Evaluation of Liver Function Tests and Their Correlation with HBV Viral Load in Patients with Hepatitis B Virus, Thi-Qar, Iraq

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Abstract

Hepatitis B virus (HBV) infection is a worldwide healthcare problem, especially in developing areas and the risk of liver cirrhosis in the individuals with chronic hepatitis B The purpose of current study was to evaluate the alterations in Alanine amino transferase (ALT), Aspartate Amino Transferase (AST), The enzymes Alkaline Phosphatase (ALP), Albumin (Alb), Total serum Bilirubin(TSB), Direct Bilirubin (DB) and In Direct Bilirubin (InB) and their correlation with HBV viral load (HBV-DNA load). These parameters were determined in 102 patients with HBV, and 60 healthy subjects, and comparing HBV-DNA loads with the rest of the biochemical parameters. The result of levels of ALT, AST, ALP, TSB, DB, InB showed a significant increase in HBV patients compared to the control group ($P \leq 0.05$) , while result of levels of Alb showed a significant decrease in HBV patients compared to the control group($P \leq 0.05$) . The presents study showed that the correlation between HBV-DNA load and the biochemical parameters. Our findings showed a results showed a positive correlation between HBV-DNA loads and (of ALT, AST, ALP, DB, InB). And negative correlation between HBV-DNA loads and (TSB and Alb).

Keywords: Hepatitis B, HBV-DNA load, Liver function tests, ALT, AST ALP, Alb TSP, DB and InB

1. introduction

Hepatitis is an inflammatory illness of the liver that is characterized by the presence of inflammatory cells in the tissues of the liver, resulting in fibrosis or cirrhosis [1] Hepatitis B is a viral ailment caused by the hepatitis B virus (HBV), which was initially discovered by Bloomberg in 1965 among Australia's Aboriginal people [2]. Human body fluids, such as blood, serum, and wound extracts, have the highest concentrations of HBV, followed by semen, vaginal fluid, and saliva, which have a moderate amount of HBV. HBV, on the other hand, is detected in small amounts in urine, feces, sweat, tears, and breast milk [3]. Viral transmission happens when infected people come into contact with normal people's blood and body fluids, resulting in cirrhosis and cancer [4]. Accidental contact with infected blood, sexual contact, public saloon haircutting and shaving, tattooing, piercing, syringe reuse, hospitalization, risky surgery, dental extraction, circumcision, and hemodialysis are all risk factors for HBV [5]. According to several research, persons who have had blood transfusions and work in hospitals have a higher incidence [6].

Geographically, there were around 95 million cases in the Western Pacific and approximately 75 million cases in Africa, indicating that these geographical areas have a substantial number of people living with chronic hepatitis B infection. Low prevalence (less than 2%) regions, such as North America, New Zealand, and Western Europe, are included in the HBV regional prevalence variations. The Eastern Mediterranean, the Middle East, and Eastern Europe are among the intermediate endemic locations, with prevalence rates ranging from 2 to 4.99 percentThe upper intermediate endemic regions, which include nations in North Africa and Central Asia, have a prevalence of 5 to 7.99 percent. Sub-Saharan Africa, China, and the Western Pacific Region are among the high endemic regions, with a prevalence of more than 8%. [7]. Hepatocytes have a variety of important roles in regulating homeostasis and overall health. These functions include the synthesis of most essential serum proteins, the production of bile and its carriers, the regulation of, and metabolism and conjugation of lipophilic compounds for excretion in the bile or urine, and the regulation of, and metabolism and conjugation of lipophilic compounds for excretion in the bile or urine. Serum bilirubin (Alb) and prothrombin time are the two most often utilized liver function tests. . Hepatic conjugation and excretion are measured by serum bilirubin, while protein synthesis is measured by serum albumin and prothrombin time [8]. Hepatic dysfunction is characterized by abnormalities in bilirubin, albumin, and prothrombin time [9]. The enzymes (ALP), (ALT), (AST), and 5′-nucleotidase are important in determining how well the liver is working and how much inflammation is present. Because the liver is involved in carbohydrate, protein, and lipid metabolism, as well as the synthesis of many proteins, bilirubin conjugation, and drug and other substance detoxification, total and direct bilirubin, total protein and albumin, cholesterol and triglycerides, and urea and ammonia, the liver can be assessed by measuring total and direct bilirubin, total protein and albumin, cholesterol and triglycerides, and urea and ammonia .The presence of increases in both total and direct bilirubin, as well as the extent of the increased alkaline phosphatase, help to identify this liver disorder as

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obstructive jaundice [10].

