Research Article

Comparative study of the molecular, biochemical, and other parameters in Iraqi hepatitis B patients

Ban M. A. Al-Kanaan[1], Mushtak T. S. Al-Ouqaili[2]*, Khalid F. A. Al-Rawi[3]

ABSTRACT

Background/Purpose: It well recognizes that hepatitis B infections are one of the most medically important problematic infections in the world. We compared the molecular, biochemical, and other parameters of two study groups of hepatitis B patients depending on the result of hepatitis B e antigen (HBeAg). **Methodology:** The serological indicators of 80 study patients with chronic hepatitis B (CHB) were investigated. The hepatitis B surface antigen (HBsAg) levels were quantified, and the hepatitis B virus (HBV) viral quantity was determined using a quantitative polymerase chain reaction. The biochemical and hematological essays of the patients, in addition to 40 healthy individuals, were also detected. **Results:** HBsAg was revealed positive in all study patients. They were quantitatively and statistically more significant in those patients positive for HBsAg than negative ones with a mean of 7779.9 ± 3898 IU/mL and 3233.8 ± 2474 IU/mL, respectively. The HBV-DNA viral quantity was also much higher in HBeAg-positive patients (35,328,825 ± 23,101,537 IU/mL) than in HBeAg-negative patients (3115.1 ± 1916.8 IU/mL). Glutamic pyruvic transaminase and glutamic oxaloacetic transaminase values were elevated in patients carrying HBeAg in comparison with other ones in contrast with gamma-glutamyl transferase (GGT), who decreased markedly. The level of prothrombin times for HBeAg-positive (15.24 ± 2.0 s) and HBeAg-negative (15.73 ± 2.4 s) patients was higher than that for the healthy individuals (13.65 ± 0.5 s). **Conclusion:** The interesting finding that emerged from this study is the reasonably substantial significant difference between both study groups of HBeAg in terms of HBsAg level and HBV-DNA load creating an impression that a high level of attention must be brought on the diagnosis and monitoring of treatment of HBeAg positive patients. Furthermore, several biochemical parameters (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were markedly increased in patients who have HBeAg in comparison with those negative for the presence and controls. In both groups of patients with CHB infections, the GGT levels were observable, increasing than those in the healthy subjects.

KEY WORDS: Biochemical investigations, Hepatitis B virus, Polymerase chain reaction

INTRODUCTION

"Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV)" that may lead to an increase in the probability of death due to liver cirrhosis and hepatocellular carcinoma.[1] A high than 2 billion individuals are detected to have HBV infections, including >240–400 million with chronic hepatitis B (CHB) infections.[2] Despite the presence of the potent anti-hepatitis B vaccine and other recommended therapy, HBV infection still a significant global health challenge.[3] In Iraq, 3–4.5% of the population are infected with HBV, including 2–3% of apparently healthy blood donors.[4] Most patients with CHB infections do not show liver disease symptoms, but 10–30% may develop liver cirrhosis that may lead to liver cancer.[5] A CHB infection is defined when a patient is serologically indicated to have hepatitis B surface antigen (HBsAg) and/or is HBV infected and remains HBV-deoxyribonucleic acid positive for ≥6 months.[6] At present, laboratory levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total serum bilirubin (TSB), and serum albumin (ALB), along with prothrombin time (PT), are essential in diagnosing and monitoring liver disease.[7,8] In addition, serological indices virus B infection, such as levels of HBsAg, hepatitis B e antigen (HBeAg), HBcAg, HBsAb, HBeAg, HBcAb, and HBV-DNA, are among the essential tests for diagnosing and determining the severity of an infection.[5] Some investigators

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[1]Department of Biochemistry, Health Office of Anbar, Iraq, [2]Department of Medical Microbiology, College of Medicine, University of Anbar, Iraq, [3]Department of Chemistry, College of Science, University of Anbar, Iraq

***Corresponding author:** Mushtak T. S. Al-Ouqaili, Department of Medical Microbiology, College of Medicine, University of Anbar, Iraq. E-mail: ph.dr.mushtak_72@uoanbar.edu.iq

