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傳統中醫藥誘生內源性細胞因子導致血清 HBsAg 水平之下降

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Original Article

Induction of Endogenous Cytokines by Traditional Chinese Medicine Leading to Reduction of Serum HBsAg Levels

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Chronic hepatitis B virus (HBV) infection continues to be a serious global health problem and current therapeutics seldom reach treatment endpoint of HBsAg seroclearance with or without Anti-HBs. In this report, it was observed that, formulated under the guiding principle of dynamic equilibrium of ying and yang, traditional Chinese medicine (TCM) treatment of chronic hepatitis B infection resulted in remarkable reduction of serum HBsAg levels. Clinical cytokine tests demonstrated that the patients who succeeded in having levels of serum HBsAg reduced were those tested to have endogenous cytokines IFN-α, IFN-γ, TNF-α and/or TGF-β_1 induced. Those cytokines are known to be able to trigger degradation of cccDNA, the HBV transcription template, in the nucleus of the infected hepatocyte noncytopathically. We conclude that TCM is a novel strategy to successfully treat chronic hepatitis B infection.

Key words: traditional Chinese medicine (TCM), chronic hepatitis B, cccDNA, endogenous cytokines

Conflict of interest: The authors declare no conflict of interest.

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Introduction

Chronic hepatitis B virus (HBV) infection continues to be a serious global health problem, with an estimated 257 million infected individuals worldwide in 2018, despite availability of an effective hepatitis B vaccine decades ago. Hepatitis B infection resulted in 887,000 deaths in 2015, mostly from complications including liver cirrhosis and hepatocellular carcinoma (HCC) [1].

Chronicity of HBV infection is due mainly to the existence of the HBV transcription template, covalently closed circular DNA (cccDNA), in the nucleus of infected hepatocyte, which is extremely difficult to be eradicated, resulting in the persistence of HBV replication [2-5]. Quantitative serum HBsAg could be used as a surrogate marker of cccDNA [6,7]. High levels of serum HBsAg increase the risk of HCC in chronic HBV infection with low viral load [8].

Interferon-α (IFN) and nucleos(t)ide analogues(NAs) are currently the therapeutics to treat chronic hepatitis B(CHB)[9,10]. However, treatment endpoint of seroclearance of HBsAg with or without Anti-HBs is seldom reached [9,10]. Long term, sometimes lifelong treatment with NAs is necessary to prevent relapse after cessation of treatment [1]. IFN-α has sometimes intolerable side effects and is less effective among Asian patients for unknown reason [11].

Besides, antiviral treatment for chronic hepatitis B carriers is not recommended, with the exceptions of older age, elevated HBV-DNA, severe liver histological lesions, family history of HCC or cirrhosis and extrahepatic manifestations, though EASL and AASLD differ somewhat in definition [9,10]. However, 15%-40% of the asymptomatic chronic hepatitis B carriers would in their lifetime develop liver cirrhosis, HCC and decompensation of the liver [12,13]. Just doing routine follow-up is not sufficient for that multitude of carriers because their risk of HCC development is almost 100 folds higher than non-carriers and once HCC is confirmed, remaining life expectancy is numbered [12]. It, therefore, is imperative to find a cure for them now instead of asking them to wait indefinitely for fulfillment of treatment qualification before medication could commence.

Recent success of direct antiviral agents (DAAs) in HCV viral eradication stimulates researchers to target viral clearance. Potential pharmacological developments including HBV entry inhibitors, 2nd generation core inhibitors, TLR agonists, anti-sense nucleotides, cccDNA degradation agents, DAAs that target HBV capsid, HBV release inhibitors etc. are in preclinical or early clinical trials [14-16]. Therapeutic application, however, has to overcome the formidable hurdle of off-target effects resulting in cellular DNA damage; efficient and targeted delivery to the infected hepatocytes and overall safety profile etc. [2]. It would be quite sometime yet for a true cure of chronic hepatitis B to be realized.

A causal relationship between TCM per se and the reduction of serum HBsAg levels requires additional research experiments which are obviously beyond the scope of the current study. On the other hand, serum HBsAg has been appreciated as the surrogate marker of cccDNA, the HBV transcription template located in the nucleus of the infected hepatocyte [6]. Degradation of cccDNA would lead to reduction of serum HBsAg levels and ultimate HBsAg seroclearance and vice versa holds true.

Meanwhile, a number of excellent experimental modalities demonstrated that cytokines IFN-α,

IFN-γ, IFN-Λ, TNF-α and TGF-β_1 would trigger the degradation of nuclear HBV cccDNA without cytolysis. Proposed mechanism briefly was that the cytokines would upregulate or activate different members of the APOBEC family in the nucleus of the infected hepatocyte, for example, IFN-α would upregulate APOBEC 3A, and make use of HBV core to access and deaminate cccDNA, which was transiently rendered single-stranded during viral transcription. Deaminated cccDNA hence became prone to degradation by nucleases. Uracils in cccDNA were recognized and excised by base excision repair (BER) enzyme uracil-DNA glycosylase (UNG), leading to the formation of apurinic/apyrimidinic (AP) sites, which were then recognized by cellular AP endonucleases, leading to cccDNA decay. Each of before-mentioned cytokines had its own distinct pathways of activation with distinct kinetics to degrade cccDNA noncytolitically [17-21].

We reported in our previous article, " Retrospective Analysis of Traditional Chinese Medicine Treating Chronic Hepatitis B and Clinical Statistics", that TCM treating CHB and chronic HBV carriers was therapeutically effective and could reach treatment endpoint of HBsAg seroclearance: 5.90% (6/102) and 16.5% (21/127) for CHB and chronic HBV carriers respectively [22].

The mechanism of TCM treating CHB and chronic HBV carriers with such a breakthrough in HBsAg seroclearance could probably be due to the restoration of the dynamic equilibrium of ying and yang which permeates every aspect of mind, spirit and body. Ying and yang are rooted mutually but are antagonistic and restrictive to each other. Dynamic equilibrium of ying and yang could lead to healing of diseases through holistic harmonization of mind, spirit and body. That could take to mean that innate and acquired immunity are activated to expel foreign and inner pathogens. That hypothesis needs further testing.

