Biochemical Assessment as Markers for Diagnosis and Evaluation Hepatitis B Virus (HBV)

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Article History

Received on: 15/6/2021

Revised on: 1/7/2021

Accepted on: 6/7/2021

Keywords:

Hepatitis B Virus (HBV),
Liver Function, Albumin
Globulin.

DOI: <https://doi.org/10.29350/jops.2021.26.4.1359>

ABSTRACT

Background. Chronic hepatitis B virus (HBV) infection is a major cause of liver morbidity and mortality worldwide. many others risk developing complications of the end-stage liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC), without intervention. This study aimed to assess biochemical markers for the development of hepatitis B viruses (HBV), in which any possible connection between certain biochemical parameters and chronic hepatitis was identified. Liver function tests help diagnose liver disease and dysfunction, assess severity, tracking treatment, and determining prognosis. Methods. A total of (200) patients with CHB were admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad from the first of March to the end of May 2021, aged from 20-65 years by mean $\pm$ SD 31y. The patients were suffering from different clinical symptoms with previous risk factors for transmission of HBV infection. compare with a total of 100 healthy HBs Ag carriers aged from 18-52 years by mean45.06y. According to the findings of biochemical studies, chronic patients have higher levels of ALP, GPT, GOT, and TSB than carriers. The outcome of biochemical tests indicates that the ALP, GPT, GOT and TSB are higher in CHB patients than carrier group and their values are ALK. phosph KAU [(22.9 $\pm$ 8.5) and (10.1 $\pm$ 3.2)], GPT IU/l [(45.2 $\pm$ 18.2) and (13.8 $\pm$ 4.0)], GOT IU/l [(30.6 $\pm$ 11.1) and (13.5 $\pm$ 4.3)] and TSB mg/dl [(3.5 $\pm$ 1.9) and (0.9 $\pm$ 0.3)] respectively with highly significant difference (P<0.001) The rise in liver enzymes strongly indicates hepatocellular damage, even though ALP, GPT, GOT, and TSB levels in the carrier group were all within normal parameters as compared to the reference group. Testing liver function in terms of protein level, in conclusion, these findings may cast new light on immune response especially related to deficiencies of liver synthesis of acute-phase proteins and antioxidant enzyme involvement in viral infections and HBV biology.

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1. Introduction

Hepatitis is a systemic disease primarily involving the liver as a main target for viral replication which characterized clinically by fever, jaundice and gastrointestinal symptoms ([8,35]. There is a variety of agents in addition to the viruses that cause liver inflammation (hepatitis) such as bacteria, parasites, fungi and chemical agents including drugs, toxins and alcohol[25, 12]. Viral hepatitis is a disease of multiple causes that has been first described in the 5th century B.C when Hippocrates described epidemic jaundice. The recognition a form of hepatitis that has been transmitted by direct inoculation of blood [47, 5] or blood products have been first document by Lurman, Germany in (1883), then the discovery of the etiological agent for hepatitis B and the development of safe and effective vaccines for this virus are among the remarkable scientific achievements of the 20th century [38,22]. Hepatitis B virus (HBV) is a member of the Hepadnavirus family and one of the several unrelated viral species which cause viral hepatitis, the family has been divided into two groups the orthohepadnaviruses were the human HBV belongs to the avian hepadnaviruses. Hepadnaviridae are grouped together with retroviridae and some plant viruses into a superfamily of retroid viruses or reversiviruses. HBV is the smallest human DNA virus and has a very compact genome [19, 58].

Hepatitis B virus, consist of an outer lipid envelope and an icosahedral nucleocapsid core, the latter being compose of both protein and DNA [50, 49]. The outer envelope contains embedded proteins which are involved in viral binding. Virion shape is generally spherical but polymorphic form exist, including filamentous forms [27, 57, 56]. The DNA genome is not segmented and partially double-stranded containing a long and short segmented which overlap approximately 240 nucleotides to form a closed circle [52, 11]. The longer strand is 3020-3320 nucleotides long and the shorter is (700-2800) nucleotides (fig: 1) [10, 18].

Hepatitis B virus is structurally complex that it belongs to a group of animal viruses known as hepadnaviridae. It is 42 nm in diameter and consists of two primary components: a DNA, viral core and an outer protein coat [50, 49]. The viral core: represents the infectious part of the virus and the outer coat carries the major antigenic determinant of the virus (HBsAg), [6, 59]. The viral core rests within the nucleocapsid of the virus. It is approximately 27 nm in diameter and contains partially

double stranded DNA, DNA polymerase, a core antigen (HBcAg) and an 'e' antigen [28, 39] and fig. (2) shows hepatitis B particle type.

In addition to HBsAg, which accounts for 80 % of the entire surface protein, three other proteins which contain additional polypeptide sequences have been identified. These proteins have been designated pre-S2 and S (middle protein), pre- S1, pre-S2 and S (large protein), and pre-S (small protein) [53, 34, 51].

