Relationship between prognosis of biliary atresia and infection of cytomegalovirus

Chun Shen, Shan Zheng, Wei Wang, Xian-Min Xiao
Shanghai, China

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-operative reflux cholangitis. Liver fibrosis seems to be more severe in biliary atresia patients with cytomegalovirus infection.

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, 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. 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 because 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 38.5°C), 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 37°C 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, 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 rehospitalized 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 38.5°C 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)