The current study attempts to correlate the teachings of Chinese and western medicine: restoration of dynamic equilibrium of ying and yang by TCM could induce endogenous cytokines IFN-α, IFN-γ, TNF-α and/or TGF-β_1 which would degrade cccDNA, leading to reduction of serum HBsAg levels.

Methods

Clinical observation of TCM treating chronic hepatitis B infection

Patients with undetectable or low levels of HBV-DNA have their serum HBsAg titre routinely assayed quantitatively in the writer's liver clinics in Malaysia in order to assess whether TCM treatment could be safely terminated. They were not intended obviously for the current study which had been conceived just recently. The data, when pooled together, would coincidentally constitute a valuable source of quantitative information.

In order to shed some light on TCM treating CHB especially in terms of reduction of serum HBsAg levels, it would be meaningful to analyze the data in a clinical setting. Realizing that their historical clinical information would be helpful in unlocking the secrets of TCM treating CHB, 84 patients came forward, agreeing to have their test results retrieved and analyzed as well as carrying out relevant clinical tests, with written consents. To ensure objectivity and continuity of data, compliance of patients in regularly taking the medication prescribed by the writer and doing blood tests on a quarterly basis was required, for the observation period of Sept. 4, 2016 thru Sept. 24, 2018, resulting in 53 patients qualified for inclusion.

Chronic hepatitis B infection is a new disease, not

known to the practitioners in the past. The treatment formulations for liver and gut diseases passed down by famed Chinese physicians over thousands of years were not specifically for CHB and carriers. Effectiveness is not just remission of manifested symptoms. It is related to the degradation of cccDNA.

Persistence of chronic hepatitis B infection is clearly related to the malfunction of HBV specific innate and acquired immune systems. After years of clinical practice and study in this field, the writer has come to the conclusion that key for a cure is the restoration of dynamic equilibrium of ying and yang, which permeate every aspect of mind, spirit and body. It is probable that, unknowingly, the HBV specific immunity is restored under this guiding principle which could lead to healing of diseases through holistic harmonization of mind, spirit and body. Induction of endogenous cytokines by the effective patients would be the most convincing testimonials conferred to TCM treating chronic hepatitis B infection.

In fact, the theory of ying and yang permeates all aspects of TCM, used to explain morphology, structure, physiological functions and pathological changes of the human body, and guide clinical diagnosis and treatment as well as health preservation.

For example, most chronic hepatitis B cases, no matter how complicated and changeable their clinical manifestations are, can be generalized and explained with the theory of ying and yang, which can be used to summarize general morbid states on the macro-level and analyze specific symptoms on the micro-levels. Prescription is also based on the theory of ying and yang, disharmony of which underpins evolution of chronic hepatitis B. Choosing of individual herb for the prescription is also coming under the theory of ying and yang, which of course, to a certain extent, is related to the writer's personal experience and preference.

The Chinese medicinal herbs used by the writer were in line with the results of a meta-analysis of TCM treating CHB with 30 most commonly used Chinese herbs as follows [23] :

astragalus (huang qi), salvia miltiorrhiza (dan shen), atractylodis rhizoma (bai shu), radix bupleuri (chai hu), polygoni cuspidati (hu zhang), oldenlandia diffusae (bai hua she she cao), radix glycyrrhizae (gan cao), herba artemisiae capillaris (yin chen), radix paeoniae rubra (chi shao), radix cureumae wenyujin (yu jin), poria (fuling), radix paoeniae alba (bai shao), radix angelice sinensis (dang gui), radix codonopsis (dang shen), radix et rhizoma rhei (da huang), radix isatidis (ban lan gen), fructus schisandrae chinensis (wu wei zi), lycium chinensis mill(gou qi zi), fructus ligustri lucidum(nu zhen zi), herba sedi sarmentosum (chui pen cao), radix sophorae flavescentis (ku shen), hawkthom (shan zha), fructus gardeniae (zhi zi), pericarpium citri reticulatae (chen pi), semem coicis (yi yi ren), phyllanthus urinaria (zhen zhu cao), carapax trionycis (bie jia), radix scutellariae baicalensis (huang qin), herba scutellariae (ban zhi lian), radix polygoni multiflori (he shou wu).

Empirically, the following Chinese medicinal herbs were also used by the writer:

epimedium brevicornum maxim (xian ling pi), cuscuta chinensis lam (tu si zi), eucommia ulmoides oliver (du zhong), achyranthes bidentata Bl. (huai niu xi), polyporus umbellatus (pers.) fries (zhu ling), fructus aurantii (zhi ke), rhizoma drynaria fortunei (ku chiu pu), cordyceps sinensis (Berk) sacc (zong zou zin zie te), caulis cistanche deserticola Y. C. M. A. (zo zung zong).

Due to a lack of data on HBsAg seroclearance

after clinical treatment on CHB and carriers, especially Chinese patients, we decided to adopt Taiwan annual spontaneous HBsAg seroclearance rate of 1.15% [24], which was higher than 0.55% and 1% suggested by EASL and AASLD respectively, as the criterion. Those patients with serum HBsAg level reduction more than 1.15% in the observation period of April 2016-September 2018 were grouped as effective. For those failing to achieve that rate or, on the contrary, experiencing an increase in serum HBsAg levels instead, or fluctuations in the observation period, were classified as non-effective.

Serum quantitative HBsAg and Anti-HBs were analyzed by Abbott Architect i2000 assay. HBV-DNA was quantified by Roche Cobas TaqMan test. Quantitative HBeAg and Anti-HBe were assayed by Roche Cobas 1601 ECLIA. ALT levels were determined according to the standard procedures of the machine manufacturer Beckman, USA. Assay of cytokines: 20-50 µl serum was obtained, diluted to 50 µl by using diluents, according to the instruction of the reagent supplier, and add 50 µl of first antibody of Biotin-conjugate, left to react for one hour under room temperature. Emptied contents and washed four times by using washing buffer. 100 µl of second antibody of Streptavidin-HRP was added and left to react for one hour under room temperature; then emptied and washed four times by using washing buffer; 100 µl of TMB substrate was added, left to react for 10-30 minutes under room temperature, then read the absorbance OD450nm. All cytokine reagent kits used in this research were obtained from eBioscience (ThermoFisher Scientific Inc., San Diego, CA, USA). Reagent for glucocorticoid testing was obtained from Bluegene (Shanghai BlueGene Biotech Co., Shanghai, China)

As for statistical analyses, continuous variables were expressed as means ± standard deviation with 95% confidence intervals, and were compared by Student Unpaired t test or Fisher Exact test depending on prevailing situation with different P values for consideration of significance. Cramer's V value was used to test coefficient of correlation of two sets of data.

