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ESTIMATION OF IMMUNOLOGICAL AND BIOCHEMICAL PARAMETERS IN HEPATITIS B POSITIVE PATIENTS

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ABSTRACT

Background: **Changes in immunological response have been reported during HBV infections, and these changes can be markers for the diagnosis and prediction of the outcome of infection The aim of this study was to measure and correlate serum levels of interleukin-2 (IL-2), C-reactive protein (CRP) Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and HBV antigens and antibodies in a sample of patients with HBV infection and in healthy controls**

Methods: **The study population consisted of 26 patients with hepatitis B infection (HBsAg seropositive), and 26 apparently healthy (HBsAg seronegative) participants as controls. Biochemical markers of liver disease were evaluated by routine methods. Hepatitis B antigens (HBVsAg, HBeAg) and antibodies (HBsAb, HBeAb, HBcAb) were determined using immunochromatographic method. Serum concentrations of IL-2, and CRP were determined using ELISA method.**

Results**: IL-2 level in HBsAg seropositive patients was found to be lower than that of control with no statistical significance while CRP level in HBV positive patients was higher than that of control with no statistical significance. HBV patients showed statistically significant difference in AST and ALT levels, compared to healthy controls. A statistically significant value was also observed between IL-2 and CRP in HBV infected individuals.**

Conclusion: **The study concluded that deranged ALT and AST values correlate with HBV infection and may be a potential tool for disease diagnosis and progression.**

Keyword: IL-2, C-reactive protein, Alanine transferase, Aspartate transaminase, Hepatitis B antigens, Hepatitis B antibodies.

INTRODUCTION

Hepatitis is inflammation of the liver and can be caused by a variety of different viruses. 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. 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. Chronic HBV infection had been found to significantly elevate the risk for developing liver cirrhosis and hepatocellular carcinoma[1]. 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[2].

Infection with HBV leads to a wide spectrum of clinical presentations ranging from an asymptomatic carrier 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. Both viral factors and the host immune response have been implicated in the pathogenesis and clinical outcome of HBV infection[3].

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). Hepatocytes that are infected in vivo by hepadnaviruses produce an excess of non-infectious viral lipoprotein particles composed of envelope proteins[2].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. The nucleocapsid contains hepatitis B core antigen (HBcAg),

a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins[4].

HBV DNA can be detected in circulation (using PCR) within 1 month of infection, 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[5].

C-reactive protein (CRP) is an acute-phase protein that serves as an early marker of inflammation or infection. The protein is synthesized in the liver and it is normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours[6]. When the inflammation or tissue destruction is resolved, CRP levels falls, making it a useful marker for monitoring disease activity[7].

IL-2 has been known for many years as a T cell growth-promoting factor. The role of IL-2 in immune tolerance appears to be twofold[8]. First, it has been shown that IL-2 is critical in programming T cells for activation-induced cell death[9]. It is likely that this function of IL-2 is dependent on its ability to increase surface expression of Fas ligand (FasL) and suppress expression of the inhibitor of apoptosis[10]. The production of IL-2has been found to bereduced in chronic viral hepatitis B and this has been used to investigate its immunomodulatory and antiviral effects. However, the role of these important immuno-modulators in hepatic injury due to HBV has not been evaluated in detail[11].

MATERIALS AND METHODS

A total of 52 participants were recruited for this study: Twenty-six (26) were hepatitis B (HBVsAg) positive patients and twenty-six (26) apparently healthy (non-Hepatitis B surface antigen) individuals that were screened and confirmed using ELISA method participated in this study.

A total of 52 participants comprising 26 cases and 26 controls were recruited for this study. They were screened for HBVsAg status by immunographic test and ELISA prior to recruitment. The cases were HBVsAg positive patients and the controls were selected by matching and seronegativity for hepatitis C and E infections (HCV, HEV)

Sample Collection and Storage

10mls 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 -20^{o}C for analysis.

Data Management and Analysis: Data was coded and entered into the spread sheet. Analysis was using (SPSS 16.0). Descriptive statistics such as frequency counts, percentages, mean ± standard deviation was used to summarize the results. Student t-test was used to compare groups while Pearson correlation test was used to determine whether the relationships between categorical variables are not statistically significant at Pd" 0.05.

