

# 12. Presentation of Human\_removed

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**RESEARCH ARTICLE**

**Presentation of Human Cytomegalovirus (HCMV) in Liver Tissues of Cholestatic Infants with Extrahepatic and Non-Extrahepatic Biliary Atresia**

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**ABSTRACT:**

**Introduction:** Human cytomegalovirus (HCMV) is associated with cholestasis in infants. Diagnosis of HCMV infection is most often based on serological anti-HCMV. Identification of HCMV in liver tissue has been rarely reported. The aims of this study were to determine the presentation of HCMV in liver tissues and to analyze its association with serological anti-HCMV of cholestatic infants with extrahepatic and non-extrahepatic biliary atresia. **Methods:** This observational study was performed during December 2017- December 2018 with ethics data from our institutions. The parents or guardians of subjects signed the informed consent. Anti-HCMV serological data were collected from patient medical records. Histopathological diagnosis and polymerase chain reaction (PCR) for HCMV were performed from liver biopsy tissues. The data were analyzed by Chi-square. **Results:** There were 47 cholestatic infants, 38.3% EBA and 61.7% non-EBA. Anti-HCMV IgM was positive in 38.3% patients and IgG was positive in 91.5% patients. Acute infection or recent infection were 38.3%, past or not acute infection were 53.1%, and uninfected or early infection were 8.5% patients. The presentation of HCMV in liver tissues was 68.1% patients, consisting of 11/18 EBA and 21/29 non-EBA and negative in 31.9% patients, consisting of 7/18 EBA and 8/29 non-EBA. There was no association between serological anti-HCMV and PCR HCMV with histopathological features. **Conclusion:** It suggests that PCR can be used as a routine tool to detect the presentation of HCMV DNA in liver tissue. Type of cholestasis in infants, both EBA and non-EBA, cannot be determined based on the serological and PCR examination, but based on histopathological features.

**KEYWORDS:** Human cytomegalovirus, Infant mortality, Liver biopsy, Biliary atresia, Infectious disease.

**INTRODUCTION:**

Human cytomegalovirus (HCMV) is the most common viral infection causing neonatal cholestasis and the most frequent congenital virus infection worldwide<sup>1,2</sup>. HCMV infects 30,000 to 40,000 infants each year in the United States<sup>3</sup>.

Infants can acquire CMV infection from their mother through intrauterine infection (congenital infection), through contact with infected genital secretions during passage through the birth canal (perinatal infection), or postpartum through breastfeeding (postnatal infection)<sup>3</sup>. Approximately 10-15% newborns show symptomatic congenital infection with the clinical manifestation including fetal growth restriction, low birth weight, and cerebral and multiple organs involvement and 85-90% is asymptomatic<sup>3,4</sup>. Approximately 10-15% of newborns with asymptomatic CMV infection develop long-term sequel, for example, progressive sensorineural hearing difficulty and psychomotor retardation<sup>4</sup>.

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Therefore, it is very important to be able to diagnose of HCMV infection early, so that it can provide the appropriate interventions to reduce the sequelae of infection.

The most common diagnosis of HCMV infection is serological examination, namely anti-HCMV immunoglobulin M (IgM) and anti-HCMV immunoglobulin G (IgG). IgM is formed 2-4 weeks after infection, persists and decreases rapidly within 2-4 months, and then is undetectable within 12 months and IgG is formed<sup>5</sup>. However, in newborns, the ability to synthesize IgM and IgG is still low. IgG for newborns is obtained maternally from the mother, while IgM is still in the learning process to synthesize slowly<sup>1</sup> so that the serological examination of IgM and IgG anti-HCMV in the first week of a baby is still unable to reflect the condition of HCMV infection in infants. Furthermore, laboratory tests of cholestasis have shown an increase in conjugated bilirubin, which is a conjugated bilirubin level of more than 20% of the total bilirubin level if the total bilirubin level is >5mg/dl or the direct bilirubin level is more than 2mg/dl if the total bilirubin is <5 mg/dl<sup>6-8</sup> but this examination only shows the condition of cholestasis without knowing the main cause of diseases. In addition, HCMV can be isolated by PCR examination of body tissues and body fluids such as tears, salivary glands, breast milk, urine, feces, semen, cervical secretions, amniotic fluid, blood, and organ transplants<sup>9-11</sup> that might be performed to explain the main cause of cholestasis.

