

Full Length Research Paper

Clinical study on the treatment of infant cytomegalovirus (CMV) hepatitis intervened by traditional Chinese medicine

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To evaluate the efficacy and safety of Chinese medicine (CM) in treating infantile cytomegalovirus hepatitis (ICH), a total of 43 infant ICH patients were randomly divided into two groups, 22 in the treatment group and 21 in the control group. The treatment group: patients were treated with Qinggan Lidan decoction (QLD) during icteric stage and Yigan Jiangmei decoction (YJD) in non-icteric hyperaminotransferase stage by oral medication. At the same time, ganciclovir was administered to the patients via intravenous dripping in dose of 5 mg/kg every 12 h for two weeks, followed by 5 mg/kg once a day for five days every week; the whole treatment course lasted 4 weeks. The control group: apart from the Ganciclovir (same to the treatment group), they took glucuro lactone of 50 mg three times per day. The efficacy of treatment was evaluated at the ends of 2nd and 4th weeks, respectively. The total effective rate was 95.45% in the treatment group and 71.43% in the control group after 2 weeks treatment; the total effective rate was 95.45% in the treatment group and 66.67% in the control group after 4 weeks treatment, so the overall curative effect in the former was superior to that in the later, showing significant difference ($P=0.021$, $P<0.05$). In case of the rate of cytomegalovirus (CMV)-DNA negative-inverted, the treatment group was 85.71% at the 4th week, and the control group was 86.67% ($P=0.941$, $P>0.05$). There are no distinct differences in the rate of CMV-DNA negative-inverted for both groups, however, in the aspects of relieving cholestasis and reviving the liver function, the treatment group was better than the control group, this was further proven by professor PEI Xue-yi's, the well-known CM doctor, experienced in clinical treatment of ICH, focused on jaundice in the earlier stage and on the increase of hepatic enzyme in the convalescence. There are two stages of the treatment: smoothing cholecyst to degrade jaundice and reinforcing liver to drop enzyme. At the stage of icterus, patients may take orally QLD; when the jaundice fades away, but the transaminase is still high, the patients may take YJD. This traditional Chinese medicine (TCM) therapy works better than the pure western conventional medicine, and clinical results show that this scheme improves the prognosis of ICH; so, this therapy is a good therapy approach for the patients of ICH.

Key words: Infantile cytomegalovirus hepatitis (ICH), Chinese medical intervened therapy, traditional Chinese medicine, treatment.

INTRODUCTION

Infantile cytomegalovirus hepatitis (ICH) is a liver disease caused by human cytomegalovirus infection in infantile

period, being the most frequently encountered virus hepatitis in newborn and infant, and the morbidity is around 60% in all infant hepatitis. The main symptoms are jaundice, hepatomegalia, splenomegalia, and abnormal liver function, with a high clinical morbidity reaching 15 to 17%. Bristow et al. (2011) opined that treatment of

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ICH is still difficult up to date, and uniform therapeutic program is lacking. A randomized controlled study was carried out by the authors to observe the clinical efficacies and related laboratory parameters change of Chinese medicine (CM) in treating ICH from January 2011 to February 2012. This study verified further the CM experienced recipes and affirmed their effectiveness in treating ICH. Meanwhile, the relationship between CM drugs therapy with cholestasis improvement and liver function recovery was probed in all sides to find out preliminarily the best therapeutic project.