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have reported that hepatic function tests, including AST, ALT, ALB, bilirubin, and the AST/ALT ratio, demonstrate noticeable variations between infected patients. Further, HBV-DNA has a fundamental role in persistent infection.[9] Thus, the coexistence of both HBV-DNA quantity and the liver damage degree or fibrosis severity has documented.[10,11] Viral hepatitis is a pantropic disease with hematological manifestations. Hematologic profile alterations may predict patients likely to have hematological complications, even after recovering from acute viral hepatitis.[12] The unstable social situation and lack of appropriate facilities in several regions of Iraq have meant that the diagnosis of HBV infections is at a preliminary stage, relying only on serum HBsAg detection.[13] The seroconversion of HBeAg from HBeAg-positive to anti-HBeAg-positive represents a significant therapeutic outcome indicator for patients with envelop antigen-positive patients, especially in chronic status. Other indices, such as HBcAb and HBV-DNA levels, are needed for correct diagnoses and confirmation of HBV infections, and these are not usually possible in Iraq.[13] Limited information about the changes in molecular and biochemical factors in both study groups of HBeAg and the availability of quantitative polymerase chain reaction testing led to this study. Thus, the study has been conducted as a comparative for the molecular, biochemical, and other parameters of both HBeAg study patients groups in Ramadi, Iraq.

METHODOLOGY

Ethical Approval Status

This study was subjected to the Ethical Approval Committee of the University of Anbar (No. 97, May 27, 2018). Written informed assent has been done for each study subject.

The Design of the Study and Population

This study was an observational, cross-sectional study conducted between February 2018 and January 2019. The participants included patients with chronic HBV infections, diagnosed by an expert clinician, and healthy participants (controls) attending the General Al-Amiri Hospital (Ramadi, Iraq). The healthy subjects were selected clinically depending on the clinical and/or laboratory indicator of hepatic disease. The study participants were confirmed not to be taking any type of drug.

Sample Collection

For patients with CHB, the samples were collected according to each patient's clinical stage of disease (HBeAg-positive or -negative), as determined by an expert hepatologist. The samples from patients with HBsAg were collected when the patients were in the chronic hepatitis phase (infection duration of >6 months). Fresh blood (total, 8 mL) has been obtained in the study patient and healthy control. The specimens were processed for complete blood counts; additionally, serum was collected and used as soon as possible for the biochemical investigations. Plasma was also collected and stored at −20°C for subsequent quantitative real-time polymerase chain reaction. Citrated blood was separated, and the plasma retained for PT and fibrinogen testing.

Serological Part of the Study

The serological parameters used in this study were first checked by HBsAg levels (it is considered positive when the level was ≥50 IU/mL). Furthermore, the hepatitis B serological marker evaluation has been done for the following: HBeAg, HBsAb, HBeAb, and HBcAb for HBsAg-positive patients using a one-step immunochromatographic assay cassette (CTK Biotech, San Diego, CA, USA).

HBV-DNA Detection

HBV-DNA was extracted using an automated machine (SaMag-12, Sacace Biotechnologies, Italy). The recovered DNA was quantified using an HBV Real-TM Quant kit (Sacace Biotechnologies).

The amplification process was started by initial denaturation at 95°C for 15 min, followed by 42 cycles for each of 20-s denaturation at 95°, and 40-s annealing and extension at 60°. The program for a polymerase chain reaction was running in a total volume of 25 µL, which contains equal volumes of template or standards, and reaction mix and fluorescent rays were detected during the stage of annealing of each cycle. The sample quantitation was performed using the internal control and the fluorescent values of both the sample and inhibitory concentration. The quantitation analyses were performed with the use of the thermo cycler's software (version 2.0, Smart Cycler II, Cepheid, Sunnyvale, CA, USA) for the healthy subjects (control) and the study patients. The quantity (load) of HBV-Deoxyribonucleic acid was determined according to the following formula.[14,15]

$$\frac{\text{HBV DNA copies / specimen}}{\text{IC DNA copies / specimen}} \times \text{coefficient*} = \text{copies HBV / mL}$$

*the coefficient is specific for each lot and is reported in the kit.