2. Materials and Methods

The AL-Hussein Teaching Hospital in Thi-Qar, the Endocrine Glands Center, the Biochemistry Laboratory, the Hormones and Immunes Laboratory, and specialty clinics were all involved in this research. There were 162 participants in total, with 60 controls and patients (102). The serum samples were separated and stored at (-20oC) after a bout (5mL) of blood samples from HBV patients and controls were obtained and allowed to clot at room temperature in empty disposable tubes centrifuged for 10 minutes at 3000 rotor per minute (rpm). HBV DNA quantification: HBV viral load was performed in all HBsAg positives and some samples HBsAg negatives as control group, because of the availability of HBV DNA quantitative regents. For this, we used the (Bioron Diagnostic GmbH ,Germany) .HBV-DNA loads in serum was analyzed by Real-time PCR. Liver function tests: Serum ALT, AST were analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, kits supplied by Randox, United kingdom. ALP, Alb TSP and DB were analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, kits supplied by Biolabo, France. InB has been calculated through the following equation

Indirect bilirubin=Total serum bilirubin - Direct bilirubin

Statistical Analysis: Statistical analysis was done using the software SPSS version 23.0.The results were expressed as mean ± standard deviations (mean ± SD) with LSD. One way ANOVA-test was used to compare parameters in different studied groups. P-values (P ≤ 0.05)

3. Results

The present study identified the effect of HBV on liver function tests and determined their association with HBV-DNA load. The result of levels of ALT, AST, ALP, TSB, DB and InB showed a significant increase in HBV patients compared to the control group (P ≤ 0.05), while result of levels of Alb showed a significant decrease in HBV patients compared to the control group(P ≤ 0.05) . The correlation between HBV-DNA load and the above parameters, the results showed a positive correlation between HBV-DNA loads and (of ALT, AST, ALP, DB, InB). and negative correlation between HBV-DNA loads and (TSB and Alb).

Table (1). The diagnostic parameters of (ALT, AST, ALP, Alb, TSB, DB and InB) in patients with HBV and the controls.

Group	NO	ALT (U/L) Mean ±SD	AST (U/L) Mean ±SD	ALP(U/L) Mean ±SD	Alb (g/dL) Mean ±SD	TSB (mg/dl) Mean ±SD	DB (mg/dL) Mean ±SD	ID (mg/dL) Mean ±SD
Controls	60	6.62±1.39 b	8.01±1.14 b	65.34±7.78 b	3.97±0.37 b	0.73±0.16 b	0.32±0.09 b	0.53±0.15 b
Patients	102	25.13±5.40 a	22.83±3.85 a	101.24±15.22 a	3.28±0.52 a	1.61±0.43 a	0.75±0.17 a	1.47±0.38 a
LSD		0.91	0.65	2.63	0.12	0.07	0.03	0.07

Note: Each value represents mean ± SD values with non-identical superscript (a, b or c…etc.), were considered significantly differences (P≤ 0.05).
-No: Number of subjects.
-SD: Standard deviation.
-LSD: Least Significant Difference.

There is a positive correlation between HBV-DNA load and ALT levels (r = 0.32) as shown in Figure(1).

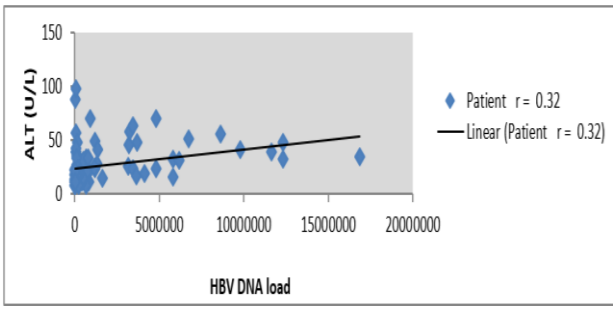

Figure (1): Correlation between HBV DNA load and serum ALT patients of HBV.

There is a positive correlation between HBV-DNA load and AST levels (r = 0.38) as shown in Figure (2).

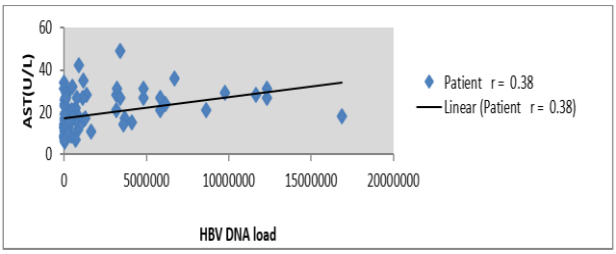

Figure (2): Correlation between HBV DNA load and serum AST in patients of HBV.

There is a positive correlation between HBV-DNA load and ALP levels (r = 0.47) as shown in Figure (3).

