Dynamic relationship between infantile hepatitis syndrome and cytomegalovirus infection

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Abstract. We investigated the correlation between cytomegalovirus (CMV) infection and infantile hepatitis syndrome and the correlation between blood ammonia levels in children with CMV-induced hepatitis syndrome and liver function indicators. To analyze the relationship between the positive-negative attributes of CMV infection and the recurrence rate of infantile hepatitis syndrome, a total of 86 cases of children with hepatitis syndrome admitted to Xuzhou Children's Hospital from January 2014 to March 2015 were selected for the study group. Furthermore, 86 cases of healthy children who visited our hospital for a physical examination during the same period were selected as the control group. From the two groups, serum CMV-immunoglobulin M (IgM) levels were determined via enzyme-linked immunosorbent assay, and urinary CMV-deoxyribonucleic acid (DNA) was ascertained by fluorescent ratio polymerase chain reaction. These levels were then compared between the two groups and analyzed. A fully automatic biochemical analyzer was utilized to evaluate the blood ammonia and liver function indicators of the children with infantile hepatitis syndrome induced by CMV infection and to analyze the correlation of these factors. A mean follow-up of 12 months after the children's discharge was adopted to observe the relationship between the negative-positive attributes of CMV infection and the recurrence rate in the children upon cure. The positive detection rate for the serum CMV-IgM was 24.4%, and that for the urinary CMV-DNA was 34.9%; both values were significantly higher than that for the control group ($P<0.05$). The blood ammonia levels of the children with infantile hepatitis syndrome induced by CMV infection were not correlated with the liver function indicators, such as total bilirubin, direct bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, total bile acid, and cholinesterase ($P>0.05$), but they were negatively correlated with blood albumin (ALB) ($P<0.05$). The recurrence rate of hepatitis syndrome among the children with negative CMV infection was 3.8%, which was significantly lower than that among the children with positive CMV infection (62.5%, $P<0.05$). A significant correlation was found between CMV infection and infantile hepatitis syndrome, with the former being a risk factor for the latter. Changes in the conditions of infantile hepatitis syndrome may be reflected by blood ammonia and ALB indicators. Through improved monitoring, these indicators facilitate the early diagnosis and treatment of children with hepatitis syndrome induced by CMV infection. Sufficient attention should be paid to preventive measures to reduce the incidence rate of infantile hepatitis syndrome.

Introduction

Infantile hepatitis syndrome is a relatively common hepatitis disease with the main symptoms being jaundice and liver and spleen enlargement (1). Once infantile hepatitis syndrome attacks, the disease should be timely and appropriately treated. Otherwise, the disease may progress to liver failure and thus seriously worsen children's quality of life. Many infectious viruses may induce infantile hepatitis syndrome; these viruses include the rubella virus, cytomegalovirus (CMV), and herpes simplex virus (2-4). This investigation revealed a correlation between CMV infection and infantile hepatitis syndrome. Further correlation between blood ammonia and liver function indicators in pediatric patients with CMV-related infantile hepatitis syndrome is determined to provide valuable reference for the early diagnosis and treatment of infantile hepatitis syndrome.

Patients and methods

Patient selection. A total of 86 cases of children with hepatitis syndrome admitted to Xuzhou Children's Hospital from January 2014 to March 2015 were selected as the study group. Among these patients, 47 were male and 39 were female. The subjects were aged 3-20 months (mean age, 1.2±0.2 years) and were of Chinese descent. Of the cases 32 showed endo-retention of damp heat, 45 cases showed splenasthenic fluid retention, and 9 showed 'qi' stagnation and blood stasis. The selected patients satisfied the diagnostic criteria for infantile hepatitis syndrome (5); the children with highly serious conditions, highly significant adverse reactions, and gastrointestinal

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comorbidities (liver and gallbladder) were excluded. The subjects whose serum CMV-immunoglobulin M (IgM) or urinary CMV-deoxyribonucleic acid (DNA) tested positive were diagnosed with CMV infection. A control group was established with a selection of 86 cases of healthy children who came to our hospital for a physical examination during the same period. No difference in general data was found between the two groups (P>0.05). This study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from the guardians of the participants before the study.