Baseline characteristics of the participants were set out in Table 1. Outstanding characteristics of participants were: majority of them were chronic hepatitis B carriers, with negativity of HBeAg and high levels of serum HBsAg, as well as of older age.

Results

TCM was effective in reducing serum HBsAg levels

Based on Table 2, Figure 1 was plotted to depict the remarkable difference between the effective and non-effective groups in terms of average serum HBsAg titre reduction. Figure 2 was another plot in linear form to demonstrate at a glance the difference between the effective and non-effective groups in terms of average serum HBsAg titre reduction. As showed in Figure 3, the differences in average and in mean serum HBsAg reduction between the effective and non-effective groups were significant (**p=0.007 by Unpaired t test). Reduction for the effective group compared to the non-effective one in terms of averages was 4.84 folds higher as showed in Table 2.

If treatment efficacy was defined as reaching a rather high but meaningful bench mark of 30% serum HBsAg titre reduction at the end of observation period, then 72.2% (26/36) of patients out of the effective group surpassed 30% while only 17.7% (3/17) of

Table 1 Baseline characteristics of participants

Parameter		Effective group	Non-effective group
		No. (%)	No. (%)
HBsAg (IU/mL)	50-100	3(8)	0(0)
	101-500	5(14)	2(12)
	501-1,000	2(5)	1(6)
	1,001-3,000	6(17)	2(12)
	3,001-5,000	6(17)	3(17)
	>5,000	14(39)	9(53)
ALT (U/L)	≤40	34(94)	15(88)
	≥41	2(6)	2(12)
HBeAg	(+)>1.0	0(0)	3(18)
	(-)<1.0	36(100)	14(82)
HBV-DNA (IU/mL)	≤10	15(42)	8(47)
	≥11	21(58)	9(53)
Gender	Male	26(72)	16(94)
	Female	10(28)	1(6)
Age (year)	<30	1(3)	0(0)
	30-39	3(8)	1(6)
	40-49	11(31)	5(29)
	50-59	13(36)	8(47)
	≥60	8(22)	3(18)
State	Malaysia	25(69)	9(53)
	Taiwan, ROC	10(28)	8(47)
	Indonesia	1(3)	
Total		36	17

Table 2 Comparison of serum HBsAg titre reduction between effective and non-effective groups

unit: IU/mL

	average	mean	standard deviation	standard deviation error	95% confidence interval	
					lower limit	upper limit
effective-starting	3858	3565	3119	519.8	2803	4913
effective-ending	2289	1041	2452	408.6	1460	3119
effective-titre reduced	1569	980	1673	278.9	1003	2135
non-effective-starting	4453	5312	2680	650.1	3075	5831
non-effective-ending	4129	4181	2537	615.3	2825	5433
non-effective-titre reduced	324.1	380	1044	253.2	-212.6	860.9

Average serum HBsAg titre reduced for the treatment effective group was 1569 IU/mL versus 324.1 IU/mL for the non-effective one, with a difference of 4.84 times.

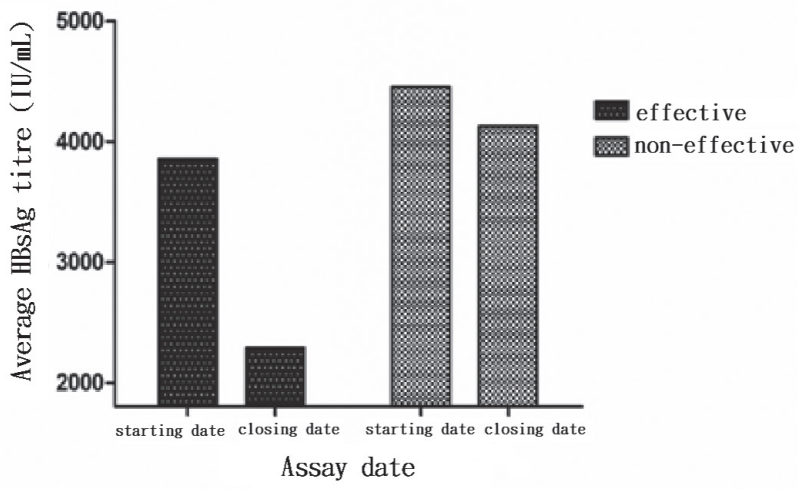

Figure 1 Comparison of average serum HBsAg titre reduction between effective and non-effective groups. Based on Table 2, the average titre at the starting date for the treatment effective and non-effective group were 3858 IU/mL and 4453 IU/mL respectively, versus 2289 IU/mL and 4129 IU/mL at closing date as simplified below. Visual effect was spectacular.

	effective group	non-effective group	
	average (95% CI)	average (95% CI)	p-value
starting	3858 (2803,4913)	4453 (3075,5831)	0.007
ending	2289 (1460,3119)	4129 (2825,5433)	
titre reduced	1569 (1003,2135)	324.1 (-212.6,860.9)	

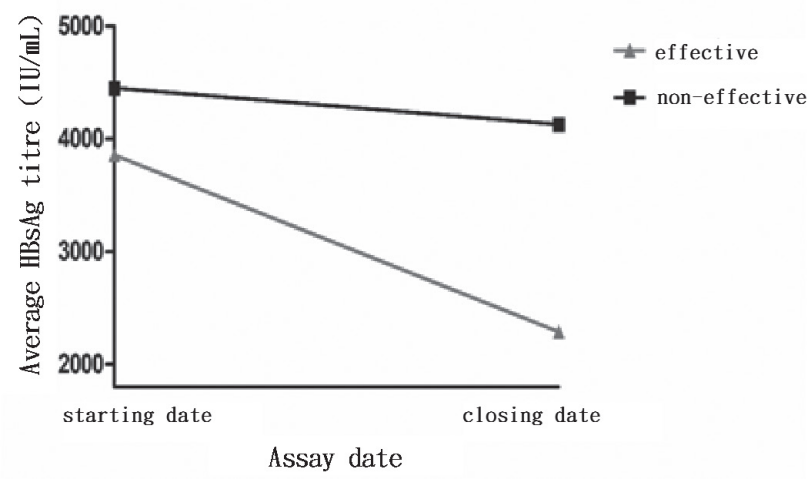

Figure 2 Comparison of average linear serum HBsAg titre reduction. Comparison of average titre reduction between the effective and non-effective groups in linear form, also based on Table 2, simplified as below. The difference was huge.