Neonatal cholestasis is often classified as either extrahepatic or intrahepatic in origin. Extrahepatic is most known as biliary atresia and intrahepatic known as neonatal hepatitis. Biliary atresia is a decrease or obstruction of the hepatic or bile ducts at any point from the porta hepatis to the duodenum with an ongoing intrahepatic bile duct damage<sup>2</sup>. Based on the histopathological features, there are two type of cholestasis, namely extrahepatic biliary atresia (EBA) and non-EBA. The histopathological features of the liver biopsy in biliary atresia are biliary duct proliferation, bile plugs, portal tract fibrosis and edema, whereas in non-biliary atresia are showing feature of viral hepatitis, such as human cytomegalovirus (HCMV) or herpes simplex, and changes thought to be due to metabolic disease<sup>12</sup>. The time of diagnosis is a critical time for disease intervention because biliary atresia rapidly leads to the liver to cirrhosis<sup>13</sup>. Percutaneous liver biopsy has a high accuracy (88.2%) for diagnosing biliary atresia<sup>14</sup> and is the best method to differentiate biliary atresia and non-biliary atresia<sup>15</sup>.

The objective was to determine the presentation of HCMV DNA in the liver biopsy tissues and to analyze its association with serological anti-HCMV in

cholestasis infants and histological features, both EBA and non-EBA. The results of this study are expected to be used as a theoretical basis for using PCR examination as a routine procedure to diagnose HCMV infection in cholestatic infants.

## MATERIALS AND METHODS:

### Sample Collections:

This research was a descriptive observational study with a cross-sectional approach at the Inpatient Clinic, Department of Pediatric, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, during December 2017-December 2018. This study has obtained ethics from the Health Research Ethics Commission, Dr. Soetomo General Academic Hospital, Surabaya, number 729/Panke.KKE/XII/ 2017. Subjects who participated in this study had obtained the parental or guardian consent by signing a written informed consent after receiving an informed consent explanation.

The sample was liver biopsy tissues obtained from cholestatic infants who showed symptoms of jaundice with conjugated bilirubin levels 20% of total bilirubin levels, if total bilirubin is  $\geq 5$  mg/dL or direct bilirubin levels  $>2$  mg/dL if total bilirubin  $<5$  mg/dL. Inclusion criteria was aged 1-12 months. Exclusion criteria were patients who had received antiviral therapy, HIV patients, miliary tuberculosis patients, malnourished patients, history of use of immunosuppressive drugs such as corticosteroids and cytostatics, platelets  $<80,000$ mg/dl, prolonged hemostasis physiology, and ascites.

### Histopathological Diagnosis:

The histopathological diagnosis of liver biopsy tissues was performed by two pathologists based on microscopic features in Hematoxylin Eosin and Masson Trichrome staining. The histopathological diagnosis consisted of extrahepatic biliary atresia and non-extrahepatic biliary atresia.

### Detection Infection HCMV:

Serological examination of HCMV infection was performed by measuring IgM and IgG levels using The VIDAS CMV IgM and IgG Assay (Biomérieux). IgM interpretation is the IgM index unit  $<0.7$  is negative,  $<0.7-0.9$  is equivocal,  $>0.9$  is positive. IgG interpretation is the IgG index unit  $<4$  is negative,  $>4 - <6$  is equivocal,  $>6$  is positive. This data were secondary data from patient medical record.

Detection of HCMV DNA in liver tissues were performed by nested polymerase chain reaction (PCR) using MIE4/MIE5 primers for the first round and IE1/IE2 primers for the second round (Table 1)<sup>5</sup>. DNA was extracted from the liver biopsy using QIAamp DNA Mini Kit (Qiagen) and used for PCR. First, PCR was

conducted for the  $\beta$ -globin gene using PCO3 + and PCO4 + primers<sup>5</sup> (Table 1) and secondly for HCMV. The master mix used PCR Master Mix (Promega), namely a reaction mix composed by 10 $\mu$ l master mix (Promega), 1 $\mu$ l forward primer (PCO3 +) 10 pmol, 1 $\mu$ l reverse primer (PCO4 +) 10 pmol, 5 $\mu$ l ddH<sub>2</sub>O (water), and 3 $\mu$ l DNA template. PCR conditions were pre-denaturation 94°C, 5 minutes for one cycle, denaturation 94°C for 30 seconds, annealing 55°C for 30 seconds, extension 72°C for 45 seconds, all for 40 cycles, then the final extension was 72°C for 7 minutes. Electrophoresis of PCR  $\beta$ -globin gene products showed a band of 110 base pair (bp). Samples with positive results for the  $\beta$ -globin gene will be followed by PCR HCMV.

