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Non invasive Markers to Predict liver Damage Among Patients with Chronic HBV, Hilla City-Iraq

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Abstract:

Objective: Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) which affects the liver. It can cause both acute and chronic infections. Chronic HBV (CHB) infection is usually defined as detectable hepatitis B surface antigenemia (HBsAg) for a period of six months or more. The current study aims to investigate the serum level of total IgA, IgM, IgG, C3, C4, GPT, GOT, GGT, Ceruloplasmin, Haptoglobin and Transferin as a predictor for chronic HBV in Hilla City- Iraq.

Methodology: Serum samples were collected from the patients who sent to the central lab in Hilla City during a period of sex months. Only samples positive for HBsAg and total anti-HBc Ab were used for the study. This study include 24 patients with age (40.85±6.87 year) and 12 healthy control (40.42±9.06 year).

Results: Measurement of serum levels for ALT, AST, GGT, Haptoglobin, Ceruloplasmin and Transferin, C3, C4, IgG, IgM and IgA were done and the results revealed significant difference of ALT level (22.90±11.16 for CHB), (18.49±2.00 for control) and AST level(32.23±16.46 for CHB), (17.78±4.39 for control). when compared between CHB and healthy control and non significant for GGT level (15.02±7.90 for CHB), (15.45±5.01 for Control). Although there is a significant differences between CHB and health control but the results remain within the normal values which indicate stable non damaged liver. Haptoglobin, Ceruloplasmin and Transferin were investigated among CHB and healthy control and the results revealed no significant differences among CHB and healthy control. (85.12±45.97 for CHB, 97.50±59.84 for control), (28.34±11.98 for CHB, 29.31±8.15 for control), (216.54±75.11 for CHB, 203.71±54.36 for control) respectively .Serum level for C3 and C4 among CHB and healthy control record no significant differences in which (88.82±35.24 and 32.00±14.85 mg/dl for CHB) and (71.61±28.43 and 23.81±16.92 mg/dl for healthy control) for C3 and C4 respectively. The serum level of IgG, IgM and IgA were significantly increased when compare between CHB and health control in which (1309.21±501.06, 144.37±67.03 and 227.58±69.95 mg/dl for CHB) and (395.83±433.54, 73.29±92.47 and 93.78±23.04 mg/dl for health control) for IgG, IgM and IgA respectively.

Conclusion: The current study conclude that the serum levels of ALT, AST, GGT, Haptoglobin, Ceruloplasmin and Transferin are very important non invasive predictor for liver damage in CHB patients in patients who refuse of afraid from liver biopsy.

Keywords: HBV, HBsAg, Anti-HBc Ab, Haptoglobin, ALT.

Introduction:

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) which affects the liver. It can cause both acute and chronic infections. Acute hepatitis B refers to newly acquired infections. In most people with acute hepatitis, symptoms resolve over weeks to months and they are cured of the infection. However, a small number of people develop a very severe, life-threatening form of acute hepatitis called fulminant hepatitis. Chronic HBV (CHB) infection is usually defined as detectable hepatitis B surface antigenemia (HBsAg) for a period of six months or more. Once the infection becomes chronic, it may never go away completely. About 350 million people around the world undergo chronic HBV and 15% to 40% of chronically infected individuals will eventually develop cirrhosis, end-stage liver disease or hepatocellular carcinoma, or require liver transplantation[1].afraid

The envelope proteins are surface glycoproteins collectively designated as hepatitis B surface antigen (HBsAg). In virusinfected liver cells, HBsAg is produced in excess and secreted into the blood, where it serves as a marker for active infection and infectivity. The core open reading frame encodes a polypeptide that is expressed as either the hepatitis B e antigen (HBeAg) or the viral capsid protein (HBcAg). The presence of detectable HBeAg in serum or plasma is associated with high levels of HBV replication, greater infectivity and an increased risk of hepatic fibrosis[2].

When the HBsAg is positive, the patient is considered HBV-infected. Chronic infection is diagnosed when the HBsAg remains detectable for greater than six months and the chronic HBV confirmed when the total anti-HBc antibody were positive [3]. Many persons infected with HBV or HCV are oblivious for infection and have clinically silent infections for decades until developing cirrhosis or hepatocellular carcinoma [4] . The current study will focus on HBV due to that Iraq considered one of the intermediate HBV endemic countries and HBV was the common among another types of hepatitis [5]. One third of population of world have HBV infection and about 400 million were HBsAg carriers. The current study aims to investigate the serum level of total IgA, IgM, IgG, C3, C4, GPT, GOT, GGT, Ceruloplasmin, Haptoglobin and Transferin as a predictor for chronic HBV in Hilla City- Iraq.