	effective group	non-effective group	
	average (95% CI)	average (95% CI)	p-value
starting	3858 (2803,4913)	4453 (3075,5831)	0.007
ending	2289 (1460,3119)	4129 (2825,5433)	
titre reduced	1569 (1003,2135)	324.1 (-212.6,860.9)	

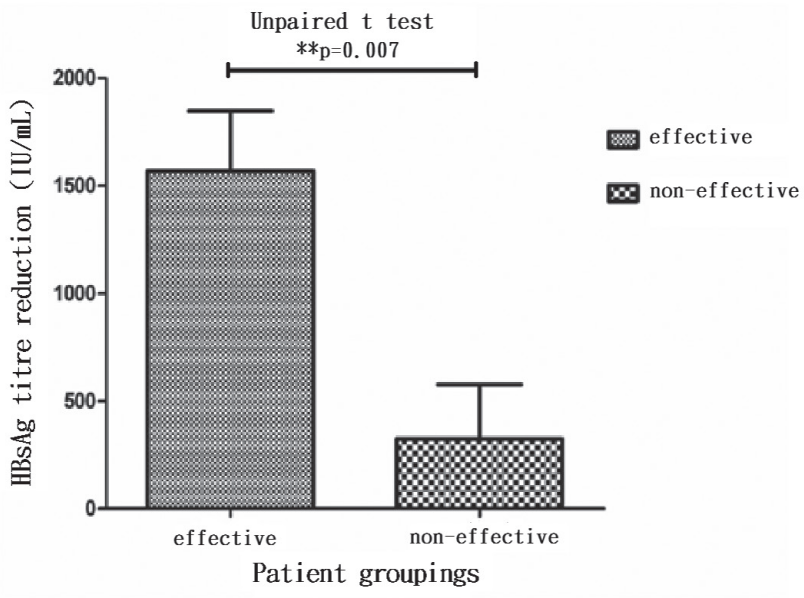

Figure 3 Unpaired t test of serum HBsAg titre reduction. Based on Table 2, or a simplified one as below, the average serum HBsAg titre reduced for the effective and non-effective group were 1569 IU/mL and 324.1 IU/mL respectively. The difference was statistically significant: **p=0.007 by Unpaired t test.

	effective group	non-effective group	
	average (95% CI)	average (95% CI)	p-value
starting	3858 (2803,4913)	4453 (3075,5831)	0.007
ending	2289 (1460,3119)	4129 (2825,5433)	
titre reduced	1569 (1003,2135)	324.1 (-212.6,860.9)	

patients from the non-effective group managed to reach that 30% target. Coefficient of correlation Cramer's V value (0-1) 0.512 demonstrated that those two sets of data were of median correlation. ***p=0.0003 by Fisher Exact test indicated that the difference between the two groups in this regard was very significant, as showed in Table 3 and Figure 4.

Ratio wise, the average serum HBsAg titre reduction between the effective and non-effective groups was very significant (***p=0.0002, by Unpaired t test as showed in Table 4 and Figure 5).

Table 3 Comparison of patients attaining efficacy of 30% reduction of serum HBsAg titre

	Reduction of HBsAg titre			
	>30%: patients (%)	<30%: patients (%)	Total (%)	p-value
effective group	26 (72.2)	10 (27.8)	36 (100)	0.0003
non-effective group	3 (17.7)	14 (82.3)	17 (100)	
total	29 (54.7)	24 (45.3)	53 (100)	

Coefficient of correlation Cramer's V value 0.512 signified median correlation.

72.2% (26/36) patients in the effective group managed to have their serum HBsAg titre reduced more than 30% versus 17.7% (3/17) for the non-effective one in April 9, 2016 — September 24, 2018 observation period. The difference is very significant, ***p=0.0003. Coefficient of correlation Cramer's V value 0.512 indicated median correlation between those two sets of data.

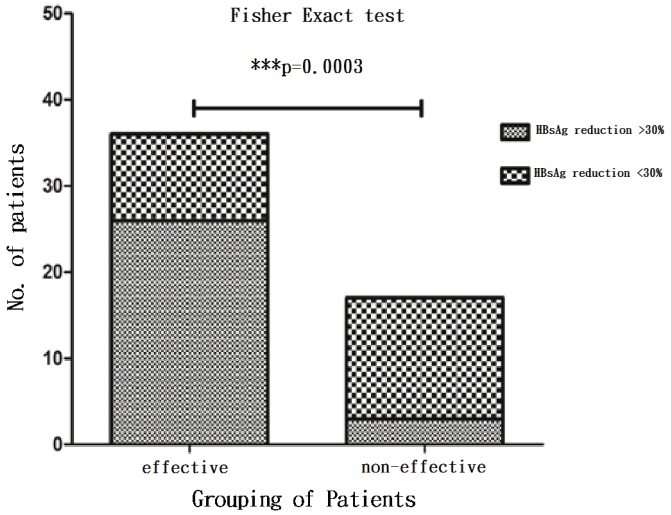

Figure 4 Comparison between number of effective and non-effective patients reaching >30% serum HBsAg reduction target. Based on Table 3, a total of 72.2% (26/36) of patients from the effective group reached the treatment target of >30% of serum HBsAg titre reduction versus 17.7% (3/17) in the non-effective group. The difference was statistically very significant: ***p=0.0003 by Fisher Exact test. Those two sets of data were of median correlation (Cramer's V value=0.512).

Table 4 Comparison of average serum HBsAg titre reduction ratio

Grouping	No. of patients	average	mean	standard deviation	standard deviation error	95% confidence interval	
						lower limit	upper limit
effective	36	51.91%	57.06%	33.63%	5.61%	40.53%	63.29%
non-effective	17	2.69%	2.29%	53.96%	13.09%	-25.05%	30.43%

Average serum HBsAg titre reduction ratio between the effective and non-effective groups was 51.91% versus 2.69%.

