

EVIDENCE-BASED CASE REPORT

**The Association Between Biliary Atresia
and Cytomegalovirus Infection****Meutia Ayuputeri,* Hanifah Oswari****Department of Pediatrics FM Universitas Indonesia-
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Abstract

Perinatal infection of cytomegalovirus (CMV) may cause cholestasis resembling biliary atresia. CMV infection is found in patients with biliary atresia. This simultaneous occurrence of biliary atresia and CMV infection prompted speculation that CMV may contribute to the progression of biliary atresia. This report has the objective to obtain the evidence regarding the association between biliary atresia and CMV infection. Two databases were searched to obtain the evidence: PubMed and Scopus. The study design which was selected for this report was case control due to its relevance to answer the clinical question. There were two case control studies which are appropriate to answer the clinical question. Both studies showed that biliary atresia is associated with CMV infection ($OR > 1$). Biliary atresia is associated with CMV infection at the time of diagnosis, therefore the presence of CMV infection in neonatal cholestasis should not delay the investigation towards biliary atresia.

Keywords: biliary atresia; CMV infection; serology.

Hubungan Atresia Bilier dan Infeksi Sitomegalovirus**Abstrak**

Infeksi sitomegalovirus perinatal dapat menyebabkan kolestasis yang menyerupai atresia bilier. Infeksi tersebut juga ditemukan pada pasien dengan atresia bilier. Kejadian atresia bilier dengan infeksi sitomegalovirus menimbulkan dugaan bahwa sitomegalovirus berperan pada terjadinya atresia bilier. Laporan kasus berbasis bukti ini bertujuan menyajikan bukti hubungan atresia bilier dengan infeksi sitomegalovirus. Laporan ini menggunakan dua basis data dalam pengumpulan bukti, yakni PubMed dan Scopus. Disain penelitian yang relevan untuk menjawab pertanyaan klinis pada kasus ini adalah case-control. Dua penelitian yang disajikan menunjukkan bahwa atresia bilier berhubungan dengan infeksi sitomegalovirus ($OR > 1$). Oleh karena itu, didaptkannya infeksi sitomegalovirus pada kolestasis neonatus tidak boleh menunda penelusuran diagnosis ke arah atresia bilier.

Kata kunci: atresia bilier; infeksi cytomegalovirus; serologi.

Introduction

Cholestasis is defined as reduced bile flow and abnormal accumulation of conjugated bilirubin, indicating impaired hepatobiliary function. Obstructive (extrahepatic, intrahepatic) or hepatocellular (infectious, metabolic, toxic, genetic or idiopathic) diseases may cause cholestasis in newborns and infants.¹ Extrahepatic biliary atresia, subsequently mentioned as biliary atresia, was reported to occur in 1 in 18.000-20.000 live births, more commonly in Asians (1 in 5.000-8.000).² The role of infectious agents in causing postnatal bile duct obstruction has been heavily suggested but remains controversial and inconclusive. Although cytomegalovirus (CMV) is known to cause intrahepatic bile duct destruction and paucity, its role as the cause of EHBA has been a topic of much debate.³

CMV is a double-stranded DNA virus of the Herpesviridae family that infects biliary epithelia, as demonstrated by CMV inclusion bodies within the epithelia. CMV infection is present in 1-2.4% North American newborns, possibly higher in developing country like Indonesia. Congenital CMV infection ranges from 0.2-2.2% of all live births and perinatal transmission is even more common (10-60% in the first 6 months of life).

The perinatal infection of CMV is commonly through genital secretion of the mother and breastmilk. Perinatal infections of CMV are usually asymptomatic, but can cause cholestasis resembling biliary atresia.⁴ Furthermore, CMV infection is also found in patients with biliary atresia. This simultaneous occurrence of biliary atresia and CMV infection prompted speculation that CMV may contribute to the progression of biliary atresia.^{5,6} However, in some cases, CMV infection in neonates may postpone investigation towards biliary atresia. A study by Tarr et al⁵ demonstrated that biliary atresia patients with ongoing CMV infection were referred later than non-infected patients.

