Relationship between prognosis of biliary atresia and infection of cytomegalovirus

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Background: **The etiology of biliary atresia is still unknown. It is generally accepted that virus infection may be one of the important causes that lead to biliary atresia. This study aimed to illustrate the relationship between infection of cytomegalovirus and prognosis of biliary atresia.**

Methods: **From January 2002 to March 2004, 27 patients who had undergone Kasai's procedure because of biliary atresia were investigated for cytomegalovirus -IgG, IgM and pp65, and their mothers were also examined for confirmation of cytomegalovirus infection. The patients were divided into three groups: infection free group, cytomegalovirus positive group and cytomegalovirus infection group. The rate of jaundice disappearance and the incidence of reflux cholangitis were analyzed statistically. The histopathological changes of the liver were also analyzed.**

Results: **The positive expression of cytomegalovirus -IgM and cytomegalovirus-pp65 in the patients was higher than that in their mothers (48% versus 14.81% and 37% versus 3.78%, respectively). Compared with the other two groups (80% in the infection free group, and 82% in the cytomegalovirus positive group), the rate of jaundice disappearance after operation in the cytomegalovirus infection group (36%) was significantly lower ($P<0.05$), and the incidence of reflux cholangitis was higher ($P<0.05$). Histopathological examination also showed that the degree of liver fibrosis and inflammation was more serious ($P<0.05$).**

Conclusions: **There is a strong correlation between cytomegalovirus infection and a lower rate of jaundice disappearance, also a higher post-operational reflux cholangitis. Liver fibrosis seems to be more severe in biliary atresia patients with cytomegalovirus infection.**

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World J Pediatr 2008;4(2):123-126

Key words: **biliary atresia; cytomegalovirus; prognosis**

Introduction

Since Morio Kasai introduced his procedure for the so-called non-correctable form of biliary atresia (BA) in 1959, Kasai's one-stage hepatoportoenterostomy (HPE) has become the first-line treatment. Though about 30% to 40% of patients will survive for more than 5 years with their native liver after Kasai procedure,[1,2] their long-term outcome remains controversial. BA is a progressive disease and hepatic fibrosis will continue even though a successful HPE was performed. The pathogenesis of progressive hepatic fibrosis and cirrhosis is still unknown.

Cytomegalovirus (CMV) hepatitis has a process of progressive hepatic fibrosis and cirrhosis.[3] We hypothesize that the prognosis of BA patients with CMV infection might not be as good as that of those without CMV infection. CMV-IgG in serum, transferred through mother's placenta, would gradually disappear after 2 years. Positive CMV-IgM in plasma indicates that the patient has been infected by the virus recently but already cured. CMV-pp65 is an antigen of the virus and its expression represents duplication of CMV. In this study, serum samples were taken from patients for examination of CMV-IgG, CMV-IgM and CMV-pp65. The rate of jaundice disappearance within 3 months after operation, the incidence of reflux cholangitis, and the degree of hepatic fibrosis in these patients were analyzed. The purpose of this study was to illustrate the relationship between CMV infection and prognosis of BA.

Methods

From January 2002 to March 2004, 64 infants with extrahepatic BA were admitted to Children's Hospital, Fudan University, Shanghai. Thirty-seven infants were ruled out of the study becasue of the incomplete

data. This study focused on 27 of the patients and their mothers were investigated for CMV-IgG, CMV-IgM and CMV-pp65. CMV-IgG and CMV-IgM were measured by ELISA. Indirect immunofluorescence test was used for the measurement of CMV-pp65 in serum.

The 27 patients were followed up at the outpatient department for 6 to 36 months after operation (every 1 to 2 months for the first 6 months; every 3 to 6 months in the later period). Data of the liver function and onset of cholangitis were collected in the follow up.

Five patients negative for CMV-IgG, CMV-IgM, and CMV-pp65 were included in CMV free group, 11 patients positive for CMV-IgG and/or CMV-IgM but negative for CMV-pp65 were regarded as CMV positive group, and 11 patients positive for CMV-pp65 in CMV infection group.

The jaundice disappearance was defined as the total serum bilirubin levels returned to 34 μmol/L (2 mg/dL) or less within 3 months after operation. Reflux cholangitis after surgery was diagnosed if the patient had unexplainable high fever (rectal temperature higher than 38.5ºC), aggravated clinical jaundice with acholic stools or increased levels of serum bilirubin. The incidence of reflux cholangitis after HPE was observed within 6 months after operation.

