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Intrahepatic Cholestasis due to Cytomegalovirus Infection and Extrahepatic Cholestasis due to Sludge Bile with Confirmed COVID-19

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ABSTRACT

Background: Human Cytomegalovirus (CMV) is a Beta-Herpes virus that leads to congenital infection in 0.4% to 2,3 % of all newborns. Diagnosis of CMV infection includes the culture of the nasopharynx, saliva, and urine. Serologic tests are also beneficial for CMV diagnosis. IgM CMV-specific antibodies can be monitored. Biliary sludge is a mixture of particulate solids that have precipitated from bile. Laparoscopic cholecystectomy offers the most definitive therapy for biliary sludge. **Case presentation:** A 2-month-old boy complained yellow appearance since the age of 1 week ago. Looks pale. Defecation was sometimes colored putty since the age of 2 weeks. Blood laboratory examination with hemoglobin 8,7 gr/dL, Prothrombin time, activated partial thromboplastin time, and liver function were increased. Bilirubin, alkaline phosphatase, and Gamma GT were increased. Two-phase and abdominal USG were biliary atresia. PCR CMV examination with urine and blood sample was positive. Head USG was consistent with CMV infection. Cholangiography showed sludge bile with liver cirrhosis. Therapy ganciclovir IV for 14 days then ganciclovir per oral for 6 months. **Conclusion:** Cholangiography is the definitive diagnosis for biliary sludge. CMV is a congenital infection that causes asymptomatic to multiple severe symptoms, so it is necessary to monitor children for adherence to treatment, improve nutritional status and reduce morbidity and mortality rates.

1. Introduction

Human Cytomegalovirus (CMV) is a Beta-Herpes virus that leads to congenital infection in 0.4% to 2,3 % of all newborns.^{1,2} Diagnosis of CMV infection includes the culture of the nasopharynx, saliva, and urine. Serologic tests are also beneficial for CMV diagnosis. IgM CMV-specific antibodies can be monitored.¹

Biliary atresia (BA) is a common cause of neonatal cholestasis, characterized by extensive fibrosing inflammation of the extrahepatic bile duct, leading to obstruction of bile flow and subsequently resulting in permanent liver damage if not early managed. Patients with BA have been tested for several viruses in an

attempt to determine the viruses associated with the disease onset. Among these viruses, Cytomegalovirus (CMV) remains the most common virus linked to BA. The gold standard of diagnosis of BA is intraoperative cholangiography. However, liver biopsy and, following histopathological examination, have quite high sensitivity of approximately 96.9%.³

This study aimed to report a case of extrahepatic cholestasis due to bile sludge which was initially suspected as biliary atresia prior to the cholangiography procedure. Aside from the extrahepatic cholestasis, the patient also suffered intrahepatic cholestasis due to CMV and was treated with intravenous ganciclovir and oral valganciclovir,

respectively, for 6 months. Accurate diagnosis is the main key to successful treatment and better outcomes.

2. Case Presentation

A 2-month-old boy with chief complaint yellow appearance since the of 1 week. It started on the face and progressively increased to the entire body. Defecation was sometimes colored putty since the age of 2 weeks, interspersed with yellow. No colored urination looks like tea. No fever, no seizure. He also did not suffer from coughs and colds. No shortness of breath, no previous vomit. No history of bleeding. No contact history with COVID-19 patient. There is no interference with the child's growth and development. Children are currently breastfed with a frequency of 10-12 times/day, a duration of 15-30 minutes/time. Maternal history of fever during pregnancy did not exist. No maternal history of jaundice previously. Never performed TORCH serology examination before. His mother had no history of consuming foods like fresh vegetables and raw meat. The parent kept the cat in the house and had a farm of ducks. The patient had already been taken to the pediatrician and performed 2 phase abdomen ultrasonography with the result of suspect biliary atresia dd/ biliary stenosis before finally being referred to Dr. M Djamil General Hospital with the description of cholestatic jaundice suspect biliary atresia. The patient has no past illness and a family illness history of the same disease. The patient's birth history was doing well with spontaneous delivery assisted by a midwife, birth weight of 3.300 gram and birth length of 49 cm, vigorous, with no congenital abnormalities found.