Biliary atresia, manifested by progressive inflammation and fibrosis of extra-hepatic and intrahepatic bile ducts, is the major cause of neonatal cholestasis in Dr. Cipto Mangunkusumo National Hospital, Jakarta, accounting for 23% cases from 2000-2003.⁷ It is a devastating disease that leads to cirrhosis and the need for liver transplantation in the majority of children.⁸ The etiology is unknown, and one proposed theory is that it may involve a primary perinatal hepatobiliary viral infection and a secondary generation of an autoimmune-mediated bile duct injury.⁴

Case Illustration

The patient was a baby girl aged 13 months old and was hospitalized with jaundice, pneumonia, GI bleeding and ascites. She was born through a caesarian section at term and weighed 3.600 g and measured 49.5 cm. She was presented with jaundice from the age of one week. At the age of three months, her direct bilirubin level was 3 mg/dL and she was tested positive with CMV infection at that time and was given anti-cholestatic treatment of ursodeoxycholic acid. She was diagnosed with biliary atresia. According to the mother, she did not receive any surgical procedure according to the infection. A liver transplantation was planned for her once her condition improved.

Clinical Question

Is there any association between CMV infection and biliary atresia?

Methods

This is an etiologic question, therefore cohort study and case control study are the suitable study design to find the answer to our clinical question. Biliary atresia is a rare disease, therefore case control study is the type of study to be used. Two databases namely Pubmed and Scopus were used to search relevant studies for our clinical question. The search was done on 7 September 2013 and was limited to articles published from 2000-2013. All articles were evaluated for suitability according to pre-defined inclusion and exclusion criteria. Biliary atresia, CMV, and etiology were used as keywords.

Results

The search yielded two cross sectional studies from the last 13 years. However, these cross sectional results are not satisfactory to answer our clinical question. Subsequent hand-search was done that resulted in one case-control study dated back to 1998. The details of the search are illustrated in Figure 1.

Critical appraisal utilises appraisal tools from the Center of EBM, University of Oxford for etiology and case control studies. Validity of the study is shown in table 1.

