Association of TNF-α, IFN-γ, IL-6, and IL-10 with different clinical manifestations of hepatitis B infection

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ABSTRACT

Cytokines have a crucial part in the pathogenesis, persistence of infection, and prognosis of hepatitis B virus (HBV) infection as HBV does not cause direct liver destruction; rather, disease-related complications and prognosis are more associated with immune system action, specifically cytokines such as TNF-α, IFN-γ, IL-6, IL-10, and other cytokines. This study sought to link TNF-, IFN-, IL-6, and IL-10 to various clinical manifestations of HBV infection. Ninety sera were taken from HBV-infected patients, 30 (33.3%) of whom had liver cirrhosis, 30 (33.3%) were HBV carriers, 19 (21.2%) were acute HBV patients, and 11 (12.2%) were recently HBV infected. ELISA was used to determine the serum levels of TNF-α, IFN-γ, IL-6, and IL-10. HBV-infected patients with liver cirrhosis had considerably higher mean serum levels of IFN-γ (P=0.005) and IL-10 (P=0.003), but TNF-α and IL-6 were significantly higher in recent HBV-infected patients (P values 0.034 and 0.004, respectively). There were substantial changes in mean serum levels of TNF-α, IFN-γ, IL-6, and IL-10 at different phases of HBV infection, implying a role for cytokines in HBV etiology, chronicity, and consequences.

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Introduction

Since hepatitis B virus (HBV) is not responsible for the direct destruction of the liver, the disease-related complications and prognosis are more related to immune system action, specifically cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), IL-10, and other cytokines.[1]

TNF- levels in the serum of HBsAg and HBeAg seropositive individuals are substantially higher than in normal controls.[2] Furthermore, the variations in TNF-α levels between chronic HBV carriers and normally immune patients imply that TNF- may be an important mediator of HBV clearance.[3] This is evidenced by a drop in TNF- during viral treatment, with a greater decrease in those responding to treatment.[4] When HBV was incubated with hepatocytes from HBV-infected patients in two different incubation conditions; with and without gamma interferon, viral antigen expression was estimated using radioimmunoassay and immunocytochemistry techniques. The experiment discovered that

IFN- suppresses the expression of all viral antigens, including HBsAg/pre-S2.[5]

Many clinical studies have found that HBV patients had greater serum levels of IL-6 than healthy people.[6] The level of IL-6 in HBV-infected patients with liver problems such as cirrhosis and hepatocellular carcinoma (HCC) is considerably higher than in those without difficulties.[7] Furthermore, the hepatitis B virus X protein can increase IL-6 production through the NF-kB pathway.[8]

IL-10 may be involved in the progression of HCC.[9] The role of effector cluster of differentiation 8 (CD8) + T cell-derived IL-10 in the enhancement of acute liver immunopathology in an acutely HBV-positive mouse model has been studied, and the conclusion is that CD8 + T cell-derived IL-10 act in an autocrine/paracrine fashion to enhance IL-2 responsiveness, thereby avoiding antigen-induced HBV-specific effector CD8 + T cell apoptosis, and they demonstrated that IL-10 produced by effector CD8+.[10] This study sought to link TNF-α, IFN-γ, IL-6, and IL-10 to various clinical manifestations of HBV infection.

Materials and Methods

Research design

The research was carried out at Ibn Sina specialty hospital and Alzaiem Alazhari University in Khartoum State from June 2016 to June 2017. The study included 90 patients that resulted positive for HBV infection indicated by being positive for HBsAg using ELISA. The population was divided into four categories: i) category I: with complications, mainly liver cirrhosis and/or hepatocellular carcinoma; category II: HBV carriers without complications; category III: Acute hepatitis B infection without complications; category IV: Recent hepatitis B infections without complications.

Inclusion criteria

Category I: HBV-positive patients with liver complications (Cirrhosis or HCC). The participants of this group were selected based on clinical examination, patient history, ultrasound image, and laboratory investigations such as liver function tests.

Category II: (HBV Carrier): HBV-positive patient without liver complications. The participants of this group were selected based on clinical examination, patient history, ultrasound image, and laboratory investigations such as liver function tests and IgG anti-HBcAg.

