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ARTICLE

Impacts of PEG-IFN-α-2b Combination Therapy on Liver Function, Immune Factors and Risk Factors in Patients With HBV Infection: A Retrospective Study

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Abstract

Aims/Background Hepatitis B virus (HBV) infection poses a challenge to global healthcare. Peginterferon alfa-2b (PEG-IFNα-2b) is an effective treatment for HBV infection. This study aimed to explore the efficacy of PEG-IFNα-2b combined with entecavir in the treatment of HBV infection, its effect on liver function and immune factors, and the risk factors affecting the prognosis of patients with HBV infection.

Methods The clinical data of 184 patients with HBV infection who were treated at Jinhua Central Hospital from January 2021 to January 2024 were collected for retrospective analysis. Patients were divided into a control group (not receiving antiviral treatment, $n = 34$), a standard treatment group (receiving entecavir, $n = 85$), and a combination treatment group (PEG-IFNα-2b and entecavir, $n = 65$) according to the treatment approach. Treatment efficacy, liver function indicators (albumin [ALB], alanine aminotransferase [ALT], and aspartate aminotransferase [AST]), immune factor indexes (tumour necrosis factor alpha [TNF-α] and interferon gamma [IFN-γ]), hepatitis B surface antigen [HBsAg] and HBV DNA levels were compared among the three groups. All patients were followed up after treatment. According to their prognosis, the patients were divided into good prognosis group ($n = 118$) and poor prognosis group ($n = 66$). Logistic regression analysis was performed to explore the risk factors affecting the prognosis of HBV patients.

Results The efficacy in the combination treatment group was higher (92.31%) than that in the control group (8.82%) and the standard treatment group (78.82%) ($p < 0.05$). After treatment, the HBsAg and HBV DNA levels were decreased in the standard treatment and combination treatment groups ($p < 0.05$). Compared with the control and standard treatment groups, the combination treatment group exhibited significantly lower HBsAg and HBV DNA levels after treatment ($p < 0.05$). Besides, the combination treatment group had lower ALT and AST levels ($p < 0.05$), and higher ALB level ($p < 0.05$), than the control and standard treatment groups after treatment. Compared with the control and standard treatment groups, the combination treatment group demonstrated decreased TNF-α level and higher IFN-γ level after treatment ($p < 0.05$). Multivariate logistic regression analysis identified family medical history as the risk factor affecting the prognosis of patients with HBV infection ($p = 0.001$, odds ratio [OR] = 3.614, 95% confidence interval [CI]: 1.685–7.750) and therapy regimen as the protective factor ($p = 0.029$, OR = 0.135, 95% CI: 0.022–0.815).

Conclusion The PEG-IFNα-2b combination therapy in patients with HBV infection significantly improves the clinical treatment efficacy, liver function, and immune factors. In addition, this study found that therapy regimen and family medical history are independent factors affecting the prognosis of HBV infection.

Key words: peginterferon alfa-2b; chronic hepatitis B virus infection; liver function; immune factors; prognosis

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Introduction

Hepatitis B virus (HBV) infection is a global public health problem. According to the World Health Organization, about 257 million people worldwide are infected with HBV, and approximately 887,000 people succumb diseases associated with HBV infection (Tan et al, 2021; World Health Organization, 2017). HBV infection is closely associated with chronic hepatitis, cirrhosis and even liver cancer, substantially threatening the physical and psychological health of patients (Martyn et al, 2023). Nucleoside (acid) analogues are the primary treatment of HBV infection by inhibiting viral replication of HBV and delaying progression of HBV-related events, offering excellent antiviral effects and good tolerance (Yang et al, 2021). However, HBV infection requires a long duration of treatment with nucleoside (acid) analogues and is prone to relapse after drug discontinuation (Ramesh et al, 2021). In recent years, immunotherapy has emerged as a new strategy for HBV infection treatment (Lang et al, 2019). For example, vesatolimod (GS-9620), a Toll-like receptor 7 agonist, has been reported as safe and effective in treating chronic HBV infection, based on findings from a phase II clinical trial (Janssen et al, 2018). But another clinical study reported that the GS-9620 did not significantly decrease the serum hepatitis B surface antigen (HBsAg) levels, even after combination with nucleoside (acid) analogues (Agarwal et al, 2018). In general, these new therapies are still in the clinical trial stage. It is necessary to explore effective treatment strategies for patients with HBV infection to improve their survival status.