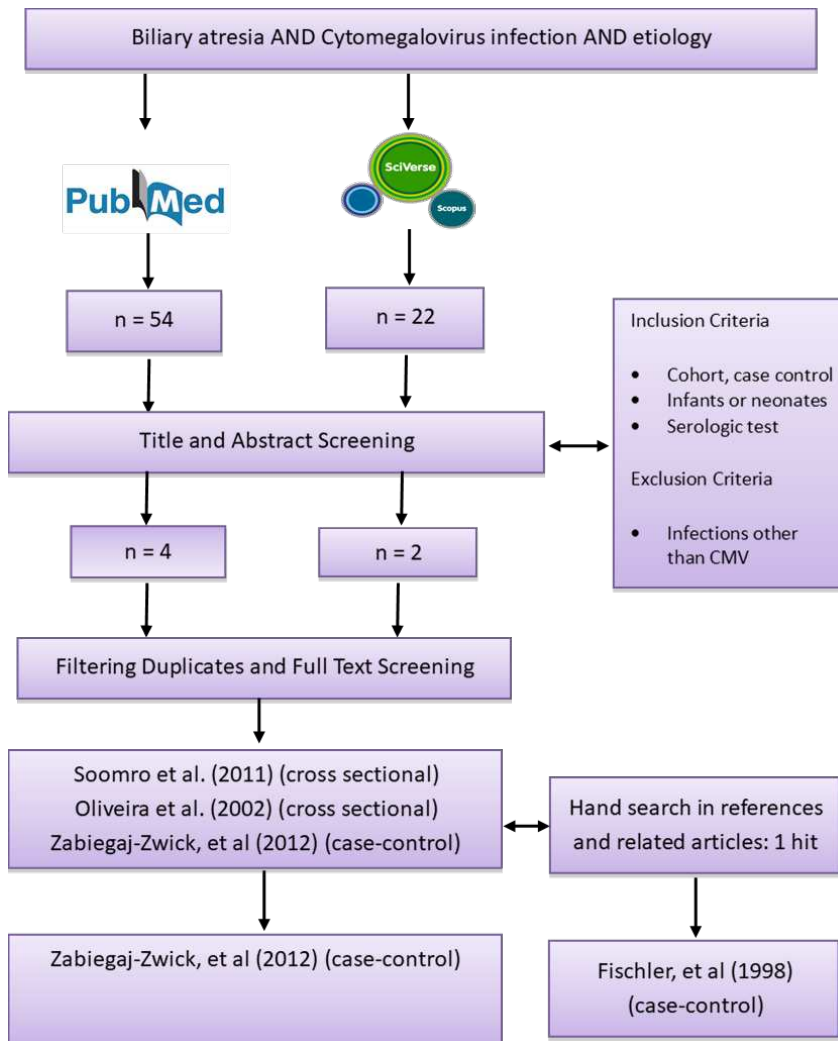


Figure 1. Flow Chart Depicting Retrieval of The Results from The Two Databases and Hand-search

Table 1. Validity of The Study

Validity	Fischler et al ⁹	Zabiegaj-Zwick et al ²
Were there clearly defined groups of patients, similar in all important ways other than exposure to the treatment or other cause?		
Were treatment exposures and clinical outcomes measured the same ways in both groups (e.g. was the assessment of outcomes either objective or blinded to exposure?)		
Was the follow-up study patients complete and long enough?	NA	NA
Do the results satisfy some “diagnostic tests for causation”?		
- Is it clear that the exposure preceded the onset of outcome?		
- Is there a dose-response gradient?		
- Is there positive evidence from a “dechallenge-rechallenge” study?		
- Is the association consistent from study to study?		
- Does the association make biological sense?		
Are the results of this harm study valid?		

The importance of this study was measured by using serology IgM as surrogate of CMV infection and biliary atresia as the outcome. The odds ratio (OR) for biliary atresia was 10.15 (95%CI: 7.63-12.68, p<005).

The study by Fischler et al⁹ also included liver biopsy from some of the cases and some of the controls. A subsequent PCR was done to detect the presence of local and ongoing CMV infection in the liver and hepatobiliary tree. This made a better surrogate to the measurement of importance. The odds ratio of 1.33 signified the importance of the

effect in this study. Infants with CMV infection was 33% more likely to develop biliary atresia than non-infected infants.

The odds ratio signified 10-fold increase risk in developing biliary atresia in infants with CMV infection. Both of the studies can be extrapolated to our patient. The patient's risk of developing biliary atresia by CMV infection (number needed to harm, NNH) was 11.23 (95% CI: 11 to 12, for IgM measurement) in Fischler et al⁹ study and 11 (95% CI: 11 to 12) in Zabiegaj-Zwick et al² study.

Table 2. Comparison between Study and Patient Characteristics

	Fischler et al ⁹	Zabiegaj-Zwick et al ²	Our patient
Entry	Jaundice (neonatal cholestasis)	Jaundice (neonatal cholestasis)	Jaundice
Age at diagnosis of biliary atresia	8 weeks (1-21 weeks)	Not mention	12 weeks
Methods of detection of CMV infection	Serology IgM Liver biopsy	Serology IgM/IgG	Blood test?
Place of treatment	Tertiary referral centre	Referral Hospital	Tertiary referral centre
Ethnicity	Caucasian	? Possibly African	Asian

Table 3. The Review of the Study According to Oxford Centre for EBM 2011 Levels of Evidence¹¹

Author	Study Design	Target Population (n)	Study Criteria	Indicators (Outcome)	Outcome Measures (Follow-up Duration)	Missing Data		Level of Evidence*
						Drop Out (%)	Loss to Follow-up (%)	
Fischler et al ⁹ (1998)	Case control	21 (cases) 35 control)	Inclusion criteria: - Infants with neonatal cholestasis at Sweden tertiary referral center between July 1988-February 1995 - Jaundiced Exclusion criteria: - Infants with other hepatotropic viral infection	Biliary atresia	NA	NA	NA	4
Zabiegaj-Zwick et al ² (2012)	Case Control	27 (cases) 31 (control)	Inclusion criteria: - Cholestatic jaundiced referred to Paediatric Surgical Unit, Tygerberg - Serologic test of CMV Exclusion criteria - Other infection	Biliary atresia	NA	NA	NA	4

Looking at our patient retrospectively, these two studies are highly applicable due to the similarities of characteristics mentioned above (Table 2). The presence of CMV infection and neonatal cholestasis in primary care setting (in the absence of other common etiology of neonatal cholestasis) should prompt to suspicion towards biliary atresia to allow prompt management of biliary atresia.