Evaluation of Biochemical and Hematological Parameters

Hepatic function indicators, ALT, AST, ALP, TSB, GGT, and total protein (TP), were evaluated using a lyophilized liver function panel (MNCHIP, Tianjin, China).[16] The total and differential leukocytes count

and platelets were performed using a differential hematology analyzer (SFRI Medical Diagnostics, St. Jean D'Illac, France).[17] The PT and fibrinogen assays were performed using the BIO-TP and BIO-FIBRI kits (Biolabo, Maizy, France), respectively.[18] The international normalized ratio (INR) was also determined using standard methods.

Statistical Analysis

"All data were analyzed using Excel (Microsoft, Redmond, WA, USA), Minitab (version 17, Minitab, State College, PA, USA), and SPSS (version 24, IBM, Armonk, NY, USA). The results are expressed as means ± standard deviation" and comparisons were also made with the use of the Chi-square (χ^2) test, Student's paired independent *t*-test. $P < 0.05$ was used as the threshold of statistical significance.

RESULTS AND DISCUSSION

A total of 80 patients with HBsAg, which were identified during the preliminary screening and 40 healthy subjects, were also included in this study, avoiding selection bias. The HBV patients were divided into those who were HBeAg-positive (20 males, 20 females; mean age, 22.6 ± 16.16 [range, 3–67] years) and those who were HBeAg-negative (22 males, 18 females; mean age, 31.5 ± 13.08 [range, 11–72] years. The healthy controls included 21 males and 19 females (mean age, 29.6 ±16.30 [range, 3–60] years).

Serological Markers

The seroprevalence of HBV into the assigned serotypes is represented in Table 1. In the HBeAg-positive patients, the mean serum HBsAg level was 7779.9 ± 3898 IU/ml. The viral load of viral-DNA (35,328,825 ± 23,101,537 IU/mL) was statistically more elevated than observed inpatients who have not HBeAg (3233.8 ± 2474 IU/mL and 3115.1 ± 1916.8 IU/mL, respectively). Anti-HBsAg antibodies were not determined in any of the patients with the infective form of hepatitis B (HBeAg-positive patients), and anti-HBeAg was only detected in 1 male; and the remaining 19 males and 20 HBe-Ag-positive females (97.5%) did not have detectable HBeAg antibodies. Similarly, in HBeAg-positive patients, anti-HBc antibodies were detected in 12 males and 15 females (67.5%); the remaining eight males and five females (32.5%) did not have detectable antibodies. In the HBeAg-negative patients, anti-HBsAg antibodies were detected in 1 (2.5%) female; however, anti-HBeAg antibodies were detected in 29.0 (72.5%) patients. Anti-HBc antibodies were detected in 39.0 (97.5%) patients [Table 1].

It is well realized that the hepatitis B infection is a life-threatening infection and it is an endemic disease in Southeast Asia in addition to the study country, Iraq, where it is an important cause of mortality.[19] "HBsAg is an important marker that indicates not only active hepatitis B infection but also predicts clinical outcomes.[20]" Therefore, the study investigated the use of plasma HBsAg values as a quantitative indicator of HBV infection. In addition, "the detection and quantitation of HBV-DNA play an essential role in diagnosing and monitoring HBV infections as well as assessing therapeutic responses.[21]"

Biochemical Investigations (Liver Enzymes)

As shown in Table 2, the mean of AST, ALT, and ALP in the patients with HBeAg-positive was higher than those observed in patients with negative or controls (both, $P < 0.01$). The HBV patients (HBeAg-positive and -negative) had higher mean GGT levels than did the healthy group ($P = 0.002$). No significant difference between the three study groups was detected for TSB, direct bilirubin, indirect bilirubin, TP, albumin, and globulin levels.

When the HBeAg-positive patients were classified into depending on the sex, significantly higher levels of TP and globulin were observed among female patients. In the HBeAg-negative patients, males demonstrated marked elevation of study enzymes, including ALT, AST, GGT, TSB, and direct bilirubin than did the female patients [Table 3].

