Journal of Pediatric Surgery 50 (2015) 1398–1404

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Review Articles

Evidence for viral infection as a causative factor of human biliary atresia☆

Takeshi Saito *, Keita Terui, Tetsuya Mitsunaga, Mitsuyuki Nakata, Sachie Ono, Naoko Mise, Hideo Yoshida

Department of Pediatric Surgery (E6), Graduate School of Medicine, Chiba University, Chiba, Japan

ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 7 April 2015
Accepted 20 April 2015

Key words:
Etiology
Reovirus
Rotavirus
Cytomegalovirus
Choledochal cyst
Mouse model

ABSTRACT

Objectives: To explore the evidence for viral infections triggering human biliary atresia (BA) by reviewing archival original articles that analyzed human samples via polymerase chain reaction (PCR) experiments, considering the recent experimental trend of extensive use of rotaviral BA animal models.
Methods: A PubMed search retrieved original articles that reported the results of PCR experiments for detecting viral DNA or RNA in patient samples as proof of past infection. Search terms included the often-debated DNA or RNA viruses and BA. Special focus was directed toward PCR analyses that targeted reovirus and rotavirus, where PCR accuracy, specimen characteristics and their interpretations were compared.
Results: Nineteen studies were conducted on 16 different kinds of viruses using PCR, with 5 studies on reovirus, 3 on rotavirus, 10 on cytomegalovirus, 5 on Epstein-Barr virus, 4 on parvovirus B19, and so on. Among the papers suggesting a possible viral link to only BA, there was no study on reovirus, 1 on rotavirus, 3 on cytomegalovirus, 1 on EB virus, and 1 on papillomavirus. Of the 6 PCR studies on *Reoviridae*, 3 on reovirus and 2 on rotavirus were evaluated rigorously for experimental accuracy, including their sensitivity. Two research groups analyzed preoperative stool samples in addition to generic hepatobiliary tissue obtained at surgery. Sample collection timing varied widely, with storage period prior to PCR experimentation not revealed in most reports on *Reoviridae*.
Conclusion: Although a considerable number of PCR studies have sought to clarify a viral role in the pathogenesis of BA using human samples, the findings have been contradictory and have not succeeded in achieving an obvious differentiation between causative and accidental infection of the focused virus. Reproducible and convincing evidence for a causative *Reoviridae* infection has been lacking based on objective data from highly sensitive PCR experiments. Even though the possibility remains of viral disappearance at the timing of collection, to avoid further ambiguous interpretations of PCR results, rigorous and meticulous collection of large numbers of specimens at carefully planned timing, along with a strictly adjusted and finely tuned PCR system, is strongly recommended for obtaining more reliable and consistent results.

Biliary atresia (BA) is a devastating infantile hepatobiliary disease that presents only in the first several months after birth. The essential pathophysiologic feature is that the intrahepatic and extrahepatic bile ducts progressively become irregularly tapered, narrowed, and finally obliterated because of severe inflammation initiated by an unknown cause. Without prompt diagnosis, meticulous portoenterostomy and delicate postoperative follow-up, the patient's liver will suffer chronic cholestasis and will eventually proceed to irreversible cirrhosis. BA patients with intractable cholangitis and deteriorated hepatic reserve will have no other option but to undergo liver transplantation.

Because of the life-threatening course of BA mentioned above, exploration of the possible causative agents in BA has been conducted extensively. Some researchers have claimed that the initiation and/or development of BA is multifactorial, and that the presence/absence, extent and balance of those factors might determine the clinical characteristics of BA. There are thought to be two types of BA, the prenatal form and the acquired form [1]. Among triggering factors, viral infection has been suggested as the possible culprit in the inflammation centering on the hepatobiliary system in BA, in combination with Landing's theory that BA, neonatal hepatitis (NH), and choledochal cyst (CC) all result from a single disease process, with the outcome dependent on the timing and location of the original insult [2]. After the appearance of several reports that reoviral infection in the weanling mouse could create pathophysiological features seemingly similar to human BA [3], Morecki's group in the 1980s repeatedly hinted at the reoviral link to BA by means of serological [3–5] and immunohistochemical [6] analyses. Other groups attempted to reproduce the findings shown by Morecki et al. with the same methodology, but could not obtain supportive results [7,8]. In the last few decades, more advanced PCR methods have been employed using a variety of human BA samples [9–12], with the aim of detecting reoviral RNA as a sign of past infection and seeking its plausibility; however, a lack of deep understanding into the experimental system of each report has confused the assessment of

Abbreviations: BA, biliary atresia; CC, choledochal cyst; CMV, cytomegalovirus; NH, neonatal hepatitis; PCR, polymerase chain reaction; RRV, rhesus rotavirus; RT-PCR, reverse transcription polymerase chain reaction.

☆ The authors report no conflicts of interest.

* Corresponding author at: Department of Pediatric Surgery (E6), Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba City, Chiba 260-8677, Japan. Tel.: +81 43 226 2312; fax: +81 43 226 2366.
E-mail address: takk@faculty.chiba-u.jp (T. Saito).

http://dx.doi.org/10.1016/j.jpedsurg.2015.04.006
0022-3468/

Table 1
Articles analyzing viral involvement in the development of BA and other cholestatic diseases using PCR with human samples.