MATERIALS AND METHODS

The cases came from the infant patients of the CM department of Beijing Children's Hospital from January 2011 to January 2012. Diagnosis standards of ICH were referred to the "Diagnostic project of cytomegalovirus infection" manufactured at November 1998 by Association of Academy of Pediatrics (Chin, 1999). At last, one of the cytomegalovirus (CMV)-DNA and CMV-immunoglobulin M (IgM) was positive, and clinical types were accorded with icteric hepatitis, cholestatic hepatitis, and cholestatic without icteric hepatitis (Hu et al., 2009). Chinese medicine syndrome classification was referred to as "Traditional Chinese Medicine Diagnosis of Disease and Syndrome Curative Standard, Paediatric Department of Traditional Chinese Medicine Diagnosis of Disease and Syndrome Curative Standard" manufactured by State Administration of Traditional Chinese Medicine. There were 43 cases in total, who were randomly assigned into two groups, 22 in the treatment group and 21 in the control group. There were 27 boys and 16 girls with a ratio of 1.6:1; the smallest was 34 days, the biggest was 145 days, and the average age was 73 ± 11.4 days; the shortest course was 5 days, the longest one was 120 days, and the average course was 57 ± 5.3 days. There were no statistically significant differences of the two groups in gender, age, course, liver function index and liver and spleen size ($P > 0.05$).

Cytomegalovirus hepatitis clinical disease and syndrome features

Admission situation

Comparative analysis of the two groups showed that the two groups came from the same community ($P > 0.05$), so the comparative study of clinical treatment is acceptable (Table 1).

First symptoms

Among the 43 infant patients, 37 came into the hospital because they had stained yell on their skin, about 86.1% of the patients' first symptom was stained yell, 4 cases suffered from fever, and 2 cases, cough. While 6 cases did not suffer from stained yell, after being in the hospital, then further examination of abnormal liver function made a definite diagnosis that they got cytomegalovirus hepatitis.

Defecate color

Among the 43 cases, 4 cases were white clay, 5 cases were yellow and white, and the others were yellow. We ruled out the possibility of extrahepatic biliary atresia by the tests of abdominal B ultrasound, nuclear magnetism and radionuclide hepatobiliary

among the abnormal 9 cases.

Amalgamative diseases

ICH has clinical characteristics of various systemic damage, including respiratory system, digestive system, circulation system, blood system, and nervous system (Table 2).

Observe index

Clinical curative effect observation index

The clinical observation includes changes in jaundice color of skin, sclera, urine and stool; character and size of liver and spleen; laboratory indexes of liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), total bilirubin (TBIL), direct bilirubin (DBIL), and serum total bile acid (TBA).

Safety observation index

Routine tests of blood, urine and stool; functions of heart, liver and kidney; as well as adverse reactions were examined for safety monitoring.

Treatment method

The treatment group: traditional Chinese medicine (TCM), according to the Chinese medicine dialectical stage, was united with western medicine comprehensive treatment. The patients were treated with QLD during icteric stage and YJD in non-icteric hyperaminotransferase stage by oral medication. QLD was prescribed based on the principle of mainly clearing damp-heat, activating blood to remove stasis with cholericics to alleviate jaundice; the prescription was *Fructus Hordei Germinatus* of 9 g, *Herba Artemisiae scopariae* of 12 g, *Medulla Tetrapanacis* of 3 g, *Herba Lysimachiae* of 9 g, *Herba Lycopi* of 9 g, *Cortex Phellodendri* of 3 g, *Radix Salviae miltiorrhizae* of 9 g, *Poria* of 9 g, *Rhizoma Atractylodes alba* of 4 g, in addition, *Indigo Naturalis*, *Resina Draconis*, *Alumen* and *Succinum* each of 0.3 g, swallowed with water; YJD was prescribed according to principles, mainly for dispelling dampness, removing toxic substances, activating blood to dredging collaterals, dispersing Liver(Gan)-qi and evacuating nodules; the prescription was *Indigo Naturalis* of 3 g, *Radix Arnebiae seu Lithospermi* of 9 g, *Herba Taraxaci* of 9 g, *Semen Coicis* of 15 g, *Herba Patriniae cum Radice* of 9 g, *Rhizoma Smilacis Glabrae* of 9 g, *Rhizoma Dryopteris Crassirhizomae* of 9 g, *Herba Portulacae* of 9 g, scrap iron of 9 g, and *Herba Hedyotis diffusae* of 9 g. The herbal drugs were boiled with water, and were taken one dose three times every day. At the same time, Ganciclovir was administered to the patients via intravenous dripping in dose of 5 mg/kg every 12 h for two weeks, followed by 5 mg/kg once a day for five days every week; the whole treatment course lasted 4 weeks. The control group: apart from the Ganciclovir (same to the treatment group), they took glucurrolactone 50 mg three times per day. The efficacy of treatment was evaluated at the ends of 2nd and 4th weeks, respectively.