Distributing the patients into six age groups (<21, 21–30, 31–40, 41–50, 51–60, and >60 years) revealed significant differences between some variables. In HBeAg-positive patients, the mean serum GGT level was highest in the >60 years group and lowered at <21 years group. The mean ALP level was the highest in the <21 years group and lowest in the >60 years group. The mean TSB values were highest in the 41–50 years group and lowest in the 31–40 years group. Further, the mean of the direct bilirubin level was highest in the 41–50 years group and lowest in the >60 years group. Although the mean indirect bilirubin level was also highest in the 41–50 years group, it was lowest in the 31–40 years group. The AST, ALT, TP, albumin, and globulin levels were not revealed any significant

Table 1: Prevalence of hepatitis B serological markers patients with HBV

Marker	HBeAg-positive patients (*n*=40)		HBeAg-negative patients (*n*=40)	
	Positive no. (%)	Negative no. (%)	Positive no. (%)	Negative no. (%)
HBsAg	40.0 (100)	0.0 (0.0)	40.0 (100)	0.0 (0.0)
HBsAb	0.0 (0.0)	40.0 (100)	1.0 (2.5)	39.0 (97.5)
HBeAb	1.0 (2.5)	39.0 (97.5)	29.0 (72.5)	11.0 (27.5)
HBcAb	27 (67.5)	13.0 (32.5)	39.0 (97.5)	1.0 (2.5)

Hbeag: Hepatitis B E antigen, Hbsag: Hepatitis B surface antigen, HBV: Hepatitis B virus

Table 2: Serum biochemical parameter levels (means±SD) in HBeAg-positive and -negative patients and in healthy controls

Chemical parameters	HBeAg-positive patients	HBeAg-negative patients	Healthy controls	*P*-value
AST (IU/L)	34.01±23.63[a]	21.18±10.3[b]	16.71±3.5[b]	0.001[**]
ALT (IU/L)	33.32±24.55[a]	22.24±9.4[b]	18.39±4.3[b]	0.001[**]
GGT (IU/L)	19.66±12.20[a]	21.56±10.4[a]	14.27±3.5[b]	0.002[*]
ALP (IU/L)	98.86±50.0[a]	67.92±36.9[b]	58.02±18.5[b]	0.001[**]
TSB (mg/dL)	0.77±0.5[a]	0.70±0.1[a]	0.69±0.1[a]	0.430[N.S]
Direct bilirubin (mg/dL)	0.192±0.22[a]	0.184±0.07[a]	0.191±0.08[a]	0.963[N.S]
Indirect bilirubin (mg/dL)	0.575±0.25[a]	0.515±0.12[a]	0.501±0.13[a]	0.141[N.S]
Total protein (g/dL)	6.223±0.54 [a]	6.200±0.48[a]	6.200±0.48[a]	0.973[N.S]
Albumin (g/dL)	3.60±0.26[a]	3.71±0.30[a]	3.74±0.31[a]	0.080[N.S]
Globulin (g/dL)	2.63±0.33[a]	2.49±0.27[a]	2.49±0.29[a]	0.071[N.S]
Albumin/globulin ratio	1.38±0.13[b]	1.50±0.16[a]	1.51±0.16[a]	0.001[**]

*significant differences ($P<0.05$), (**) highly significant differences ($p<0.01$). †Means that do not share a letter (horizontally) are significantly different (according to the least significant test). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen

Table 3: Differences in serum biochemical parameters among hepatitis B patients grouped by sex

Parameters	HBeAg-positive patients		HBeAg-negative patients	
	Males (*n*=20)	Females (*n*=20)	Males (*n*=22)	Females (*n*=18)
AST (IU/L)	27.60±17.6[a]	40.40±27.4[a]	24.64±12.35[a]	16.95±4.39[b]
ALT (IU/L)	27.50±19.30[a]	39.20 ±28.2[a]	26.07±10.93[a]	17.54±3.78[b]
GGT (IU/L)	21.19±15.63[a]	25.25±9.61[a]	26.18±11.75[a]	15.91±4.29[b]
ALP (IU/L)	102.8±53.0[a]	94.90±47.8[a]	64.16±34.54[a]	72.52±40.06[a]
TSB (mg/dL)	0.706±0.10[a]	0.83±0.63[a]	0.744±0.17[a]	0.64±0.08[b]
Direct bilirubin (mg/dL)	0.146±0.07[a]	0.150±0.04[a]	0.206±0.08[a]	0.158±0.04[b]
Indirect bilirubin (mg/dL)	0.55±0.09[a]	0.601±0.34[a]	0.54±0.15[a]	0.486±0.08[a]
Total protein (g/dL)	6.05±0.43[b]	6.40±0.58[a]	6.21±0.47[a]	6.189±0.50[a]
Albumin (g/dL)	3.55±0.21[a]	3.65±0.30[a]	3.77±0.31[a]	3.63±0.28[a]
Globulin (g/dL)	2.49±0.27[b]	2.75±0.34[a]	2.44±0.25[a]	2.55±0.30[a]
Albumin/globulin ratio	1.43±0.12[a]	1.33±0.13[b]	1.55±0.16[a]	1.43±0.15[b]