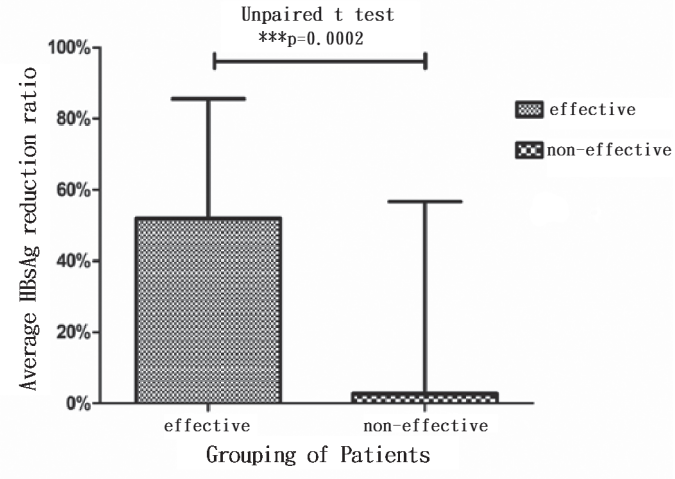

Figure 5 Comparison of average serum HBsAg reduction ratios between the effective and non-effective groups. 51.91% of average serum HBsAg reduction ratio for the effective group versus 2.69% for the non-effective one. The difference was statistically very significant, ***p=0.0002 by Unpaired t test.

TCM was effective in treating chronic hepatitis B carriers

In terms of serum HBsAg titre reduction, TCM was effective in treating chronic hepatitis B carriers, even for the non-effective group of carriers, because the average titre reduced was higher for the carrier group than the entire non-effective group: 506.3 IU/mL Vs 324.1 IU/mL. That narrowed the gap between the effective and non-effective carriers: 2.88 folds instead of 4.84 folds for the entire groupings. Detailed data were showed in Table 5.

The ratio of serum HBsAg reduction between the effective carriers group and the non-effective one was significant (*p=0.03, by Unpaired t test, Figure 6) though less spectacular than the overall study groups (***p=0.0002).

Negativity of HBeAg posed no problem for TCM treatment since 100% of the effective group of patients were negative in HBeAg. Non-effective treatment of TCM was not due to negativity of HBeAg per se. Other more imposing factors were involved.

Table 5 Comparison of serum HBsAg titre reduction between effective and non-effective chronic hepatitis B carriers

unit: IU/mL

	average	mean	standard deviation	standard deviation error	95% confidence interval	
					lower limit	upper limit
effective-starting	3816	3511	3198	548.4	2701	4932
effective-ending	2359	1041	2493	427.5	1489	3228
effective-titre reduced	1458	833.5	1656	283.9	880.2	2036
non-effective-starting	4563	5312	2689	694.3	3074	6052
non-effective-ending	4057	4181	2642	682.3	2593	5520
non-effective-titre reduced	506.3	320	901.6	232.8	6.97	1006

Average serum HBsAg titre reduced for the group of treatment effective chronic HBV carriers was 1458 IU/mL versus 506.3 IU/mL for the non-effective ones with a difference of 2.88 times.

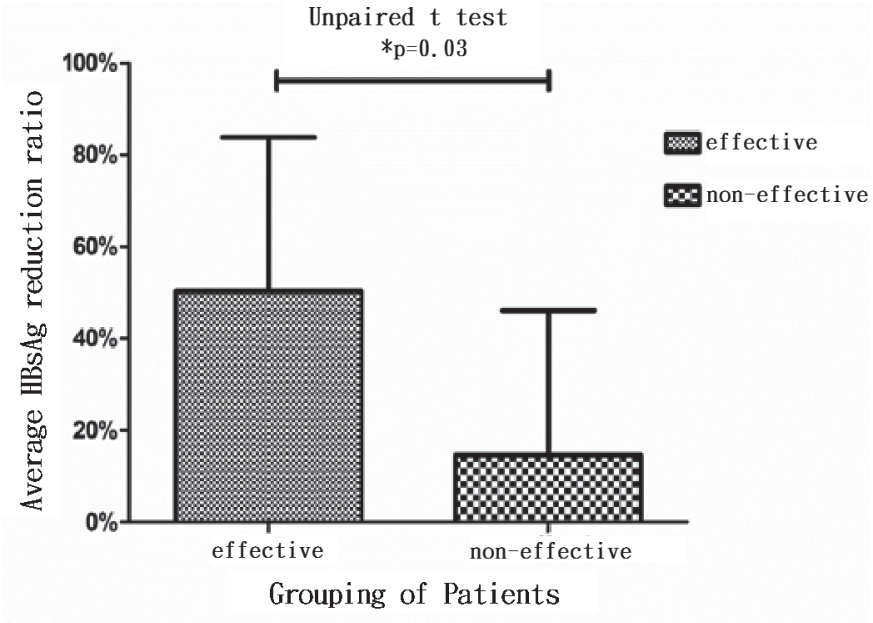

Figure 6 Comparison of average serum HBsAg reduction ratios between the effective and non-effective groups of chronic hepatitis B carriers. 50.26% of average serum HBsAg reduction ratio for the effective group of chronic HBV carriers versus 14.62% for the non-effective one. The difference was statistically significant, *p=0.03 by Unpaired t test.

Uni- and multi-variate analyses demonstrated that factors of serum HBsAg levels, normality/abnormality of ALT, positivity/negativity of HBeAg, HBV-DNA levels, gender, age and locality of patients were not associated with the effectiveness of TCM treatment of chronic hepatitis B infection.

Stress adversely impacted TCM effectiveness

Among all the factors that adversely affect the immune system, stress is the most outstanding and well-documented. The intensely contested Malaysia general election on May 9, 2018 was well worth mentioning in this regard.

As showed in Figure 6A, 4 out of the effective group of patients continued to have decrease of serum HBsAg titres, versus 9 having their downward trend reversed during the election period. Statistically the difference was quite significant (**p=0.0036, Fisher Exact test in Figure 6A) with Cramer's V value of 0.632 demonstrating median correlation between the two sets of data. Figure 7, 8 and 9 showed graphically the twists and turns of serum HBsAg levels experienced by six effective patients in such a scenario.

However, lacking the cytokine levels before and after the election, we could only speculate that the stress resulting from the political syndrome was the underlying factor.