Category III: Acute HBV-positive patients or recently infected patients without liver complications. The participants of the acute subgroup of this group were positive for IgM anti-HBcAg with normal liver ultrasound. The participants of the recently infected subgroup tested positive for HBsAg for the first time after previous negative tests.

Category IV: Recently infected patient tested positive for HBsAg for the first time within 45 days after previous negative HBsAg tests. All of them are asymptomatic, without liver complications, and positive for IgM anti-HBcAg with normal liver ultrasound.

Exclusion criteria

Category I: HBsAg seronegative individuals, patients with liver cirrhosis or HCC without HBV infection, patients with alcoholic liver cirrhosis, and individuals diagnosed with any other known type of cancer rather than HCC were excluded.

Category II, III, and IV: HBsAg seronegative individuals and individuals diagnosed with liver cirrhosis, HCC, or any other known type of cancer were excluded.

Ethical considerations

The ethical considerations and conformity to individuals in this study were considered by approving of Ethical committees of Alzaiem Alazhari University and Ibn Sina specialized hospital, in addition to using documented agreement within the questionnaire signed by the participants.

Methods

Fortress Diagnostics (www.fortressdiagnostics.com) provided an ELISA kit for HBsAg detection and analysis. IFN-, TNF-, IL-6, and IL-10 levels were measured using an ELISA kit from abcam® (www.abcam.com).

Data analysis

Mean, standard deviation and mean difference were computerized and calculated by the statistical package for social science (SPSS®) program 21.

Results

This study was conducted at Ibn Sina specialized hospital in Khartoum state in Sudan and the practical work at Alzaiem Alazhari University. The study included 90 participants. All the participants were HBV positive indicated by HBsAg. The participants were divided into two main groups. One group included 30 participants with liver cirrhosis. The second group was 60 HBV-positive participants without liver cirrhosis. The last group included 30 HBV carriers, 19 acute hepatitis, and 11 newly diagnosed HBV patients.

The mean serum level of IFN-γ was found to be markedly high among HBV-positive patients with liver cirrhosis (369.775±528.818) pg/ml, followed by

those with recent HBV infection (140.883±107.007) pg/ml, then those with acute hepatitis (110.967± 152.045) pg/ml. The differences between the mean serum levels of IFN-γ were statistically significant (P=0.005) (Table 1).

Figure 1 showing the levels of tumor necrosis factor-α, interferon-γ, interleukin-6, and interleukin-10 in various clinical presentations of hepatitis B virus infection.

The mean serum level of IFN-γ was found to be insignificantly higher among patients with recent BV infection than those with acute HBV infection (P=0.809), and HBV carriers (P=0.612). On the other hand, the serum level of IFN-γ was significantly higher in patients with liver cirrhosis than in recent HBV-infected patients (P=0.05), and acute HBV infection (P=0.008) and HBV carriers (P=0.001) (Table 2).

There was an increase in the mean serum level of TNF-α among the recent HBV-infected group (482.972±803.873 pg/ml), the mean serum concentration went down among acute HBV infection (334.579±312.008 pg/ml) then HBV carriers (249.758±478.886 pg/ml) and HBV with liver cirrhosis (80.425±77.169 pg/ml). The difference between the mean serum levels of TNF-α was statistically significant (P=0.034) (Table 1).

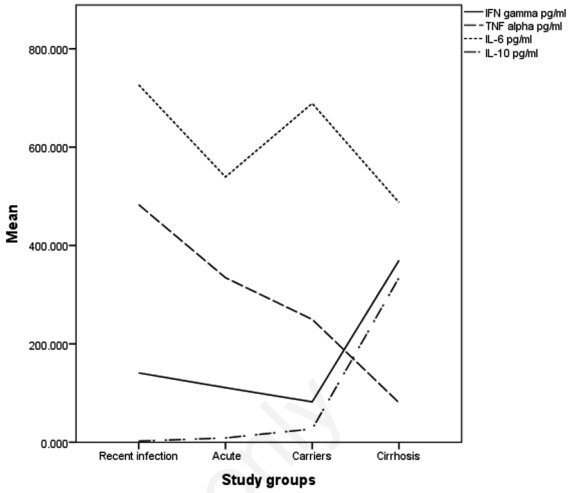

Figure 1. Line graph shows means of tumor necrosis factor-α, interferon-γ, interleukin-6 and interleukin-10 within different clinical manifestations of hepatitis B virus infection.