Laboratory Procedure: ELISA method was used to measured CRP and IL-2 while ALT and AST were measured spectrophotometrically. Hepatitis B (HBsAb, HBeAg, HBeAb, HBcAb) serology test was done using immunochromatic method with a multi HBV test cassette consisting of 5 chromatographic strips.

RESULT

Table 1 describes the analysis of mean between cases and control of all biochemical parameters (mean ± *SD*) as well as comparison between the two groups. There was no significant difference between the C-reactive protein (CRP) level of hepatitis B positive patients and the control population (2.96±0.60; 2.68±0.54ng/dl). There was difference seen between the values of interleukin- 2 (IL-2) in patients and control samples (1879±1829; 2599±2573pg/ml) with the control showing a higher value than that of the test. Values of Alanine Transaminase (ALT) were higher in test than in controls (5.07±8.7; 2.76±2.6UI) and Aspartate Transaminase was considerably higher in test population than in the control population (18.32±22.00; 6.90±3.98UI).

Table 1: Comparison of biochemical profiles of HBsAg seropositive patients and controls

Parameters	Cases	Control	F	P value
CRP (mg/dl)	2.96±0.60	2.68±0.54	3.60	0.296
IL-2 (pg/mL)	1879±1829	2599±2573	0.22	0.091
ALT (U/I)	5.07±8.7	2.76±2.60	1.78	0.205
AST(U/I)	18.3±22.0	6.9±3.68	3.10	0.015

Table 2: Pearson correlation between biochemical tests in HBsAg seropositive patients

		IL-2	AST	CRP	ALT
IL-2	**r**	1	-0.197	0.403	-0.020
	P		0.335	0.041*	0.923
AST	**r**	-0.197	1	0.39	0.850
	P	0.335		0.849	0.000**
CRP	**r**	0.403	0.039	1	0.156
	P	0.041*	0.849		0.445
ALT	**r**	-0.020	0.850	0.156	1
	P	0.923	0.000**	0.445	

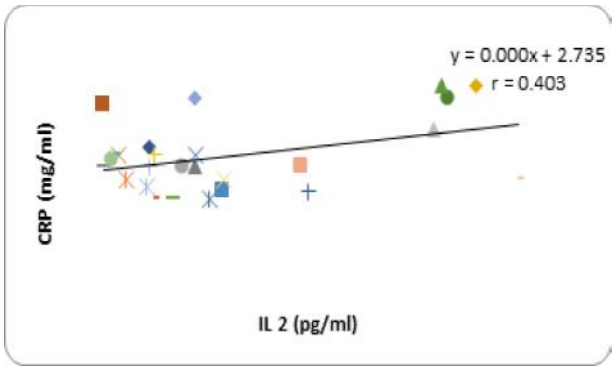

Figure 1: Correlation pot between CRP and IL-2 in HBsAg seropositive patients

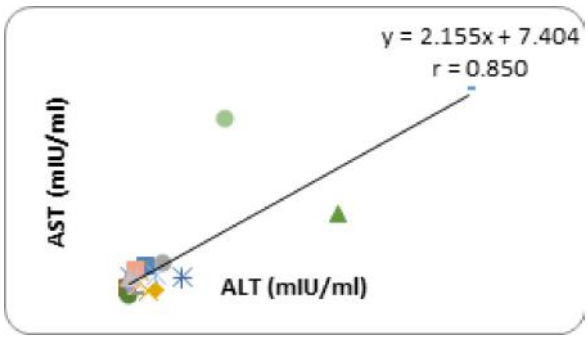

Figure 2: Correlation Plot between AST and ALT in HBsAg Seropositive Patients

Using Pearson's correlation, the levels of IL-2, CRP, AST and ALT were compared as represented in Table 2. There was a statistically significant positive correlation was observed between IL-2 and CRP (r = 0.403). And a strong statistically significant positive correlation was observed between ALT and AST (r = 0.850) Meanwhile, there was a negative correlation between AST and IL-2 (r = -0.197) but not statistically significant and also another negative non statistically significant correlation between ALT and IL-2 (r = -0.020).