Statistics method

Adopting Statistical Package for Social Sciences (SPSS) 11.5 software, data were expressed by mean \pm standard deviation ($\pm s$); the comparison between groups was conducted by t-test, and the

Table 1. Comparison of the basic condition about the two groups.

Group	N	Score			Bilirubin (umol/L)			ALT (U/L)		
		Average	Standard deviation	P	Average	Standard deviation	P	Average	Standard deviation	P
Treatment	22	11.5	1.07	0.9045	108.48	14.0	0.3738	128.09	26.58	0.0991
Control	21	11.4	1.2		101.48	17.5		129.24	18.70	

Table 2. Amalgamative diseases of the 43 cases.

S/N	Amalgamative diseases	NNT	Percentage
1	Patent foramen ovale	30	69.77
2	Ventricular septal defect	1	2.33
3	Patent ductus arteriosus	1	2.33
4	Alveobronchiolitis	25	58.14
5	Left room inside diameter increase	1	2.33
6	Myocardial damage	5	11.63
7	Bronchitis	4	9.30
8	T thrush	7	16.28
9	Dysaudia	13	30.23
10	Granulocytopenia	6	13.95
11	Agranulocytosis	1	2.33
12	Moderate anemia	6	13.95
13	Infantile diarrhea	4	9.30
14	Abnormal head CT	12	27.91

comparison of rates was performed by χ^2 test; $P < 0.05$ was regarded as the difference having statistical significance.

The comparison of two groups' quantitative data was checked with t-test or rank-sum test; the comparison in each group' quantitative data was checked with χ^2 ; $\alpha < 0.05$ was the significant inspection standard.

RESULTS

Standard for efficacy assessment

As regard the "Standard for diagnosis and efficacy evaluation of CM diseases and syndromes, Pediatrics", effectiveness of treatment was divided into 4 grades: cured, which means jaundice color in skin and sclera completely faded away with full normalized liver function; markedly effective, which means jaundice color in skin and sclera completely faded away with obvious normalized liver function; effective, which means jaundice color in skin and sclera faded away to certain degree with a few restoration of liver function; and ineffective, which means not reaching the aforementioned criteria.

Treatment results

Total curative effect evaluation

The treatment group: total effective rate was 95.45%,

cure rate 27.27%, markedly effective rate 40.91%, effective rate 27.27%, and ineffective rate 4.55%. The rates in the control group were 66.67, 9.52, 19.05, 38.10, and 33.335% respectively. $F = 0.034$, efficacies between the two groups were different significantly, and the overall effectiveness in the treatment group was better than that in the control group (Table 3).

Comparison of reduction of bilirubin levels

The treatment group is shown in Table 4, and the rank-sum test of the two groups could draw $Z = 0.0543$ more than $Z = 0.05$; no statistical significance was observed, and the curative effect of dropping bilirubin for the two groups were equal in the 4th weeks. However, the average of bilirubin reduction in the treatment was (91.43 umol/L) higher than that in the control group (51.61 umol/L), showing that the range of bilirubin reduction in the treatment was greater (Table 4).

Comparison of stained yellow on skin and sclera

It was different between the two groups in the treatment of stained yellow on skin and sclera in the 2nd week, and the rate of stained yellow reduction in the treatment group was apparently higher than that in the control group.

Table 3. Comparison of total effectiveness between two groups after 4 weeks.

Group	Cured (%)	Markedly effective (%)	Effective (%)	Ineffective (%)	Total effective (%)	F
Treatment	6 (27.27)	9 (40.91)	6 (27.27)	1 (4.55)	95.45	0.0343
Control	2 (9.52)	4 (19.05)	8 (38.10)	7 (33.33)	66.67	

Table 4. Comparison of bilirubin reduction between two groups after 2 weeks and 4 weeks.

