Original Article

Liver Histopathological Features Influencing HBeAg Seroconversion in Patients with HBeAg-positive Chronic Hepatitis B Treated with Pegylated Interferon α

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Objective To investigate the efficiency of pegylated interferon α therapy for patients with HBeAg-positive chronic hepatitis B (CHB) and explore whether liver histopathological features and other factors might influence HBeAg seroconversion.

Methods Total of 80 HBeAg-positive CHB patients who received liver puncture were treated with pegylated interferon α once a week for 48 weeks. The rate of HBeAg seroconversion was determined after therapy, and the factors influencing HBeAg seroconversion were analyzed.

Results The rate of HBeAg seroconversion was 30.00% at the end of treatment. The rate of HBeAg seroconversion gradually increased with the elevation of liver inflammatory activity ($\chi^2 = 9.170$, $P = 0.027$). But liver fibrosis has little correlation with the rate of HBeAg seroconversion ($\chi^2 = 5.917$, $P = 0.116$). Except HBeAg, other baseline indexes including gender, age, serum ALT and serum HBV DNA levels had no statistical difference between the patients with HBeAg seroconversion and the patients without HBeAg seroconversion. By binary logistic regression analysis, liver inflammation and HBeAg were influencing factors for HBeAg seroconversion.

Conclusions Pegylated interferon α therapy induces a higher rate of HBeAg seroconversion in HBeAg-positive chronic hepatitis B patients with severe liver inflammation, so the liver biopsies should be performed in time.

Key words: Chronic hepatitis B; Pegylated interferon α ; Hepatitis B e antigen; Seroconversion

Serological conversion of HBeAg is a satisfactory treatment goal in HBeAg-positive patients with chronic hepatitis B. It is generally considered that a higher efficacy of interferon treatment can be achieved in patients with severer liver inflammation.¹ Currently, alanine aminotransferase (ALT) is the most widely used serological marker to reflect the liver inflammation. However, the ALT level is found to be inconsistent with the inflammatory activity of liver in some patients.² In addition, it has been observed that the inflammatory activity of the liver tissues is the only factor affecting the therapeutic efficacy of interferon in treatment of patients with chronic hepatitis B, while the ALT level has little effects on interferon effect.³ Recently, the use of pegylated interferon for

the treatment of chronic hepatitis B has been paid much attention in clinical practices. However, there is little knowledge on the association between the pathological characteristics of liver and serological conversion of HBeAg in HBeAg-positive patients with chronic hepatitis B. In the current study, the HBeAg-positive patients with chronic hepatitis B confirmed by liver biopsy were treated with pegylated interferon, so as to evaluate the effect of the pathological characteristics of liver on the serological conversion of HBeAg in HBeAg-positive patients.

PATIENTS AND METHODS

Patients

The HBeAg-positive patients with chronic hepatitis B undergoing antiviral therapy with pegylated interferon α in Ningbo No. 2 Hospital (Ningbo, China) during the period from March, 2009 to June, 2011 were enrolled in this study. The participants involved

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58 males and 22 females, aged 20-45 years, with a mean age of 30.00 ± 5.80 years. All patients met the diagnostic criteria of chronic hepatitis B defined in the Guideline for Prevention and Control of Chronic Hepatitis B jointly revised by the Hepatology Branch and Infectious Diseases Branch of Chinese Medical Association in 2005.¹ All patients had no decompensated liver cirrhosis or co-infection of hepatitis C virus (HCV) or HIV, and those who met the following criteria were excluded from the study: (1) patients with alcohol-induced liver diseases; (2) patients with drug-induced liver diseases; (3) autoimmune liver diseases; and (4) pregnant or lactating women. All patients had no contraindications of interferon therapy.

Interferon therapy

All patients were subcutaneously injected with pegylated interferon α -2a (Shanghai Roche Pharmaceuticals Ltd., Shanghai, China) at doses of 135 μ g or 180 μ g related to individual body weights or pegylated interferon α -2b (Shanghai Schering-Plough Pharmaceutical Co., Ltd., Shanghai, China) at doses of 50 μ g, 80 μ g or 100 μ g related to individual body weights once a week, with a treatment duration of 48 weeks. In addition, some patients with HBV DNA viral load of $\geq 1 \times 10^8$ copies/ml underwent antiviral therapy with interferon and nucleotide analogues initially or 24 weeks post-treatment. A 24-week follow-up was performed after the drug withdrawal. This study was approved by the Ethics Review Committee of Ningbo No. 2 Hospital, and informed consent was obtained from all participants.

