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• *BRIEF REPORTS* •

Traditional Chinese medicine syndromes of chronic hepatitis B with precore mutant

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Abstract

AIM: This study aims at exploring the distribution of TCM syndromes in CHB patients with HBV pre-core mutation (1896) and the relationship between pre-core mutation and T lymphocytes subgroup, through which to provide objective data on clinical syndrome differentiation of TCM, and further to suggest the therapeutic principle and guide clinical treatment.

METHODS: One hundred and forty CHB patients were evenly divided into two study groups, HBV pre-core mutant group and HBV pre-core wild-type group. Besides, 30 healthy blood donors were selected as a healthy control group. HBV-labeled compound, T lymphocytes subgroup, and HBV-DNA pre-core mutant were tested in the study groups. T lymphocytes subgroup were also tested in the control group. All the patients were both diagnosed by syndrome differentiation of TCM and western medicine.

RESULTS: The most common syndrome in mutant group was damp-heat combined with blood stasis, and the most common syndrome in the wild-type group was damp-heat stasis in the middle-jiao. There were more cases of medium and severe hepatitis in mutant group than that in wild-type group. The content of CD4+ lymphocytes and CD4+/CD8+ ratio were decreased gradually (healthy control group>wild-type group>mutant group). In the wild-type group, severe and medium CHB patients had considerably lower level of them than mild CHB patients. However, in the mutant group, the opposite result appeared. Meanwhile, the content of HBV-DNA in mutant group was higher than that in wild-type group.

CONCLUSION: Damp, heat, toxin and blood stasis were the basic pathogens of CHB, whether pre-core mutant or not. CHB with precore mutant may lead to more severe hepatitis. The decreased content of CD4+ lymphocytes and ratio of CD4+/CD8+ may be taken as one of the indices in confirming the deficiency syndrome of CHB patients with pre-core mutation.

Key words: Traditional Chinese medicine; Syndrome differentiation; Chronic hepatitis B; Pre-core mutant; T lymphocytes subgroup

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INTRODUCTION

With the development of research on HBV pre-core gene mutation (1896), it is noticed that pre-core mutant cases were frequently encountered in clinics. One of the studies in China showed that the rate of pre-core mutation was 56% in CHB patients with anti-HBe antibody in serum[1]. Taiwanese researchers reported a rate of 67% in CHB patients with HBeAg-negative[2]. In a study by Japanese scholars, the rate of pre-core mutation in 46 patients with HBsAg and anti-HBe-positive was up to 100%[3]. One study showed that the fibrosis state of liver in CHB patients with pre-core mutant was more severe when compared with those without mutation, hence it was considered that the mutation may be related to disease progression as well as the lack of patients' response to therapy[4]. Studies also found that CHB patients without pre-core mutant strain before treatment was more responsive to IFN-α[5]. In one sequential combination therapy, most of the patients with pre-core mutation relapsed within 4 mo after treatment, suggesting that trials of patients with HBeAg negative should be based on characterization of HBV mutants[6]. Many studies supported that HBV pre-core mutant was closely correlated to the occurrence of severe hepatitis and hepatocellular carcinoma (HCC)[7-10]. Moreover, researchers have pointed out that the relapse rate was considerably higher among patients with the pre-core mutant than the ones with non-precore mutant after medical operation[11]. Studies also showed that there was close relationship between HBV pre-core mutation and severe hepatitis in those HCC patients involved in HBV infection during the chemo-therapy[12,13]. Among those patients with

severe CHB, studies revealed a mutation rate as high as 88.89%, with a relatively higher content of HBV-DNA in serum[14]. At present, much work has been done about the study on HBV pre-core mutation, and quite many diseases related to HBV infection were found aggravated due to HBV pre-core mutation, which has brought a new challenge to the prophylaxis and therapies of hepatitis B and HBV-related diseases[15-18]. Since there is less work done on the study of HBV pre-core gene mutation (1896) with CHB patients in the way of TCM sciences, we made the study on CHB patients in respect of HBV pre-core gene mutation, syndrome types of TCM, diagnosis of western medicine, content of HBV-DNA, T lymphocytes subgroup and virus replication capacity. The distribution characteristics of TCM syndromes in the patients and the pathogenesis according to Chinese medical theories were studied so as to provide objective data to clinical syndrome differentiation of TCM, and further to suggest the therapeutic principle of TCM and guide the treatment in clinic.