Putative Virus	Number (Articles)	Viral Involvement in BA and Other		Viral Involvement in BA Only	
		+	−	+	−
Reovirus	5	1	4	0	5
Rotavirus	3	1	2	1 (?)	2
CMV	10	6	4	3	7
Epstein-Barr virus	5	1	4	1	4
Parvovirus B19	4	0	4	0	4
Papillomavirus	3	1	2	1	2
Hepatitis C virus	3	0	3	0	3
Adenovirus	3	0	3	0	3
Herpes simplex virus	2	0	2	0	2
Varicella zoster virus	2	0	2	0	2
Hepatitis B virus	2	0	2	0	2
Coxsackie B4 virus	1	0	1	0	1
Echovirus	1	0	1	0	1
Enterovirus 68/69	1	0	1	0	1
Human herpes virus	1	1	0	0	1
BK virus	1	0	1	0	1

PCR, polymerase chain reaction; BA, biliary atresia; CMV, cytomegalovirus.
? means that the distinct involvement of rotavirus in the etiology of only BA was fairly questionable because hepatobiliary samples from patients with what they called 'BA' included samples from patients with actual choledochal cyst (CC).

the plausibility of the reoviral infection theory. As for other DNA and RNA viruses, the circumstances around research into their etiologic involvement have been similar to those of the reovirus. To date, the viral infection theory has not always been accepted without any contradictions [9,11,13,14].

Alternatively, skipping the time-consuming and steady process of seeking evidence for viral causative footprints in patients' samples, the experimental BA mouse model (first created via peroral inoculation of group A rhesus rotavirus (RRV) by Riepenhoff-Talty et al. [15] and afterwards modified into intraperitoneal inoculation by Petersen [16]) has swept broadly through the research field, with the most focus on replicating and understanding the immunobiological events that might follow the assumed viral infection [17,18]. More recently, a mouse model produced by cytomegalovirus (CMV) infection has been introduced [19]. However, if future studies clearly rule out the involvement of viruses in the onset of BA, the currently inferred process and mechanism of the biliary system being obstructed through a number of experiments using mouse models might more or less depreciate.

The present study was conducted in an attempt to collect objective evidence for the viral causative infection theory through reviewing the previous original scientific papers that reported the performance of PCR experiments using only human BA samples. In particular, we sought to validate the evidence level of archival papers targeting *Reoviridae* infection. A further goal was to specify points to remember in collecting and storing samples for investigation into the viral infection theory by PCR methods.

1. Methods

A PubMed search was performed in November 2013 to select previous original articles dealing with PCR experiments and investigating the plausibility of a viral infection theory of BA through the use of human samples. The search words were "reovirus"; "rotavirus"; "cytomegalovirus (CMV)"; "papillomavirus"; "Epstein-Barr virus"; "hepatitis A, B or C virus"; "herpes simplex virus"; "human herpesvirus 6 or 7"; "varicella zoster virus"; "parvovirus"; "adenovirus"; "polyomavirus"; "Coxsackie virus"; "echovirus"; "enterovirus"; "*Toxoplasma*" or "rubella virus"; and "biliary atresia". Original papers dealing with BA mouse models produced by inoculation of the specific virus were excluded.

First, the numbers and outlines of those articles in accordance with each virus were recognized. Then, among them, the papers suggesting a viral link to the initiation of infantile cholestasis including BA, and those claiming its link to that of only BA were reviewed. Last, in terms of the articles on PCR analyses targeting *Reoviridae* (specifically, reovirus and rotavirus) and CMV, the validity of the experimental system with respect to setting positive and negative controls and conducting sensitivity testing, characteristics of the samples, and the detection rates of viral nucleic acids in patients with disorders were compared. Here, "sensitivity testing" means that the experiment clearly depicted how small an amount of viral copies, load, or titer the PCR system could detect, mostly by using serial dilutions of viral nucleotides.

2. Results

2.1. Number of original articles on PCR analyses targeting specific viruses

The total number of original articles reporting on the performance of PCR studies and analysis of viral involvement in the etiology of BA with human samples was 19, in which 16 putative causative DNA or RNA viruses were mentioned. The number of papers in connection with each virus is listed in Table 1.

There were 5 articles on the reovirus, with only one article suggesting its involvement in the infantile cholestatic diseases including BA. However, none of those 5 articles referred to the possible relevance of the reovirus to the onset of only BA. Among 3 articles on rotavirus, one study hinted at its involvement as the trigger of BA and other diseases.

Although CMV was the most frequently analyzed virus, the majority of studies did not support a link between CMV and the development of only BA except 3 articles revealing positive detection rates of 22%, 42%, and 60% for CMV infection in BA patients [20–22]. There was one paper each suggesting the relevance of the Epstein-Barr virus and the human papillomavirus to the cause of BA, describing detection rates of 11% [20] and 89% [23], respectively. A considerable number of studies explored the possible etiologic role of other viruses such as *Herpesviridae* (including the Epstein-Barr virus, the herpes simplex virus and the varicella zoster virus), the hepatitis B and C viruses, parvovirus B19, human papillomavirus, and so on; however, the number of articles corroborating their link to only BA was quite limited.

Table 2
PCR assays for reovirus infection in tissues from BA patients.

Author	Year	RT-PCR	Target Prototype	Target Gene	Sensitivity Check[a]	Control	
						Positive	Negative
Steele [9]	1995	Nested	T3D	M3	+	+	+
Riepenhoff-Talty [24]	1996	Seminested	T3D	S3	n.m.	n.m.	n.m.
Tyler [10]	1998	Nested	T1L, T2J, T3D, T3A	L1	+	+	+
Saito [11]	2004	Nested	T1L, T2J, T3D, T3A	L3	+	+	+
Rauschenfels [12]	2009	Nested	T1L, T2J, T3D, T3A	L3	n.m.	n.m.	n.m.

PCR, polymerase chain reaction; BA, biliary atresia; RT-PCR, reverse transcription polymerase chain reaction; n.m., not mentioned.
[a] Sensitivity check means that the manuscript clearly depicts how small an amount of viral copies, load, or titer the PCR system could detect, mostly using serial dilutions of the viral nucleotides.