The PCR master mix (Promega) for identification HCMV at the first round was 10 $\mu$ l master mix PCR, 1 $\mu$ l the 10 pmol MIE4 primer, 1 $\mu$ l the 10 $\mu$ mol MIE5 primer, 4 $\mu$ l ddH<sub>2</sub>O, and 4 $\mu$ l DNA template. The PCR conditions were pre-denaturation 94°C for 5 minutes, denaturation 94°C for 30 seconds, annealing 55°C for 30 seconds, extension 72°C for 45 seconds for 40 cycles. The final extension was 72°C for 7 minutes. For the second round, the PCR conditions were the same as the first round PCR, but the template was 1 $\mu$ l of the first round PCR product and PCR was performed by IE1/IE2 primers. PCR results were electrophoresed on 2% acrylamide gel and documented.

**Table 1. Oligonucleotide primers for PCR amplification of HCMV**

Primer Name	Position	Sequence	Product (base pair)
PCO3+	Forward	5'-CCT CTG ACA CAA CTG TGT TCA CTA GC-3'	110 bp
PCO4+	Reverse	5'-TCA CCA CCA ACT TCA TCC ACG TTC ACC-3'	
MIE4	Forward	5'-CCA AGC GGC CTC TGA TAA CCA AGC C-3'	435 bp
MIE5	Reverse	5'-CAG CAC CAT CCT CCT CTT CCT CTG G-3'	
IE1	Forward	5'- CCA CCC GTG GTG CCA GCT CC-3'	161 bp
IE2	Reverse	5'-CCC GCT CCT CCT GAG GAC CC-3'	

**Statistical analysis:**

The association between the serological examinations of HCMV with PCR examination of liver biopsy tissue from infants with cholestasis was done by Chi-square, if significantly different, the strength of the relationship was known by contingency coefficient

**RESULT:**

Samples were 47 infants with cholestasis symptoms, consisting of 27 boys and 20 girls, aged between 1-12

months (mean $\pm$ SD = 3.28 $\pm$ 2.214). Patients consisted of 39 patients aged under 4 months and 8 patients aged over 5 months. The direct/conjugated bilirubin (D Bil) level was 7.9430 $\pm$ 4.13824 (mean $\pm$ SD) and the total bilirubin (T Bil) was 11.0828 $\pm$ 5.50279 (mean $\pm$ SD) (Table 2).

Histopathological diagnosis of liver biopsy tissue from infants with cholestasis showed that there were 29 (61.7%) non-extrahepatic biliary atresia patients and 18 (38.3%) extrahepatic biliary atresia patients (Table 3, Figure 1).

Serological examination showed that anti-HCMV IgM was positive in 18/47 (38.3%) patients and anti-HCMV IgG was positive in 43/47 (91.5%) patients, including 17 EBA and 26 non-EBA. In the IgM+ and IgG+ groups (acute or recent infection of HCMV groups), there were 18 (38.3%) patients consisting of 10/18 the EBA patients and 8/18 the non-EBA patients. In the IgM- and IgG + groups (past or not acute infection of HCMV groups), there were 25 (53.2%) patients consisting of 7/25 the EBA patients and 18/25 the non-EBA patients. In the IgM- and IgG- group (uninfected or early infection of HCMV), there were 4 (8.5%) patients (Table 3, 4, Figure 2).

**Table 2. Characterization of patients**

Age (mean $\pm$ SD)	3,28 $\pm$ 2,214
Age (n / %)	
1-2 month	23/48,9
3-4 month	16/34,1
5-6 month	4/8,5
>6 month	4/8,5
Sex	
Male (n / %)	27/57,4
Female (n / %)	20/42,6
D Bil (mean $\pm$ SD)	7.9430 $\pm$ 4.13824
T Bil (mean $\pm$ SD)	11.0828 $\pm$ 5.50279

D Bil = the direct / conjugated bilirubin level, T Bil = total bilirubin level

**Table 3. The histopathological diagnosis, serological examination, and HCMV PCR in cholestatic infants**

		n	Percentage
Histopathological diagnosis	Extrahepatic biliary atresia	18	38,3%
	Non-extrahepatic biliary atresia	29	61,7%
Serological examination	IgM+	18	38,3%
	IgG+	43	91,5%
	IgM+, IgG+ (recent or past/recurrent infection)	18	38,3%
	IgM-, IgG+ (past infection)	25	53,2%
	IgM-, IgG- (uninfected or early infected)	4	8,5%
PCR HCMV	Positive	32	68,1%
	Negative	15	31,9%