Table 1. The mean serum level of interferon-γ, tumor necrosis factor-α, interleukin-6, and interleukin-10 for the different study groups.

Parameters	Patients with recent HBV infection	Patients with acute HBV infection	HBV carriers	HBV positive patients with cirrhosis	P-value
IFN-γ pg/ml	140.883±107.007	110.967±152.045	82.264±133.637	369.775±528.818	0.0051
TNF-α pg/ml	482.972±803.873	334.579±312.008	249.758±478.886	80.424±77.169	0.0341
IL-6 pg/ml	726.645±75.544	539.347±284.723	688.920±120.881	487.433±328.158	0.0042
IL-10 pg/ml	2.313±.712	8.765±28.255	27.150±81.238	334.554±623.086	0.0032

The Kruskal-Wallis test was used to calculate the P-value; the ANOVA test was used to calculate the P-value; P-value less than 0.05 is considered significant; mean±standard deviation. HBV, hepatitis B virus; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL, interleukin.

Table 2. Comparison of the mean serum level of interferon-γ among the study population.

Variable	Recent HBV infection	Acute HBV infection	HBV carriers	HBV infection with liver cirrhosis	P-value
Mean IFN-γ pg/ml of serum	140.883±107.007	110.967±152.045			0.809
	140.883±107.007		82.264±133.637		0.612
	140.883±107.007			369.775±528.818	0.050
Variable	**Acute HBV infection**	**HBV carriers**	**HBV infection with liver cirrhosis**		**P-value**
Mean IFN-γ pg/ml of serum	110.967±152.045	82.264±133.637			0.765
	110.967±152.045		369.775±528.818		0.008
Variable	**HBV carriers**	**HBV infection with liver cirrhosis**			**P-value**
Mean IFN-γ pg/ml of serum	82.264±133.637	369.775±528.818			0.001

Independent t-test was used to calculate P-value; P-value less than 0.05 is considered significant; mean±standard deviation. HBV, hepatitis B virus; IFN, interferon-γ.

Table 3 shows significant mean differences in the mean of TNF α of HBV with liver cirrhosis group with recent HBV infection (P=0.008) and acute HBV infection (P=0.041).

Table 1 shows that the mean serum level of IL-6 was higher among the recent HBV-infected group (726.645±75.544 pg/ml), followed by HBV carriers (688.920±120.881pg/ml), then acute hepatitis B (539.347±284.723 pg/ml) and HBV infected with liver cirrhosis (487.433±328.158 pg/ml). The difference between these means was found to be statistically significant (P=0.004).

Table 4 shows statistically significant differences in the mean serum level of IL-6 of the recent HBV-infected group in comparison to acute hepatitis B (P=0.045) and HBV with liver cirrhosis group (P=0.006). Statistics significant differences were found between the mean serum level of IL-6 of HBV carriers with acute hepatitis B (P=0.038) and HBV infection with liver cirrhosis (P=0.002).

The mean serum level of IL-0 was highly increased among the group of HBV infection with liver cirrhosis (334.554±623.086 pg/ml), then HBV carriers (27.150± 81.238), acute hepatitis B (8.765±28.255 pg/ml) and recent HBV infected (2.313±.715 pg/ml). The difference between the means was statistically significant (P=0.003) (Table 1).

The mean serum level of IL-10 among HBV with liver cirrhosis was significantly elevated in comparison with the recently infected group (P=0.012), acute hepatitis B infection (P=0.003), and HBV carriers group (P=0.002) (Table 5).

Discussion

In this study, HBV patients with liver cirrhosis had considerably higher mean serum levels of IFN-γ than those with recent HBV infection, acute HBV infection, or HBV carriers. This could imply that IFN-γ has

Table 3. Comparison of the mean serum level of tumor necrosis factor-α among the study population.