Peginterferon alfa-2b (PEG-IFNα-2b) has immunomodulatory and anti-HBV effects, but its monotherapy effect is less than satisfactory (Shen et al, 2016; Wu et al, 2023). Therefore, there have been proposals of combining it with other therapies to maximize their therapeutic effectiveness against HBV infection. Wen et al (2024) reported that 52.9% of patients achieved HBsAg clearance in chronic HBV infection by 48 weeks of PEG-IFNα-2b combined with nucleoside analogs treatment. Another study found a significant improvement in liver inflammation and fibrosis in patients with chronic HBV infection after PEG-IFNα-2b and nucleoside analogs combination therapy (Li et al, 2019). A meta-analysis found that the combination of nucleoside analogues and interferon (IFN) increased the HBsAg clearance rate in chronic HBV infection (Liu et al, 2020). At present, studies analyzing the influence of combination therapy on liver function and immune factors of patients with chronic HBV infection, as well as their prognosis, remain scarce. Therefore, this study aimed to explore the effects of PEG-IFNα-2b combination therapy on the efficacy, liver function, and immune factors of patients with chronic HBV infection, and investigate the related factors affecting their prognosis. The findings would provide a basis for the clinical personalized treatment plan and insights into the formulation of more optimized treatment strategies for HBV-infected patients, with the aim to improve treatment success rate.

Methods

General Information

In this retrospective study, a total of 184 HBV-infected patients admitted to Jinhua Central Hospital from January 2021 to January 2024 were recruited as research subjects. Patients were divided into three groups according to the treatment approach: control group (n = 34, not receiving antiviral treatment), standard treatment group (n = 85, receiving entecavir), and combination treatment group (n = 65, receiving combination of PEG-IFNα-2b and entecavir).

Patients who met the inclusion criteria were included: (1) patients' condition complied with the diagnostic criteria for chronic HBV infection (Vittal and Ghany, 2019), being positive for HBsAg and/or HBV DNA for more than six consecutive months; (2) patients aged ≥18 years old; (3) patients with complete clinical data, including the history of disease, symptoms, signs, laboratory test results, etc., and (4) patients who had given informed consent for research participation. Individuals meeting the following conditions are excluded: (1) pregnant and lactating; (2) comorbid with other viral infections, such as hepatitis C virus, human immunodeficiencyvirus (HIV), etc., (3) comorbid with liver cancer, and drug-, fatty- and alcohol-induced liver diseases; (4) comorbid with blood disease, such as leukemia, coagulation dysfunction, and aplastic anemia; (5) comorbid with immune diseases, such as asthma, rheumatoid arthritis, and systemic lupus erythematosus; (6) received immunotherapy within the past 6 months; and (7) loss of follow-up. Fig. 1 shows the flow chart of inclusion procedures. This study has been approved by Jinhua Central Hospital Ethics Review Committee (Approval No. 2022-44) and was conducted in strict adherence to the Declaration of Helsinki.

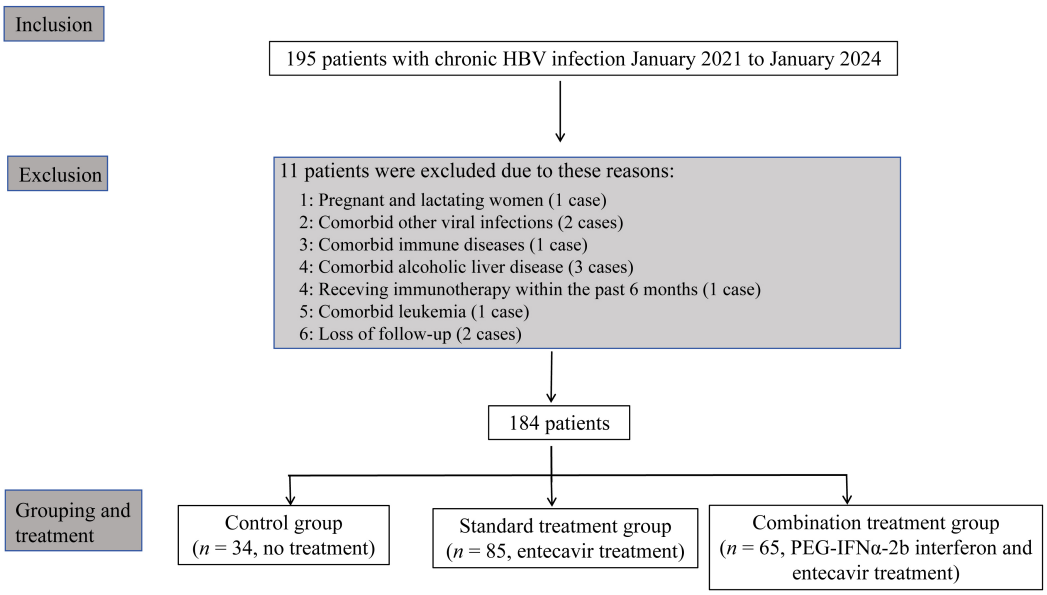

Fig. 1. Flowchart of patient inclusion, exclusion and grouping. HBV, hepatitis B virus; PEG-IFNα-2b, peginterferon alfa-2b.