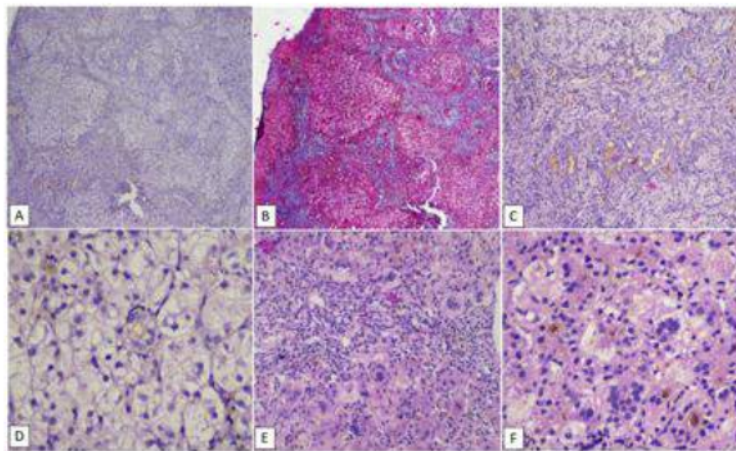


Figure 1. Histopathological features of extrahepatic biliary atresia and non-extrahepatic biliary atresia. (A) Disrupted hepatic parenchymal architecture in EBA (HE, 40x), (B) Biliary cirrhosis in EBA (MT,40x), (C) Bile ductular proliferation containing bile plug in widened portal area displayed in EBA (HE, 100x), (D) Dilated canaliculi containing bile pigmen and ballooning degeneration of hepatocytes documented in EBA (HE, 400x), (E), (F) Numerous multinucleated giant cell hepatocytes accompanied with lobular inflammation in Non-EBA (HE, 200x and 400x). MT: Masson Trichrome, HE: Hematoxylin Eosin.

17 HCMV DNA was detected in liver biopsy specimens from 32 of 47 (68.1%) (Table 4, Figure 2) that consisted of 11/18 (61.1%) EBA patients and 21/29 (72.4%) non-EBA patients. In 11 EHBA patients with the HCMV DNA positive in their liver showed that 7 patients had anti-HCMV IgM and IgG in their sera and the rest showed anti-HCMV IgG in their sera. The 21 non-EHBA patients with the HCMV DNA positive in their liver showed that 5 patients had positive for anti-HCMV IgM and IgG, 15 had positive for anti HCMV IgG, and 1 patient had negative for anti-HCMV IgM and IgG (Table 4, Figure 2).

17 HCMV DNA was not detected in liver biopsies specimens from 15 of 47 (31.9%) patients that consisted of 7/15 (46.7%) EBA patients and 8/29 (53.3%) non-EBA patients. In 7 EHBA patients with the HCMV DNA negative in their liver showed that 3 patients had anti-HCMV IgM and IgG in their sera and 3 had positive for anti HCMV IgG, and 1 patient had negative for anti-HCMV IgM and IgG. In 8 non-EHBA patients with the HCMV DNA negative in their liver showed that 3 patients had anti-HCMV IgM and IgG in their sera and 3 had positive for anti HCMV IgG, and 2 patients had negative for anti-HCMV IgM and IgG (Table 4, Figure 2).

12 There was no association between serological anti-HCMV and PCR HCMV liver with histopathological diagnosis, neither EBA nor non-EBA ( $p=0.151$ ,  $p=0.627$ ) (Table 4). There was no association between serological anti-HCMV with PCR HCMV liver specimens in cholestatic infants ( $p=0.106$ ) (Table 5).

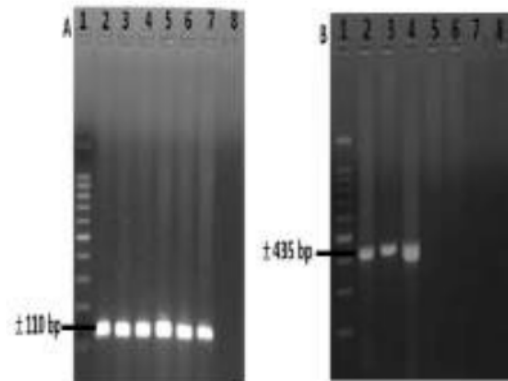


Figure 2. PCR  $\beta$ -globin with the oligonucleotide primer PCO3+/PCO4+ (A) and HCMV with the oligonucleotide primer MIE4/MIE5 (B) from the liver tissue of cholestatic infant. (A) lane 1 = marker, lane 2, 3, 4, 5, 6, 7 = specimens showed positive ( $\pm 110$  bp), lane 8 = positive control. (B) lane 1 = M, lane 2, 3, 4 = specimens showed positive ( $\pm 435$  bp), lane 5, 6, 7 = specimens showed negative, lane 8 = positive control.