Data are presented as means and standard deviations, †Means that do not share the same letter (horizontally) within study groups are significantly different (according to independent *t*-test). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen

difference among the age groups. In HBeAg-negative patients, the mean AST level was highest in the 51–60 years group and lowest in the 31–40 years group. Similarly, the mean GGT level was also highest in the 51–60 years group but was lowest in the <21 years group. The mean ALP level was the highest in the <21 years group and the lowest in the >60 years group. The mean indirect bilirubin level was highest in the 51–60 years group and lowest in the <21 years group. The other parameters did not exhibit significant differences among the age groups [Table 4].

Increases in the levels of both ALT and AST strongly suggest hepatocellular injury. AST is released from the damaged muscle tissues, red blood cells, and hepatocytes,[22] and ALT is "released by hepatocytes during liver injury, usually reflecting the degree of liver damage. The ALT level is commonly used to assess the liver disease activity and to identify patients who require treatment. However, ALT may be influenced by various factors, making it an imperfect surrogate marker."[23] In our study, the AST and ALT levels in patients who have to envelop antigens were higher than those who not have such antigens and controls, similar to a previous study.[22] The previous study also demonstrated that the "AST and ALT levels in HBeAg-positive, CHB patients were higher than in HBeAg-negative CHB patients." Such increased enzyme activity may be the result of liver cell destruction and the subsequent release of enzymes.[24] In hepatitis, ALT and AST levels become elevated as the liver disease progresses, likely as a result of direct hepatocellular damage and membrane leakage.[25] GGT, the most accurate bio indicator of liver disease, is most available in cells of the liver, kidney, and also the intestine. The majority of serum-detected GGT is hepatic in origin.[26] In our study, the GGT values markedly increased in two study group patients of HBeAg, compared with healthy controls. This result is congruous with the results from another study that demonstrated that GGT levels are statistically elevated than in sera from patients with hepatitis B than in controls.[27] High serum GGT levels are indicative of advance fibrosis in hepatitis B patients.[8] Elevated ALP levels may be associated with many parenchymal disorders of the liver, including hepatitis.[22] In our

Table 4: The distribution of liver function parameters among hepatitis B surface antigen-positive and negative according to age groups

