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Original Article

TCM constitution yin deficiency affects treatment response of Peg-IFNα in patients with HBeAg-positive chronic hepatitis B and is related to HLA-DQA1 gene polymorphisms

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Abstract: Objective: The aim of this study was to observe the relationship of Yin deficiency with peg-IFN treatment response in patients with HBeAg positive chronic hepatitis B (CHB) and polymorphisms of human leukocyte antigen (HLA) DQA1 genes. Methods: A total of 120 patients with HBeAg-positive CHB, undergoing peg-IFN treatment, were collected and divided into two groups, the yin-deficiency group (YD, 59 patients) and non-yin-deficiency group (nYD, 61 patients). They were selected and divided according to the criteria of Wangqi constitution classification for detection and comparison of HLA-DQA1 genotypes by polymerase chain reaction-sequence specific primer (PCR-SSP). Efficacy was evaluated at the end of treatment and intergroup differences in gene frequency and constitution distribution were compared. Results: The effective rate in Group YD (complete response (CR) + partial response (PR)) was lower than in Group nYD (61.0% vs 78.7%, $P<0.05$). The HLA-DQA1*0501 gene frequency (14.8%) in Group CR was lower, while the frequency of HLA-DQA1*0601 genes (18.5%) was higher than in the non-response group (NR) (30.6% and 5.6%, respectively, $P<0.05$, $Pc>0.05$). The frequency of HLA-DQA1*0501 genes in Group YD (33.9%) was higher, while HLA-DQA1*0301 (2.5%) was lower than in Group nYD (18.9% and 9.8%, respectively, $P<0.05$, $Pc>0.05$). Conclusion: TCM constitution and HLA-DQA1 polymorphisms affect the treatment response to peg-IFNα in patients with HBeAg-positive CHB. Yin deficiency and HLA-DQA1*0501 genotypes are not conducive to the response, but the relationship requires further examination.

Keywords: Chronic hepatitis B, Chinese medicine constitution, yin deficiency, human leukocyte antigen, HLA-DQA1

Introduction

Chronic hepatitis B (HBV) viral infection is a serious disease burden, causing approximately 1 million liver disease-related deaths, worldwide, each year [1, 2]. The interferon represented by the pegylated interferon (Peg-IFN)-α has certain advantages, such as a definite treatment course, avoiding antiviral-induced resistance, and realizing the seroconversion of hepatitis B surface antigen (HBsAg) in certain patients. Therefore, it is the preferred treatment for young HBeAg-positive CHB patients without contraindications [3, 4]. However, the overall efficacy of interferon is far from satisfactory and there are many adverse effects [5-7]. It is of great significance to understand the reasons for response differences among individuals, thus guiding individualized treatment. This is particularly important in the era of chasing functional cures of hepatitis B infections [6, 7].

The constitution of Chinese medicine describes characteristics of personal constitution in terms of morphological structure, physiological function, psychological characteristics, and reactive states. The TCM constitution theory is a high generalization of the host's genetic features, from the perspective of TCM, and has significant implications for TCM practice. Thus, it is one of the hot spots in clinical research of integrated traditional Chinese and Western medicines [8-12].

Human leukocyte antigens (HLAs) are a group of cell-surface and antigen-presenting proteins encoded by genes in the human major histocompatibility complex region. The diversity of HLA polymorphism makes it a useful genetic

marker for various diseases, including HBV infections [13]. Given the shared features of HLA polymorphism and TCM constitution in disease susceptibility, their intrinsic relationship has long been hypothesized and identified in the general population from China. Genetic correlation has been deemed to be an important means to bridge the language gap between TCM and modern Western biomedicine [14]. A series of studies targeting the relationship between TCM constitution and outcomes of chronic HBV infections in recent years have found that TCM constitution types can influence the pathological progression and disease progression of patients with chronic HBV infections. They are also related, to a certain degree, to the polymorphism of immunoregulatory genes represented by HLA-DQA1 genes. Of these, yin deficiency is related to various adverse clinical outcomes of chronic HBV infections, as well to HLA-DQA1*0501 genotypes [15, 16]. HLA is an important host factor affecting outcomes of HBV infections [17]. However, there have been few reports observing differences in IFN response from the perspective of TCM constitution. Therefore, based on previous works, this study chose HLA-DQA1 as a genetic marker to compare differences in the response to Peg-IFNα treatment between HBeAg-positive CHB patients with/without yin deficiency and the relationship with HLA-DQA1 gene polymorphisms. The current study aimed to explore the predictive value of yin deficiency toward the response to Peg-IFNα treatment in treating CHB and its partial molecular biological basis. This study also aimed to provide a basis for individualized interferon therapy for CHB patients.