Observation index

Routine blood test, serum biochemical indexes, thyroid function, serological markers of HBV (HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) and HBV DNA viral load were determined once every month for 3 successive times post-treatment, followed by once every 3 months after 3 months of treatment. The biochemical indexes were determined using a fully automatically biochemical analyzer (Olympus, Japan), and the serological markers of HBV were detected using a chemiluminescence assay (Abbott Laboratories, Abbott Park, IL, USA). HBV DNA viral load was determined using a real-time fluorescence quantitative PCR assay (DaAn Gene Co., Ltd. of Sun Yat-sen University, Shenzhen, China). The pathological examinations of the liver tissues were performed one week prior to the treatment, and the inflammatory activity and fibrosis of

the liver was evaluated by pathologists.

Statistical analysis

The therapeutic efficacy was evaluated based on the 24-week follow-up following the 48-week therapy, and the effects of baseline levels of various parameters, liver inflammatory activity and degree of liver fibrosis on the serological conversion of HBeAg were assessed. Serological conversion of HBeAg was defined as disappearance of HBeAg with presence of anti-HBe, while the serum HBV DNA viral load was $< 1 \times 10^3$ copies/ml and/or the ALT level $< 1 \times$ the upper limit of the normal range (ULN). All measurement data were expressed as mean $\pm$ standard deviation (SD). The difference of the means of normally distributed data was compared using Student *t* test, while non-parametric test was used to compare the non-normally distributed data. The count data were presented as number or percentage, and differences of proportions were compared using chi-square test. The factors affecting the serological conversion of HBeAg were investigated using a multivariate binary-classification logistic regression analysis. All statistical analyses were performed using the statistical software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA), and a *P* value < 0.05 was considered statistically significant.

RESULTS

Correlation between baseline levels of parameters and serological conversion of HBeAg

Of the 85 patients enrolled in this study, 3 cases were withdrawn from the study due to occurrence of adverse reactions (one case with hyperthyroidism and two cases with severe liver function injury) during the treatment, and 2 cases were lost to follow-up. Finally, a total of 80 cases completed the 48-week therapy. Following the treatment, 24 patients developed serological conversion of HBeAg, and 56 cases did not develop serological conversion of HBeAg, with a serological conversion rate of 30.00%. Except HBeAg level, there were no significant differences in the baseline levels of various markers between the two groups (all *P* values > 0.05) (Table 1).

Correlation between liver inflammatory activity and serological conversion of HBeAg

Of the 80 patients with chronic hepatitis B, 22 cases had grade 1 inflammatory activity, with a HBeAg

Table 1. Comparison of baseline characteristics between the patients with HBeAg seroconversion and without HBeAg seroconversion

Characteristics	Patients without HBeAg seroconversion (n = 56)	Patients with HBeAg seroconversion (n = 24)	Statistics
Male	42/56	16/24	$\chi^2 = 0.585$, $P = 0.044$
Age (years)	30.12 $\pm$ 6.48	29.12 $\pm$ 5.01	$t = 0.746$, $P = 0.459$
TBil ($\mu\text{mol/L}$)	17.97 $\pm$ 10.58	16.98 $\pm$ 9.79	$z = -0.688$, $P = 0.492$
A/G	1.49 $\pm$ 0.24	1.90 $\pm$ 2.06	$z = -0.357$, $P = 0.721$
AST (U/L)	100.88 $\pm$ 121.07	107.71 $\pm$ 64.11	$z = -1.486$, $P = 0.137$
ALT (U/L)	271.21 $\pm$ 36.24	212.70 $\pm$ 43.42	$z = -0.940$, $P = 0.347$
ALP (U/L)	84.02 $\pm$ 27.54	83.79 $\pm$ 22.85	$z = 1.045$, $P = 0.296$
GGT (U/L)	70.96 $\pm$ 70.54	81.67 $\pm$ 82.59	$z = -0.436$, $P = 0.663$
WBC ($10^9/\text{L}$)	5.18 $\pm$ 1.15	4.90 $\pm$ 0.93	$t = 1.158$, $P = 0.252$
N ($10^9/\text{L}$)	2.78 $\pm$ 0.90	2.56 $\pm$ 0.95	$z = -1.203$, $P = 0.229$
L ($10^9/\text{L}$)	1.92 $\pm$ 0.54	1.94 $\pm$ 0.53	$t = -0.163$, $P = 0.871$
RBC ($10^{12}/\text{L}$)	4.81 $\pm$ 0.49	4.74 $\pm$ 0.62	$t = 0.470$, $P = 0.641$
HBG (g/L)	146.16 $\pm$ 16.35	145.21 $\pm$ 15.37	$t = 0.249$, $P = 0.804$
PLT ($10^9/\text{L}$)	172.75 $\pm$ 48.84	161.17 $\pm$ 38.11	$z = -0.971$, $P = 0.331$
HBeAg ($\log_{10} \text{S/CO}$)	2.28 $\pm$ 0.87	1.58 $\pm$ 1.34	$t = 2.665$, $P = 0.012$
HBV DNA ($\log_{10} \text{copies/ml}$)	6.56 $\pm$ 1.14	6.37 $\pm$ 1.27	$t = 0.618$, $P = 0.540$