Biochemical indicator	Age mean±SD	HBeAg-positive patients					HBeAg-negative patients				
		<21	21–30	31–40	41–50	51–60	>60	<21	21–30	31–40	41–50	51–60	>60
AST IU/L	Mean±SD	38.64a*±26.98	24.01a±8.25	24.68a±5.70	20.13a±1.59	-	41.20a ±2.97	20.21bc±5.29	20.00bc±7.87	14.92c±2.19	28.03ab±12.00	42.70a±27.44	22.90abc±0.00
ALT IU/L	Mean±SD	37.09a±26.82	23.25a±5.86	23.68a±7.06	30.37a±2.83	-	30.75a±0.07	22.81a±4.30	21.77a±10.20	17.42a±4.23	26.27a±12.09	36.00a±20.08	21.10a±0.00
GGT IU/L	Mean±SD	15.05c±5.74	15.28c±5.30	28.85b±5.47	30.87ab±13.05	-	44.80a±26.87	15.83b±3.04	18.59b±8.25	21.47b±11.45	27.70ab±10.99	39.80a±14.28	30.80ab±0.00
ALP IU/L	Mean±SD	129.65a±36.36	56.36c±13.32	68.65bc±7.66	124.5ab±100.95	-	46.60c±7.78	121.09a±59.64	57.05b±16.45	55.03b±17.67	58.98b±10.59	61.05b±23.55	44.20b±0.00
TSB mg/dL	Mean±SD	0.74b±0.08	0.65b±0.09	0.61b±0.01	1.67a±1.59	-	0.66b±0.09	0.69a±0.05	0.69a±0.19	0.70a±0.11	0.64a±0.04	0.96a±0.06	0.70a±0.00
Direct bilirubin mg/dL	Mean±SD	0.15b±0.04	0.17b±0.09	0.17b±0.04	0.62a±0.76	-	0.13b±0.01	0.14b±0.02	0.18b±0.05	0.18b±0.08	0.20ab±0.06	0.33a±0.18	0.19ab±0.00
Indirect bilirubin mg/dL	Mean±SD	0.59b±0.08	0.48b±0.09	0.44b±0.05	1.04a±0.83	-	0.53b±0.10	0.55a±0.06	0.51a±0.18	0.52a±0.07	0.44a±0.08	0.64a±0.12	0.51a±0.00
Total protein g/dL	Mean±SD	6.25a±0.54	6.09a±0.21	6.03a±0.87	6.93a±0.67	-	6.00a±0.42	6.44a±0.30	6.09a±0.48	6.33a±0.55	6.00a±0.40	6.45a±0.07	5.40a±0.00
Albumin g/dL	Mean±SD	3.62a±0.26	3.59a±0.19	3.48a±0.28	3.83a±0.40	-	3.35a±0.21	3.80a±0.12	3.59a±0.33	3.82a±0.35	3.65a±0.26	3.99a±0.16	3.40a±0.00
Globulin g/dL	Mean±SD	2.64a±0.30	2.50a±0.13	2.55a±0.60	3.10a±0.46	-	2.65a±0.21	2.64a±0.32	2.51a±0.27	2.51a±0.24	2.35a±0.24	2.46a±0.23	2.00a±0.00
Albumin/ globulin ratio	Mean	1.37a	1.44a	1.40a	1.25a	-	1.27a	1.45a	1.44a	1.52a	1.56a	1.64a	1.70a

*Means that do not share a letter are significantly ($P<0.05$) different (horizontally) within each group, according to the least significant difference (LSD) test. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transpeptidase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen

study, the ALP levels were significantly higher in HBV patients than in healthy subjects. A similar result was previously reflected in the serum of CHB study patients.[27] "ALP, in the liver, is located in the cell membranes of the hepatic sinusoids and the biliary canaliculi. Accordingly, levels rise with intrahepatic and extrahepatic biliary obstruction and with sinusoidal obstruction, as occurs in infiltrative liver disease."[19] Because these enzymes are found in hepatocytes, they can "leak" into the blood if the hepatocytes are damaged.[28]

Hematological Parameters

The mean PTs and INRs for the HBeAg-positive and negative patients were statistically more increased than those for the healthy controls ($P = 0.001$). The mean platelet count for patients has HBeAg that was higher than for either the patients who do not the antigen or controls ($P = 0.002$). However, the white blood cell counts, and the levels of fibrinogen and hemoglobin were similar among the three groups of individuals [Table 5]. When the patients were grouped by sex, significant differences in the hemoglobin levels were seen among the HBeAg-positive patients. Females were observed to have significantly less hemoglobin (12.45 ± 1.31 g/dL) than males (13.71 ± 1.72 g/dL). A similar difference was also seen between female () and male () patients who were HBeAg-negative. In addition, female HBeAg-negative patients had significantly higher fibrinogen (298.5 ± 85.6 mg/dL) than males (234.8 ± 66.6 mg/dL) and platelets (246.7 ± 69.0 × 10^3) than did male (214.1 ± 68.1 × 10^3) HBeAg-negative patients. Reviewing the data for the HBeAg-positive and -negative patients, grouped by age, as done for Table 4, also revealed significant differences. In HBeAg-positive patients, the PT, INR, WBCs, platelet count, and levels of fibrinogen and hemoglobin were the same in the different age groups. However, in the patients do not have the envelope antigen, the PT was highest in patients >60 years old (mean, 25.4 ± 0.00 s) and lowest in those 31–40 years old (mean, 14.85±1.4 s). Similarly, the INR was highest in the oldest group of patients (mean, 2.7 ± 0.00) and lowest in the 31–40 years group (mean, 1.27 ± 0.29). There were no significant differences between the study group in fibrinogen and hemoglobin levels or white blood cell and platelet counts.