On physical examination, the patient was alert with a moderately ill presentation, blood pressure of 80/50 mmHg, heart rate of 100 beats per minute, respiratory rate 25 times per minute, and body temperature was 36,8°C. The patient was on a good nutritional status with a bodyweight (BW) of 5,1 kg and body length (BL) of 54 cm. The skin looks jaundiced on the entire body, with no edema, no cyanosis, and no pallor. The head was normocephal (head circumference of 40 cm), large

fontanel was flat and not tense. The conjunctiva was anemic with jaundice sclera and isochor pupil (diameter of 2 mm/2 mm), as well as a positive normal light reflex. Tonsils T1-T1 were not hyperemic, and the pharynx was not hyperemic. Lung and heart on physical examination were normal. There was no abdominal distension and abdominal circumferences of 36 cm. The liver was palpable 1/4-1/4, with flat surfaces, sharp edge, and chewy consistency. Lien was not palpable. Percussion timpani, peristaltic sound was normal. Extremities were warm with good capillary refilling. No abnormality was found in the genitalia.

Laboratory investigation revealed haemoglobin count of 8,7 gr/dL, leucocyte 8.680/mm³, trombosit 435.000/mm³, erythrocytes 2,87 million/mm³, hematocrit 25 %, differential count 0/4/1/20/67/8, IT ratio was 0,05. MCV 86 fl, MCH 30 pg, MCHC 34%, prothrombin time (PT) 47,5 second (increased), activated partial thromboplastin time (APTT) 75,1 second (increased), Sodium (Na) 136 mmol/L, potassium (K) 4.2 mmol/L, chloride 109 mmol/L, albumin 3,9 gr/dL, Glucose 109 mg/dL, SGOT 51 U/L (increased), SGPT 23 U/L, Ureum 12 mg/dL, creatinine 0,2 mg/dL. Total bilirubin 17,3 mg/dL, indirect bilirubin 3,2 mg/dL, direct bilirubin 14,2 mg/dL. Alkaline phosphatase 827 U/L (increased), Gamma GT 320 U/L (increased), HbsAg non-reactive, Anti HAV negative, Anti HCV negative, Anti HBC IgM negative, HBeAg negative, Anti HBS, and Anti HAV reagen not available. PCR SARS-CoV 19 negative. The urinalysis showed yellow colour, bilirubin (+1), urobilinogen (+), protein (-), reduction (-), leukocytes 2-3/ LPB, erythrocytes 0-1 / LPB. Bilirubinuria impression. The patient's stool examination showed a normal result. Result Two phases USG is suspected biliary stenosis/atresia. The liver, pancreas, and spleen showed no abnormalities. Abdominal USG found a hyperechoic tubular lesion that appears in the portal vein region (triangular sign), the gall bladder not visualized with impression was biliary atresia (figure 1). Tohoku Congenital Biliary Test Score System showed a score of 6 that confirmed the biliary atresia

in the patient. TORCH Serology Resulted: Rubella IgG was 74,55 IU/mL/Reactive (< 10 non-reactive, ≥10 reactive), Rubella IgM was 0,282 COI/Non-reactive (<0,8 non-reactive, ≥0,8- <1 indeterminate, ≥1 reactive), CMV IgG was 20,43 U/mL/Reactive (<0,5 non-reactive, ≥0,5- <1 indeterminate, ≥1 reactive),

CMV IgM was 2,24 U/mL/ Reactive (<0,7 non-reactive, ≥0,7- <1 indeterminate, ≥1 reactive), with a conclusion of CMV and Rubella infection on patient. The PCR CMV examination with urine and blood samples resulted in positive CMV.



Figure 1. Abdominal USG

Head USG found sulci and gyri are good. Pathological lesions were seen in the form of diffuse hyperechoic patches in the subcortical white matter

and periventricular and thalamus of both hemispheres. Impression: consistent with CMV infection (figure 2).

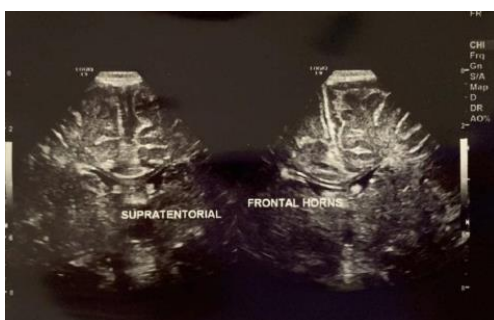


Figure 2. Head USG

BERA (performed in Otolaryng Outpatient Clinic), Tympanometry (right and left ear was normal). The patient has consulted to ophthalmology division with the result is anterior segment was within the normal limit, posterior segment can't be assessed.