Table 2. Relationship between liver inflammation activity and HBeAg seroconversion

Liver inflammation activity	HBeAg seroconversion	
	Without (n = 56)	With (n = 24)
G1	20 (25.00%)	2 (2.50%)
G2	26 (32.50%)	12 (15.00%)
$\geq G3$	10 (12.50%)	10 (12.50%)
Statistics	$\chi^2 = 8.435$, $P = 0.015$	

Table 3. Relationship between ALT level and HBeAg seroconversion in patients with severe liver inflammation ($\geq G2$)

ALT level	Patients without HBeAg seroconversion (n = 36)	Patients with HBeAg seroconversion (n = 22)
$< 2 \times \text{ULN}$	12 (20.69%)	8 (13.79%)
$> 2 \times \text{ULN}$	24 (41.38%)	14 (24.14%)
Statistics	$\chi^2 = 0.056$, $P = 0.816$	

serological conversion rate of 9.09%; 38 cases had grade 2 inflammatory activity, with a HBeAg serological conversion rate of 31.58%; 19 cases had grade 3 inflammatory activity, with a HBeAg serological conversion rate of 47.3%; and 1 case had grade 4 inflammatory activity, with successful HBeAg serological conversion. The serological conversion rate of HBeAg gradually increased with the elevation of the inflammatory activity ($\chi^2 = 8.435$, $P = 0.015$; considering only one case with grade 1 inflammatory activity, the case was involved in the subjects with grade 3 inflammatory activity for analysis). The patients with grades 2, 3 and greater inflammatory activities had higher serological conversion rates of HBeAg than the patients with grade 1 inflammatory

activity (both P values < 0.05), while no significant difference was observed in patients with grade 2 inflammatory activity and grade 3 and greater inflammatory activities ($P > 0.05$) (Table 2).

Effects of severe liver inflammation (grade 2 or greater) and various ALT levels ($< 2 \times \text{ULN}$ and $> 2 \times \text{ULN}$) on serological conversion of HBeAg

The patients with severe liver inflammation (grade 2 or greater) were assigned into two groups according to the ALT level. The serological conversion rates of HBeAg were 40.00% (8/20) and 36.84% (14/38) in the subjects with ALT levels of $< 2 \times \text{ULN}$ and $> 2 \times \text{ULN}$, respectively, and no significant difference was detected ($P > 0.05$) (Table 3).

Table 4. Relationship between the extent of liver fibrosis and HBeAg seroconversion

The extent of liver fibrosis	HBeAg seroconversion	
	Without (n = 56)	With (n = 24)
S0	6 (7.50)	3 (3.75)
S1	32 (40.00)	7 (8.75)
S2	10 (12.50)	9 (11.25)
S3	8 (10.00)	5 (6.25)
Statistics	$\chi^2 = 5.917, P = 0.116$	

Table 5. Multivariate logistic regression analysis results of HBeAg seroconversion

	B	S. E.	Wald	Sig.	Exp (B)	95.0% CI for EXP (B)	
						Lower	Upper
Gender	0.94	0.974	0.929	0.335	2.555	0.379	17.221
Age	-0.08	0.062	1.620	0.203	0.924	0.818	1.044
G	1.11	0.518	4.613	0.032	3.041	1.102	8.392
S	0.17	0.419	0.165	0.684	1.186	0.522	2.694
TBil	-0.05	0.044	1.498	0.221	0.948	0.870	1.033
A/G	1.37	1.406	0.543	0.461	2.819	0.179	44.335
AST	0.00	0.006	0.284	0.594	0.997	0.984	1.009
ALT	0.00	0.002	0.589	0.443	1.002	0.997	1.006
ALP	-0.02	0.016	1.140	0.286	0.983	0.952	1.015
GGT	0.01	0.007	1.230	0.267	1.008	0.994	1.022
WBC	-1.49	1.353	1.207	0.272	0.226	0.016	3.207
N	1.47	1.486	0.978	0.323	4.348	0.236	80.034
L	1.99	1.449	1.877	0.171	7.276	0.425	124.450
RBC	-0.47	0.905	0.274	0.601	0.623	0.106	3.672
HBG	0.01	0.036	0.117	0.733	1.012	0.944	1.086
PLT	0.00	0.008	0.004	0.952	1.001	0.984	1.017
HBeAg	0.00	0.001	4.226	0.040	0.998	0.997	1.000
HBV DNA	-0.14	0.288	0.227	0.634	0.872	0.495	1.534
Constant	-0.48	6.476	0.006	0.941	0.618		