Methods. Evaluation of serum CMV-IgM levels: Enzyme-linked immunosorbent assay (ELISA) was employed to ascertain the serum CMV-IgM levels of the subjects in the two groups. The approach was conducted by centrifuging 2 ml of venous blood drawn from the subjects in the early morning. The supernatant was obtained and stored at 4˚C for later use. The method was carried out in strict accordance with instructions from the human anti-CMV antibody IgM ELISA kit (purchased from Shanghai Yaji Biotech Co., Ltd., Shanghai, China). IgM levels were regarded as positive if they exceeded 20 arbU/m.

Evaluation of urinary CMV-DNA: Fluorescence ratio polymerase chain reaction (PCR) was adopted to determine the urinary CMV-DNA levels of the subjects in the two groups. The test was performed by first collecting 10 ml of urine samples from the individuals and using a PCR amplification device (produced by Thermo Fisher Scientific Arktik, New York, NY, USA). The applied PCR kit was then purchased from Guangzhou Dongsheng Biotech Co., Ltd. (Guangzhou, China).

Tests on blood ammonia and liver function indicators: A fully automatic biochemical analyzer (produced by Beckman Coulter, Inc., Tokyo, Japan) was utilized to determine liver function indicators, such as ammonia, total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), total bile acid (TBA), cholinesterase (CHE), and albumin (ALB). The related products for quality control, calibration, and reagents were used.

Coloring method for liver tissue immunohistochemistry Streptavidin-perioxidase-biotin (SP) in children: After obtaining signed informed consent from the guardians, percutaneous biopsy guided by type-B ultra-sonic (provided by Wuxi Cansonic Medical Science and Technology Co., Ltd., Wuxi, China) was performed on the participating children to collect 2 cm of liver tissue. The tissue samples were then fixed with 10% formalin and prepared via paraffin sectioning. Immunohistochemistry (SP) staining was performed with a relevant kit provided by Shanghai Ke Min Biotech Co., Ltd. (Shanghai, China). Instead of a primary antibody, phosphate-buffered saline (PBS) was used as negative control while observing the distribution of CMV early antigen in liver tissue via light microscopy (magnification, x400).

Observation indicators. Positive detection rates for serum CMV-IgM and urinary CMV-DNA in the study and control groups were compared and analyzed. The correlation between blood ammonia and hepatic function indicators, such as TBIL, DBIL, ALT, AST, γ-GT, TBA, and CHE in the children with infantile hepatitis syndrome induced by CMV infection was analyzed. The children were followed up for a 1-year observation of the correlation between the negative-positive attributes of CMV infection and the recurrence rate upon cure.

Table I. Comparison of serum CMV-IgM and urinary CMV DNA between the two groups [no. (%)].

Groups	No.	Serum CMV-IgM (+)	Urine CMV-DNA (+)
Study	86	21 (24.4)[a]	30 (34.9)[a]
Control	86	1 (1.2)	1 (1.2)

[a]P<0.05 when compared with the control group. CMV, cytomegalovirus; IgM, immunoglobulin M.

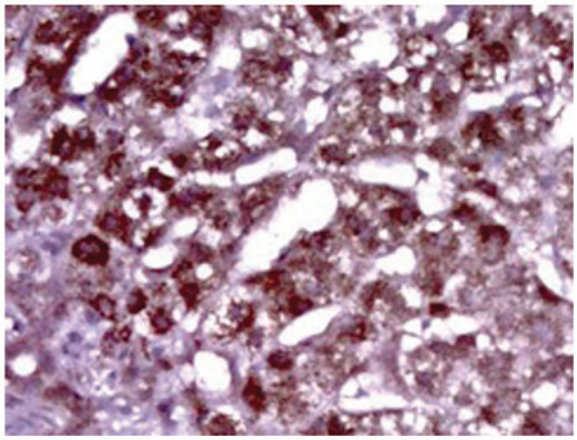

Figure 1. Distribution of cytomegalovirus early antigen in liver tissue (magnification, x400; streptavidin-perioxidase-biotin).

