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Original Article

Serum alpha-fetoprotein level is correlated with the level of inflammatory markers in the immune-clearance phase of chronic hepatitis B in Eastern China

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Abstract: Purpose: To clarify the association of serum alpha-fetoprotein (AFP) with inflammatory markers interleukin (IL)-6 and tumor necrosis factor (TNF)-α in patients with chronic hepatitis B (CHB) during the immune-clearance phase in Eastern China. Methods: This research selected 60 CHB patients during the immune clearance phase who tested positive for AFP, including 32 cases treated by non-antiviral therapy (experimental group) and 28 cases treated by antiviral therapy (positive control group). Another 30 cases tested negative for AFP were set as a negative control group. The correlations of serum AFP with IL-6 and TNF-α in patients were analyzed. Results: HBV DNA clearance in patients receiving antiviral therapy, in both the positive or negative control groups, was not significantly related to other clinical data. In the experimental group, a positive correlation of HBV DNA clearance with serum AFP level (r=0.5126, P=0.0027), alanine aminotransferase (r=0.3924, P=0.0263), and total bilirubin (r=0.5126, P=0.0027) was found. The experimental and positive control groups exhibited elevated serum IL-6 and TNF-α contents versus the negative control group (P<0.05). A positive association of AFP with IL-6 and TNF-α was also identified. Conclusion: Serum AFP level is positively related to IL-6 and TNF-α levels in CHB patients during the immune-clearance phase.

Keywords: Immune-clearance phase, alpha-fetoprotein, HBV DNA, IL-6, TNF-α

Introduction

Hepatitis B refers to a liver infection induced by hepatitis B virus (HBV) - a double-stranded DNA virus of the family Hepeviridae [1]. Based on World Health Organization (WHO) statistics, there are over 2 billion HBV-infected cases globally, 350 million of which were chronic, and an estimated 65 million of the chronically infected die from chronic hepatitis B (CHB)-induced liver disease [2]. According to the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2022 edition)" [3] revised by the Hepatology and Infectious Diseases Branch of the Chinese Medical Association, CHB can be divided into four phases based on the integration of virological, biochemical and histological features, namely hepatitis B e antigen (HBeAg)-positive chronic HBV infection (previously known as immune tolerance, chronic HBV carrier status), HBeAg-positive CHB (previously known as immune-clearance [IC], immune activity), HBeAg-negative chronic HBV infection (previously known as immune control, inactive hepatitis B surface antigen [HBsAg] carrier status), and HBeAg-negative CHB (also known as reactivation). The IC phase begins with the host's immune response to the infected hepatocytes (HCs). At this time, elevated serum alanine aminotransferase (ALT: higher levels indicate more intense responses and more severe HC injury) and chronic active hepatitis visible on liver ultrasound scan (USS) or biopsy can be observed, while the immune system can gradually clear HBV DNA along with increased levels of liver inflammation [4, 5].

Once they are CHB-infected, patients will develop chronic inflammation that is linked to certain liver complications [6]. The immune system consists of various molecules and cells involved in the induction of inflammation [7], among which interleukin (IL)-6 [8] and tumor necrosis factor (TNF)-α [9] are the major innate immunocytokines that trigger liver inflammation and immunoreactions. Inflammation in the liver leads to extracellular matrix accumulation, causing fibrosis. Therefore, it is crucial to identify inflammation in time for early antiviral therapy [10]. As an embryo-specific glycoprotein comprising nearly 580 amino acids and 3-5% carbohydrates, alpha-fetoprotein (AFP) is the most extensively used serum biomarker for cirrhosis, which is synthesized by the yolk sac and liver (1-2 months) and then predominates in the human liver [11]. AFP is the most extensively applied serum biomarker for hepatocellular carcinoma (HCC) surveillance in China [12, 13], with its elevated serum levels associated with acute HCC. Antiviral drugs are known to be effective in inhibiting not only HBV replication but also hepatitis activity, which may reduce the false-positive rate of AFP testing. However, some patients still develop HCC during antiviral therapy [14-16]. Therefore, it is clinically important to distinguish between the high AFP level associated with HBV infection and that which occurs with early-stage HCC, especially in those on antiviral therapy for elevated AFP.