Electron microscopy of hepatitis B virus- positive serum has revealed that three morphologically distinct forms of particles:

1. Small 22nm spherical or tubular forms comprise of virus surface proteins which are synthesized in excess of the 42 nm complete virions.
2. Complete 42 nm virion (Dane particle). The HBsAg differs from the HBsAg found in the 22 nm particles in that pre- S1 epitopes are present.
3. The 27 nm nucleocapsid comprises of the DNA genome are surrounded by a second protein, the HBcAg [39].

Fig. (1). Schematic representation of the HBV genome [10,18].

Fig. (2): - A simplified drawing of the HBV particle and surface antigen [60].

Diagnosis and Criteria of HBV infection

The diagnosis of hepatitis is made by biochemical assessment of liver function, initial laboratory evaluation should include total and direct bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, globulin, complete blood count, erythrocyte sedimentation rate (ESR) and coagulation profile [23, 45]. Viral hepatitis B diagnosis is confirmed by detection of specific markers (antigens and/or antibodies) in the serum of infected patients [24, 14].

Aims of the study: The present study aimed to fulfill and to determine the best marker to be used as predictive parameter in the prognosis and disease progression in Iraqi hepatitis patients.

2. Methodology

Subject

Patients groups include the following:

Chronic hepatitis B patient's groups

A total of (200) patients with CHB who were admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad, aged from 20-65 years by mean $\pm$ SD 31y. The patients were suffering from different clinical symptoms with previous risk factors for transmission of HBV infection.

Control groups

A- A total of 100 healthy HBs Ag carriers were discovered accidentally through attending blood bank for donation of blood, aged from 18-52 years by mean $\pm$ SD, 45.06y.

Patients and carriers blood samples were submitted for the following:

Biochemical tests.

Total serum bilirubin (TSB) determination:

The estimation of TSB was done by using the diazo reagent. Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid, in the presence of Dimethyl sulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin while in the absence of Dimethyl sulfoxide, only direct bilirubin reacts with diazotized sulfanilic acid to form azobilirubin color formation was measured by a spectrophotometer (wave length 555 nm) [21].

Aminotransferases determination:

According to the method of Lee, *et al.*, [33], for colorimetric determination of serum alanine aminotransferase (ALT=GPT) and aspartate aminotransferase (AST=GOT).

Principle of ALT:

Glutamic Pyruvic Transaminase(GPT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4dinitrophenyl-hydrazine; absorbance was measured by spectrophotometer (wave length 540 nm) [48, 13].

Principle of AST:

Glutamic Oxaloacetic Transaminase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4dinitrophenyl-hydrazine, absorbance was measured by spectrophotometer (wave length 540 nm) [48, 41].

Alkaline phosphatase determination (ALP)

According to the method of Turan *et al.*, [55], for the determination of plasma phosphatase enzymes.

Principle:

Colorimetric determination of alkaline phosphatase activity according to the following reaction:

ALP

PH 10

The liberated phenol is measured, in the presence of 4-aminoantipyrine and potassium ferricyanide, by spectrophotometer (wave length 510 nm).

Total serum protein (TSP)

The procedure of determination of serum protein was according to Coeurdacier *et al.*, [15].

Principle:

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex which was measured by spectrophotometer of wave length 546 nm [48, 7].

Serum Albumin

According to Metz and Schuttre [40]. The albumin specifically binds to green bromocresol (GBC) with acid pH, the green color given by GBC was proportional to the albumin contained in the sample. Absorbance was read at 650 nm wavelength of spectrophotometer [48,32,17]

Serum Globulin

Determination of globulin was calculated from the difference between total protein and albumin according to the Buzanovskii, [7]

Results

Biochemical parameters.

The outcome of biochemical tests indicates that the ALP, GPT, GOT and TSB are higher in CHB patients than carrier group and their values are ALK. phosph KAU [(22.9±8.5) and (10.1±3.2)], GPT IU/l [(45.2±18.2) and (13.8±4.0)], GOT IU/l [(30.6±11.1) and (13.5±4.3)] and TSB mg/dl [(3.5±1.9) and (0.9±0.3)] respectively with highly significant difference ($P<0.001$). Furthermore, the estimation of serum protein profile can provide helpful clues to diagnosis, hence the total protein, serum albumin and serum globulin give the contradict value. There has been an increase in mean level of protein profiles of carrier group in comparison with chronic group with highly significant difference ($P<0.001$), as mention in Table (1)

Table 1: The comparison between chronic hepatitis B patients and carrier group according to biochemical parameters.