Materials and methods

Diagnostic and exclusion criteria

Diagnosis of CHB used the relevant "Guidelines for the Prevention and Treatment of Chronic Hepatitis B" in China in 2005 [18]. Exclusion criteria: (1) Age <18 years or >60 years; (2) Combined with severe organ injury (the heart, brain, kidney, or blood system) or mental illness, etc.; (3) In pregnancy or lactation; (4) Combined with autoimmune liver disease, alcoholic liver disease, Wilson's disease, or other specific reason-related chronic liver diseases; (5) Progression to cirrhosis by histological or imaging confirmation; and (6) HBeAg-negative CHB and HBeAg-positive CHB with obvious clinical symptoms that may cause the confusion of "symptoms" with "essence", affecting the judgment of constitutional type. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the ethics committee of Xixi Hospital of Hangzhou. Written informed consent was obtained from all participants.

Judgment of TCM constitution types

Referred to the criteria of Wangqi TCM constitution [8].

Clinical data

A total of 120 patients with HBeAg-positive CHB, hospitalized and treated with peg-IFNα antiviral treatment in Xixi Hospital of Hangzhou, from June 2010 to December 2013, were selected. Patients included 83 males (69%), aged 19-46 years, with a mean age of (32.87±6.55) years old. Baseline HBV DNA was 7.23 Log10 IU/mL and alanine aminotransferase (ALT) was 246 IU/L.

Treatment program

Peg-IFN α-2b (1.0 μg/kg body weight) or 180 μg of Peg-IFN α-2a was subcutaneously injected once a week. Efficacy was judged after 6 months of initial treatment. Patients that responded were then assigned to complete the one-year treatment course. Routine blood tests were performed 4 weeks before treatment. During treatment, hepatic function was tested once every 4 weeks and hepatitis B-related items, HBV DNA, thyroid function, urine routine, and blood glucose, were measured once every 12 weeks. At the end of treatment, treatment response was judged: 1. Complete response (CR) refers to the occurrence of HBeAg seroconversion, HBV DNA turning negative, and normal liver function; 2: Partial response (PR) refers to HBV DNA turning negative and normal liver function, with no occurrence of HBeAg seroconversion; and 3. Non-response (NR) refers to none of the above criteria [18].

Judgment of constitution type and grouping

Each patient was comprehensively judged on five aspects, namely physical characteristics, common performance, psychological characteristics, morbidity, and adaptability to external environment. Tongue and pulse patterns were viewed and judged based on the difference

principles of three chief physicians. In principle, the judgment should avoid the conclusion of the combination of various constitutions. When it was difficult to judge, the person in charge of the study re-arranged experts, finally confirming the main constitution type.

Specimen collection

A total of 10 mL of venous blood was sampled from each patient. Of which, 5 mL was used to separate the serum for detection of HBV markers and liver function. The other 5 mL was stored in EDTA anticoagulant at -70°C for DNA extraction and HLA-DQA1 genotype detection.

Polymerase chain reaction-sequence specific primer (PCR-SSP)

This procedure was the same as in previous studies [15, 16]. Specific primers were designed based on references [19] and the HLA-DQA1 genotype was identified using PCR-SSP.