Moreover, studies have demonstrated increased serum AFP levels with the worsening of pathologic inflammation and fibrosis in CHB [17]. However, a relationship between serum levels of AFP and inflammation-related factors in different phases of CHB, especially the IC phase, has not been reported. Therefore, in this study, IC-phase CHB patients were enrolled to clarify the correlation of AFP with inflammatory indicators.

Materials and methods

Participants

In this retrospective study, the clinical files of initially infected IC-phase CHB inpatients at the Taizhou People's Hospital or Taizhou Hospital of Traditional Chinese Medicine between January, 2019 and January, 2021 were analyzed. Inclusion criteria: (1) All cases met the diagnostic standards for IC phase CHB [18]; (2) All cases were serum HBsAg positive and HBeAg positive for more than 6 months; (3) All cases had a serum HBV DNA level of >2000 IU/mL for more than 6 months; (4) All cases with ALT >5× ULN or persistently elevated (>2× ULN for more than 3 months); (5) All cases with complete clinical files and follow-up data. Exclusion criteria: (1) Patients with other viral hepatitis, fatty/alcoholic/drug-induced hepatitis, or autoimmune hepatitis; (2) Patients with primary hepatic carcinoma; (3) Patients receiving nucleotides, interferon or thymidine; (4) Patients with incomplete clinical files and follow-up data. The patients were classified as either AFP negative (≤20 ng/mL) or positive (AFP >20 ng/mL) with the acknowledged upper limit of normal AFP level of 20 ng/mL as the threshold [19]. A total of 60 AFP positive cases were included with 32 cases treated by non-antiviral therapy (experimental group) and 28 cases treated with antiviral therapy (positive control group). Besides, another 30 AFP negative CHB cases were used as a negative control group. No statistical differences were identified in sex, age, height, or weight among the groups. This study was approved by the ethics committee of Taizhou Hospital of Traditional Chinese Medicine.

Data collection

After hospitalization, patients were examined once every two weeks for ALT, total bilirubin (TBil), HBsAg, HBeAg titer, HBV DNA, AFP quota, IL-6 and TNF-α. The positive and negative control groups received antiviral therapy with nucleotides analogues (Entecavir, Tenofovir disoproxil fumarate, telbivudina, adefovir dipiboxil, and Lamivudine), while the experimental group received none. Patients were closely observed for their treatment and conditions throughout their hospitalization and underwent a ≥2-year follow-up after hospital discharge.

Outcome measures

The primary outcome measure was the AFP level. The secondary outcomes measures were the differences in the AFP level and other indicators (ALT, total bilirubin [TBil], HBsAg, HBeAg titer, HBV DNA, HBV DNA AFP quota, IL-6 and TNF-α).

Statistical analyses

SPSS 20.0 software was used for statistical analyses. Measured data were expressed by mean ± standard deviation (SD); Inter-group

Table 1. Baseline information

Group	Experimental group (n=32)	Positive control group (n=28)	Negative control group (n=30)	F/χ^2	P
Age (year)	41.6±13.2	40.3±18.3	43.1±17.5	0.2126	0.8089
Gender, male (%)	18 (56.3)	16 (57.1)	17 (56.7)	0.0048	0.9976
BMI (kg/m^2)	22.6±2.4	23.1±2.8	22.9±3.0	0.2553	0.7753
TBil (μmol/L)	172.6±71.3	179.3±68.3	176.1±77.5	0.0640	0.9381
ALT (U/L)	573.2±249.1	527.6±310.9	601.9±191.2	0.6300	0.5350
AFP (ng/mL)	369.1±184.1	348.6±176.6	24.1±2.8*,#	51.8600	<0.0001
HBV DNA (log)	5.72±2.87	5.96±2.13	5.94±2.76	0.0794	0.9237
HBsAg (ng/mL)	3437.1±1964.1	3988.6±2276.2	3816.5±2862.0	0.4236	0.6560
HBeAg (IU/mL)	756.7±591.8	693.5±669.1	782.1±610.4	0.1546	0.8570

Notes: BMI, body mass index; TBil, total bilirubin; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; *P<0.05 *vs* experimental group; #P<0.05 *vs* positive control group.