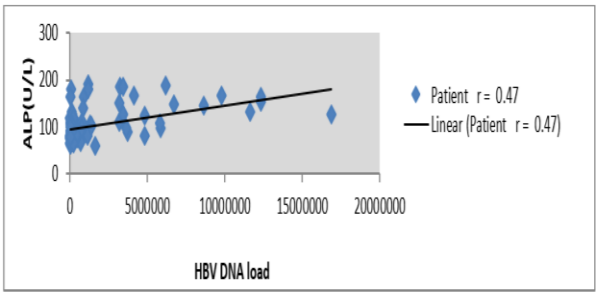

Figure (3): Correlation between HBV DNA load and serum ALP in patients of HBV.

There is negative correlation between HBV-DNA load and Alb levels (r = - 0.45) as shown in Figure (4).

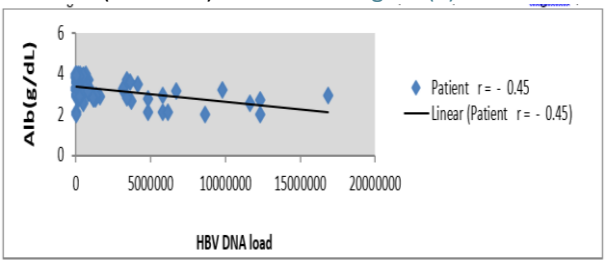

Figure (4): Correlation between HBV DNA load and serum Alb in patients of HBV.

There is a negative correlation between HBV-DNA load

and TSB levels (r = -0.64) as shown in Figure (5).

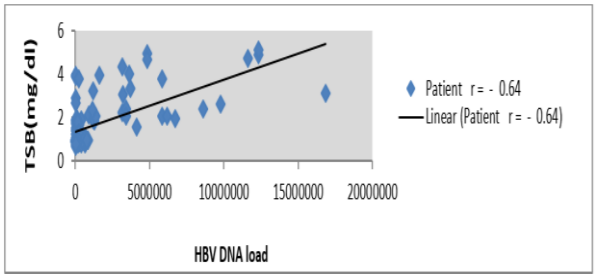

Figure (5): Correlation between HBV DNA load and TSB in patients of HBV.

There is a positive correlation between HBV-DNA load and DB levels (r = 0.60) as shown in Figure(6).

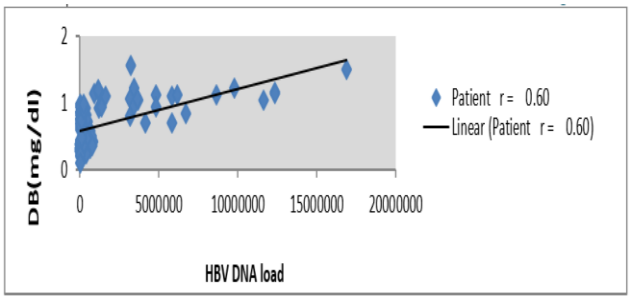

Figure (6): Correlation between HBV DNA load and serum DB in patients of HBV

There is a positive correlation between HBV-DNA load and InB levels (r = 0.56) as shown in Figure(7).

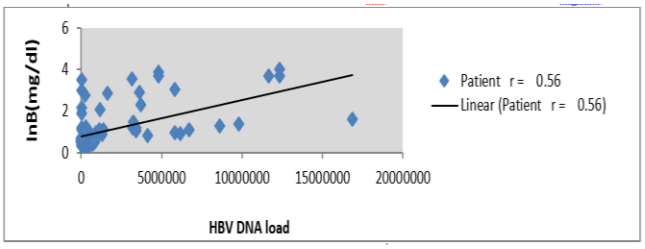

Figure (7): Correlation between HBV DNA load and serum InB in patients of HBV.

4. Discustion

Many haematological and biochemical problems have been well documented in HBV infection Hepatocellular function is measured by serum AST, ALT, ALP, and TSB. Increased circulating liver enzymes could be attributable to increased synthesis and secretion, or reduced catabolism [11]. The concentration of liver intracellular enzymes that have seeped into the circulation is indicated by serum amino transferase such as ALT and AST. These are the hepatocellular injury markers [12, 13]. Because ALT has a cytoplasmic distribution and a longer half-life in the blood (approximately 50 hours) than AST, it is the most reliable biochemical measurement for detecting liver impairment in patients with acute and chronic viral hepatitis (about 16 hours) [14, 15]. In HBV, high significant elevations in ALT were detected at (P ≤ 0.05) when compared to control; these findings are consistent with those of others. This finding is comparable to those of [3, 16, 17]. These findings are not surprising, given that the liver is a target organ for HBV replication, resulting in the destruction of hepatocytes and, as a result, an increase in the release of these molecules [12]. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and ALT levels [18].