MATERIALS AND METHODS

Subjects

Random sampling of 70 HBV-DNA pre-core mutant patients and 70 wild-type patients were taken as two study groups, 30 healthy blood donors as healthy control group. The ratio of sex, mean age and its range in three groups were shown in Table 1. No significant difference of age and sex was between three groups (P>0.05). In the two study groups, the selected 140 patients were in accord with the following criteria: (1) All the patients sought medical advice in the TCM Department of the Third Affiliated Hospital to Sun Yat-Sen University during the period from February, 1999 to December, 2002; (2) patients' western medicine diagnosis was based on the diagnostic standards defined by three joint committees of Chinese Medical Association: Infectious Diseases Committee, Parasitic Disease Committee and Hepatopathy Committee[19].

Methods and items of examination

The indicators of HBV, HBV-DNA precore mutant and the HBV-DNA content were analyzed.

HBV-DNA content The method of fluorescence quantitative PCR was applied in HBV-DNA content. The reagent was provided by Da An Gene Corp Affiliated to Sun Yat-Sen University, using USA PE5700 automatic analytical machine. The sensitivity is 1×10^4 copy/mL. See the principle and methods in Ref.[20].

Test of HBV-DNA pre-core mutation (1896) PCR method was used, primer was designed by the laboratory of infection department in our hospital, and synthesized by Shanghai Biological Institute. See the principle and methods in the reference[21].

T lymphocytes subgroup (CD4+,CD8+,CD4+/CD8+) check up APAAP techniques[22] were employed. The reagent was supplied by Fundamental Medicine Institution, Academy of Military Medical Sciences.

Traditional chinese medicine differential syndromes

TCM differential syndromes referred to the viral hepatitis diagnostic standards defined in December, 1991 by Internal Medicine Hepatopathy Committee of Chinese Traditional Medicine Association[23]. Patients with viral hepatitis were accordingly, classified into five subtypes: (a) Damp heat stasis in the middle-jiao; (b) Liver Qi stagnation and spleen deficiency; (c) Yin deficiency of liver and kidney; (d) Blood stasis into collateral; (e) Yang deficiency of spleen and kidney. See the diagnostic standards in Ref.[23]. Besides, based on the clinical manifestation, a new type of damp heat complicated with blood stasis was added.

Statistical analysis

SPSS 11.0 statistic software was adopted in analyzing all the materials. Different groups of variable were explored by the method of variant analysis. When variant fluctuation was limited, LSD method was applied, otherwise, Dunnett's T3 check was used.

RESULTS

Distribution of syndrome-types based on TCM and western medical diagnosis in mutant and wild-type groups (Table 2)

Distribution of TCM syndromes was apparently different between the two study groups ($\chi^2 = 7.44$, $P = 0.006$). The most common syndrome in mutant group was damp-heat combined with blood stasis ($n = 25$), and the most common syndrome in the wild-type group was damp-heat stasis in the middle-jiao ($n = 36$). There was no significant difference in the other syndromes. The cases including medium and severe hepatitis were 29 in wild-type group, however, which were up to 47 in mutant group ($P = 0.004$).

Table 1 The cases, sex, and age of three groups

	n	Ration of sex (M:F)	Age (yr)	
			Mean	Range
Mutant	70	11:3	29.10±1.02	18-56
Wild-type	70	61:9	28.96±0.83	17-57.5
Healthy control	30	5:1	28.85±1.40	17-55

M: male; F: female.

Table 2 Distribution of syndrome-types based on TCM in mutant and wild-type groups

Subtype	Pre-core mutant	
	-	+
DHSM	36	17
LSSD	7	12
YDLK	7	5
BSIC	4	7
DHBS	11	25
YDSK	5	4
Mild hepatitis	41	23
Medium hepatitis	22	39
Severe hepatitis	7	8

DHSM: damp-heat stasis in the middle-jiao LSSD: liver Qi stagnation and spleen deficiency YDLK: Yin deficiency of liver and kidney BSIC: blood stasis into collateral DHBS: damp heat complicated with blood stasis YDSK: Yang deficiency of spleen and kidney.

T lymphocytes subgroup and HBV-DNA

The content of CD4+ lymphocytes and the ratio of CD4+/CD8+ in mutant group were less than that in wild-type group ($P<0.05$) and the control group ($P<0.01$). The content of HBV-DNA in mutant group was higher than that in wild-type group ($P<0.05$) (Table 3).

T lymphocytes subgroup comparison in wild-type group itself (Figure 1)

The content of CD4+ lymphocytes and the ratio of CD4+/CD8+ in mild and medium hepatitis were significantly higher than that in severe hepatitis ($P<0.001$). No significant statistical difference in CD8+ lymphocytes ($P>0.05$) was found.

T lymphocytes subgroup comparison in mutant group itself (Figure 2)

Figure 2 showed that on the contrary of the wild-type group, the content of CD4+ lymphocytes and the ratio of CD4+/CD8+ in medium and severe hepatitis were significantly higher than that in mild hepatitis ($P<0.001$). No significant statistic difference in CD8+ lymphocytes ($P>0.05$) was found.