The patient was diagnosed with intrahepatic cholestasis due to CMV and Rubella infection, extrahepatic cholestasis due to suspect biliary atresia and normocytic normochromic anemia due to suspected chronic disease. The patient was treated by breastfeeding on demand, given ganciclovir 2 x 40 mg IV for 14 days, then ganciclovir 2x40 po, vitamin A 1 x

6000 IU p.o, vitamin D 1 x 400 IU po, vitamin E 1 x 200 IU po, vitamin K 1 x 2,5 mg po and urdafalk 3 x 20 mg po.

After several days of admission, another patient in the same room as our patient was confirmed positive for COVID-19. Even though our patient had no respiratory symptoms and had a normal respiratory examination, PCR SARS-CoV 19 test was revealed positive, and the patient was then admitted to the isolation ward until the next PCR SARS-CoV 19 test was confirmed negative and the patient parents' do Discharge Against Medical Advice (DAMA/PAPS).

On the second admission, a patient came with jaundice all over his body and pale-yellow-coloured defecation like before. Other significant symptoms were not found. His overall vital sign and physical examination were normal besides the full body skin icteric. His conjunctiva was no longer anemic. The

patient performed an abdominal CT Scan with an impression non visualized gallbladder, suggestive of biliary atresia. We performed cholangiography shown inspissated bile/ sludge bile with liver cirrhosis (figure 3).

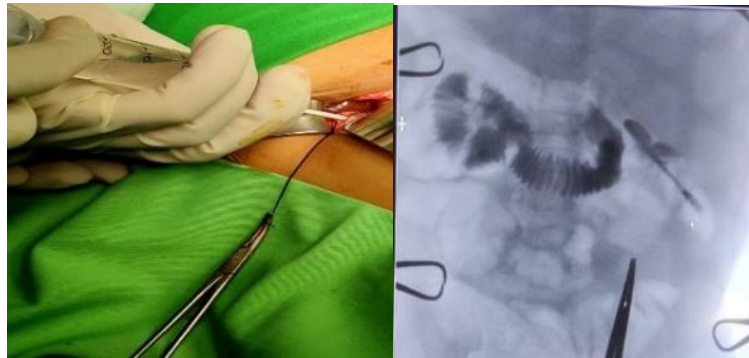


Figure 3. Insertion contrast on cholangiography

Anatomical pathology examination (Figure 4) resulted: in pieces of liver tissue consisting of hepatocytes arranged trabecular with sinusoids. There appears to be marked fibrosis between forming hepatocytes containing bile pigments, including hepatocytes seen in the infiltration of fat cells (steatosis). In the portal area, the bile ducts appear to

have proliferated. The ducts are lined with cuboidal epithelium with conclusion extrahepatic biliary atresia with liver cirrhosis. The patient was diagnosed with extrahepatic cholestasis due to sludge vesica fellea, intrahepatic cholestasis due to CMV, and Rubella infection. The patient was discharged, and the treatment still continued.

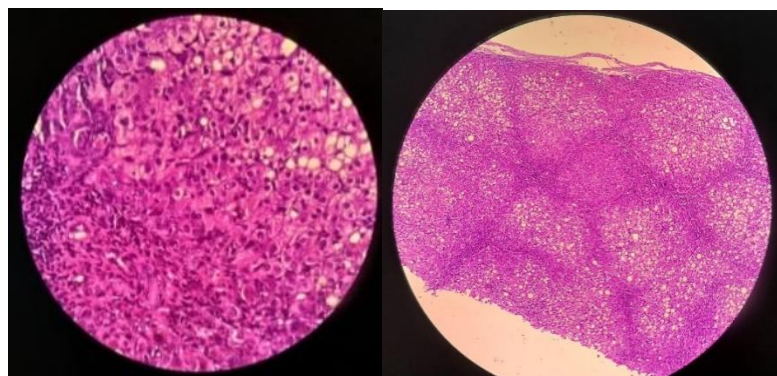


Figure 4. Histopathology of the liver

A patient going outpatient clinic control, the patient was no longer icteric with no other complaint. The vital sign and physical examination reminded normal. His laboratory examination resulted hemoglobin 10.7 gr/dL, white blood cells 12.170/mm³, platelets 452.000/mm³, Albumin 3,7 mg/fL, Total bilirubin 1,9

mg/dL, indirect bilirubin 0,4 mg/dL, direct bilirubin 1,5 mg/dL, gamma GT 27 U/L (normal). Blood PCR and Urine samples were negative for CMV. The patient has consumed Valganciclovir for 6 months, so we stop it.

3. Discussion

There have been reported cases of a 2-month-old boy baby has been hospitalized with a diagnosis of intrahepatic cholestasis due to cytomegalovirus and rubella infection, extrahepatic cholestasis due to biliary sludge. The working diagnosis was established based on the detailed history of jaundice from the age of 2 weeks. There was the acholic stool, hepatomegaly, hyperbilirubinemia, abdominal USG, head USG, and abdominal CT scan. There was the presence of IgM CMV and rubella in serum examination and PCR CMV on the urinary and blood samples.