Study Methods

Treatment Plan

In the standard treatment group, the subjects took oral entecavir (H20052237, Shanghai Squibb Pharmaceutical Co., Ltd., Shanghai, China), 0.5 mg/day, for 3 consecutive months. The patients in the combination treatment group received both PEG-IFNα-2b and entecavir therapy, using similar entecavir dosage applied in the standard treatment group and subcutaneous injection of 180 μg PEG-IFNα-2b (S20160001, Xiamen Tebao Biological Engineering Co., Ltd., Xiamen, China), once weekly, for 3 consecutive months. The control group patients did not receive any antiviral treatments. Relevant clinical data were collected from all subjects in these three groups at pre-treatment, post-treatment and follow-up.

Serum Collection

A total of 5 mL of elbow venous blood was extracted from all the participants in the fasting state, and centrifuged for 10 min at 3500 r/min. Next, serum was collected for laboratory analysis.

Observation Indicators

The primary outcomes of this study were treatment efficacy and the serum HBsAg and HBV DNA levels, while the secondary outcomes included liver function and inflammatory markers.

Efficacy

Treatment efficacy noted in the three patient groups was divided into several categories:

(1) Markedly effective: restoration of alanine aminotransferase (ALT) to normal levels, and HBsAg level turning negative;

(2) Improvement: restoration of ALT to normal levels or reduction by 50% compared to level prior to treatment, or HBsAg level turning negative;

(3) Ineffective: no significant improvement in ALT and HBsAg levels, or symptom persistence.

The total effective rate was determined using the following formula:

Total effective rate (%) = (Markedly effective + Improvement)/Ineffective × 100.

HBsAg and HBV DNA Testing Assays

Elecsys® HBsAg II quant (20143405198, Roche Diagnostics, Mannheim, Germany) was adopted for the detection of HBsAg in accordance with the manufacturer's operating procedures. HBV DNA was quantified by fluorescence quantitative polymerase chain reaction (PCR) according to the operation instructions of HBV DNA detection kit (20143402366, Mole bioscience, Taizhou, China). Specifically, the DNA in the blood sample was extracted. A specific fragment of HBV DNA was then amplified by PCR system (ABI7500, Applied Biosystems, Foster City, CA, USA). In the amplification process, fluorescent probe was combined with the amplification product to produce fluorescence signal. The primers used in the

PCR are: forward primer 5′-TAGGAGGCTGTAGGCATAAATTGG-3′; reverse primer 5′-GCACAGCTTGGAGGCTTGA-3′. The content of HBV DNA was calculated with a standard curve.

Index of Liver Function

Liver function-related indicators, including albumin (ALB), ALT, and aspartate aminotransferase (AST) levels, were measured by automated biochemical analyzer (AU5800, Beckman Coulter, Brea, CA, USA).

Immune Factor Index

Enzyme-linked immunosorbent assay (ELISA) kits for tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) (EHC103a and EHC102g, respectively, NeoBioscience Technology, Shenzhen, China) were used to test the serum TNF-α and IFN-γ levels according to manufacturer's instructions.

Follow-up and Prognosis

All patients were followed up for 6 months after treatment, with the follow-up of last case ending in September 2024. Patients were followed up every 2 months through outpatient visits. The endpoint events of follow-up include sustained elevation of HBV DNA level and deterioration of liver function (liver function indicators were increased), or the occurrence of liver disease associated with HBV infection (such as cirrhosis and liver cancer). Poor prognosis was defined as sustained elevation of HBV DNA and deterioration of liver function. Following categorization, there were 118 cases in the good prognosis group and 66 cases in the poor prognosis group.

Statistical Methods

SPSS 26.0 software (IBM, Armonk, NY, USA) was for statistical processing. Shapiro-Wilk test was adopted to assess data normality. Normally distributed data are expressed as mean $\pm$ standard deviation (SD), and the inter-group comparisons were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (among multiple groups) or *t*-test (between two groups). Data with skewed distribution are represented as median and quartile, and differences between two groups were compared using the Mann-Whitney *U* test. Categorical data are expressed as count and percentage, and chi square tests were performed for comparison. Logistic regression analysis was conducted to explore risk factors affecting the prognosis of patients infected by HBV. Specifically, clinical variables with significant differences among the three groups were included in a univariate logistic regression analysis. Subsequently, variables with $p < 0.05$ from the univariate analysis were incorporated into a multivariate analysis to explore the risk factors influencing the prognosis of HBV infection. The $p < 0.05$ was set as criteria for determining statistical significance.