Immunohistochemical staining was performed blindly. The tissues of the triangular cord of the porta hepatis and liver were obtained from the patients during the Kasai procedure. The sections were deparaffinized and incubated with CMV-pp65 mouse monoclonal antibodies (IQ Cooperation, Netherland) at 37ºC for 60 minutes. After 3 washes with PBS, the sections were incubated with FITC-conjugated sheep anti-mouse immunoglobulin (IQ Cooperation, Netherland) further for 60 minutes, then mounted with glycerin after another 3 washes with PBS. Stained sections were examined with fluorescence microscopy. The cells with cytoplasm showing bright green-yellow fluorescence were regarded as positive cells.

Pathological slices of the liver in the 27 patients after operation were reviewed blindly. According to Scheuer's classification of chronic viral hepatitis,[4,5] we proposed a scoring criterion for evaluating the degree of liver inflammation and fibrosis. Three important parameters included inflammation of the portal area, intralobular inflammation and liver fibrosis (Table 1).

Statistical analysis was performed using Fisher's exact *t* test for the rate of jaundice disappearance and the incidence of reflux cholangitis. Wilcoxon's rank-sum test was used for comparison of the degree of liver inflammation and fibrosis. A *P* value less than or equal to 0.05 was considered statistically significant.

Results

In the 27 patients after Kasai procedure, jaundice and acholic stool were noticed on 19 days on average (range: 14-28 days); the average age at operation was 73 days (42-105 days), and the average weight at operation was 4850 g (3000-5800 g). All patients were suspected of BA with obstructive jaundice before operation. The average level of total bilirubin was 174 μmol/L (range: 98-217 μmol/L), and the average level of direct bilirubin was 119 μmol/L (range: 78-178 μmol/L).

The positive expression of CMV-IgM and CMV-pp65 in patients with BA was relatively high. The positive rates of CMV-IgG, CMV-IgM, and CMV-pp65 in patients were 21/27 (78%), 13/27 (48%) and 10/27 (37%) respectively, but 22 of the 27 mothers were CMV-IgG positive (81.42%), 4 were CMV-IgM positive (14.81%) and only 1 was CMV-pp65 positive (3.78%). This finding suggested that the rate of current CMV infection was higher in BA patients than in their mothers.

Rate of jaundice disappearance within 3 months after HPE

Four patients (80%, 4/5) in the CMV free group showed normal level of serum bilirubin 3 months after operation. Nine patients (82%, 9/11) in the CMV positive group had normal levels of bilirubin within 3 months. In the CMV infection group, 4 (36%, 4/11) showed normal levels of bilirubin. There was no significant difference between the CMV free group and the CMV positive group, but the rate of jaundice disappearance in the CMV infection group was obviously lower than those in the other groups ($P<0.05$).

Incidence of reflux cholangitis after HPE

One (20%) patient in the CMV free group was re-hospitalized for treatment of reflux cholangitis 6 months after operation. Two (18%) patients in the CMV positive group were hospitalized 2 to 3 times for increased levels of serum bilirubin with rectal temperature higher than 38.5ºC with unknown reasons. Four (36%) patients in the CMV infection group had reflux cholangitis in 6 months post-operation. There was no significant difference between the CMV non-infection group and the CMV positive group, but the incidence of reflux cholangitis in the CMV infection group was obviously higher than those in the other two groups ($P<0.05$).

Immunohistochemical study of liver samples

CMV-pp65 antigen immunohistochemical staining was performed on samples of the triangular cord of the porta hepatis and liver from 27 patients. None (0/5)

Table 1. Grade point criteria of liver inflammation and fibrosis

Score	Inflammation of portal area	Intralobular inflammation	Liver fibrosis
1	Inflammation limits in one-third of portal area, no or little range of the limiting plate destroyed	Degeneration of liver cells and spotty necrosis limits in one-third intralobule	Limiting plate light enlarged, few fibrosis
2	Inflammation limits in one-third to half of portal area, apparent limiting plate destroy, less than 50%	Increased spotty and focal necrosis, advanced degeneration of liver cells	Limiting plate apparently enlarged and middle degree hyperplasia of fibrosis
3	Obvious inflammation in half to two-third of the portal area, more than 50% of the limiting plate destroyed	Bridge-form necrosis widen and increased, intralobular structure in disorder, multi-lobular necrosis	Obvious fibrosis and fibrous spetum form, lobular structure still kept
4	-	-	Serious liver fibrosis and lobular structure disappeared with pseudo-lobular form

Table 2. Grade point criteria of liver inflammation and fibrosis in the three groups

Groups	Limiting plate inflammation	Intralobular inflammation	Liver fibrosis	Total score
Non-infection	2.00	1.60	2.60	6.20
Positive	1.84	1.19	2.97	6.00
Infection	2.80*†	2.16†	3.19	8.15*†

*: $P<0.05$ (infection group vs non-infection group); †: $P<0.05$ (infection group vs positive group).

was positive in the CMV free group, while the positive rate was 36.36% (4/11) in the CMV-positive group and 72.7% (8/11) in the CMV infection group.

