysis of mean between cases and control of the serum alkaline phosphatase concentration (mean ± SD). Serum alkaline phosphatase concentration as affected by study groups are presented in table 2. Serum ALP levels were in patients with chronic hepatitis B virus significantly (P≤0.01) higher than those of apparently healthy subjects (123.32 ± 1.75 versus 65.39 ± 1.91 U/L, respectively). Also, serum ALP levels were in patients with cirrhosis significantly (P≤0.01) higher than those of apparently healthy subjects (133.01 ± 2.16 versus 65.39 ± 1.91 U/L, respectively). Patients with undetected virus also have significantly (P≤0.01) high levels of ALP in serum when compared with those of apparently healthy subjects (79.15 ± 3.68 versus 65.39 ± 1.91 U/L, respectively). Serum levels of ALP were in patients with cirrhosis significantly (P≤0.01) higher than those of patients with chronic hepatitis B virus and patients with undetected virus (133.01 ± 2.16 versus 123.32 ± 1.75 and 79.15 ± 3.68 U/L, respectively). While, serum ALP levels in patients with undetected virus were significantly (P≤0.01) lower than those of patients with chronic hepatitis B virus (79.15 ± 3.68 versus 123.32 ± 1.75 U/L, respectively).

Table (2): Comparison of patient status groups in SALP

Study groups	ALP (male 40-129 U/L Female 35-104 U/L)
Apparently healthy subjects (n=50)	65.39 ± 1.91 d
Patients with chronic hepatitis B virus (n=50)	123.32 ± 1.75 b
Patients with cirrhosis (n=50)	133.01 ± 2.16 a
patients with undetected virus (n=50)	79.15 ± 3.68 c
LSD value	6.968 **
***p*-value**	0.0001

Means with different letters in the same column differed significantly. Mean significant **(P≤0.01) level

3- Distribution of sample study according to serum GPT in difference groups:

Table (3) describes the analysis of mean between cases and control of the serum GPT concentration (mean ± SD). Serum GPT concentration as affected by study groups are presented in table 3. serum GPT levels were in patients with chronic hepatitis B virus significantly (P≤0.01) higher than those of apparently healthy subjects (38.26 ± 0.79 versus 31.72 ± 0.77 U/L, respectively).

Also, serum GPT levels were in patients with cirrhosis significantly (P≤0.01) higher than those of apparently healthy subjects (43.10 ± 0.70 versus 31.72 ± 0.77 U/L, respectively). Patients with undetected virus also have significantly (P≤0.01) same levels of GPT in serum when compared with those of apparently healthy subjects (32.66 ± 0.80 versus 31.72 ± 0.77 U/L, respectively).

Serum levels of GPT were in patients with cirrhosis significantly (P≤0.01) higher than those of patients with chronic hepatitis B virus and patients with undetected virus (43.10 ± 0.70 versus 38.26 ± 0.79 and 32.66 ± 0.80 U/L, respectively). While, serum GPT levels in patients with undetected virus were significantly (P≤0.01) lower than those of patients with chronic hepatitis B virus (32.66 ± 0.80 versus 38.26 ± 0.79 U/L, respectively).

Table (3): Comparison of patient status groups in SALT

Study groups	SALT (SGPT) (Male up to 41 U/L Female up to 33 U/L)
Apparently healthy subjects (n=50)	31.72 ± 0.77 c
Patients with chronic hepatitis B virus (n=50)	38.26 ± 0.79 b
Patients with cirrhosis (n=50)	43.10 ± 0.70 a
patients with undetected virus (n=50)	32.66 ± 0.80 c
LSD value	2.153 **
***p*-value**	0.0001

Means with different letters in the same column differed significantly. Mean significant **(P≤0.01) level

4- Distribution of sample study according to serum GOT in difference groups:

Table (4) describes the analysis of mean between cases and control of the serum GOT concentration (mean ± SD). serum GOT concentration as affected by study groups are presented in table (4). serum GOT levels were in patients with chronic hepatitis B virus significantly (P≤0.01) higher than those of apparently healthy subjects (39.82 ± 0.65 versus 26.36 ± 1.10 U/L, respectively).

Also, serum GOT levels were in patients with cirrhosis significantly (P≤0.01) higher than those of apparently healthy subjects (41.05 ± 0.73 versus 26.36 ± 1.10 U/L, respectively). Patients with undetected virus also have significantly (P≤0.01) high levels of GOT in serum when compared with those of apparently healthy subjects (32.46 ± 0.79 versus 26.36 ± 1.10 U/L, respectively). Serum levels of GOT were in patients with cirrhosis significantly (P≤0.01) same with patients of chronic hepatitis B virus and higher than those of patients with undetected virus (41.05 ± 0.73 versus 39.82 ± 0.65 and 32.46 ± 0.79 U/L, respectively).

Table (4): Comparison of patient status groups in SGOT

Study groups	SAST (SGOT) (Male up to 40 U/L Female up to 32 U/L)
Apparently healthy subjects (n=50)	26.36 ± 1.10 c
Patients with chronic hepatitis B virus (n=50)	39.82 ± 0.65 a
Patients with cirrhosis (n=50)	41.05 ± 0.73 a
patients with undetected virus (n=50)	32.46 ± 0.79 b
LSD value	2.336 **
***p*-value**	0.0001

Means with different letters in the same column differed significantly. mean significant **(P≤0.01) level

Discussion

Inflammation, fibrosis, regeneration and, ultimately, cirrhosis are the responses of the liver to chronic ongoing injuries. ALT, AST, ALP and TSB levels were observed to be higher in test participants than in control, this is similar to the earlier reported study (7). A strong statistically significant positive correlation was observed between ALT and AST, this is consistent with the findings of earlier study in Hepatitis B infection (8). Levels of serum alkaline phosphatase (ALP) and total serum bilirubin (TSB) were significantly higher among those with chronic hepatitis B compared with healthy individuals (24). The study concluded that deranged AST, ALT, TSB and ALP values correlate with HBV infection and may be a potential tool for disease diagnosis and progression.

The serum concentrations of alkanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and direct bilirubin (DB) were significantly increased in chronic HBV patient group compared to the healthy control group, indicating the hepatocytes injury caused by HBV infection. This result is in agreement Cheng.

The outcome of biochemical tests indicates that the ALP, GPT, GOT and TSB are higher in chronic patients than carrier group. The increase of liver enzymes strongly suggest hepatocellular injury(27, 28), beside that the level of ALP,GPT,GOT and TSB in patients with undetected virus within normal range as compared to reference value of liver function test (29,30).

Conclusion

This study showed that the increased production of liver enzymes (AST, ALT, ALP and TSB) was due to ongoing destruction of hepatocytes as the disease progresses, thus liver enzymes are still more important in diagnosis of Hepatitis B infection.

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