Table 2. Comparison of average length of day and hospitalization costs

Group	Average length of day	Average hospitalization costs
Experimental group (n=32)	45.3±14.2	22178.4±3670.9
Positive control group (n=28)	46.7±13.1	21712.3±4460.1
Negative control group (n=30)	44.1±14.8	22809.8±3955.9
F	0.2472	0.5453
P	0.7815	0.5817

and multi-group comparisons were conducted using the Student's t-test and the variance analysis (ANOVA) following with Bonferroni post-hoc test, respectively. Counted data were described as number (percentage) and analyzed using the Chi-square test. Pearson correlation analysis was used for two-factor correlation analysis. P<0.05 was considered significant.

Results

Baseline information and prognosis

There were 32, 28, and 30 cases in the experimental, positive control, and negative control group, respectively (**Table 1**). The enrolled patients were all discharged from hospital after their condition improved without death. There was no proof for liver tumors in patients with increased AFP. Three patients from the control group were excluded due to no apparent drop in virus after four weeks of liver protection treatment. The auto clearance ratio reached 90.32% (28/31). The average length of stay and hospitalization costs of each group are listed in **Table 2**, which showed no significant difference among the groups.

The association of HBV DNA clearance with clinical data

The correlation of HBV DNA concentration with all the concerned clinical data are presented in **Tables 3**, **4** and **Figure 1**, which only identified a correlation of HBV DNA clearance with TBil (r=0.5126, P=0.0027) and ALT (r=0.3924, P=0.0263).

Association of AFP with inflammatory indicators

Markedly elevated serum IL-6 and TNF-α levels were determined in the experimental and positive control groups compared to the negative control group (P<0.05; **Table 5**). AFP was statistically correlated with IL-6, TNF-α and HBV DNA (**Figure 2**).

Discussion

The IC phase is the second phase of HBV infection [4]. It is characterized by high serum HBV DNA concentration, persistent or indirect increase of ALT/AST, and necro-inflammatory changes of the liver [20]. Due to the multiple replications of HBV in the body, the immune system is activated and launches an immune attack against HBV, resulting in an active immune response of the organism. When the immune system attacks HBsAg on the liver cell

Table 3. HBV DNA, AFP, TBil, ALT, HBsAg and HBeAg of experimental group

	HBV DNA (log)	AFP (ng/mL)	TBil (μmol/L)	ALT (U/L)	HBsAg (ng/mL)	HBeAg (IU/mL)
0 w	5.72±2.87	369.1±184.1	172.6±71.2	573.2±249.0	3437.8±1964.3	756.7±591.2
2 w	5.86±2.40	1083.1±591.2[a]	203.1±87.7	221.7±165.6[a]	3675.4±2016.0	741.6±513.5
4 w	4.45±3.02[b]	689.6±100.9[a,b]	143.7±91.5[b]	40.3±5.8[a,b]	3859.9±2103.5	801.2±555.4
6 w	3.57±1.58[a,b]	355.2±173.4[b,c]	50.6±45.4[a,b,c]	27.7±3.9[a,b,c]	3457.1±2045.8	792.8±590.0
8 w	3.24±1.66[a,b]	108.3±65.5[a,b,c,d]	30.1±17.3[a,b,c,d]	26.5±4.8[a,b,c]	3760.5±1962.0	789.6±603.3.
16 w	3.01±0.64[a,b]	31.1±10.3[a,b,c,d,e]	16.1±9.8[a,b,c,d,e]	28.1±6.2[a,b,c]	3578.1±1960.7	748.7±619.2
20 w	2.97±0.71[a,b]	6.5±2.0[a,b,c,d,e,f]	14.6±5.9[a,b,c,d,e]	21.5±3.0[a,b,c,d,e,f]	3428.9±1994.1	785.5±590.3
24 w	2.59±0.42[a,b,c,d,f,g]	3.2±2.1[a,b,c,d,e,f,g]	8.1±6.0[a,b,c,d,e,f,g]	21.6±3.2[a,b,c,d,e,f]	3635.6±1987.6	761.9±568.2

Notes: HBV, hepatitis B virus; AFP, alpha-fetoprotein; TBil, total bilirubin; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen. [a]P<0.05 *vs* 0 w in the same group; [b]P<0.05 *vs* 2 w in the same group; [c]P<0.05 *vs* 4 w in the same group; [d]P<0.05 *vs* 6 w in the same group; [e]P<0.05 *vs* 8 w in the same group; [f]P<0.05 *vs* 16 w in the same group; [g]P<0.05 *vs* 20 w in the same group.