Some of these results have also been represented in correlation plots; Figure 1 is a correlation plot between CRP and IL-2 in HBs Agseropositive patients. Figure 2 is a correlation plot between AST and ALT in HBsAg positive patients.

Table 3 shows the result of the HBV serological test. The table shows the presence of HBeAb in 24 (92.3%) patients and its absence in 2 (7.7%) patients this is also the same with HBcAb with 24 (92.3%) patients positive and 2 (7.7%) negative. However, all hepatitis B positive patients (26) showed the absence of both HBeAg and HBsAb but were seropositive for HBsAg.Table 3.1 Hepatitis B Serological Assay Tests in seronegative patients (Controls).

Table 4 and 5 showed the comparison of biochemical parameters (mean ± *SD*) with HBeAb and HBcAb serological test respectively. The mean interleukin-2 (1971.04±1879.82pg/mL) of patients with a positive HBeAb and HBcAb was observed to be higher than that of patients with negative HBeAb and HBcAb (775.00±35.35pg/mL). The mean value of CRP in HBeAb and HBcAb positive patient (3.00±0.56) has no significant difference from that of HBeAb and HBcAb negative patients (2.55±0.35). ALT mean value was observed to be higher in patients positive for

Table 3: Hepatitis B serological assay tests in HBsAg seropositive patients

N = 26	HbsAg	HBsAb	HBeAg	HBeAb	HBcAb
POSITIVE	26 (100%)	0 (0%)	0 (%)	24 (92.3%)	24 (92.3%)
NEGATIVE	0(%)	26 (100%)	26 (100%)	2 (7.7%)	2 (7.7%)

Table 3.1: Hepatitis B serological assay tests in seronegative patients (Controls)

N = 26	HbsAg	HBsAb	HBeAg	HBeAb	HBcAb
POSITIVE	0 (%)	0 (0%)	0 (%)	0(%)	0 (%)
NEGATIVE	26(100%)	26 (100%)	26 (100%)	26 (100%)	26 (100%)

HBsAg- Hepatitis B surface Antigen
HBsAb- Hepatitis B surface Antibody
HBeAg- Hpatitis B envelope Antigen
HBeAb- Hepatits B envelope Antibody
HBcAb- Hepatitis B core Antibody

TABLE 4: Comparisons of biochemical parameters and HBeAg serological results in HBsAg seropositive patients

	HBeAb	N	Mean	F	P-value
IL2	POS	24	1971.0 ± 1876.8	3.82	0.005*
	NEG	2	775.0 ± 35.35		
CRP	POS	24	3.0 ± 0.56	0.56	0.283
	NEG	2	2.55 ± 0.35		
ALT	POS	24	5.37 ± 9.03	0.87	0.054
	NEG	2	1.50 ± 0.71		
AST	POS	24	19.08 ± 22.90	0.71	0.094
	NEG	2	9.50 ± 3.50		

TABLE 5: Comparisons of biochemical parameters and Hbcab serological results in hepatitis B positive patients

	HBcAb	N	Mean	F	P-value
IL2	POS	24	1971.0 ± 1876.8	3.82	0.005*
	NEG	2	775.0 ± 35.35		
CRP	POS	24	3.0 ± 0.56	0.56	0.283
	NEG	2	2.55 ± 0.35		
ALT	POS	24	5.37 ± 9.03	0.87	0.054
	NEG	2	1.50 ± 0.71		
AST	POS	24	19.08 ± 22.90	0.71	0.094
	NEG	2	9.50 ± 3.50		

TABLE 6: Summary of pearson correlations between biochemical parameters and serological results in hepatits B positive patients