Table 1. Comparison of general data among three groups.

Metric	Control group (n = 34)	Standard treatment group (n = 85)	Combination treatment group (n = 65)	F/χ^2	p
Sex, n (%)				0.769	0.681
Male	20 (58.82%)	55 (64.71%)	44 (67.69%)		
Female	14 (41.18%)	30 (35.29%)	21 (32.31%)		
Age (year)	43.85 ± 6.96	41.72 ± 7.47	43.62 ± 7.96	1.578	0.209
Smoking history, n (%)				0.817	0.665
Yes	15 (44.12%)	45 (52.94%)	34 (52.31%)		
No	19 (55.88%)	40 (47.06%)	31 (47.69%)		
Overweight, n (%)				0.111	0.946
Yes	12 (35.29%)	31 (36.47%)	22 (33.85%)		
No	22 (64.71%)	54 (63.53%)	43 (66.15%)		
History of drinking, n (%)				0.876	0.645
Yes	18 (52.94%)	47 (55.29%)	40 (61.54%)		
No	16 (47.06%)	38 (44.71%)	25 (38.46%)		
Family medical history, n (%)				0.605	0.739
Yes	11 (32.35%)	25 (29.41%)	23 (35.38%)		
No	23 (67.65%)	60 (70.59%)	42 (64.62%)		
Hepatitis[#], n (%)				2.942	0.230
G0–G2	28 (82.35%)	59 (69.41%)	43 (66.15%)		
G3–G4	6 (17.65%)	26 (30.59%)	22 (33.85%)		
Liver fibrosis, n (%)				3.036	0.219
S0–S2	29 (85.29%)	60 (70.59%)	46 (70.77%)		
S3–S4	5 (14.71%)	25 (29.41%)	19 (29.23%)		

Notes: [#] Viral hepatitis; overweight is defined as body mass index ≥24 kg/m^2.

Results

Comparison of General Data

There were no significant differences in age, sex, smoking history, overweight, drinking history, hepatitis, liver fibrosis and family medical history among three groups ($p > 0.05$) (Table 1).

Comparison of Treatment Efficacy

The difference in treatment efficacy was statistically significant among three groups ($p < 0.001$), as detailed in Table 2. The efficacy of the combination treatment group was higher (92.31%) than that in the control (8.82%) and standard treatment groups (78.82%).

Comparison of HBsAg and HBV DNA Levels Before and After Treatment

Before treatment, there were no significant differences in HBsAg and HBV DNA levels among the three groups ($p > 0.05$). After treatment, the control group recorded a decreased level of HBsAg ($p < 0.05$) and an unchanged HBV DNA level

Table 2. Comparison of treatment efficacy among the three groups.

Group	Markedly effective	Improvement	Ineffective	Total effective rate
Control group (n = 34)	1 (2.94%)	2 (5.88%)	31 (91.18%)	3 (8.82%)
Standard treatment group (n = 85)	20 (23.53%)	47 (55.29%)	18 (21.18%)	67 (78.82%)
Combination treatment group (n = 65)	35 (53.85%)	25 (38.46%)	5 (7.69%)	60 (92.31%)
χ^2	30.880	24.791	80.122	80.122
p	<0.001	<0.001	<0.001	<0.001

Table 3. HBsAg and HBV DNA levels among the three groups.

Group	HBsAg (Log_{10} IU/mL)		HBV DNA (Log_{10} IU/mL)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control group (n = 34)	2.35 ± 0.35	2.21 ± 0.19[a]	2.91 ± 0.42	2.89 ± 0.51
Standard treatment group (n = 85)	2.40 ± 0.24	2.01 ± 0.22[a]*	3.04 ± 0.53	2.60 ± 0.41[a]*
Combination treatment group (n = 65)	2.42 ± 0.31	1.24 ± 0.17[a]*&	3.11 ± 0.58	2.03 ± 0.35[a]*&
F	0.663	378.637	1.586	59.141
p	0.517	<0.001	0.208	<0.001

[a]$p < 0.05$ compared with before treatment in the same group; *$p < 0.05$ compared with control group during the same period; &$p < 0.05$ compared with standard treatment group during the same period. HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

($p > 0.05$), while both the standard treatment and combination treatment groups exhibited reduced HBsAg and HBV DNA levels ($p < 0.05$). Compared with the control and standard treatment group, the HBsAg and HBV DNA levels were significantly reduced in the combination treatment group post-treatment ($p < 0.05$) (Table 3).