CMV is the largest herpes virus with a diameter of 200 nm in the form of double-stranded DNA. CMV during pregnancy will transfer to the fetus, and 5 to 10% of infected fetuses would cause disease. CMV can cause a congenital infection during pregnancy through the placenta, during delivery through cervical secretions or blood, and postnatally through breast milk. CMV infection via breast milk in preterm infants is possible with different disease patterns. CMV infection in pregnant women can be due to a primary infection or a secondary infection (reinfection or viral reactivation).^{5,6,7,8}

Examination of CMV-specific IgG or IgM may help the diagnosis. The diagnosis of intrauterine CMV infection was detected CMV in the first 3 weeks after birth, with the discovery of specific CMV-IgM positive on umbilical blood or blood baby. The presence of CMV-specific IgM was detected only in 70% of infants infected with congenital. Serologic tests over the age of 3 weeks, it is difficult to distinguish whether the infection occurred prenatal or postnatal. Serologic tests are recommended to be repeated in the first 2-3 weeks of the examination. We found IgM and IgG anti-CMV in this patient. This finding guided us to congenital CMV.^{5,6,7,10}

CMV infection in developed countries occurs with an incidence between 0.3% and 2.4% of all live births. Mother-to-child transmission is mainly the result of primary maternal CMV infection, which carries a risk of transmission varying from 24% to 75% (mean value of 40%). Cases of CMV transmission due to non-

primary infection have been reported in 1-2.2% of cases. Ten to fifteen percent of congenitally infected infants will have symptoms at birth, including intrauterine growth restriction, microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, anemia, and 20% to 30% of them will die, mostly of disseminated intravascular coagulation, hepatic dysfunction, or bacterial superinfection. Most of the congenitally infected infants (85-90%) have no signs or symptoms at birth, but 5% to 15% of them will develop sequelae such as sensorineural hearing loss, delay of psychomotor development, and visual impairment.^{2,4,13}

The clinical manifestations of congenital CMV infection was asymptomatic infants at birth 10-17% may develop hearing loss and neurologic sequel. At the same time, 5-10% of babies will suffer irreversible central nervous system disorders. The most common clinical manifestation are chorioretinitis, microcephaly, cerebral ventriculomegaly, intracranial calcification, usually periventricular in distribution, motoric disorders, hearing loss, other than that obtained by other symptoms like hepatosplenomegaly, thrombocytopenia, petechiae, yellow, and seizures. We found a few symptoms in this patient; jaundice, hepatosplenomegaly, and the suggestion of periventricular calcification. Suspicion of congenital CMV infection in newborns when found jaundice (62%), petechiae (58%), and hepatosplenomegaly (50%). We must be aware of the systemic feature of CMV infection, such as chorioretinitis, hearing impairment, and bleeding. We have done the screening for chorioretinitis by consultation with the ophthalmologic department, and there was no sign of chorioretinitis. For hearing impairment, we also have consulted to ENT department, and we must perform OAE and BERA examination to screen the SNHL in this patient. We had to perform BERA in the outpatient clinic.⁷

Unfortunately, we don't yet perform the BERA examination. For a while, we could not exclude SNHL in this patient. On the other hand, we also can perform

a simple examination to evaluate the hearing impairment in this baby by performing a hearing test. We can see his response to voice and noise. If he is sleeping and he wakes up because of noise and voices, it means his hearing is in good condition. In this case, the baby responds to the voice and noise around him. SNHL is discovered in approximately 10% to 15% of childhood with congenital CMV infection. The general public of children with CMV-associated hearing loss. The pathogenesis of congenital CMV infection and the mechanisms of SNHL in a child with this intrauterine contamination have now not been defined. The presence of microcephaly, seizures, strange tone, or chorioretinitis inside the newborn duration has been proven to expect cognitive and motor deficits. Extra new reports have established that expanded amniotic fluid CMV burden was predictive of intrauterine transmission. Infants with symptomatic congenital CMV infection excrete greater CMV in urine within the first few months of life and exhibit better peripheral blood viral load than those with asymptomatic infection.^{7,11}