As a matter of fact, most of the patients in the non-effective group were stressed in one way or the other: taking care of elderly sick relatives, financially burdened, nagging spouse, mean boss etc. The political syndrome of the effective group objectively demonstrated that the non-effective patients need to find a way out of their dilemma. They were always counselled to look on the bright side of life, accept life as it is, forget about the Joneses, practice qigong and

meditation etc. during their visit to the writer's clinics in order to uplift themselves and boost the immune system.

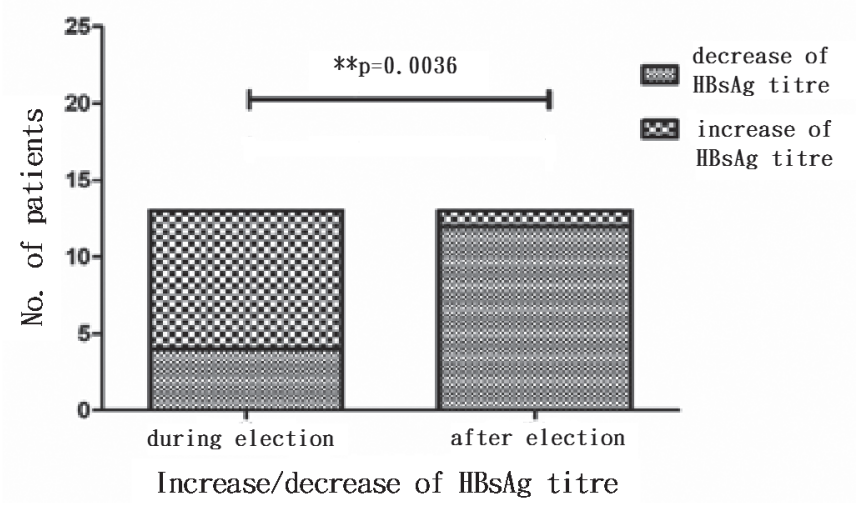

Figure 6A Comparison of Changes of serum HBsAg titre during and after Malaysia election by Fisher Exact test. During the election period, 4 patients in the effective group experienced decrease of serum HBsAg titre versus 9 with increment. The trend was reversed after the election: 12 decrease versus 1 increase. The difference was quite significant, **p=0.0036 by Fisher Exact test and Coefficient of correlation Cramer's V value of 0.632 indicated median correlation between those two sets of data.

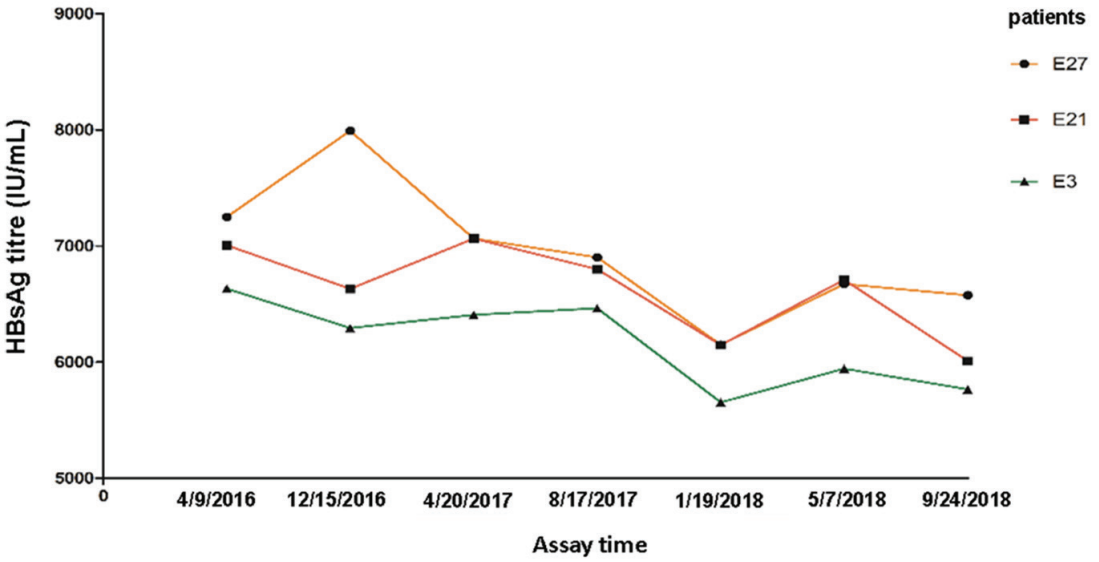

Figure 7 Change of trend direction during and after Malaysia election for 3 patients. The long term downward trend of serum HBsAg titre reduction for patients Nos. E27, E21 and E3 reached the lowest point of 6150 IU/mL, 6148 IU/mL and 5654 IU/mL respectively. Due possibly to stress resulted from political syndrome, serum HBsAg titre increased to 6673 IU/mL, 6710 IU/mL and 5944 IU/mL respectively. The titre gradually reduced to 6577 IU/mL, 6011 IU/mL and 5767 IU/mL for the three effective patients after the election.