Axygen® AxyPrep™ Blood Genomic DNA Miniprep Kit (AP-MN-BL-GDNA-50) was purchased from Axygen (Hangzhou, China), 2 × Taq PCR Mastermix (KT201) was obtained from TIANGEN BIOTECH (Beijing, China), and primers were purchased from Thermo Fisher SCIENTIFIC (Shanghai, China).

Statistical analysis

Experimental results were processed using SPSS13.0 statistical software. Intergroup comparisons of the measurement data used one-way analysis of variance. Intergroup comparisons of frequency were performed using Chi-squared test. *P*-values, OR-values, and 95% CIs were recorded. Bonferroni's method was used to correct *P* values (Pc), with P<0.05 indicating statistical significance (the Pc value was the *P* value multiplied by the number of the alleles actually detected HLA-DQA1).

Results

Treatment response

At the end of treatment, 27 patients achieved CR, 57 patients achieved PR, and 36 patients achieved NR. Comparison of gender, age, and baseline laboratory parameters among the three groups showed that ALT values in groups CR and PR were significantly higher than in group NR (P<0.05). There were no statistical differences in gender, age, and HBV DNA values between the three groups (P>0.05).

Comparison of treatment response between Group YD and Group nYD

Subject to sample size, the patients were grouped according to their constitutions for comparison. Results revealed that 59 patients exhibited yin deficiency, while 61 patients exhibited non-yin deficiency. There were no significant differences in gender (39 (66%) males in group YD and 44 (72%) in group nYD, respectively) and age ((33.31±6.68) yrs in group YD and (32.44±6.45) yrs in group nYD, respectively) between the two groups (P>0.05). Neither ALT nor HBV DNA of the patients indicated significant differences (P>0.05). There were 11 cases of CR and 25 cases of PR in group YD. There were 16 cases of CR and 32 cases of PR in group nYD. The effective rate in Group YD (CR+PR) was significantly lower than that in Group nYD (χ^2=4.460, P=0.035).

Comparison of frequency distribution of HLA-DQA1 alleles between Group YD and Group nYD

The frequency of HLA-DQA1*0301 in Group YD was 2.5%, significantly lower than that in Group nYD (9.8%, P=0.020, OR=0.239, 95% CI=0.07-0.87). The frequency of HLA-DQA1*0501 in Group YD was 33.9%, significantly higher than that in Group nYD (18.9%, P=0.008, OR=2.207, 95% CI=1.22-3.99). Corrected Pc values were both >0.05 (**Table 1**).

Comparison of frequency of HLA-DQA1 alleles between Group CR and Group NR

The frequency of HLA-DQA1*0501 in group CR was 14.8%, significantly lower than that in group NR 30.6% (P=0.04, OR=0.395, 95% CI=0.16-0.98). Corrected Pc value was >0.05. The frequency HLA-DQA1*0601 in group CR was 18.5%, significantly higher than that in group NR 5.6% (P=0.022, OR=3.864, 95% CI=1.14-13.09). Corrected Pc value was >0.05 (**Table 2**).

Comparison of frequency of HLA-DQA1 alleles between Group PR and Group NR

There was no statistical significance in the frequency of HLA-DQA1 alleles between Group PR and Group NR (P>0.05) (**Table 3**). There was also no statistical significance in the frequency

Table 1. Comparison of frequency of HLA-DQA1 alleles between Group YD and Group nYD

HLA-DQA1	YD (%) (n=118£)	nYD (%) (n=122£)	χ^2	P	OR	95% CI
*0101	7 (5.9)	9 (7.4)	0.201	0.654	0.792	0.29-2.20
*0102	11 (9.3)	13 (10.7)	0.119	0.731	0.862	0.37-2.01
*0103	20 (18.5)	23 (18.5)	0.004	0.948	0.978	0.50-1.90
*0104	7 (5.9)	6 (4.9)	0.120	0.729	1.219	0.40-3.74
*0201	17 (14.4)	22 (18.0)	0.579	0.447	0.765	0.38-1.53
*0301	3 (2.5)	12 (9.8)	5.446	0.020	0.239	0.07-0.87
*0401	1 (0.8)	2 (1.6)	F#	1.000	0.513	0.05-5.73
*0501	40 (33.9)	23 (18.9)	7.014	0.008	2.207	1.22-3.99
*0601	12 (10.2)	12 (9.8)	0.007	0.931	1.038	0.45-2.41

Note: £, the number of alleles; #, the *P* value was measured by the Fisher's exact probability.