Materials and Methods:

Sample collection:

Serum samples were collected from the patients who send to the central lab in Hilla City during a period of sex months. Only samples positive for HBsAg and total anti-HBc Ab were used for the study. This study include 24 patients and 12 healthy control. The specimen of choice for the diagnosis of HBV infection is blood. Serological tests for viral antigens and antibodies are typically used for diagnostic screening and can be performed on either serum or plasma. Both HBV antigens and antibody are stable at room temperature for days, at 4°C for months, and frozen at –20°C to –70°C for many years.

Qualitative ELISA test for HBsAg and total anti-HBc Ab:

Qualitative Foresight ELISA kit (Acon /USA) were used to detect Hepatitis B surface antigens and total Hepatitis B core antibodies according to the instruction of the manufacturer. The ELISA system used in measurement is reader Elx800 and washer Elx50 (Biotek/USA).

Measurement the level of ALT, AST and GGT:

Serum level of ALT, AST and GGT (u/l) were measured using semi automated Humalyzer 2000 (Human/Germany).

Single Radial immunodifusion (sRID):

The serum level of total IgA, IgM, IgG, C3, C4 in (mg/dl) and GPT, GOT, GGT, Ceruloplasmin, Haptoglobin and Transferin (g/l) (Liofilchem/Italy) were determined using sRID by recording the precipitation zone and compared them with the value of interpretation result chart.

Statistical analysis:

The statistical analysis were done using IBM SPSS statistic ver.20 using t test (P value=0.05). Statistically the difference was significant (P<0.05).

Results:

Diagnosis of Chronic Hepatitis B:

The ages of the patients included in this study were (40.85±6.87 year) and for control were (40.42±9.06 year) . This results in accordance with many studies who state that the most of the patients with chronic hepatitis B virus were in the fourth decade of age [6,7]. All patients were positive for both HBsAg and anti-HBc Ab while the control negative for both. The positivity of both HBsAg and anti-HBc Ab indicate a chronic hepatitis B status [8,9,10,11].

Serum levels of ALT, AST and GGT:

The results revealed significant difference of ALT level and AST level when compared between CHB and healthy control and non significant for GGT level table (1). Although there is a significant differences between CHB and health control but the results remain within the normal values which indicate stable non damaged liver. The current results are in accordance with Zeng et. al. (2013)[12] who found a positive correlation between liver damage and elevation of ALT, AST and GGT.

Table (1): Serum levels of ALT, AST and GGT

Marker	N		Mean	Std. Deviation	Sig.
ALT (u/l)	Patients	24	22.9000	11.16563	*0.017
	Control	12	18.4917	2.00837	
AST(u/l)	Patients	24	32.2375	16.46854	*0.000
	Control	12	17.7833	4.39604	
GGT(u/l)	Patients	24	15.0292	7.90583	0.308
	Control	12	15.4583	5.01424	

*mean significant difference when compared with control at P value=0.05

Serum levels of Haptoglobin, Ceruloplasmin and Transferin:

The serum levels of Haptoglobin, Ceruloplasmin and Transferin were investigated among CHB and healthy control and the results revealed no significant differences among CHB and healthy control table (2). Among patients of CHB who develop liver damage the serum level of Haptoglobin, Ceruloplasmin and Transferin must be decreased and so there is negative correlation between there levels and liver damage and fibrosis[12].

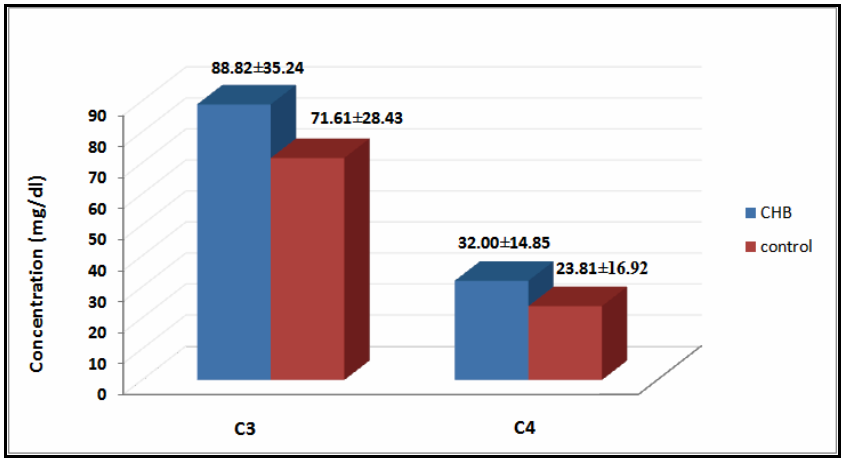
Table (2): Serum levels of Haptoglobin, Ceruloplasmin and Transferin