Comparison of DNA content in mutant group and wild-type group (Table 4)

Although mutant group had a relatively stronger HBV-DNA copy capability than wild-type group, there was no significant difference between the two groups according to the severity of hepatitis ($P>0.05$) (Table 4).

DISCUSSION

Numerous studies on HBV precore mutation are being made in the way of modern medicine. It has been found that the patients with HBV precore mutation are subjected to develop not only severe hepatitis but also HCC[3,24-26]. Therefore when doctors treat these patients, they should pay attention to more questions. It is considered in modern medical sciences that HBV precore mutation is mainly caused by high immune pressure of human organism. The HBV are not eliminated but continue to reduplicate through mutation and evasion of the distinction of host immune system. In traditional Chinese medicine, HBV are regarded as a mixture of damp, heat and toxin, which restrains Yang Qi, goes deeply into the blood, internal organs and collaterals. A disease caused by these pathogens has usually a long course and is often lingering and difficult to cure. Therefore, damp, heat, and toxin are the basic pathogens and exist during the whole development course of CHB. Our study showed that, in the mutant group, damp-heat combined with blood stasis was mostly often seen, followed by damp-heat stasis in the middle-jiao. These two syndromes occupied 67% (47/70) of the total cases in this group. These two syndromes also occupied 60% (42/70) of the total cases in wild-type group. So, our study showed that damp, heat, toxin and blood stasis were the basic pathogenic factors of CHB, whether pre-core mutant or not. According to the western medical diagnosis of two study groups, our study found that there was close relationship between pre-core mutant and the severity of hepatitis, just like quite other studies that had already reported[4,11,14]. These patients usually had a long course and presented hypochondriac pain, jaundice, dark urine, loose stool, *etc*. Therefore, we consider that the nature of damp, heat, toxin and blood stasis does not change though with HBV mutant, and the mutation is possibly the result of the conflict of antipathogenic Qi and pathogenic Qi.

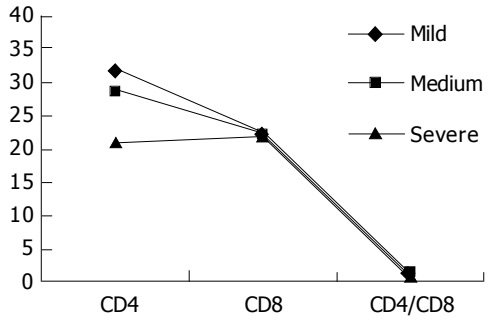

Figure 1 T lymphocytes subgroup comparison in wild-type group itself.

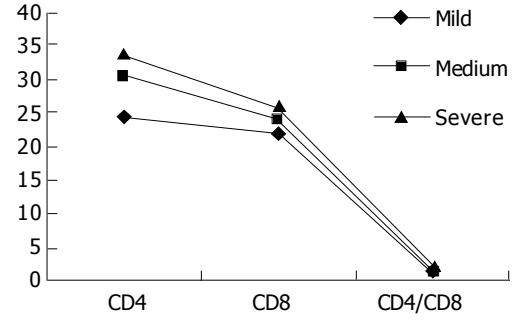

Figure 2 T lymphocytes subgroup comparison in mutant group itself.

Concerning the question of why hepatitis B could easily turn into CHB, most of the researchers believe that it is because of the host's immune malfunction and partial or full immunologic tolerance to various HBV antigens. Therefore, T lymphocytes play a very important role in the process of HBV chronic infection. CD4+ cells and CD8+ cells are a main part in the T lymphocytes subgroup. Under normal conditions, they are in a state of balance. If the balance is broken, it will lead to cell immunopotency decrease, immune regulation dysfunction, and consequently, HBV chronic infection. In severe hepatitis-B, the infective rate of HBV mutant strain was high. The mutant strain induces more severe immune disorders in host, resulting in the release of cytokines, so liver damage is more prominent[13]. Studies in modern TCM clinical treatment support that the cellular immune function of human body accord with "antipathogenic Qi". The "coexistence of weakened antipathogenic Qi and excessive pathogenic Qi" is one of the manifestations of decreased cellular functionality. Vigorous antipathogenic Qi results in a more intense conflict between the two Qi, and a more violent immune reaction which explains the formation of acute disease. On the other hand, deficient antipathogenic Qi and decreased immune functionality cause the disease prolonged uncured and turned to be chronic and lingering. Da and Chen[27] classified CHB patients as deficiency syndrome, excess syndrome and deficiency complicated with excess syndrome. He made a comparison among the three syndromes in respect of cellular immune functionality.