We screened neurological impairment by performing head USG. The result showed calcification of bilateral periventricular, the gyri were still intact, and there was no ventriculomegaly. Even though a maximum of the inflamed newborns has an asymptomatic ailment, most children who are afflicted by symptomatic CMV infection and, even more importantly, 10-15% of children with asymptomatic CMV disease will develop excessive neurological impairments including deafness, intellectual and psychomotor retardation, blindness, microcephaly, hydrocephalus, and cerebral calcifications. The exact direction of CMV infection to growing CNS remains insufficiently described. Several mechanisms of virus entry into the brain parenchyma were proposed for distinct neurotropic viruses, such as herpes viruses, based totally on the facts extrapolated from each in vitro experiment and the ones done on animals fashions. Following contamination of an infant, CMV establishes viremia and colonizes exceptional organs, which includes the brain. Mechanisms of virus entry

into the CNS are nevertheless insufficiently described, and numerous routes have been proposed: infection of endothelial cells forming the blood-brain barrier (BBB) and viral unfold to astrocyte tactics; virus spread via cerebrospinal liquor and contamination of epithelial cells of the choroid plexus, and infection via infiltration of monocytes. In early postnatal length, monocytes populate the mind to grow to be microglia cells. When considering that CMV replicates in monocytes, those cells ought to serve as a provider of the virus entry into the growing CNS. Furthermore, CMV infection of endothelial cells induces monocyte extravasations and infection, which helps virus propagation into the growing mind. For special viruses, which include CMV, the functionality to infect endothelial cells, the main structural elements of BBB, pronouncedly facilitate the course for getting into mind parenchyma. Altered expression of tight junctions, disintegrating the BBB, is determined following viral contamination of endothelial cells in diverse viral infections. However, records concerning BBB disruption in the course of congenital CMV contamination are rare.¹²

Some literature reports that the administration of intravenous ganciclovir for 3-4 weeks reduced transmission of CMV. Ganciclovir is an artificial acyclic nucleoside analog, structurally much like guanine. We used the ganciclovir protocol 7,5 mg/kg/dose every 12 hours for 2 weeks, followed by oral valganciclovir 13,2 mg/kg in two doses orally for 6 months. Treatment of congenital CMV infection with antivirals should be instituted in infants with evidence of central nervous system (CNS) involvement, including SNHL, and should be considered in infants with serious end-organ disease (hepatitis, pneumonia, thrombocytopenia). The cornerstone of antiviral therapy is ganciclovir, which was the first compound licensed specifically for the treatment of CMV infections.^{1,2,9,10}

Valganciclovir may be very nicely absorbed following oral administration. It's far swiftly metabolized following oral dosing into ganciclovir. A suspension component is licensed and available, and although now not certified for the remedy of congenital

CMV, its use may be considered as an alternative to intravenous therapy. Research in neonates has confirmed strong drug levels following oral dosing. Presently, data from a medical trial of 6 weeks versus 6 months of valganciclovir completed by using the Collaborative Antiviral examine group is being analyzed in the direction of the goal of figuring out whether lengthy-time period therapy confers additional neurodevelopmental blessings to toddlers. Even though anecdotal reviews from out-of-control research suggest that lengthy-term oral remedy is nicely tolerated and in all likelihood effective, there's insufficient proof of this factor to advocate an extended-time period (6 months) course of therapy for toddlers with congenital CMV contamination. Similarly, it remains unclear if treatment initiated beyond the neonatal period affords an advantage with appreciation to neurodevelopmental outcomes, even though additional research on this question is warranted.^{10,13,14}

Biliary atresia can be difficult to distinguish from other causes of neonatal cholestasis because of similar manifestations. Cytomegalovirus infection may be one of the most confounding factors. Previous studies have focused on the etiologic association between CMV and biliary atresia; however, the suggestion that CMV infection may trigger biliary atresia is still controversial.

Biliary sludge is a mixture of particulate solids that have precipitated from bile. The diagnosis of biliary sludge is almost always based on ultrasonographic findings. On transabdominal ultrasonography, biliary sludge has been defined as an amorphous mixture of particulate matter and bile, which occurs when various solutes in bile precipitate. Biliary sludge is echogenic and does not cast an acoustic shadow. Although sludge is dependent on gravity, it slowly moves to the dependent portion of the gallbladder. Laparoscopic cholecystectomy offers the most definitive therapy for biliary sludge. On this patient, we initially suspected biliary atresia on 2 phase abdominal USG result, but after we performed cholangiography, we found sludge, but the bile ductus

was still intact.¹⁵

4. Conclusion

The case was initially suspected of biliary atresia, but after cholangiography, the definitive diagnosis is biliary sludge. Intrahepatic cholestasis due to CMV was treated with intravenous ganciclovir and oral valganciclovir, respectively, for 6 months. Accurate diagnosis is the main key to successful treatment and better outcomes.

CMV is a congenital infection that causes asymptomatic to multiple severe symptoms and presents with microcephaly, liver disease with jaundice, retinitis, hearing and vision loss, and developmental and motor delays, so it is necessary to monitor children for adherence to treatment, improve nutritional status and reduce morbidity and mortality rates.

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