Comparison of Liver Function Indicators

Before treatment, there were no significant differences in liver function index among the three groups ($p > 0.05$). Compared with before treatment, patients in the control group showed an increase in ALB and a decrease in ALT levels ($p < 0.05$), but no significant change in AST levels after treatment ($p > 0.05$). The post-treatment significant increase in ALB level and the notable decrease in ALT and AST levels were observed in the standard treatment and combination treatment groups compared to their levels before treatment ($p < 0.05$). In addition, the combination treatment group had significantly lower ALT and AST levels ($p < 0.05$) and higher ALB level compared with the control and standard treatment groups ($p < 0.05$) (Table 4).

Comparison of Immune Function Indicators

Before treatment, there were no significant differences in TNF-α and IFN-γ levels among the three groups ($p > 0.05$). After treatment, the TNF-α and IFN-γ levels were significantly decreased and increased, respectively, in the standard treatment and combination treatment groups (all $p < 0.05$). In the control group,

Table 4. Liver function indicators in the three groups.

Group	ALB (g/L)		ALT (U/L)		AST (U/L)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control group (n = 34)	30.58 ± 5.01	33.98 ± 4.92^a	69.95 ± 6.67	61.43 ± 7.06^a	55.68 ± 5.12	55.24 ± 5.35
Standard treatment group (n = 85)	32.59 ± 4.34	41.56 ± 5.78^a*	70.87 ± 5.97	36.18 ± 6.71^a*	54.89 ± 4.47	35.85 ± 3.16^a*
Combination treatment group (n = 65)	31.47 ± 3.98	52.37 ± 6.89^a*$^{\&}$	68.52 ± 6.30	24.90 ± 9.86^a*$^{\&}$	56.75 ± 4.95	27.53 ± 2.64^a*$^{\&}$
F	2.924	115.429	2.632	231.999	2.805	698.472
p	0.056	<0.001	0.075	<0.001	0.063	<0.001

$^a p < 0.05$ compared with before treatment in the same group; $^* p < 0.05$ compared with control group during the same period; $^{\&} p < 0.05$ compared with standard treatment group during the same period. ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 5. Comparison of TNF-α and IFN-γ levels among the three groups.

Group	TNF-α (pg/mL)		IFN-γ (pg/mL)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control group (n = 34)	61.45 ± 7.21	60.33 ± 7.24	73.33 ± 7.20	75.43 ± 6.59
Standard treatment group (n = 85)	60.48 ± 6.89	46.61 ± 5.94^a*	70.35 ± 7.58	89.58 ± 8.72^a*
Combination treatment group (n = 65)	61.11 ± 6.54	34.06 ± 3.42^a*$^{\&}$	71.83 ± 7.23	100.74 ± 7.94^a*$^{\&}$
F	0.301	265.651	2.127	111.291
p	0.740	<0.001	0.122	<0.001

$^a p < 0.05$ compared with before treatment in the same group; $^* p < 0.05$ compared with control group during the same period; $^{\&} p < 0.05$ compared with standard treatment group during the same period. TNF-α, tumour necrosis factor alpha; IFN-γ, interferon gamma.

Table 6. Comparison of clinical characteristics between good prognosis group and poor prognosis group.

Metric	Good prognosis group (n = 118)	Poor prognosis group (n = 66)	$t/Z/\chi^2$	p
Age (year)	42.49 ± 8.06	43.30 ± 6.66	0.694	0.488
Sex, n (%)			0.048	0.826
Male	77 (65.25%)	42 (63.64%)		
Female	41 (34.75%)	24 (36.36%)		
Smoking history, n (%)			0.008	0.931
Yes	60 (50.85%)	34 (51.52%)		
No	58 (49.15%)	32 (48.48%)		
Overweight, n (%)			2.270	0.132
Yes	37 (31.36%)	28 (42.42%)		
No	81 (68.64%)	38 (57.58%)		
History of drinking, n (%)			2.747	0.097
Yes	62 (52.54%)	43 (65.15%)		
No	56 (47.46%)	23 (34.85%)		
Therapeutic regimen, n (%)			27.766	<0.001
No treatment	13 (11.02%)	21 (31.82%)		
Standard treatment	48 (40.68%)	37 (56.06%)		
Combination treatment	57 (48.30%)	8 (12.12%)		
Family medical history, n (%)			8.470	0.004
Yes	29 (24.58%)	30 (45.45%)		
No	89 (75.42%)	36 (54.55%)		
Hepatitis, n (%)			0.214	0.644
G0–G2	82 (69.49%)	48 (72.73%)		
G3–G4	36 (30.51%)	18 (27.27%)		
Liver fibrosis, n (%)			0.022	0.883
S0–S2	87 (73.73%)	48 (72.73%)		
S3–S4	31 (26.27%)	18 (27.27%)		
HBsAg after treatment (Log_{10} IU/mL)	1.69 (1.24, 2.07)	2.05 (1.88, 2.21)	4.253	<0.001
HBV DNA after treatment (Log_{10} IU/mL)	2.40 ± 0.50	2.54 ± 0.56	1.744	0.083
ALB after treatment (g/L)	44.48 (38.79, 52.20)	40.36 (36.14, 47.64)	2.336	0.019
ALT after treatment (U/L)	34.81 (24.41, 42.62)	35.36 (29.46, 56.43)	2.092	0.036
AST after treatment (U/L)	34.11 (27.45, 38.70)	34.93 (31.94, 50.31)	2.723	0.006
TNF-α after treatment (pg/mL)	40.14 (33.85, 48.07)	49.54 (40.71, 55.64)	4.274	<0.001
IFN-γ after treatment (pg/mL)	93.43 ± 11.73	86.39 ± 11.27	3.959	<0.001