Group	N	2 weeks			4 weeks		
		Average (umol/L)	S	Z	Average (umol/L)	S	Z
Treatment	22	85.79	15.32	0.0062 (<0.05)	91.43	12.22	0.0543 (>0.05)
Control	21	37.65	17.51		51.61	13.56	

Table 5. Comparison of stained yellow reduction between two groups after 2 weeks and 4 weeks.

Group	2 weeks (P=0.0448) (<0.05)				4 weeks (P=0.2691) (>0.05)			
	Fade away	No.	Total	Rate (%)	Fade away	No.	Total	Rate (%)
Treatment	11	8	19	57.89	16	3	19	84.21
Control	4	14	18	22.22	12	6	18	66.67

Table 6. Comparisons of liver shrink between the two groups after 2 weeks and 4 weeks.

Group	N	2 weeks		4 weeks	
		Average (cm)	Z	Average (cm)	Z
Treatment	17	1.1±0.38	0.1590 (0.05)	1.9±0.23	0.0227 (0.05)
Control	13	0.9±0.21		1.0±0.17	

Curative effect of stained yellow reduction of two groups was equal in the 4th week. In conduction, the course of stained yellow reduction in the treatment was less than that in the control group (Table 5).

Comparison of liver shrink checked by B ultrasound

In two weeks, there was no statistical significance, showing that curative effect of liver shrink of the two groups was not distinct apparently. In the 4th week, the effective rate of curative effect of liver shrinks in the treatment was higher than that in the control group, and with Chi-square test, $Z=0.0227$, <0.05 , there was statistical significance, showing that curative effect of liver shrink of two groups was different, and the treatment group was senior to the control group (Table 6).

Comparison of spleen shrink checked by B ultrasound

Comparisons of spleen shrink checked by B ultrasound after 2 weeks and 4 weeks showed that there was no

statistical significance, so it could be concluded that curative effect of spleen shrink of the two groups was not distinct apparently (Table 7).

Comparison of ALT reduction between the two groups

In two weeks, the rank-sum test of ALT reduction showed that $Z=0.4202$ was more than 0.05, no statistical significance was observed, so the curative effect of reducing ALT of the two groups was not distinct apparently. In four weeks, the rank-sum test of ALT reduction showed that $Z=0.0437$ which was less than 0.05, thus the curative effect of reducing ALT of the two groups was distinct. The average of ALT reduction in the treatment group was 75.16 U/L, 51.63/L in the control group, showing that the curative effect of ALT reduction in the treatment was greater (Table 8).

Comparison of CMV-IgM and CMV-DNA negative-inverted rate of the two groups after 4 weeks

The CMV-IgM and CMV-DNA negative-inverted rates of

Table 7. Comparison of spleen shrinks between two groups after 2 weeks and 4 weeks.

Group	N	2 weeks		4 weeks	
		Average (cm)	Z	Average (cm)	Z
Treatment	8	1.3±0.42	0.9606 (>0.05)	2.1±0.37	0.7358 (>0.05)
Control	11	1.4±0.27		1.5±0.43	

Table 8. Comparison of ALT reduction of two the groups after 2 weeks and 4 weeks.

Group	N	2 weeks			4 weeks		
		A (U/L)	S	Z	A (U/L)	S	Z
Treatment	22	58.96	17.73	0.4202 (>0.05)	75.16	23.46	0.0437 (<0.05)
Control	21	41.66	13.55		51.63	18.45	

Table 9. Comparison of CMV-IgM negative-inverted rate of the two groups after 4 weeks.

Group	Negative-inverted	No change	In total	Rate (%)	P
Treatment	10	8	18	55.56	0.2964
Control	5	10	15	33.33	

Table 10. Comparison of CMV-DNA negative-inverted rate of the two groups after 4 weeks.

Group	Negative-inverted	No change	In total	Rate (%)	P
Treatment	12	2	14	85.71	0.9418
Control	13	2	15	86.67	

the two groups were not distinct apparently, and Chi-square test showed that, both P of the two groups were more than 0.05, showing that curative effect of CMV-IgM and CMV-DNA negative-inverted rate of two groups was not distinct apparently after 4 weeks treatment (Tables 9 and 10).