The findings show that patients have higher levels of AST than healthy people, which is consistent to the findings of a previous study [16, 17, 19]. AST is found in the liver, heart, muscle, kidney, brain, pancreas, and lungs. Because of the vast variety of organs that contain AST, it is a less specific sign of liver damage than ALT. In myocardial infarction and chronic liver illness, an increase in mitochondrial AST in the blood is highly predictive of tissue necrosis. More than 80% of the liver AST activity is contributed by mitochondrial form of the isoenzymes, while the circulating AST in the blood is contributed by cytoplasmic form of AST. AST is especially markedly raised in those with liver cirrhosis.(Shivaraj et al., 2009). ALT is distributed in liver cell plasma, and AST is distributed in liver cells and mitochondria. In the early stages in HBV serum ALT levels rise more than serum AST levels, but in advanced stages, liver cell damage is serious and mitochondria have also been severe damage, therefore, serum AST levels rise more than the ALT. The most sensitive assays for hepatocyte necrosis are ALT and AST values. Hepatocellular injury is indicated by high levels of these enzymes, which are secreted by injured hepatocytes. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and AST levels [18].

In HBV, high significant increases in ALP were seen at (p0.05) when compared to control; these findings are consistent with those of others [16, 19, 20]. The enzyme alkaline phosphatase (ALP) is found in the small intestine mucosal epithelium, proximal convoluted tubule of the kidneys, bone, liver, and placenta. It's also located in the cells that line the liver's bile ducts, therefore liver damage creates the high levels of ALP. ALP levels are normally normal or slightly elevated in acute viral hepatitis. Hepatitis A, for example, causes a rise in ALP due to cholestasis. Cirrhosis and hepatitis can cause a mild increase in ALP Gowda et al. [21]. Among the possible mechanisms are: When ALP excretion in the bile is diminished, the hepatic sinusoid regurgitates the enzyme into circulation [22]. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and ALP levels [18].

The findings show that patients have lower levels of Alb than healthy people, which is similar to the findings of another study [17]. Reduced Alb levels in patients with liver cirrhosis usually indicate a problem with liver function and a poor prognosis [13]. Other causes of low serum Alb may include liver disease (decreased synthesis), the enhanced turnover resulting from either increased catabolism or enhanced loss of albumin into the urine or intestine [23] .The acute phase response, of which interleukin-6 is a potent inducer, to infectious conditions, neoplastic growth, or immunological disorders, is also associated with inhibition of liver protein synthesis in animal studies [24]. As a result, a decrease in serum Alb may indicate the persistent inflammation that has been linked to HBV infection. Low serum Alb levels could be the result of different immune activation pathways that are less directly linked to the inflammatory marker interleukin-6 (IL-6). Finally, serum Alb may be

protective due to its antioxidant activity, the binding capacity of endogenous and exogenous substances (e.g., fatty acids and carcinogens), or its antithrombotic actions [25, 26]. Similar to the findings of a prior study, there is a negative connection between HBV DNA load and Alb levels [18].

TSB levels were significantly higher in HBV patients compared to controls, which accords with other research [16, 17, 19]. This reflects the use of TSB as a measure of liver injury [27]. Increased serum TSB due to liver problems that manifest as bilirubin metabolism abnormalities (e.g., reduced hepatocyte absorption, impaired bilirubin conjugation, and reduced hepatocyte production of bilirubin), such as cirrhosis and viral hepatitis [28]. Elevated serum bilirubin levels generally reflect an imbalance between production and conjugation followed by excretion [29]. The liver is in charge of removing unconjugated bilirubin from the bloodstream by 'conjugating' it (modifying it to make it water-soluble) with an enzyme called uridine 5'-diphospho UDP-glucuronyl-transferase. The deposition of bilirubin in the sclera, skin, and mucous membranes causes these areas to turn yellow [21]. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and TSB levels [18].

A substantial increase in serum direct bilirubin was seen, which is similar with findings from another study [17]. Hepatocellular dysfunction or cholestasis is indicated by an increase in direct bilirubin (conjugated bilirubin). The amount of conjugated bilirubin that rises is proportional to the amount of hepatocyte damage. Conjugated bilirubin levels can rise as a result of viral hepatitis [30]. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and DB levels [18]. The high level of indirect bilirubin in patients with HBV is consistent with the results of a previous study [17]. These results indicate the presence of viral infection of liver cells. The liver is responsible for clearing the blood of unconjugated bilirubin, and about 30% of it is taken up by a normal liver on each pass of the blood through the liver .The increase in predominantly unconjugated bilirubin is due to overproduction, reduced hepatic uptake of the unconjugated bilirubin and reduced conjugation of bilirubin. Overproduction can occur as a result of a haematoma's reabsorption and inadequate erythropoiesis, which results in increased red blood cell death. The UDP-glucuronyltransferase enzyme is defective in Gilbert's syndrome and Crigler–Najjar syndrome, which affects bilirubin conjugation [21]. Similar to the findings of a prior study, there is a positive connection between HBV DNA load and InB levels [18].

5. Conclusion

In patients with HBV, we find a significant rise levels of ALT, AST, ALP, TSB, DB and InB and a significant reduction in Alb

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