the TNF-α and IFN-γ levels did not undergo significant changes after treatment ($p > 0.05$). Compared with control and standard treatment groups, the combination treatment group experienced a drop in TNF-α level and a surge in IFN-γ level post-treatment (all $p < 0.05$) (Table 5).

Comparison of Clinical Data of Patients With Different Prognosis Status

Patients were divided into good prognosis group (n = 118) and poor prognosis group (n = 66) based on their follow-up results, and their clinical data, including

Table 7. Assignment scale in multivariate analysis.

Factor	Assignment
Dependent variable	
Prognosis of HBV-infected patients	Poor prognosis = 1; Good prognosis = 0
Independent variables	
Therapeutic regimen	No treatment or standard treatment = 0; Combination treatment = 1
Family medical history	No = 0; Yes = 1
HBsAg after treatment	Original value
ALB after treatment	Original value
ALT after treatment	Original value
AST after treatment	Original value
TNF-α after treatment	Original value
IFN-γ after treatment	Original value

baseline characteristics and post-treatment laboratory data, were compared. There were no significant differences in age, sex, smoking history, overweight, history of drinking, hepatitis, liver fibrosis and HBV DNA level after treatment between the two prognosis groups (both $p > 0.05$). The significant differences between the two groups were observed in therapeutic regimen, family medical history, and post-treatment levels of HBsAg, ALB, ALT, AST, TNF-α and IFN-γ ($p < 0.05$) (Table 6).

Logistic Regression Analysis of Risk Factors for Prognosis of HBV-Infected Patients

The logistic regression analysis was conducted to explore the risk factors for prognosis of HBV-infected patients. The factors with $p < 0.05$ in Table 6 were selected as the potential risk factors for logistic regression. Table 7 shows the variables used for logistic regression analysis. Univariate analysis results showed that the risk factors affecting prognosis of HBV-infected patients included therapeutic regimen ($p < 0.001$, odds ratio [OR] = 0.148, 95% confidence interval [CI]: 0.065–0.336), family medical history ($p = 0.004$, OR = 2.557, 95% CI: 1.348–4.853), ALB after treatment ($p = 0.039$, OR = 0.964, 95% CI: 0.931–0.998), ALT after treatment ($p = 0.014$, OR = 1.026, 95% CI: 1.005–1.047), AST after treatment ($p = 0.004$, OR = 1.045, 95% CI: 1.015–1.077), HBsAg after treatment ($p < 0.001$, OR = 5.670, 95% CI: 2.567–12.526), TNF-α after treatment ($p < 0.001$, OR = 1.061, 95% CI: 1.030–1.094), and IFN-γ after treatment ($p < 0.001$, OR = 0.949, 95% CI: 0.924–0.976) (Table 8).

Variables with $p < 0.05$ in the univariate analysis were included in the multivariate analysis to further analyze risk factors with an influence on the prognosis of patients with HBV infection. The multivariate analysis identified family medical history as the risk factor affecting the prognosis of patients with HBV infection ($p = 0.001$, OR = 3.614, 95% CI: 1.685–7.750) and therapeutic regimen as the protective factor ($p = 0.029$, OR = 0.135, 95% CI: 0.022–0.815) (Table 8).

Table 8. Logistic regression analysis of risk factors affecting prognosis in patients with HBV infection.