Adverse reaction and results of following-up study

There were no obvious adverse reactions in two groups. There were 3 cases in the treatment group whose stool frequency increased apparently, and after they took smecta and zhengchangsheng, the symptom was in control. There was 1 case in the control group whose granulocyte decreased, and after he took the leucogen, his granulocyte level became normal.

DISCUSSION

The incidence of infantile cytomegalovirus hepatitis (ICH) is rather higher, accounting for 62.86% in infantile hepatitis syndrome. The clinical symptoms appear very early. Foreign investigation showed that the via-placental

dissemination rate of CMV was 20 to 40% (Hu et al., 2009); most of the congenital infections originated from the re-activation of latent virus; while in 50% newborns, it may be caused by fetus gulping down the infected reproductive organs discharge from mother in labor. Besides, breast feeding is regarded as another path of peri-natal period infection; about 32% mothers with viremia could secrete virus into breast milk to form viro lactia. The infection of baby is closely correlated with the DNA load in milk (Klaus et al., 2005); the infected rate in infants fed with virus-contaminated breast milk may reach 69%. Although, the virus titer in mother's milk was generally rather low, infection could yet occur through long-term feeding due to the accumulative effect, especially threatening premature infant or newborn of low birth weight. The clinical symptoms may be present in 10 to 15% congenital ICH patients, and severe symptoms frequently appeared in the infants whose mother suffered from primary CMV infection (Soley et al., 2007). Liver is an organ liable to be involved by CMV infection; the main manifestations of ICH are jaundice, hepatomegaly, splenomegaly, and liver function abnormality. In the progress of the disease, further developed pericholangitis could produce fibrous degeneration, which blocks the bile duct gradually, leading to bile excreting path obstruction

and finally the secondary biliary atresia and cholestasis cirrhosis. CMV-IgM and CMV-DNA test are common methods of diagnosis, and conventional polymerase chain reaction (PCR) detection CMV-DNA is simple and quick. But it is easy to lead false positive results (Wang and Mao, 2007). Now we mainly use the real-time fluorescent quantitative fluorescence PCR method, dynamically monitoring the CMV in the body (Yang, 2006). A research has shown that (Lazzarotto, 2010; De Tommaso et al., 2005), we can detect free virus and cells virus from the whole blood samples which is used for HCMV-DNA quantitation to improve the sensitivity, and it can reflect the active level of virus replication.

Treatment of ICH is still difficult up to date. Ganciclovir is the commonly chosen anti-viral agent. Its long time application can bring about drug-resistant virus strain, presenting the early descent but late ascent of CMV copy amount. Uniform of dosage and therapeutic course for applying ganciclovir are still lacking so far at home and abroad; eight weeks or so is the recommended period (Hu and Jiang, 2005). Although, ganciclovir could improve the clinical symptoms of ICH, it is disregarded in clinical practice due to its inconvenient administration (must be given by intravenous dripping in hospital), since the bioavailability and effectiveness of its dosage-form for orally taken are very low. Additionally, long-term medication of ganciclovir would bring about leucopenia and drug-resistance, thereby it could not satisfy the requirement of ICH treatment, and a chronic course needs long time therapy.

The well-known CM doctor, PEI Xue-yi has been engaged in clinical study of ICH since early 1970s. Through probing in the characteristics of clinical symptoms, prognosis and outcomes of ICH, he formulated the two experienced recipes, QLD and YJD, for the treatment, which had good curative effect. This study showed that the total effective rate in the treatment group was 95.45%, apparently higher than that in the control group. In the treatment group the serum bilirubin dropped faster and greater so that the cirrhotic risk caused by long-hour cholestasis, and liver damage could be reduced, and the prognosis of ICH was improved. It could say that ME had taken some effects in the treatment of ICH, further proving professor PEI Xue-yi's, the well-known CM doctor, clinical treatment experiences of infantile cytomegalovirus hepatitis, which are focused on jaundice in the earlier stage and then on the increasing of hepatic enzyme in the convalescence. There are two stages of the treatment: smoothing cholecyst to degrade jaundice and reinforcing liver to drop enzyme.