Table 3 Variant analysis between three groups and Dunnett-*t* test

	Groups	Cases	mean±SD
CD4+	Control group	30	38.27±4.00
	Wild-type group	70	29.9±6.14[a,b]
	Mutant group	70	28.69±5.38[b]
CD8+	Control group	30	23.9±2.96
	Wild-type group	70	22.4±4.60
	Mutant group	70	23.53±4.80
CD4+/CD8+	Control group	30	1.59±0.163
	Wild-type group	70	1.36±0.31[a,b]
	Mutant group	70	1.26±0.28[b]
DNA	Wild-type group	70	4.94±1.16[a]
	Mutant group	70	5.89±1.88

One-way ANOVA variant analysis was made between the three groups. During Dunnett-*t* test control group was taken as object group, other groups comparing with the object group. [a]$P<0.05$ Comparison between two study groups, [b]$P<0.01$ comparison made to control group.

Table 4 Comparison of DNA content in mutant group and wild-type group

		mean±SD
Mutant group	Mild	5.934±1.936
	Medium	5.558±1.687
	Severe	6.661±2.128
Wild-type group	Mild	4.664±1.1361
	Medium	4.999±1.196
	Severe	5.445±0.975

Results showed a significantly higher content of CD4+ lymphocytes and CD8+ lymphocytes in excess syndrome group, but there was no significant difference between deficient and excess groups in contrast with the deficiency complicated with excess syndrome group, suggesting a possibly lowered functional level of the assistant T lymphocytes in deficiency syndrome and a predominant immune reaction depending on the cytotoxic T lymphocytes in excess syndrome. Numerous research have showed that the content of CD4+ lymphocytes and the ratio of CD4+/CD8+ could be greatly increased by strengthening antipathogenic Qi of the CHB patients. Zhang *et al*[28], prescribed Ginseng-Astraglus Four Ingredients Decoction to cure CHB patients, which significantly increased the content of CD4+ lymphocytes and the ratio of CD4+/CD8+. He pointed out that the CHB patients could be differentiated as blood deficiency syndrome and treated with Chinese herbs functioning in supplementing antipathogenic Qi and blood. Our research demonstrated considerably higher content of CD4+ lymphocytes and ratio of CD4+/CD8+ in control group than that in two study groups, which was in line with most of the document reported. Low content of CD4+ lymphocytes and ratio of CD4+/CD8+ of the CHB patients was regarded as one of the indices of deficiency syndrome. Lack of antipathogenic Qi is the essential reason of human disease. Cite the chapter "Discussion on Acupuncture Method" in plain question: "Pathogenic Qi is difficult to invade if antipathogenic Qi is sufficient in human body". Only when antipathogenic Qi is relatively weak and unable to defeat pathogenic Qi, the invasion of pathogenic Qi becomes possible, which leads not only to the imbalance of Yin and Yang but also the dysfunction of viscera and meridians, consequently, the occurrence of diseases. For the CHB patients with precore mutation, antipathogenic Qi is not strong enough to fight against pathogenic Qi, so that HBV could evade immune attack through mutating and is not eliminated in time and its reduplication multiplies, which can explain why HBV-DNA content is higher in pre-core mutant positive group than in negative group. Meanwhile, the intensity of the conflict of the antipathogenic Qi and the pathogenic Qi, together with the power of antipathogenic Qi, determines the state of patients' condition. The more intense the fight, the stronger the immune system reacts, and the liver cells are greatly damaged. During this stage, the excess syndrome is predominant and vice versa. With long-term conflict between the antipathogenic Qi and the pathogenic Qi, the antipathogenic Qi becomes weaker and weaker, which can explain why the content of CD4+ lymphocytes and the ratio of CD4+/CD8+ in the mutant group was less than that in the wild-type group. For CHB patients with precore mutation, it is the common case that the weakness of antipathogenic Qi and the vigor of pathogenic Qi gradually develop into blood stasis, even liver cirrhosis and HCC come into existence. Added with the imbalance of the seven emotional factors (joy, anger, melancholy, brooding, sorrow, fear and shock) and the imbalanced daily regimen, patients' condition will soon deteriorate if no prompt medical treatment or erroneous treatment is given. It may explain why the patients with severe hepatitis is more often seen in those patients with precore mutation. Therefore, we consider that those patients with precore mutation should be differentiated as deficiency complicated with excess syndrome. Because of the coexistence of weakened antipathogenic Qi and excessive pathogenic Qi, HBV can evade from immune through mutation and the HBV reduplication capacity becomes vigorous, which result in disease lingering. When patients' condition is in the mild stage, the syndrome of deficiency complicated with excess should be considered, while in severe stage, excess syndrome manifested should be focused on. Clinical treatment should rely on the indices of CD4+ and CD4+/CD8+, strengthening antipathogenic Qi and eliminating pathogenic factors according to the state of the patients. Attentively, tonic herbs should not be misused, which may aggravate patients' condition.

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