Variables	Univariate analysis					
	β	SE	Wald	OR	95% CI	*p*
Therapeutic regimen (=1)	–1.913	0.420	20.776	0.148	0.065–0.336	<0.001
Family medical history (=1)	0.939	0.327	8.254	2.557	1.348–4.853	0.004
ALB after treatment	–0.037	0.018	4.267	0.964	0.931–0.998	0.039
ALT after treatment	0.026	0.010	6.076	1.026	1.005–1.047	0.014
AST after treatment	0.044	0.015	8.495	1.045	1.015–1.077	0.004
HBsAg after treatment	1.735	0.404	18.413	5.670	2.567–12.526	<0.001
TNF-α after treatment	0.060	0.015	14.913	1.061	1.030–1.094	<0.001
IFN-γ after treatment	–0.052	0.014	13.640	0.949	0.924–0.976	<0.001
	Multivariate analysis					
	β	SE	Wald	OR	95% CI	*p*
Therapeutic regimen (=1)	–2.001	0.916	4.767	0.135	0.022–0.815	0.029
Family medical history (=1)	1.285	0.389	10.891	3.614	1.685–7.750	0.001
ALB after treatment	0.059	0.031	3.637	1.061	0.998–1.127	0.057
ALT after treatment	–0.017	0.023	0.561	0.983	0.939–1.029	0.454
AST after treatment	–0.001	0.039	0.000	0.999	0.926–1.079	0.987
HBsAg after treatment	0.579	0.894	0.419	1.784	0.309–10.294	0.518
TNF-α after treatment	0.020	0.029	0.493	1.020	0.964–1.080	0.483
IFN-γ after treatment	–0.032	0.021	2.301	0.968	0.928–1.010	0.129

OR, odd ratio; CI, confidence interval.

Discussion

HBV is a non-cytopathic hepatotropic virus that damages liver cells, triggering hepatocellular inflammation, necrosis or fibrosis, which in turn impairs liver function (Iannacone and Guidotti, 2022). Both nucleoside analogues and IFN are used as clinical antiviral treatment of HBV infection. In our study, PEG-IFNα-2b combined with entecavir, which is a nucleoside analogue, resulted in a total effective rate of 92.31% in chronic HBV infection, which was higher than the rate attributed to entecavir monotherapy or no antiviral treatment. Meanwhile, HBV DNA and HbsAg levels were significantly decreased after combination therapy was administered. It was suggested that the PEG-IFNα-2b combined with entecavir treatment could inhibit HBV replication, owing to its satisfactory therapeutic efficacy in chronic HBV infection, which was higher than that of entecavir monotherapy. Xie et al (2022) reported that PEG-IFNα combined with entecavir reduced the HBsAg and HBV DNA levels in chronic HBV patients, which was comparable to our results. In addition, a previous study's results are also consistent also with ours (Wen et al, 2024). On the contrary, a prospective, randomized controlled trial reported contrasting results, revealing that PEG-IFNα-2a combined with entecavir did not achieve HBsAg seroconversion in treatment-naïve chronic HBV patients and showed no significant difference in terms of treatment efficacy when compared

to entecavir monotherapy (Yang et al, 2020). But another study reported excellent therapeutic effects stemming from the combination treatment in terms of liver function and histopathology (Yang et al, 2020). This may be related to individual differences among patients included in the study. Another plausible reason may be related to the difference in the PEG-IFNα drugs. From a mechanistic perspective, entecavir directly inhibits the activity of HBV DNA polymerases, thus inhibiting viral replication; moreover, it also interrupts HBV DNA synthesis by competing with natural nucleoside substrates (Pan et al, 2023). PEG-IFNα possesses antiviral activity and regulates immune functions. It could act on the cycle proteins related to viral replication to prevent the replication of HBV DNA and simultaneously regulate immune cell activity, including natural killer cells and T lymphocytes (Cao et al, 2022; Zhao et al, 2024). Thus, the combination of the PEG-IFNα-2b and entecavir could exert a synergistic effect in inhibiting the replication of virus.

Liver function was impaired in patients with HBV infection due to the virus-mediated destruction of hepatocytes. The ALT and AST levels were increased, and ALB levels were decreased in HBV-infected patients as compared to normal healthy volunteers (Tian et al, 2019). Our study reported that combination of PEG-IFNα-2b and entecavir significantly reduced the levels of AST and ALT, and increased ALB level in patients and chronic HBV infection, indicating an improvement in liver function. The combination therapy was more effective than entecavir monotherapy. The improvement of liver function was associated with the inhibitory effect of combination therapy on HBV. The inhibition of HBV virus minimizes the virus-mediated hepatocyte damage, thereby improving the patients' liver function, a conclusion concordant with a previously reported study (Lin et al, 2020).