Table 2. Comparison of frequency of HLA-DQA1 alleles between group CR and group NR

HLA-DQA1	CR (%) (n=54)	NR (%) (n=72)	χ^2	*P*	OR	95% CI
*0101	4 (7.4)	5 (6.9)	F#	1.000	1.072	0.27-4.20
*0102	6 (11.1)	8 (11.1)	0.000	1.000	1.000	0.33-3.07
*0103	12 (22.2)	5 (6.9)	0.997	0.318	1.584	0.64-3.93
*0104	2 (3.7)	4 (5.6)	F#	0.700	0.654	0.12-3.71
*0201	8 (14.8)	12 (19.4)	0.079	0.778	0.870	0.33-2.30
*0301	3 (5.6)	5 (6.9)	F#	1.000	0.788	0.18-3.45
*0401	1 (1.9)	1 (1.4)	F#	1.000	1.340	0.08-21.91
*0501	8 (14.8)	22 (30.6)	4.215	0.04	0.395	0.16-0.98
*0601	10 (18.5)	4 (5.6)	5.250	0.022	3.864	1.14-13.09

Note: #, the *P* value was measured by the Fisher's exact probability.

Table 3. Comparison of frequency of HLA-DQA1 alleles between Group PR and Group NR

HLA-DQA1	PR (%) (n=114)	NR (%) (n=72)	χ^2	*P*	OR	95% CI
*0101	7 (6.1)	5 (6.9)	F#	1.000	0.877	0.27-2.88
*0102	10 (8.8)	8 (11.1)	0.276	0.599	0.769	0.29-2.05
*0103	20 (17.5) 11 (15.3)	11 (15.3) 11 (15.3)	0.163	0.686	1.180	0.53-2.63
*0104	7 (6.1)	4 (5.6)	F#	1.000	1.112	0.31-3.94
*0201	19 (16.7)	12 (19.4)	0.200	0.655	0.833	0.38-1.85
*0301	7 (6.1)	5 (6.9)	F#	1.000	0.877	0.27-2.88
*0401	1 (0.9)	1 (1.4)	F#	1.000	0.628	0.04-10.21
*0501	33 (28.9)	22 (30.6)	0.055	0.815	0.926	0.49-1.76
*0601	10 (8.8)	4 (5.6)	0.656	0.418	1.635	0.49-5.42

Note: #, the *P* value was measured by the Fisher's exact probability.

of HLA-DQA1 alleles between Group CR+PR and Group NR (**Table 4**).

Discussion

Results of the current study suggest that TCM constitution was related to the HLA-DQA1 gene polymorphism and treatment response to peg-IFNα in patients with HBeAg-positive CHB. Yin deficiency was unfavorable for the response and HLA-DQA1*0501 was not, while HLA-DQA1*0601 was favorable for CR. The HLA-DQA1*0501 allele was relatively frequent, while the HLA-DQA1*0301 allele was relatively rare in patients with yin deficiency.

Immune regulation is the theoretical basis for IFN, helping some CHB patients achieve a "functional cure". IFNα is known to upregulate expression of HLA-DR and other immune molecules in CHB patients, while HLA-II allele polymorphism influences outcomes of HBV infections and the efficacy of IFNα, to some extent [20-22]. Studies mainly focusing on the sub-region of HLA DQ and DR have shown that HLA-DRB1*04, DQA1*0303, and DQB1*07 are associated with non-response to IFNα therapy in patients with CHB. However, HLA-DQA1*0505, HLA-DQA1*0303, and HLA-DRB1*14 tend to promote IFNα response [24, 25]. Several studies have shown that HLA-DQA1*0501 is a susceptibility gene for CHB but HLA-DQA1*0301 is a resistance gene, suggesting that the HLA-DQA1 polymorphism may result in different clinical outcomes of HBV infections and participate in the formation of differences in interferon response through immunoregulating the host's anti-HBV ability [26, 27]. Results of the current study show that the carrying rate of HLA-DQA1*0501 in group CR