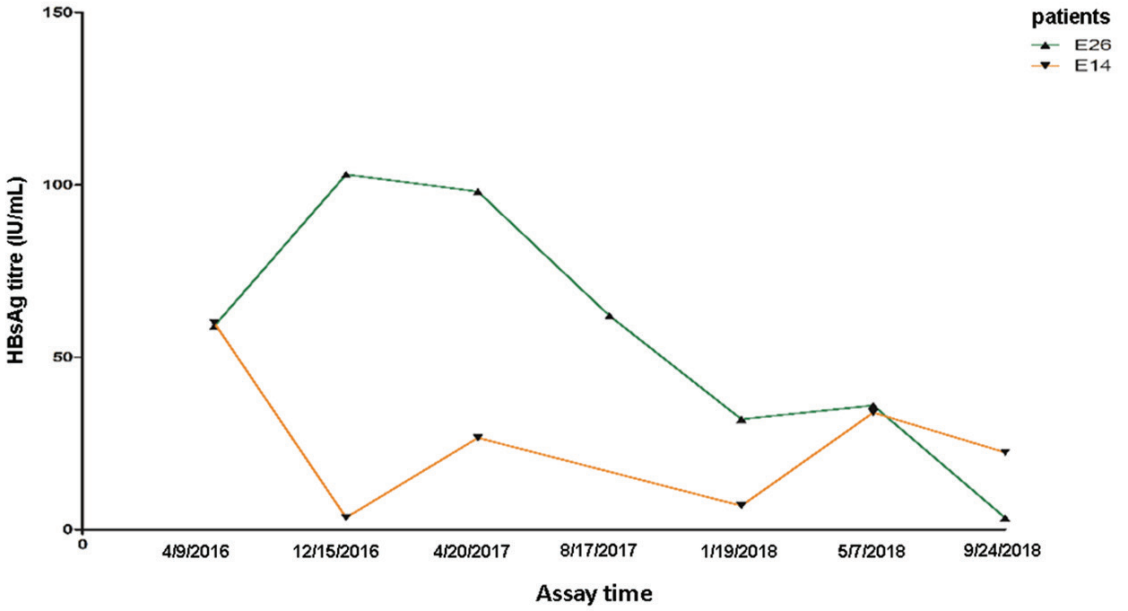

Figure 8 Change of trend direction during and after Malaysia election for 2 patients. Treatment effective patients Nos. E26 and E14 experienced increase of serum HBsAg titre from a respective low of 32 IU/mL and 6.91 IU/mL to 36 IU/mL and 33.9 IU/mL possibly due to stress resulting from the political syndrome. Resumption of downward trend continued to 22.4 IU/mL and 3.4 IU/mL respectively for them after the election.

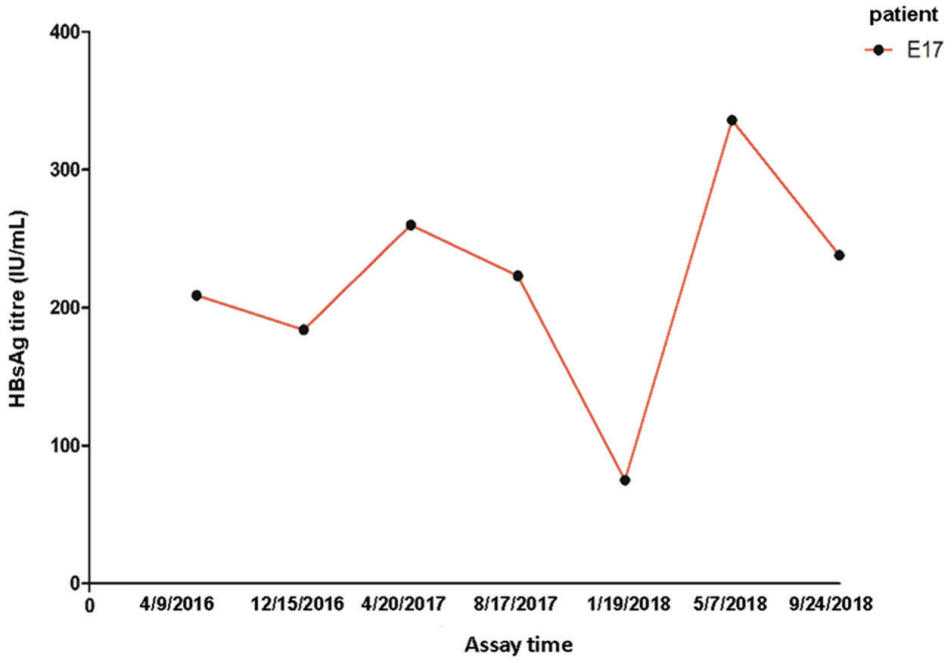

Figure 9 Change of trend direction during and after Malaysia election for patient No. E17. Upward and downward serum HBsAg titre reduction was very dramatic for treatment effective patient No. E17: increased to 336 IU/mL from a low of 75 IU/mL during the election. It decreased slightly to 238 IU/mL after the election.

Induction of endogenous cytokines IFN-α, IFN-γ, TNF-α and/or TGF-β_1 by TCM

Clinical cytokine measurement of IFN-α, IFN-γ, TNF-α and/or TGF-β_1 were carried out in April, 2018 for the 53 patients with their letters of consent.

It was observed that 94.3% (33/35) of the effective patients tested were found to have at least 1 cytokine induced, out of the 4 cytokines emphasized above. The patient from Indonesia missed the tests. Besides, 5.9% (1/17) of the non-effective patients was found to have a cytokine induced. The Cramer's V value of 0.858 signified high correlation between those 2 sets of data showed in Table 6 and the difference is very significant (***p<0.0001 by Fisher Exact test, as showed in Figure 10).

TCM could induce more than 1 cytokine simultaneously for the same patient during the course of treatment with 31.4% (11/35) and 22.8% (8/35) of the effective patients had 2 and 3 cytokines simultaneously induced, respectively. Among them, 1 effective patient extraordinarily succeeded in inducing 4 cytokines, resulting in 90.3% of serum HBsAg reduction, demonstrating synergistic effects of different cytokines. Another patient with 2 cytokines induced, the resulting serum HBsAg reduction was 99.56%, and the one with 3 cytokines, the rate of serum HBsAg reduction was 99.73%.

Some non-effective patients managed to have their serum HBsAg levels reduced, without the presence of tested cytokines. One plausible explanation for this phenomenon is the induction of IFN-Λ which was not assayed in the current study.

Table 6 Difference in number of patients succeeded in cytokine induction between the effective and non-effective groups

	cytokine induction			
	patients with (%)	patients without (%)	Total (%)	p-value
effective group	33 (94.3)	2 (5.7)	35 (100)	<0.0001
non-effective group	1 (5.9)	16 (94.1)	17 (100)	
total	34 (64.2)	18 (35.8)	53 (100)	

Coefficient of correlation Cramer's V value of 0.858 signified high correlation between those two sets of data.

Around 94.3% (33/35) of patients in the treatment effective group had at least one cytokine induced, out of the 4 cytokines emphasized, versus 5.9% (1/17) for the non-effective ones. The difference was very significant, ***p<0.0001 by Fisher Exact test. Coefficient of correlation Cramer's V value of 0.858 signified high correlation between those two sets of data.