Correlation between liver fibrosis degree and serological conversion of HBeAg

Of the 80 patients with chronic hepatitis B, 9 cases had stage 0 hepatic fibrosis, with a HBeAg serological conversion rate of 33.33%; 39 cases had stage 1 liver fibrosis, with a HBeAg serological conversion rate of 17.95%; 19 cases had stage 2 liver fibrosis, with a HBeAg serological conversion rate of 47.37%; and 13 case had grade 3 liver fibrosis, with a HBeAg serological conversion rate of 38.46%. The liver fibrosis degree had no significant correlation with the serological conversion rate of HBeAg ($\chi^2 = 5.917, P = 0.116$) (Table 4).

Multivariate logistic regression analysis

Multivariate logistic regression analysis revealed

that among the factors including gender, age, liver inflammatory activity, liver fibrosis degree, ALT level, AST level and HBV DNA viral load, only liver inflammatory activity was included in the regression equation, indicating that liver inflammatory activity is a major factor affecting the serological conversion of HBeAg (Table 5).

DISCUSSION

Interferon exhibits the effects of both antiviral activity and immune regulation. Interferon therapy usually results in a high serological conversion of HBeAg or HBsAg, and does not induce resistance of drugs used for antiviral therapy. Pegylated interferon α has been recommended as a preferable drug for the treatment

of chronic hepatitis B in the Guidelines for Prevention and Management of Hepatitis B.⁵⁻⁷ However, interferon therapy does not achieve a satisfactory efficacy for treatment of chronic hepatitis B in China, and exploring the factors affecting the interferon therapy of chronic hepatitis B and predicting the efficacy of interferon therapy is a hot topic in clinical research.

All patients enrolled in this study had pathological data of the liver and reliable diagnosis. After treatment with pegylated interferon α for 48 weeks, the serological conversion rate of HBeAg was 30% among the 80 HBeAg-positive patients with chronic hepatitis B, which was similar to that reported by Lau et al.⁸ It is considered that a high baseline ALT level ($> 3 \times \text{ULN}$) and a low HBV DNA viral load are important predictors of HBeAg serological conversion,⁹⁻¹⁰ and the serological conversion and virological response of HBeAg is reported to be associated with the baseline ALT level.¹¹ The present study showed no significant differences in gender, ALT level and HBV DNA viral load between HBeAg serological conversion group and the HBeAg non-serological conversion group except the HBeAg level. It is indicated that a higher efficacy of interferon therapy can be achieved in patients with severe liver inflammation and mild liver fibrosis.³ Our findings showed that the liver inflammation was closely associated with the serological conversion of HBeAg. A higher serological conversion rate of HBeAg was observed in patients with severer inflammatory activities, while the degrees of liver fibrosis had no significant association with the serological conversion of HBeAg. Similar results were observed from the study by Mao et al.,¹ which targeted the HBeAg-negative patients with chronic hepatitis B undergoing therapy with normal interferon. Multivariate logistic regression analysis revealed that the liver inflammatory activity is a major factor affecting the serological conversion of HBeAg in HBeAg-positive patients with chronic hepatitis B treated with pegylated interferon α .

Our findings showed that the ALT level had little effects on the serological conversion of HBeAg in patients with severe liver inflammation (grade 2 or greater inflammatory activity), which further confirmed the limitation of use of ALT level to reflect the degree of liver inflammation. This finding was consistent with the results from our previous study.¹² The liver biopsy has a big problem of invasiveness, which limits its application. Therefore, the enrollment

of chronic hepatitis B patients for antiviral therapy still depends on the detection of serological markers till now. Pegylated interferon α has been shown effective in antiviral therapy for chronic hepatitis B; however, treatment with pegylated interferon α has a high cost and long treatment course, which would certainly increase the economic burden. Therefore, it is of great significance to accurately identify the patients with indications for therapy with pegylated interferon α . Liver biopsy is therefore suggested to identify the liver inflammatory activity in patients requiring treatment with pegylated interferon α .

In summary, liver inflammatory activity is a major factor affecting the serological conversion of HBeAg in HBeAg-positive patients with chronic hepatitis B receiving treatment with pegylated interferon α , which has a better value for predicting the serological conversion of HBeAg as compared to other serum biochemical indices including ALT. However, only 85 patients were enrolled in this study, and there was bias in the enrolled subjects due to loss of follow-up and adverse reactions. Further large-scale and multi-center trials are required to validate this conclusion drawn from this study.

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