Table 4. Comparison of frequency of HLA-DQA1 alleles between Group CR+PR and Group NR

HLA-DQA1	CR+PR (%) (n=168)	NR (%) (n=72)	χ^2	*P*	OR	95% CI
*0101	11 (6.5)	5 (6.9)	F#	1.000	0.939	0.31-2.81
*0102	16 (9.5)	8 (11.1)	0.141	0.707	0.842	0.34-2.07
*0103	32 (19.0)	11 (15.3)	0.487	0.485	1.305	0.62-2.76
*0104	9 (5.4)	4 (5.6)	F#	1.000	0.962	0.29-3.23
*0201	27 (16.1)	12 (19.4)	0.013	0.909	0.957	0.46-2.02
*0301	10 (6.0)	5 (6.9)	F#	0.775	0.848	0.28-2.58
*0401	2 (1.2)	1 (1.4)	F#	1.000	0.855	0.08-9.59
*0501	41 (24.4)	22 (30.6)	0.985	0.321	0.734	0.40-1.35
*0601	20 (11.9)	4 (5.6)	2.257	0.133	2.297	0.76-6.98

Note: #, the *P* value was measured by the Fisher's exact probability.

was lower than in group NR, but the frequency of HLA-DQA1*0601 was higher than in group NR. This suggests that the HLA-DQA1 gene polymorphism was involved in the formation of differences in peg-IFNα response. Results suggest that the smaller sample size was related to non-statistically significant corrected Pc values.

Results of constitution analysis showed that the response rate in group YD was lower than in group nYD, suggesting that TCM constitution factors were involved in the formation of peg-IFNα response differences. Combined with previous analysis of constitution with liver pathology and disease outcomes in patients with chronic HBV infections, results suggest that the relatively heavier liver fibrosis may be one of the reasons for the poor response to interferon therapy in group yin deficiency [15, 16]. From the narrative point of view, immunomodulator interferon could have different efficacies in individuals with different constitutional types. Body constitutions do affect the titer of neutralizing antibodies against liver-tropic adeno-associated virus serotype vectors, according to a recent study [28]. Because of the small sample size which came from a single center, the influence of TCM constitution on the response to interferon requires further research and verification. Analysis, targeting the association between constitutional type and HLA-DQA1 genotype, revealed the following trends. The HLA-DQA1*0501 allele is relatively more, while the HLA-DQA1*0301 allele is relatively rare in patients with yin deficiency. This is consistent with previous studies targeting the impact on outcomes of HBV infections. The distribution frequency of DQA1*0501 in yin deficient populations prone to development of CHB and yin deficiency HBV carriers prone to hepatic fibrosis is high [15, 16]. The correlation between yin deficiency and HLA-DQA1*0501 alleles is consistent with results obtained by Chen et al. [28] in the general population in Southern China. Their results support the intrinsic relationship between TCM constitution and polymorphisms of HLA-II gene. Results of the current study suggest HLA-DQA1*0501 as partial molecular biological basis in a Han population with yin deficiency. This may also be another important reason for the poor response to interferon therapy in patients with yin deficiencies. However, despite increasing clinical research on TCM constitution, basic research on the molecular biology of yin deficiency is rare. More studies are necessary for verification [8, 29-31].

In summary, TCM constitution and HLA-DQA1 gene polymorphisms influence the response to peg-IFNα in HBeAg-positive CHB patients. Yin deficiency constitution and HLA-DQA1*0501 genes are not conducive to the response, but their relationship must be further studied. The relationship between TCM constitution and gene polymorphisms of other loci of HLA, in this context, also requires more investigation. An important host factor, the predictive value of TCM constitutional analysis in interferon therapy against CHB warrants further attention.

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Disclosure of conflict of interest

None.

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