Statistical analysis. SPSS 20.0 (IBM SPSS, Armonk, NY, USA) statistical analysis software was utilized to process the data. Quantitative data are presented in terms of rate and then tested by χ^2 test, measurement data were expressed as mean ± standard deviation, and statistical analysis was carried out by t-test. Pearson's correlation was used in the correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of serum CMV-IgM and urinary CMV-DNA in the two groups. In the study group, the positive detection rate for serum CMV-IgM was 24.4%, and that for urinary CMV-DNA was 34.9%; both values were significantly higher than those in the control group (P<0.05; Table I).

Distribution of CMV early antigen in liver tissue. CMV antibodies were mainly located in the nucleus of the antigen-positive cells and with a small amount of cytoplasmic staining; the epithelial cells of the portal area and vascular endothelial cells were involved (Fig. 1). Fig. 2 presents the negative control.

Table II. Correlation between blood ammonia levels in children with hepatitis syndrome induced by CMV infection and liver function indicators (n=30).

Parameters	Blood ammonia TBIL (μmol/l)	Blood ammonia DBIL (μmol/l)	Blood ammonia ALT (μ/l)	Blood ammonia AST (μ/l)	Blood ammonia AST (γ-GT)	Blood ammonia TBA (μmol/l)	Blood ammonia CHE (μ/l)	Blood ammonia ALB (g/l)
Pearson relevance quotient	0.192	0.213	0.056	0.161	0.087	0.012	-0.152	-0.265
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05

TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; TBA, total bile acid; CHE, cytomegalovirus; ALB, albumin.

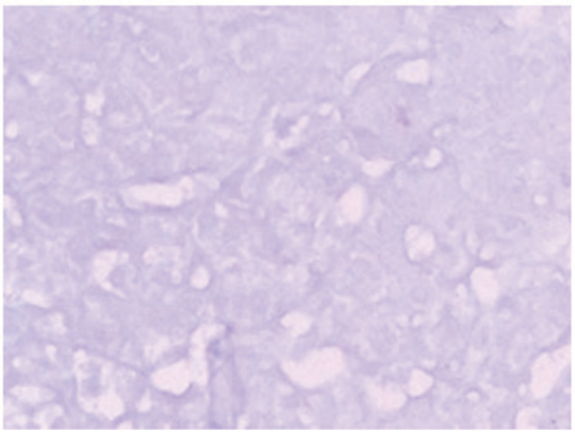

Figure 2. Negative control of phosphate-buffered saline replacing primary antibody.

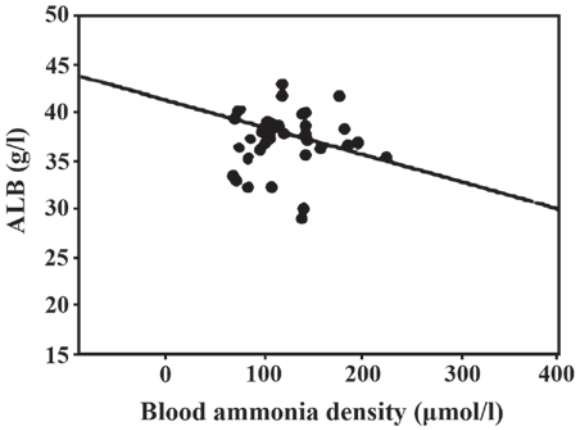

Figure 3. Correlation between blood ammonia and albumin (ALB) levels.

Correlation between blood ammonia in children with hepatitis syndrome induced by CMV infection and liver function indicators. Among the 86 cases of children with infantile hepatitis syndrome, 30 cases were diagnosed with infantile hepatitis syndrome induced by CMV infection. Through a correlation analysis of blood ammonia levels and liver function indicators, we found that blood ammonia levels were irrelevant to the levels of TBIL, DBIL, ALT, AST, γ-GT, TBA, and CHE (P>0.05), but they were negatively correlated with blood ALB (Table II and Fig. 3).