Variable	Recent HBV infection	Acute HBV infection	HBV carriers	HBV infection with liver cirrhosis	P-value
Mean TNF-αpg/ml of serum	482.972±803.873 482.972±803.873 482.972±803.873	334.580±312.008	 249.758±478.886	 80.425±77.169	.352 .117 .008
Variable	**Acute HBV infection**	**HBV carriers**	**HBV infection with liver cirrhosis**		**P-value**
Mean TNF-αpg/ml of serum	334.580±312.008 334.580±312.008	249.758±478.886	 80.425±77.169		.491 .041
Variable	**HBV carriers**	**HBV infection with liver cirrhosis**			**P-value**
Mean TNF-αpg/ml of serum	249.758±478.886	80.425±77.169			.120

Independent t-test was used to calculate P-value; P-value less than 0.05 is considered significant; mean±standard deviation. HBV, hepatitis B virus; TNF-α, tumor necrosis factor-α.

Table 4. Comparison of the mean serum level of interleukin-6 among the study population.

Variable	Recent HBV infection	Acute HBV infection	HBV carriers	HBV infection with liver cirrhosis	P-value
Mean IL-6 pg/ml of serum	726.645±75.544 726.645±75.544 726.645±75.544	539.347±284.723	 688.920±120.881	 487.433±328.158	0.045 0.660 0.006
Variable	**Acute HBV infection**	**HBV carriers**	**HBV infection with liver cirrhosis**		**P-value**
Mean IL-6 pg/ml of serum	539.347±284.723 539.347±284.723	688.920±120.881	 487.433±328.158		0.038 0.468
Variable	**HBV carriers**	**HBV infection with liver cirrhosis**			**P-value**
Mean IL-6 pg/ml of serum	688.920±120.881	487.433±328.158			0.002

Independent t-test was used to calculate P-value; P-value less than 0.05 considered significant; mean±standard deviation. HBV, hepatitis B virus; IL, interleukin.

a beneficial effect in reducing HBV and the development of associated liver cirrhosis.

Weng and his colleagues determined that IFN-γ has a superior anti-hepatic fibrosis impact in patients with chronic hepatitis B. Our findings corresponded with other studies that showed the role of IFN- in the treatment of HBV infection and related fibrosis.[11] IFN-γ treatment for nine months improves fibrosis score in chronic hepatitis B patients, the action may be as an antagonistic effect to profibrogenic transforming growth factor beta [12], and nine months of IFN-γ treatment significantly improves fibrosis score in chronic HBV infected patients.[13] All of these findings demonstrated the potential utility of IFN-γ gene therapy for the treatment of HBV infection.[14]

This study found an insignificant negative connection between HBV viral load and IFN-γ, indicating that this cytokine has an inhibitory effect. This was consistent with the findings of Lau and colleagues, who discovered that IFN- inhibits the production of all HBV antigens, including HBsAg/pre-S2.[5] Another study found that higher levels of IFN-γ were obtained from the recovered group in response to different hepatitis B antigens HBsAg ad, HBsAg ay, and HBcAg, indicating a protective role, particularly in patients who are unable to develop anti-HBV antibodies,[15] and the specific expression of hepatocytes human IFN- effectively controlled HBV replication in HBsAg secreting hepatocytes without cell toxicity.[16,17]

Contrarily to positive outcomes such as viral clearance and replication, Toyonaga *et al*. discovered that liver-specific interferon-γ production is sufficient to produce chronic inflammatory illness in a transgenic model of chronic active hepatitis.[18]

The current investigation found statistically significant differences in mean serum TNF-α levels across study groups. The group of patients with recent HBV infection had the highest mean serum level of TNF-α, followed by those with acute HBV infection, HBV carriers, and HBV infected with liver cirrhosis.

When compared to patients with recent HBV infection and acute HBV disease, the mean serum level of TNF-α was considerably lower in HBV-infected persons with liver cirrhosis. This could indicate that TNF-α has an inhibiting effect on HBV in the early stages of the disease. Our findings agreed with those of Sheron *et al.*, as well as Cagri *et al*. The first found that HBsAg and HBeAg seropositive patients had significantly higher serum TNF-α levels than normal controls, whereas Cagri *et al*. found that differences in TNF- levels between chronic HBV carriers and naturally immune subjects suggest that TNF- may be a critical mediator of HBV clearance.[2,3]

TNF-α is significantly reduced during virus treatment, and HBV was activated after employing TNF-α inhibitors.[4,19,20] Biermer *et al*. discovered that TNF-α can impede HBV replication,[21] while Tzeng *et al*. discovered that TNF blockade increased serum HBV viral levels while maintaining HBV viral gene expression in the liver.[22]