		IL2	AST	CRP	ALT	HBeAb	HBsAb	HBeAg
IL2	Pearson Correlation	1	-.197	.403	-.020	-.178	.[a]	.[a]
	Sig. (2-tailed)		.335	.041*	.923	.385	.	.
	N	26	26	26	26	26	26	26
AST	Pearson Correlation	-.197	1	.039	.850	-.118	.[a]	.[a]
	Sig. (2-tailed)	.335		.849	.000**	.567	.	.
	N	26	26	26	26	26	26	26
CRP	Pearson Correlation	.403	.039	1	.156	-.219	.[a]	.[a]
	Sig. (2-tailed)	.041*	.849		.445	.283	.	.
	N	26	26	26	26	26	26	26
ALT	Pearson Correlation	-.020	.850	.156	1	-.121	.[a]	.[a]
	Sig. (2-tailed)	.923	.000**	.445		.557	.	.
	N	26	26	26	26	26	26	26
HBeAb	Pearson Correlation	-.178	-.118	-.219	-.121	1	.[a]	.[a]
	Sig. (2-tailed)	.385	.567	.283	.557		.	.
	N	26	26	26	26	26	26	26
HBsAb	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.	.	.		.
	N	26	26	26	26	26	26	26
HBeAg	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.	.	.	.	
	N	26	26	26	26	26	26	26
HBcAb	Pearson Correlation	-.178	-.118	-.219	-.121	1.000	.[a]	.[a]
	Sig. (2-tailed)	.385	.567	.283	.557	.000**	.	.
	N	26	26	26	26	26	26	26

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

a. Cannot be computed because at least one of the variables is constant.

HBcAb and HBeAb as compared with patients negative for it (5.37±9.03mIU/mL; 1.50±0.71mIU/mL). The same trend of higher and lower AST level was observed for HBcAb and HBeAb positive (19.0±22.9mIU/mL) and negative (9.50±3.50mIU/mL) patients respectively.

Table 6 shows the summary correlation of all analyzed parameters and serological tests of Hepatitis B positive patients. A strong statistically significant difference was observed between Hepatitis B core Antibody (HBcAb) and Hepatitis B envelope Antibody (HBeAb) (r = 1.000).

A negative non statistically significance was observed between all measured parameters (IL-2, CRP, AST and ALT) and both B core Antibody (HBcAb) and Hepatitis B envelope Antibody (HBeAb) in Hepatitis B positive patient.

DISCUSSION

Inflammation, fibrosis, regeneration and, ultimately, cirrhosis are the responses of the liver to chronic ongoing injuries. IL-2 is produced by T cells that make up part of resident lymphoid population in the liver. C-reactive protein (CRP) is a non-specific marker of inflammation and a predictor of a coronary heart disease, cardiovascular disorders, sub-clinical vascular diseases[12]. Levels of CRP raise parallel with the chronic liver disease progression, such as chronic hepatitis and liver cirrhosis, as well as before the progression beginning, therefore it is a useful prognostic parameter[13].

It was observed in this study that interleukin 2 (IL 2) mean was slightly higher in HBsAg seronegative individuals than in Hepatitis B positive patients, however there was no statistically significant difference. This is similar to the observation of earlier studies that reported no difference between patients and control [11, 14, 15]. However, the results of this study are different from one which reported that there was a higher interleukin-2 level in chronic Hepatitis B infection[16]; this may not be unconnected with the different assay method used in both studies.

This study observed a statistically significant correlation between interleukin 2 and C-reactive protein. However, there was no statistically significant difference observed between CRP level in HBV seropositive and seronegative participants. The mean CRP of test population was slightly higher than that of control population. This is consistent with previous study[13] that reported that the expression of C- reactive protein in HBsAg seropositive individuals correlates with progression of the disease.

ALT and AST levels were both observed to be higher in test participants than in control, this is similar to the earlier reported study[17]. A strong statistically significant positive correlation was observed between ALT and AST with AST having higher values (mean) than ALT, this is consistent with the findings of earlier study in Hepatitis B infection[18].

In this study, serological test of HBsAg seropositive patients revealed two persons (7.7%) with (HBsAg+, HBeAg-, HBeAb-) while the remaining 24 (92.3%) with HBsAg+, HBeAg- HBeAb+. All test samples were found to be HBsAb negative. CRP, ALT and AST levels in sample negative for HBeAb and HBcAb were observed to be higher than that of the other patients and this is similar to findings from earlier reported studies[13, 16]. However, IL-2 in patients negative for HBeAb and HBcAb were observed to be lower than values of HBeAb and HBcAb positive.

CONCLUSION

This study showed that the increased production of CRP and liver enzymes (AST and ALT) was due to ongoing destruction of hepatocytes as the disease progresses and that interleukin 2 production levels are reduced thus liver enzymes are still more important in diagnosis of Hepatitis B infection.

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