This study also found that PEG-IFNα-2b combined with entecavir treatment has an important regulatory effect on the inflammatory factors in the HBV infection context. Combination treatment significantly reduced serum TNF-α and increased IFN-γ levels. IFN-γ secreted by immune cells, including natural killer cells and T lymphocytes, inhibits virus replication and participates in HBV clearance, thereby exerting antiviral effects (Hillaire et al, 2023). Wang et al (2020) found that in chronic HBV infection, TNF-α-producing cells were the dominant population of HBV-specific CD4 T cells. It also found that in patients with hepatitis B flare, TNF-α-producing CD4 T cells were associated with liver damage, and HBV-specific IFN-γ-producing CD4 T cells were related to HBV viral clearance (Wang et al, 2020). These findings indicated that the immune function was affected by HBV infection, and these changes were related to liver function and virus clearance in patients with HBV infection. Very few studies have reported the effects of PEG-IFNα-2b combined with entecavir treatment on immune function and related factors. Our study analyzed the impact of PEG-IFNα-2b combination treatment on immune factors in chronic HBV infection. The impacts of combination treatment on immune factors may be related to the immune regulatory effect of PEG-IFNα, whose regulatory role on the activation of natural killer cells has previously been reported (Nishio et al, 2021). Another study found that PEG-IFNα could upregulate the exosomal microRNAs, and IFN-α-related miRNAs were transferred from macrophages to HBV-infected hepatocyte through exosomes, thereby inhibiting the

expression and replication of HBV and exerting antiviral activity (Wu et al, 2021). In addition, our study also depicted the potential alterations in immune factors due to entecavir treatment. A previous study revealed that entecavir downregulated serum interleukin (IL)-6, IL-8, and TNF levels in chronic HBV infection, suggesting a role of the gut microbiota in these changes of these immune factors (Lu et al, 2021). The combination treatment amalgamates the regulatory effects of PEG-IFNα-2b and entecavir on immune function, jointly exerting a synergistic effect in improving immune function.

Regarding the prognosis of HBV infection, the combination treatment stands as a protective factor. PEG-IFNα-2b combined with entecavir treatment could effectively inhibit viral replication, significantly improving the prognosis of HBV-infected patients. Family history represents an important risk factor for the outcome of patients with HBV infection, primarily attributed to genetic susceptibility and intra-family transmission, which predisposes hepatocytes of certain individuals to damage in response to HBV infection or the virus-activated immune system. Close contact and lifestyle similarities among family members are elements in the context of family history elevating the risk of viral transmission. Single nucleotide polymorphisms or haplotypes in cytokine genes, such as those of chemokines and human leukocyte antigens, have been found to be related with susceptibility to HBV infection (Xu et al, 2021). A study has reported that the family history of hepatitis is an influencing factor of persistent positive HBsAg status (Hu et al, 2021), concurring with our analysis. A previous study has reported that serum cytokine profiles, especially IL-7, IL-18, and IFN-γ at the end of therapy, were predictors for viral infection recurrence and chronic hepatitis B relapse in patients after using nucleoside analogues-based therapies (Lin et al, 2023). However, our results were slightly different from this prior study. Although there were significant differences in TNF-α and IFN-γ levels after treatment between the poor and good prognosis groups, the results of logistic regression analysis did not portray a conspicuous relation of inflammatory factors to prognosis of HBV infection.

This study has some limitations. The study was constrained by the retrospective research design and small sample size, which reduced statistical power of the study. In addition, other factors that may influence the prognosis of HBV infection were not thoroughly investigated. Therefore, future research should expand the sample size and consider more factors for analysis. In addition, a large-cohort prospective study is warranted for validating clinical value of the tested indicators.

Conclusion

In summary, the combination of PEG-IFNα-2b and entecavir significantly improve the clinical therapeutic effect, liver function and immune factor levels in patients with HBV infection. Additionally, therapeutic regimen and family medical history, which are independent factors affecting the prognosis of HBV infection, should be taken into account while formulating appropriate treatment strategies.

Key Points

- The combination of PEG-IFNα-2b and entecavir constitutes an improved therapeutic effect in patients with chronic HBV infection and reduces HBsAg and HBV DNA levels, outperforming the entecavir monotherapy.
- The combination of PEG-IFNα-2b and entecavir improves liver function of patients with chronic HBV infection.
- The combination of PEG-IFNα-2b and entecavir improves inflammatory response in patients with chronic HBV infection.
- Therapeutic regimen and family medical history are associated with the prognosis of patients with chronic HBV infection.

Availability of Data and Materials

All data included in this study are available from the corresponding author upon reasonable request.

Author Contributions

JingZ designed the research. JunZ prepared relevant materials and collected the clinical data. JunZ analyzed the raw data. JingZ wrote the first draft. Both authors contributed to the important editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study has been approved by Jinhua Central Hospital Ethics Review Committee (Approval No. 2022-44) and was conducted in strict adherence to the Declaration of Helsinki. The patients themselves included in the study have signed the informed consent form.

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Conflict of Interest

The authors declare no conflict of interest.

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