Table 4. Significant changes in HBV DNA, AFP, TBil, ALT, HBsAg, and HBeAg over time

Source	Type III Sum of Square	Mean Square	F	*P* value
HBV DNA	3947.61	3947.61	1156.52	<0.001
Error	105.81	3.413		
AFP	28016365.07	28016365.07	723.827	<0.001
Error	1199882.15	38705.88		
TBil	1639508.99	1639508.99	550.539	<0.001
Error	92318.230	2978.01		
ALT	3688368.26	3688368.26	317.399	<0.001
Error	360239.22	11620.62		
HBsAg	333826976782	333826976782	1935.409	<0.001
Error	53387267.76	1722169.93		
HBeAg	153036893.67	153036893.67	647.823	<0.001
Error	7323208.62	236232.536		

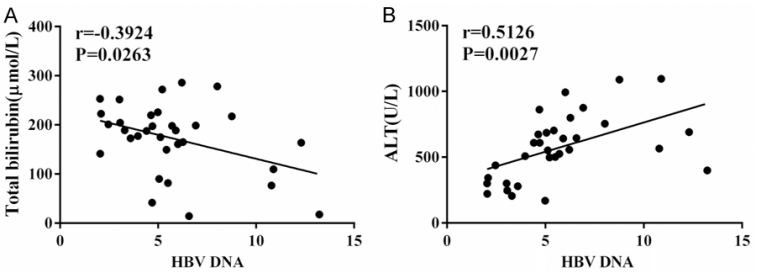

Figure 1. Correlation of HBV DNA level and other indicators. A: Correlation of HBV DNA level and TBil level; B: Correlation of HBV DNA level and ALT level. Notes: HBV, hepatitis B virus; TBil, total bilirubin; ALT, alanine aminotransferase.

membrane, a large number of HCs are damaged or even become necrotic, accompanied by liver cell reproduction [21]. At the same time, there may be a rise in jaundice and abnormalities of aminotransferase clinically. CHB is a persistent HBV infection-induced chronic necroinflammatory liver disease [22]. The difference in the CHB phase may depend on the dynamic interplay between the virus and the hepatic microenvironment composed of hepatic parenchymal, non-parenchymal and local immune cells. Lifelong quiescence may occur in some patients, but others can experience severe complications featuring HBV DNA and ALT level fluctuations [23]. In our study, HBV DNA was also in direct proportion to ALT levels in IC-phase CHB.

AFP is a bioprotein that is generated under pathologic state during the embryonic phase [24]. When cell division enters an exuberant state, the body grows mature, resulting in a decrease in AFP secretion. However, in the case of liver cancer or liver damage, HCs will secrete AFP in great quantities. Since the identification of the correlation between AFP and liver cancer

Table 5. Comparison of HBV DNA, AFP, IL-6 and TNF-α levels

Group	AFP (ng/mL)	IL-6 (ng/L)	TNF-α (ng/L)
Experimental group (n=32)	369.1±184.1	42.6±7.2	73.1±24.9
Positive control group (n=28)	348.6±176.6	43.1±7.7	71.7±19.6
Negative control group (n=30)	24.1±2.8*,#	27.7±9.5*,#	40.3±10.6*,#
F	51.86	34.23	27.41
P	<0.0001	<0.0001	<0.0001

Notes: AFP, alpha-fetoprotein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; *P<0.05 *vs* experimental group; #P<0.05 *vs* positive control group.