Correlation between positive CMV infection and recurrence rate among patients upon cure. All of the 86 cases were discharged after being cured, but 8 cases of hepatitis syndrome induced by CMV infection showed positive CMV test results. After hospital departure, a mean follow-up of 12 months was performed for all cases. Follow-up findings revealed that 3 out of the 78 cases with negative CMV infection experienced recurrence (accounting for 3.8%). Furthermore, 5 out of the 8 cases with positive CMV infection also experienced recurrence (accounting for 62.5%). Hence, we concluded that the recurrence rate of hepatitis syndrome in the children with negative CMV infection was significantly lower than that in the patients with positive CMV infection (P<0.05).

Discussion

Infantile hepatitis syndrome is a common infantile jaundice with complicated etiology and long course. Study (6) has shown that infectious diseases are a major factor that induces infantile hepatitis syndrome. CMV infection accounts for a large proportion of infantile hepatitis syndrome infectious diseases (7-9). If specific types of infectious diseases can be diagnosed early, corresponding antiviral drugs can be used immediately as proper remedies, thereby improving cure rates and reducing mortality.

In clinical practice, serum CMV-IgM and urinary CMV-DNA are frequently used for CMV infection in infantile hepatitis syndrome (10-13). Results of this study show that the serum CMV-IgM positive detection rate was 24.4% and that the urinary CMV-DNA positive detection rate was 34.9% in the study group. Both detection rates were significantly higher than those in the control group ($P<0.05$) and can serve as evidence of the close correlation between CMV infection and infantile hepatitis syndrome. In particular, the positive rate in urine CMV-DNA was significantly higher than that in the serum CMV-IgM ($P<0.05$). As the human urinary tract epithelial cells show a high degree of susceptibility to CMV and CMV infection in the urine is relatively stable, the positive rate of urinary CMV-DNA testing is high (14).

Under normal physiological conditions, blood ammonia levels are usually relatively low. However, some studies (15) reported that rising blood ammonia levels indicate the incidence of diseases, such as organic aciduria, uremia, liver failure, and hepatitis. The main factor inducing the rise in blood ammonia levels is the accumulation of blood ammonia that cannot be removed timely from the kidney because of the obstruction of the urea cycling pathway resulting from the difficult formation of urea from the blood ammonia released as a product of high protein metabolism in the body caused by certain damage to organic liver functions; as a result, blood ammonia levels increase (16-18). The rising blood ammonia levels gradually develop into hepatic encephalopathy by hampering normal brain function. Therefore, infantile hepatitis syndrome can be diagnosed, and its prognosis can be assessed by blood ammonia levels. In this study, the correlation between blood ammonia in children with infantile hepatitis syndrome induced by CMV infection and liver function indicators was analyzed. We found that blood ammonia levels in the children with infantile hepatitis syndrome induced by CMV infection were not correlated with liver function indicators, such as TBIL, DBIL, ALT, AST, γ-GT, TBA, and CHE ($P>0.05$) but were correlated with blood ALB ($P<0.05$). The capacity for human liver synthesis can be reflected by hemoglobin with a density that decreases as the liver synthesis ability declines. However, some research (19) reported that because of a long half-life generally ranging from 17 to 23 days, ALB does not significantly change during the early stages of liver disease. Given that the synthetic ability of the liver can be reflected by blood ammonia and that few factors affect blood ammonia levels, the condition change of infantile hepatitis syndrome can be sensitively and accurately revealed through the combined testing of blood ammonia and serum ALB.

Previously, a study (20) also showed that the probability of infantile hepatitis syndrome increases significantly after CMV infection. In the present study, we demonstrated that the recurrence rate of hepatitis syndrome in children with CMV infection was 3.8%, which was significantly lower than those with a positive CMV infection rate of 62.5% ($P<0.05$). Therefore, a close correlation was noted between positive CMV infection and recurrence of infantile hepatitis syndrome.

In conclusion, we found an obvious correlation between CMV infection and infantile hepatitis syndrome, with the former being a risk factor for the latter. Being capable of reflecting changes in the condition of infantile hepatitis syndrome, the indicators of blood ammonia and hemoglobin facilitated the early diagnosis and treatment of the children with hepatitis syndrome when monitoring was strengthened. Sufficient attention should be paid to preventive measures against CMV infection to lower the incidence rate of infantile hepatitis syndrome.

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