Marker	N		Mean	Std. Deviation	Sig.
Haptoglobin (g/l)	Patients	24	85.1292	45.97330	0.715
	Control	12	97.5083	59.84557	
Ceruloplasmin (g/l)	Patients	24	28.3417	11.98430	0.085
	Control	12	29.3167	8.15596	
Transferin (g/l)	Patients	24	216.5417	75.11034	0.085
	Control	12	203.7167	54.36881	

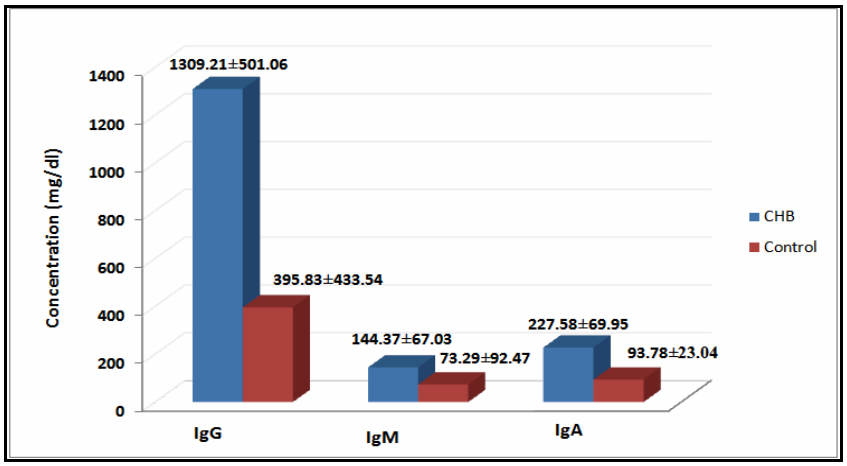
*mean significant difference when compared with control at P value=0.05

Serum levels of total IgA, IgM, IgG, C3 and C4:

Results of serum level for C3 and C4 among CHB and healthy control record no significant differences in which (88.82±35.24 and 32.00±14.85 mg/dl for CHB) and (71.61±28.43 and 23.81±16.92 mg/dl for healthy control) for C3 and C4 respectively figure (1). The serum level of IgG, IgM and IgA were significantly increased when compare between CHB and health control in which (1309.21±501.06, 144.37±67.03 and 227.58±69.95 mg/dl for CHB) and (395.83±433.54, 73.29±92.47 and 93.78±23.04 mg/dl for health control) for IgG, IgM and IgA respectively figure (2). The current results in agreement with Ahmed et al(2014)[13] who found significant increase of immunoglobulin among CHB and disagreed with same researcher in which who found significant decrease of C3 and C4 among CHB.



Figure(1): Serum level of C3 and C4 among CHB and Health control.



Figure(2): Serum level of IgG, IgM and IgA among CHB and Health control.

Discussion:

The chronic hepatitis B be dominant among aged HBV patients and this may be attributed to the sleighed activity and lowering in the defense immunity [7]. The presence of HBsAg in the blood for longer than six months along with total Anti-HBc Ab can be confirmed indicator for CHB. The existence of HBsAg reveal presence of virus and the positivity to total anti-HBc Ab occur in patients that have had HBV infection but not vaccinated. If the anti-HBc-Total test is negative, the patient has no evidence of current or remote HBV infection. If the anti-HBc-Total test is positive, this is compatible with current or resolved HBV infection. A negative HBsAg confirms a resolved infection. HBV vaccination does not induce anti-HBc-Total[14].

Both ALT and AST are usually present in the serum at low concentrations, below 30 to 40 U/L, and their normal range oscillates amongst laboratories. The level of ALT and AST among CHB are about two-fold the upper limit of normal value and this can be used as an indicator for chronic hepatitis B while the elevation in

the level of ALT and AST ten-fold the upper limit of normal value can be used as an indicator for acute hepatitis B. The high level of ALT, AST and GGT plasma level, are well known as surrogate markers of advanced liver disease and thus of significant or severe fibrosis[15].

Haptoglobin, Ceruloplasmin and Transferin are synthesized in the liver and play a vital role in acute phase reactions in which there serum levels elevated during inflammation and decreased during sever liver damages[16-19]. Normal level of C3 and C4 reveal normal liver function among CHB and the lowering in the level of them may be interoperated decreasing in the complement synthesis due to sever hepatocellular injury[20].

The results of immunoglobulin were in accordance with many studies who state the elevation in IgG, IgM and IgA among CHB[13,21]. The raised immunoglobulin levels in liver disease may be due to the inability of kupffer cells to sequester exogenous antigen from the gastrointestinal tract[22].

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