CM holds that the main symptom of ICH is jaundice in the early stage, and the basic pathogenesis is inheritance of dampness-heat poisonous substances from mother to fetus, which affects liver (Gan)-gallbladder (Dan) to cause bile overspill, causing stained yellow on skin. The color of yellow likes orange, belonging to TCM jaundice of Yang-Huang; or due to Qi-Xue deficiency of mother and

connatural deficit of fetus, spleen(Pi)-deficit induced dampness to cause cold-dampness blocked Middle-Jiao, Liver purging function disability, bile juice overflow, causing stained yellow on skin, thus forming jaundice. The color of yellow is dam, likes being fumigated by smoke, belonging to TCM jaundice of Yin-Huang. If the jaundice could not be cured after a long time, it becomes more serious, and the colour becomes yellow and black with abdominal fullness and distention, and breast hard lump.

The pathogenic factor would develop deeply, damage the blood vessels, and block the channels and collaterals, showing the symptom of blood stasis yellow.

In the recovery stage, most of the infant patients' jaundice fades away; however, the level of liver enzyme rises up repeatedly, so the dampness-heat poisonous substances stay in the body, fighting for many days, congesting the liver meridian, and inhibiting the liver function of catharsis. QLD, which was used in the jaundice stage, was prescribed mainly by the principle of clearing damp-heat, activating blood to remove stasis with cholericotics to alleviate jaundice; the prescription was *Fructus Hordei Germinatus*, *Herba Artemisiae scopariae*, *Medulla Tetrapanacis*, *Herba Lysimachiae*, *Herba Lycopi*, *Cortex Phellodendri*, etc. If the infant patients suffered from cholestasis, the main symptoms would be dam yellow and light yellow or ashen defecate, this prescription should plus *Herba Lycopi*, *Radix Salviae miltiorrhizae*, *Resina Draconis*, and *alum* to activate blood, remove stasis, dispel stagnancy and resolve lump. If there were abdominal apophysis, blood vessel exposure, and obvious enlargement of liver and spleen, this prescription should plus safflower, semen persicae, pericarpium arecae and carapax trionycis, activating blood to dredging collaterals and evacuating nodules. Yigan Jiangmei decoction (TJD) was used in recovery stage (non-icteric hyper-aminotransferase stage) for dispelling dampness, removing toxic substances, activating blood to dredging collaterals, dispersing liver-qi and evacuating nodules, in which, all the herbal drugs used are *Indigo Naturalis*, *Radix Arnebiae seu Lithospermi*, *Herba Taraxaci*, *Semen Coicis*, *Herba Patriniae cum Radice*, *Rhizoma Smilacis Glabrae*, *Rhizoma Dryopteris Crassirhizomae*, *Herba Portulacae*, *scrap iron*, *Herba Hedyotis diffusae*, etc. Modern pharmacological research approved that *Fructus Hordei Germinatus*, *Rhizoma Dryopteris Crassirhizomae*, *Herba Taraxaci*, *Herba Viola*, *Rhizoma Polygoni Cuspidati*, *Radix Arnebiae seu Lithospermi*, and *Herba Hedyotis diffusae* can reduce the level of aminotransferase, cure acute/chronic hepatitis; *Herba Artemisiae scopariae*, *Herba Lysimachiae*, *Cortex Phellodendri*, *Medulla Tetrapanacis*, *Herba Lycopi alum*, etc., have the tendency of promoting bile secretion and excretion, can suppress inflammatory response, improve microcirculation, dilate vessels, inhibit blood coagulation, advance liver cell regenerating, recover the affected hepatocytes and

anti-fibrosis. In the treatment process of infantile cytomegalovirus hepatitis, the TCM has many advantages (Jiangsu new medical college, Chinese medicine dictionary, 1995); however, the mechanism and principle about TCM treatment of cytomegalovirus hepatitis are waiting for further study.

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