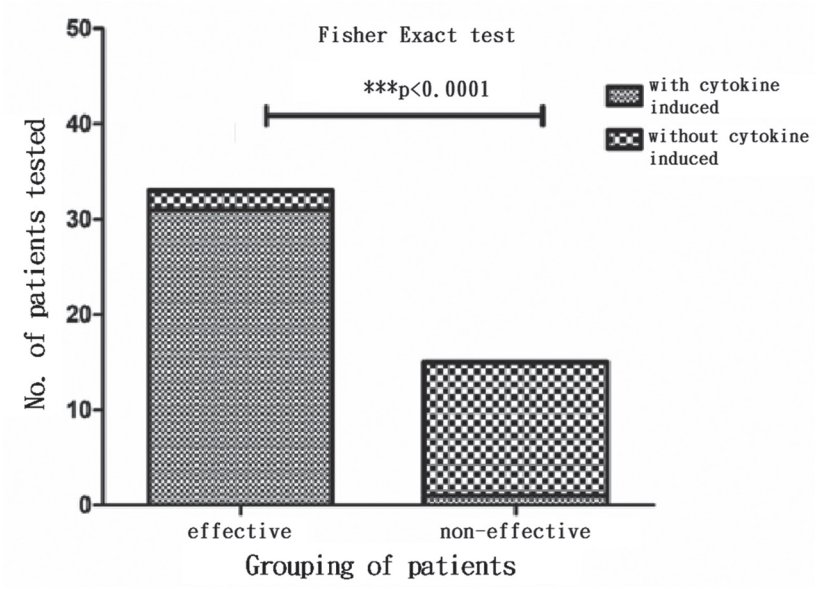

Figure 10 Difference in number of patients succeeded in cytokine induction between the effective and non-effective groups. This figure was based on Table 6, showing 94.3% (33/35) of the treatment effective patients succeeded in inducing cytokines in varied number, versus 5.9% (1/17) of the non-effective group. Difference was very significant, ***p<0.0001 by Fisher Exact test. Coefficiant of Cramer's V value of 0.858 signifies high correlation between those two sets of data.

Discussion

Chronic hepatitis B infection remains to be a serious global health problem with an estimated 250 million infected individuals worldwide in 2018. NAs could control replication of HBV by targeting reverse transcription of the virus, but, rarely reach treatment endpoint of HBsAg seroclearance. IFN-α has direct antiviral function as well as immune modulatory effects, may result in serum HBsAg loss, but, its clinical application is constrained by sometimes intolerable side effects. Besides, IFN-α is less effective among Asian patients for unknown reason. Potential pharmacological developments are in early preclinical or early clinical trials. Therapeutic application would be possible only after resolving the problem of off-target effects and targeted delivery system to the infected hepatocytes. It would still be quite some time to realize a true cure of chronic hepatitis B and problems of chronic hepatitis B carriers linger on.

It has been demonstrated that antiviral cytokines IFN-α, IFN-γ, IFN-Λ, TNF-α and TGF-β_1 could trigger the degradation of the HBV transcription template in the nucleus of the infected hepatocytes, cccDNA, non-cytolytically, in different experimental modalities. Only IFN-α has been licensed for therapeutic application thus far. However, the cccDNA purging mechanism of antiviral cytokines provides valuable insight into developing other means of treating HBV. Activation of cytokines in the liver is an option currently explored. Small molecule toll-like receptor (TLR) agonists have been developed for this purpose. However, its therapeutic effect could not be hepatocyte-specific due to TLR-7 expression profiles, and further clinical trials are warranted.

Our current study demonstrated that TCM,

formulated under the guiding principle of dynamic equilibrium of ying and yang, could induce endogenous cytokines IFN-α, IFN-γ, TNF-α and/or TGF-β_1. Besides, more than 1 cytokine could be induced simultaneously with synergistic effects. Correlation of cytokine induction between effective and non-effective groups of patients treated with TCM is high and the difference is very significant.

That being said, we can now conclude that the mechanism of TCM reaching the treatment endpoint is induction of endogenous cytokines IFN-α, IFN-γ, TNF-α and/or TGF-β_1. They are safe and effective in leading to reduction of serum HBsAg levels and the possible mechanisms such as degradation of cccDNA need to be explored in the future. However, to be effective, the TCM has to be formulated under the guiding principle of dynamic equilibrium of ying and yang.

Needless to say, the delicate state of dynamic equilibrium of ying and yang is not that easy to achieve and maintain, but, not beyond reach. The road to recovery thus abounds with poor performers due to their inability to reconcile with themselves as well as the environment and make necessary adjustments.

One other possible mechanism of TCM treating chronic HBV infection effectively would be invigorating kidney and strengthening spleen under the guiding principle of dynamic equilibrium of ying and yang. But that needs further study to ascertain.

Besides taking TCM regularly, practicing qigong, meditation, yoga etc. are observed to have additive or complementary effect. This warrants further study, however.

It is interesting to note in passing that TCM might also activate the innate immune system to control and eliminate HBV in a non-cytolytic manner, through the induction of IL-4 and IL-6 known to be able to control transcription of HBV-RNAs. Those two cytokines could work in concert to augment the effect of before-mentioned endogenous cytokines induced to control and eliminate HBV safely.

Even before proving that TCM could have other functions in controlling HBV, based on the before-mentioned key findings and discussion, we suggest that TCM is a novel strategy to successfully treat chronic hepatitis B infection.

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原始論文

傳統中醫藥誘生內源性細胞因子導致血清 HBsAg 水平之下降

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慢性 B 型肝炎病毒（HBV）感染持續為一種嚴重的世界性健康衛生問題，而現有的治療方案鮮能達致清除血清 HBsAg，同時產生或未產生 Anti-HBs 的治療終點。本報告觀察到在陰平陽謐指導原則下，傳統中醫藥（TCM）治療慢性 B 型肝炎感染導致血清 HBsAg 水平顯著下降。細胞因子的臨床實驗表明血清 HBsAg 水平成功降低的病患即那些誘生了一種或多種內源性細胞因子 IFN-α, IFN-γ, TNF-α 及 TGF-β_1。而那些細胞因子已知能導致受感染肝細胞核中的 HBV 複製模板 cccDNA 的降解，但不傷及肝細胞。我們因而論定 TCM 是一種能有效治療慢性 B 型肝炎感染的新穎策略。

關鍵字：傳統中醫藥、慢性 B 型肝炎、閉鎖環狀共價鍵去氧核醣核酸、內源性細胞因子

利益衝突：作者群表示無利益衝突。

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