This study found statistically significant differences in the mean serum level of IL-6 among the three groups of HBV-positive individuals. The mean blood IL-6 level in recent HBV-infected patients was considerably higher than in acute HBV patients and HBV infected with liver cirrhosis. There was a statistically significant difference in mean blood IL-6 levels between HBV carriers and individuals with acute hepatitis B and liver cirrhosis. In the study population, there was no significant negative connection between IL-6 and viral load. The increase in mean blood IL-6 levels among newly infected HBV patients may imply that IL-6 has an inhibitory effect on HBV in the early stages of the disease. And the negative correlation with viral load may indicate the inhibitory effects of IL-6.

Table 5. Comparison of the mean serum level of interleukin-10 among the study population.

Variable	Recent HBV infection	Acute HBV infection	HBV carriers	HBV infection with liver cirrhosis	P-value
Mean IL-10 pg/ml of serum	2.313±.715	8.765±28.255			0.963
	2.313±.715		27.14963±81.238		0.847
	2.313±.715			334.55396±623.085892	0.012
Variable	**Acute HBV infection**	**HBV carriers**	**HBV infection with liver cirrhosis**		
Mean IL-10 pg/ml of serum	8.765±28.255	27.14963±81.238			0.864
			334.55396±623.085892		0.003
Variable	**HBV carriers**	**HBV infection with liver cirrhosis**			
Mean IL-10 pg/ml of serum	27.14963±81.238	334.55396±623.085892			0.002

Independent t-test was used to calculate P-value; P-value less than 0.05 considered significant; mean±standard deviation. HBV, hepatitis B virus; IL, interleukin.

The current study found the lowest mean blood IL-6 level in HBV patients with liver cirrhosis. These findings contradict the ones of Tang *et al.*; the level of IL-6 is significantly higher in patients with liver problems such as cirrhosis and HCC.[7] Our findings were consistent with the findings of Bouezzedine *et al.*, who found that IL-6 limits HBV entry into liver cells,[23] and that IL-6 has a repressive effect on HBV and blocks its reproduction.[24]

In contrast, our findings contradicted those of Galun *et al.*, who found that IL-6 promotes HBV infection *in vitro* and *in vivo*, and that IL-6 may be a putative mediator of HBV entrance into hepatocytes.[25] Furthermore, IL-6 plays a role in the development of HBV-induced liver cirrhosis and aggravates liver injury.[26] Another study conducted in China by Zhang *et al*. looked at the positive and negative effects of IL-6 on HBV and came to the following conclusion: elevated plasma sIL-6R is probably associated with HBV elimination, and CD4+ IL-6R+ T cells in peripheral blood may contribute to the pathogenesis of liver injury in CHB patients.[27]

The current study found a statistically significant difference in the mean blood level of IL-10 among different study populations (P=0.003), with a dramatic increase in IL-10 among HBV infection with cirrhosis, followed by HBV carriers, acute HBV, and recent HBV infected. Cirrhosis and recent HBV infection, acute HBV infection, and HBV carriers all had significant mean differences. These data suggest that IL-10 concentrations rise with infection and that IL-10 has a direct immuno-inhibitory effect, which is linked to the development of liver cirrhosis. Our findings were consistent with the findings of Tülek *et al.*, who determined that serum interleukin-10 levels were considerably greater in chronic hepatitis and asymptomatic carriers than in others,[28] and agreed with Wang *et al*., who thought IL-10 was an early predictor of HBV-acute-on-chronic liver failure.[29]

Our findings support the idea that IL-10 is an inhibitory cytokine capable of inhibiting multiple immune functions and plays an important role in the oncogenetic and metastatic potential of neoplasms.[30] IL-10 may have a role in the pathogenesis of HCC,[9,10] and improves acute hepatic immunopathology. In patients with persistent HBV infection, HBcAg promotes IL-10 production, which suppresses antiviral immune responses and leads to viral persistence.[31] Furthermore, Zgüler *et al.* discovered a negligible positive connection between high levels of HBV DNA and IL-10 in chronic hepatitis B patients.[32]

Conclusions

At different stages of HBV infection, mean serum levels of TNF-, IFN-, IL-6, and IL-10 changed significantly, revealing a role for cytokines in HBV etiology, chronicity, and consequences.

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