Biomarker Correlations

A correlation study involving the HBeAg-positive and negative patient biomarkers revealed no between viral DNA levels and hemoglobin levels (r = 0.33) in the HBeAg-positive patients. In HBeAg-negative patients, there was a strong positive correlation between HBsAg and fibrinogen levels (r = 0.38) and between viral surface antigen levels and WBC counts (r = 0.31). There was also no correlation between

Table 5: Differences in hematological parameters among patients with hepatitis B virus infections and healthy controls

Hematological parameters	HBeAg-positive patients	HBeAg-negative patients	Healthy controls	*P*-value
Fibrinogen (mg/dL)	276.39±75.4 a	263.47±81.3 a	242.76±26.0 a	0.074
Prothrombin time (s)	15.24±2.0 a	15.73±2.4 a	13.65±0.5 b	0.001
INR	1.31±0.3 a	1.40±0.4 a	1.06±0.1 b	0.001
WBC ($\times 10^3$)	8.33±2.5 a	7.42±1.9 a	7.49±1.3 a	0.076
Hemoglobin (g/dL)	13.08±1.6 a	13.60±1.9 a	12.92±1.3 a	0.154
Platelets ($\times 10^3$)	302.63±91.8 a	236.8±72.3 b	258.4±81.8 b	0.002

Data are presented as means and standard deviations; means that do not share a letter (horizontally) are significantly different, according to the least significant difference test. INR: International normalized ratio, WBC: White blood cell

HBsAg and INR levels (r = −0.31). Furthermore, the current results revealed that HBV-DNA correlates with both the albumin level (r = 0.31) and the albumin/globulin ratio (r = 0.38) and no correlation with fibrinogen levels (r = −0.35).

"Hematological parameters provide information regarding the status of bone marrow activity and hemolysis."[29] "The liver plays a key role in hemostasis as most of the coagulation factors, anticoagulant proteins, and components of the fibrinolytic system are synthesized by the hepatic parenchymal cells."[18] In our study, the fibrinogen levels were not observable between the CHB patients and the normal subjects, unlike in previously published reports where the fibrinogen values are highly elevated.[18]

In our study, the PTs were markedly longer in HBeAg-positive and -negative patients than in controls, as also previously reported.[18] This type of change can be explained by the diseased state of the liver, which is responsible for synthesizing clotting factors.[30] Similarly, in our study, the INR was significantly higher in the HBeAg-positive and negative patients than in the controls. Our results agree with those reported by Balkan *et al*.,[31] who concluded that the INR is significantly lower in inactive carriers than in those with CHB infections. The best application of INR in patients with the hepatic disease is for monitoring the degree of synthetic function weakness and predicting mortality. In patients with hepatic disease and abnormal coagulation testing results, INR and PT may supply information that the synthetic function of the liver but does not assess the hemorrhagic risk.[32] WBC counts are normally elevated due to infectious disease and the resulting inflammation.[33] In our study, the WBC counts revealed no huge difference between patients with HBV infections and healthy controls. The result was unpredictable since another group[34] demonstrated a clear similarity between patients with HBV infections and healthy controls. Several reasons have the role in the "incidence of unusual hematology result, according to new studies that indicate that the existence of hematological cytopenias is linked to poor prognosis in cirrhosis."[35] The research suggested that HBV-DNA viral loads and HBsAg levels are significantly elevated in HBeAg-positive patients than in those of HBeAg-negative patients creating an impression high level of attention must be brought on the clinical investigation, and therapeutic follow-up of HBeAg positive patients. Furthermore, several biochemical parameters, including hepatic function parameters, are markedly elevated in HBeAg-positive patients, compared with HBeAg-negative patients and controls in both groups of patients with CHB infections.

CONCLUSION

The interesting finding that emerged from this study is the reasonably substantial significant difference between both study groups of HBeAg in terms of HBsAg level and HBV-DNA load creating an impression that a high level of attention must be brought on the diagnosis and monitoring of treatment of HBeAg positive patients. Furthermore, several biochemical parameters (AST, ALT, and ALP) were markedly increased in patients who have HBeAg in comparison with those negative for the presence and controls. In both groups of patients with CHB infections, the GGT levels were observable, increasing than those in the healthy subjects.

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