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Detection of hepatotropic viruses has no impact on the prognosis after Kasai procedure

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Abstract

Background/Purpose: A viral origin of biliary atresia (BA) is discussed, and several studies have demonstrated different viral strains in liver biopsies of patients undergoing Kasai portoenterostomy. We hypothesized that the presence of hepatotropic viruses in patients undergoing portoenterostomy contributes to the progression of the disease and negatively affect the outcome.

Methods: Liver biopsies were prospectively taken from 70 patients undergoing portoenterostomy in our department from April 1996 to April 2004. Samples were screened by polymerase chain reaction for all common hepatic viruses. Primary outcome parameter was survival with the native liver. Secondary parameters were postoperative serum activity of liver enzymes and serum bilirubin levels at different time points. Patients underwent regular follow-up until October 2008.

Results: Twenty-eight patients (40%) were positive for 1 or more hepatotropic viruses. Four patients were lost to follow-up. In the remaining 66 patients, there was no significant difference in survival with their native liver between virus-positive and virus-negative patients. After a mean follow-up of 7.7 years (range, 4.6-16.1 years), 15 (23%) of 66 patients still lived with their native liver. There was no difference in liver enzymes, C-reactive protein, or bilirubin at any time point between both groups.

Conclusion: A significant number of our patients tested positive for hepatotropic viruses in liver biopsies at the time of the Kasai procedure, but the presence of virus had no influence on the course of BA. This suggests that the ongoing inflammatory process of BA leading to liver cirrhosis in most Kasai-treated patients is not affected by hepatotropic viruses. Our data question the necessity to aggressively screen for and treat viral infections in patients with BA.

Biliary atresia (BA) is the most common neonatal cholestatic disorder, occurring in approximately 1 of 8000 (Asian countries) to 1 of 18,000 (European countries) live births [1], with a female preponderance, characterized by complete fibrotic obliteration of the lumen of all or part of the extrahepatic biliary tree within 3 months of life.

The etiology of BA remains unclear. Several factors have been suggested in the pathogenesis of extrahepatic BA. Since the findings of Landing [2] that indicated that BA could represent the result of a virally induced process of the liver and the hepatobiliary tree, several observations have pointed

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toward a potential association between viral infection and the development of BA. Riepenhoff-Talty et al [3] detected group C rotavirus in infants with extrahepatic BA, and a viral infection was considered as a potential initiator for the obliteration of the bile ducts caused by progressive fibrosing inflammation [4-6].

This hypothesis has been supported by findings of individual viral strains in patients with BA. In liver samples of patients undergoing the Kasai procedure, cytomegalovirus (CMV) [7-11], human papilloma virus [8], reovirus type 3 [12-14], and rotavirus [3,15] were observed.

In a large retrospective study of 74 patients, we detected hepatotropic viruses in only 50% of the patients at the time of the Kasai procedure, and those patients with detectable viral RNA/DNA in their liver biopsies were significantly older than virus-free patients [16], suggesting that viral infection could be a secondary phenomenon.

However, it remains disputed whether the presence of virus in livers of patients with BA at the time of the Kasai procedure could negatively affect the outcome [17,23]. If the presence of viruses at the time of the Kasai operation would influence the outcome, this would be a rational for screening for hepatotropic viruses and eventually using antiviral medication. This clinically relevant aspect was not sufficiently answered by our previous study looking at the viral incidence in Kasai patients. Therefore, in this study, we followed up these patients to investigate whether viral presence at the time of the Kasai procedure affects the outcome of patients with BA.

Table 1 Summary of the different hepatotropic virus strains in patients with BA at the time of the Kasai procedure

Detection of virus strains		
Reovirus positive	20/70	29%
CMV positive	8/70	11%
Adenovirus positive	2/70	3%
Enterovirus positive	1/70	1%
Virus positive	26/70	37%

Patients and methods

The study design was reviewed and approved by the local ethics committee.

Patient samples

Biopsies from 70 patients with BA (30 male and 40 female) treated at the Hannover Medical School, Germany, between April 1996 and April 2004 were prospectively included in the study. Biliary atresia was diagnosed after the exclusion of other cholestatic diseases of the newborn by preoperative endoscopic retrograde cholangiopancreatography, as described previously [18]. The diagnosis was confirmed by intraoperative cholangiography in doubtful cases. All patients underwent our in-house workup for neonatal cholestasis including blood samples for direct and indirect serum bilirubin, liver enzymes, blood count, coagulation parameters, α-1–antitrypsin, hepatitis, and TORCH (Toxoplasmosis, Others, Rubella, Cytomegalovirus, Herpes Simplex) serology. Abdominal and cardiac sonography was performed to exclude associated malformations. Biliary atresia was then confirmed by performing endoscopic retrograde cholangiopancreatography in all patients preoperatively. During the Kasai procedure, liver biopsies were taken in all patients. Specimens were fixed in 4% buffered formalin for immunostaining or immediately snap frozen in liquid N_2 and stored at −80°C for polymerase chain reaction (PCR) analysis. The samples were screened by PCR for common hepatic viruses: herpes simplex virus, Epstein-Barr virus (EBV), varicella zoster virus (VZV), CMV, adenovirus, parvovirus B19, enteroviruses, papillomavirus, rotavirus, and reovirus 3, as previously described [16], and detection of DNA viruses (herpes simplex virus, EBV, VZV, CMV, adenovirus, parvovirus B19, and human papillomavirus). The DNA of 50 mg of frozen liver tissue was extracted using a commercial kit (DNeasy Tissue Kit; Qiagen, Duesseldorf, Germany), and the resuspended DNA was stored at −20°C. A LightCycler (Roche Molecular Systems, Pleasanton, USA) was used for real-time quantitative PCR. Detection of RNA viruses (enterovirus, rotavirus, and reovirus 3); RNA was extracted using TRIzol reagent (Gibco BRL, San Francisco, CA, USA) using 25 to 50 mg of frozen liver tissue.

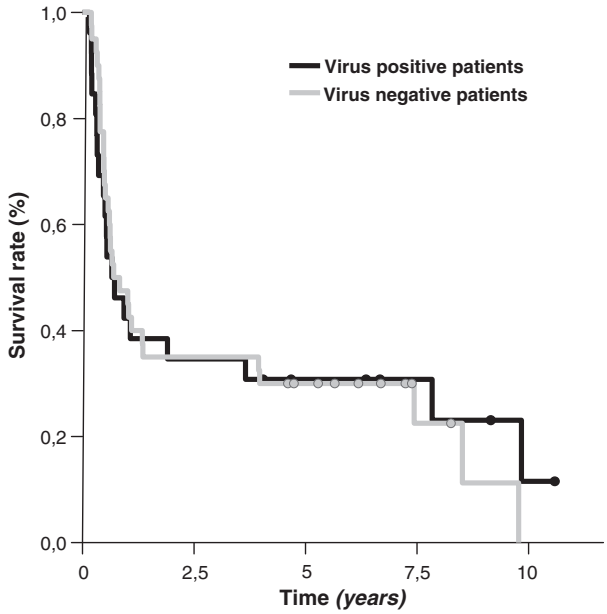

Fig. 1 Kaplan-Meier survival curve. Displayed is the time of survival of patients with BA with their native liver in years after the Kasai procedure. The points indicate the end of follow-up. Log-rank test, $P = .907$; there is no statistically significant difference.