Discussion

Fischler et al⁹ conducted a case-control study to investigate the association between CMV infection and biliary atresia. The study demonstrated that the OR of biliary atresia were 33% higher in patient with CMV infection detected through liver. This OR was statistically significant because the 95% confidence interval (CI) of this estimate (95% CI: 1.07-1.59) did not include 1.

Subsequent study was done by the same group in 2005 analysing liver biopsy from 18 biliary atresia patients and 6 control patients without liver disease. The study showed higher serum CMV-IgM levels in infants with biliary atresia and greater amounts of immunoglobulin deposits on the canalicular membrane of the hepatocytes in infants with biliary atresia with ongoing CMV infection. This finding suggests the possibility that immunologic mechanisms are of importance in the pathogenesis of biliary atresia and that a CMV infection may trigger such mechanisms.¹⁰

The studies by Fischler et al⁹ and Zabiegaj-Zwick et al² were limited by a small sample size and larger studies may be needed to detect a difference. In addition, the nature of this study (case control) prevents determination of the exact timing of CMV infection in subjects. It would be interesting to see future case control studies in this area especially considering subsequent studies (1998-now) are mostly cross sectional in design.

The study by Zabiegaj-Zwick et al² which tried to observe the correlation between CMV exposure/infection and biliary atresia affected the clinical outcome (OR: 10.06, 95% CI: 7.60 to 12.52). In developing countries like South Africa, CMV seropositivity (IgG) was high (up to 90%). On top of that, The South African population has a high background CMV infection rate in pregnant women. Therefore, in this context, it is difficult to determine when CMV infection is the unequivocal cause of biliary atresia.

Another principal difficulty in making the connection between CMV infection and biliary atresia is that no single test is 100% sensitive

and/or specific for CMV infection. Differences in sampling and diagnosis method may account for variations and conflicting results in existing studies.

Proposed Mechanism of Bile Duct Injury Due to Hepatotrophic Virus

One could postulate that if the newborn was exposed to low levels of virus in the perinatal period, the neonatal immune system could elicit a Th1 cellular response with production of virus-specific memory T cells that, upon re-stimulation with virus would become activated and secrete Th1 cytokines (IFN- γ). Brindley et al¹² analyzed the liver memory T cell response to a variety of viruses from infants with newly diagnosed biliary atresia in order to ascertain if a recent hepatobiliary virus infection had occurred.

Liver T cells from biliary atresia and control patients were cultured with antigen presenting cells in the presence of a variety of viral proteins including CMV, Epstein-Barr virus, reovirus and rotavirus. Fifty-six percent of biliary atresia patients had significant increases in IFN- γ -producing liver T cells in response to CMV homogenate and CMV-pp65 antigen, compared to minimal/no biliary atresia responses to other viruses or the control group CMV response. The robust liver T cell memory response to CMV suggested that perinatal CMV infection had occurred and that CMV infection was a plausible initiator of the bile duct damage in biliary atresia.¹³

Another possible explanation is that innate immune response in biliary atresia at diagnosis is similar to that seen with viral infections. Virus infection of bile duct epithelia activates TLRs on dendritic cells, macrophages and bile duct epithelia, leading to upregulation of MxA transcription factor and downstream stimulation of type 1 interferons (IFN). Concurrently, macrophages are activated, with increased expression of CD14 and production of pro-inflammatory cytokines. Both type 1 IFNs and inflammatory cytokines play a direct role in bile duct epithelial injury and apoptosis. It is theorized that a sustained induction of the innate response, without the development of tolerance, could explain the chronic inflammation and bile duct destruction found in biliary atresia.¹³

Conclusion

CMV infection is associated with biliary atresia in neonates and infants at the time of diagnosis. CMV infection is postulated to mediate immune response to cause damage to the structure of liver and biliary tree, causing biliary atresia. Although most CMV infection in infants and neonates are

asymptomatic, the establishment of CMV infection should not deter nor delay the investigation for biliary atresia, as it needs prompt intervention.

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