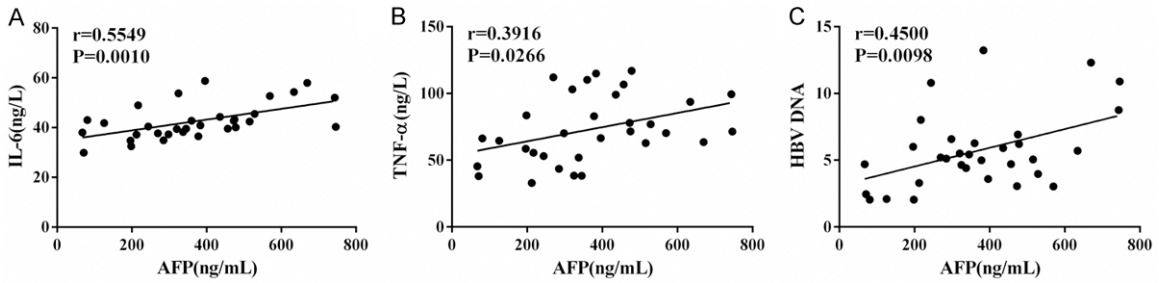

Figure 2. Correlation of AFP level and other indicators. A: Correlation of AFP level with IL-6 level; B: Correlation of AFP level with TNF-α level; C: Correlation of AFP level with HBV DNA level. Notes: AFP, alpha-fetoprotein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; HBV, hepatitis B virus.

in the 1960s, AFP has been widely used in HCC screening, diagnosing, early diagnosing, therapeutic evaluation, and prognosis prediction, [25]. However, analyses in recent years have shown that dramatic increases in AFP during acute or chronic hepatitis outbreaks may not be associated with tumors. In hepatocirrhosis patients with acute liver injury, AFP is elevated but tumors do not develop [26]. The research by Ahn [27] and He [28] came to the conclusion that AFP level elevation was strongly linked to myofibrosis progression in hepatitis C patients without hepatocarcinogenesis. Uslu [29] and his collaborators reached the same conclusion in hepatitis B patients. Our research found that the decrease in HBV DNA concentration had a significant relationship with AFP levels in IC-phase CHB patients with rising AFP, possibly due to the reproduction of HCs when virus-infected HCs are damaged or necrotic or because ALT and TBil are significantly associated with decreased HBV DNA concentrations. Our research did not find a high correlation of HBV DNA and HBeAg with decreasing HBV DNA concentrations.

The immune system is composed of several molecules and cells involved in inflammation induction [30]. IL-6 and TNF-α affect inflammatory responses [31] and participate in the regulation of important pathophysiologic processes [32]. Increased IL-6 production is associated with the pathogenesis of HCC in animal models. Previous studies have shown that high serum IL-6 levels predate HCC carcinogenesis in CHB patients and are moderately accurate in predicting future cancer [33]. Short-term IL-6 production may be the body's natural response to liver injury and may even be protective, but long-term exposure to high levels of IL-6 may increase the likelihood of developing liver damage and HCC. TNF-α system activity gets elevated in cirrhosisand, is acknowledged to be linked to several known cirrhosis-associated adverse events, such as hyperkinetic circulation, infection predisposition, and hepatic encephalopathy [34, 35]. As has been observed in multiple chronic inflammatory disorders including CHB, activation of the cytokine system may lead to energy expenditure enhancement and nutrient intake reduction [36]. The endogenous TNF-α elevation in advanced hepatopathy patients is widely acknowledged to be a result of chronic liver failure, which is related to endotoxin-dependent macrophage stimulation and reduced cytokine clearance [37]. TNF-α and IL-6 may be critical mediators of viral mass processes, although this association has yet to be fully

established [38]. Our data revealed evidently higher serum IL-6 and TNF-α in the experimental and positive control groups versus the negative control group, demonstrating their roles as two important risk factors for CHB infection. In addition, a close relationship was determined between serum AFP and IL-6 and TNF-α levels.

In recent years, antiviral therapy for CHB has received increasing attention. It is believed that hepatitis B should be given positive antiviral therapy [39, 40]. Our research revealed that the probability of HBV DNA clearance in quite higher in IC-phase CHB patients tested positive for AFP (28/31), with no effect on patient prognosis, length of stay,orhospitalization costs. The condition of such patients should be closely observed, or antivirus therapy can be given when needed. Because this clinical research followed up the patients only short term, further research is needed to observe long-term outcomes.

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Disclosure of conflict of interest

None.

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