Biochemical Parameters		Number	mean	SD	P-Value	Sig
ALK.phosph	CHV	200	10.12	3.29	0.00	HS
	HBV	100	22.96	8.51		
	Total	300				
GPT	CHV	200	13.86	4.01	0.00	HS
	HBV	100	45.28	18.23		
	Total	300				
GOT	CHV	200	13.52	4.32	0.00	HS
	HBV	100	30.62	11.1		
	Total	300				
TSB	CHV	200	0.914	367	0.00	HS
	HBV	100	3.586	1.915		
	Total	300				
globulin	CHV	200	3.024	0.679	0.00	HS
	HBV	100	2.586	0.737		
	Total	300				
albumin	CHV	200	3.826	511	0.00	HS
	HBV	100	3.422	516		
	Total	300				
Total protein	CHV	200	6.85	787	0.00	HS
	HBV	100	6.026	1.128		
	Total	300				

Note: - ALP: Alkaline Phosphatase, GPT: Glutamic Pyruvic Transaminase, GOT: Glutamic Oxaloacetic Transaminase and TSB: Total Serum Bilirubin

Normal value: ALK. phosph: 3-13 king Armstrong unit. GPT: up to 13 u/l., GOT: up to 15 u/l. TSB: up to 1.0 mg/dl. Total protein: 6.5- 8.5 g/dl. Albumin: 3.8- 5.5 g/dl. Globulin: 2.9-3.6 g/dl

Fig. 3: The relationship between chronic hepatitis B patients and carrier group according to biochemical Parameters

3.Discussion

Chronic hepatitis B infection is a common disease wide world, 5% of the world's population are chronic carriers [3,61], Chronic hepatitis B also believed to be common in Iraq as well as in many other developing countries [43, 42,46 ,54, 31].

This investigation covers a wide range of parameters including virological ,immunogenetics and molecular that may have a possible relationship with infection and investigation was done on 200 patients with CHB infection and 100 individuals with HBV carrier. In fact, age at hepatitis B infection seemed to be the most influencing factor in prognosis, the results of this study indicated that the mean age for chronic patients was 45.06y and for carrier group was 35.1y. These results coincide with the previous studies done in Iraq as [2] who establish that 45y was the mean age for CHB patients and [1] reported that the mean age was 38 y for carrier group, also on other hand Dienstag and Rayan in [16] registered that the mean age for carrier and chronic groups was 37y and 42.6y respectively. The mean age group affected of CHB patients was higher than the mean age of carrier group which might be due to early exposure to HBV [16 ,37].

This work is conducted to figure out any possible relationship between some biochemical parameters and chronic hepatitis. Liver function tests are useful in diagnosis, evaluating severity, monitoring therapy and assessing the prognosis of liver disease and dysfunction. The outcome of biochemical tests which demonstrated in fig. (3), indicates that the ALP, GPT, GOT and TSB are higher in chronic patients than carrier group as also its shown in table (1). The increase of liver enzymes strongly suggests hepatocellular injury [4], beside that the level of ALP, GPT, GOT and TSB in carrier group within normal range as compared to reference value of liver function test [48,30].

Regarding the level of protein, albumin and globulin, it is clear that a decrease in concentration of protein profiles in chronic patients than carrier group. The significant decrease of serum proteins profiles will become apparent in severe or long-standing hepatic disease [44, 29]. On other hand albumin is synthesized exclusively by the liver, in some inflammatory condition the release of tumor necrosis factor inhibits albumin synthesis, but induce the synthesis of acute phase response, hypoalbuminemia is multifactorial but in liver disease the hepatic synthesis of albumin is decreased [36, 9].

Moreover, Gheorghe *et al.* [20] found that in young adults, medical procedures such as injections, dental treatment, surgery and acupuncture are important route of HBV transmission.

After all, hepatitis B infection is considered to be one of the important causes of chronic liver disease all over the world. The possible routes of transmission of HBV is a multifactorial process, some of them are still controversial.

4. Conclusions

This paper summarizes assessment biochemical markers of the development of hepatitis B viruses (HBV), in which any possible connection between certain biochemical parameters and chronic hepatitis was identified. Liver function tests are helpful in diagnosing liver disease and dysfunction, assessing severity, tracking treatment, and determining prognosis. According to the findings of biochemical studies, chronic patients have higher levels of ALP, GPT, GOT, and TSB than carriers. The rise in liver enzymes strongly indicates hepatocellular damage, despite the fact that ALP, GPT, GOT, and TSB levels in the carrier group were all within normal parameters as compared to the reference group. Testing liver function in terms of protein level, albumin, and globulin, the concentration of protein profiles in chronic patients is clearly decreasing as compared to the carrier group. In severe or long-lasting liver disease, the significant decreasing profile of serum proteins is evident in some conditions, the release of tumor necrosis factor hampers the synthesis of albumin, however, induce the synthesis of the acute phase response, hypoalbuminemia is multifactorial, while

the hepatic synthesis of albumin is decreased in liver disease. These findings may cast new light on immune response especially related to deficiencies of liver synthesis of acute-phase proteins and antioxidant enzyme involvement in viral infections and HBV biology. as well as depend on the biochemical marker as predictive criteria in the evaluation of cirrhosis, fibrosis, and later liver cancer, in a recommendation, several promising developments of HCC risk scores and biomarkers are underway, and they are expected to transform the “one-size-fits-all” strategy and contribute to the substantial improvement of the poor prognosis of HCC patients in the foreseeable future.

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