Inflammation and fibrosis of the liver

In the CMV free and positive groups, mild to moderate degree of bile canaliculus hyperplasia could be seen in pathological slices of limiting plate. The inflammatory reaction and liver fibrous degree of the two groups were milder than those of the infection group. Hyperplasia of bile canaliculus in the infection group was heavy and inflammatory zone was wider than in the other two groups. This was supported by inflammation in one-third to one-half of the portal area, with apparent limiting plate destruction not exceeding 50% (middle to serious degree of debris necrosis), accompanied by increased liver fibrosis, disappearance of lobular structure, and numerous pseudo-lobular form (Table 2).

Discussion

BA is one of the leading causes of obstructive jaundice in newborns, but the etiology is still unknown.[6] Therefore the diagnosis and treatment of BA remain difficult. The reported 5-year survival rate was about 78.3%.[2] A comparative study by two centers in Japan and UK revealed that the 10-year jaundice-free survival rate was only 18% after HPE because of ongoing liver fibrosis.[7] It is generally accepted that virus infection may be one of the important causes that lead to BA by destroying the bile duct. Animal models have been established by injecting rotavirus, reovirus into the abdominal cavity of pregnant rats to produce BA in newborn rats.[8,9] Clinically, Szavay et al[8] revealed that 68% of BA patients have higher reovirus titer (more than 1:10) in serum. In China, the rate of CMV infection is high. Some researchers suggest that severe CMV infection in the liver may lead to BA.[10-12] A high CMV-IgM positive expression in serum indicates a new CMV infection has taken place. The expression of CMV-pp65 represents duplication of virus. In this study, we verified a higher expression of CMV-IgM and CMV-pp65 in BA patients than in their mothers. This demonstrated that the rate of current CMV infection was higher in these patients. In the majority of patients, symptoms such as jaundice and acholic stool were noted within 1 month after birth. This indicated that severe perinatal CMV hepatitis with intra- and extra-hepatic bile duct fibrosis might cause bile duct obstruction, even BA.

The rate of jaundice disappearance within 3 months after operation is one of the important indicators for the prognosis of BA after HPE. In this study, the rate of jaundice disappearance in the CMV infection group was significantly lower than those in the other groups, indicating that virus infection has negative effect on prognosis. CMV-IgG in serum comes from mothers through placenta and is not related to prognosis. Positive CMV-IgM indicates that the patient has been infected by virus recently but cured, thus it has little effect on prognosis. Positive CMV-pp65 indicates continuous infection causing persistent destruction of the bile duct, which might be related to low

disappearance rate of jaundice.

Postoperative reflux cholangitis is one of the major complications of BA. Repeated reflux cholangitis impacts the bile duct which opened during operation. The bile duct was narrowed again because of inflammation, directing toward atresia again step by step, which in turn leading to repeated increase of jaundice and deteriorated cirrhosis, even hepatic failure.[13,14] High incidence of reflux cholangitis is an indicator for poor prognosis. In our study, the CMV infection group had a higher incidence of reflux cholangitis. The immunity susceptibility might be the cause of CMV infection, which also indicates that virus infection is related to poor prognosis in BA.

In BA patients, impeded bile excretion and deposited bile lead to hepatic fibrosis and cirrhosis after a long-term course of the disease. Low-grade hepatic fibrosis and ductulus proliferation have a positive effect on the outcome although there is no statistical significance demonstrated in larger series.[15] Combined with bile deposition, viral hepatitis can cause increased inflammatory reaction of the portal area and accelerate the progression of hepatic fibrosis and cirrhosis. We found that hepatic fibrosis and cirrhosis in the CMV infection group was more serious than those in the other groups. Even in patients with ideal bile flow within a short period after operation, the progressive fibrosis and cirrhosis would continue, contributing to a poor prognosis. We suggest that BA patients with CMV infection should undergo regular antiviral treatment after operation and be followed up for a long period to examine the signs of CMV infection. Effective control and alleviation of viral hepatitis may prevent cirrhosis and improve the prognosis of BA.

Apparently, BA patients with CMV infection have a low rate of jaundice disappearance and a higher incidence of postoperative reflux cholangitis. Bile deposit combined with virus infection leads to more severe inflammation in the portal area and intralobular structure, accelerates hepatic fibrosis and cirrhosis, and is related to poor prognosis of BA. The effect of CMV infection on BA prognosis remains to be determined.

Funding: This study was supported by grants from the Science and Technology Committee of Shanghai Municipality (No. 044119602).

Ethical approval: This study was approved by Institute of Review Board of Fudan University, China.

Competing interest: None declared.

Contributors: Shen C wrote the main body of the article under the supervisor of Zheng S. Xiao XM and Wang W contributed to the intellectual content and improved the final version.

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Received March 28, 2007
Accepted after revision August 28, 2007