Table 4. Association of HCMV infections based on the results of serological examination and PCR from liver tissues in extrahepatic biliary atresia and non-extrahepatic biliary atresia

	EBA	Non-EBA	Total	P value
<b>Serology</b>				0.151
IgM+ IgG+	10	8	18	
IgM- IgG+	7	18	25	
IgM- IgG-	1	3	4	
Total	18	29	47	
<b>PCR</b>				0.627
Positive	11	21	32	
Negative	7	8	15	
Total	18	29	47	

Note: EBA = extrahepatic biliary atresia, non-EBA = non-extrahepatic biliary atresia

**Table 5. Association between HCMV identification by serological anti-HCMV and PCR HCMV**

Serology anti-HCMV	The PCR HCMV of Liver						Total	p value
	Positive			Negative				
	EBA	Non-EBA	Total	EBA	Non-EBA	Total		
IgM+, IgG+	7	5	12	3	3	6	18	0.106
IgM-, IgG+	4	15	19	3	3	6	25	
IgM-, IgG-	0	1	1	1	2	3	4	
Total	11	21	32	7	8	15	47	

Note: EBA = extrahepatic biliary atresia, non-EBA = non-extrahepatic biliary atresia

**DISCUSSION:**

Neonatal cholestatic syndrome may be a result of intrahepatic alterations caused by metabolic, toxic medication, hereditary, anatomic, and idiopathic infections. It is also the result of extrahepatic alterations caused by extrahepatic biliary atresia (EBA) and choledochal cysts. EBA is the final result of a destructive inflammatory process that affects the intrahepatic and extrahepatic biliary ducts leading to fibrosis and obliteration of the biliary tract at some point between the porta hepatis and the duodenum<sup>16</sup>. Histological assessment of the liver tissue from cholestatic patients revealed mainly mixed lymphocytic/granulocytic inflammatory infiltrations, giant cell transformation and fibrosis<sup>17</sup>. This study was conducted on the liver biopsy specimens from infants with cholestasis. All specimens diagnosed as EBA and non-EBA based on histopathological appearance, 18/47 (38.3%) patients referred as EBA, and 29/47 (61.7%) patients referred as non-EBA. The microscopic features of EBA are ductular reaction, ductular cholestasis, portal edema, and fibrosis<sup>18</sup>. Some of liver cells infected with HCMV showed the intranuclear and intracytoplasmic inclusions bodies and inflammatory cell aggregates<sup>19</sup>. Microscopic feature other than those characteristics are categorized as non-EBA<sup>18</sup>. It is also associated with intrahepatic bile duct destruction and ductal paucity, indicating a possible role in the pathogenesis and progression of extrahepatic BA<sup>20</sup>.

Human CMV is associated with cholestasis in infants. Diagnosis of HCMV infection is most often based on serological anti-HCMV. Serological examination is used to detect the specific reaction of antigen and antibody. HCMV serological examination is intended to detect the presence of anti-CMV specific antibodies in the serum<sup>21</sup>. In this study, 18/47 (38.3%) patients were positive for anti-HCMV IgM and 43/47 (91.5%) patients were positive for anti-HCMV IgG. Other studies showed that anti-HCMV IgM was positive in 13/31 (42%) patients, anti-HCMV IgG was positive in 26/31 (84%) patients<sup>24</sup>. IgM positive for CMV in neonatal cholestasis in Sweden was 19/59 (32.2%), IgG positive was 53/59 (90%), IgM-

and IgG- was in 6/59 infants<sup>22</sup>. Neonatal cholestasis in Egypt, IgM CMV was positive in 23/185 (12.4%), that consisted of 4/94 (4.3%) in EHBA and 19/91 (20.9%) in non-EHBA<sup>23</sup>.

Serological tests are also very useful to determine whether a patient has had CMV infection in the past, by evaluating anti-HCMV IgG. Detection of IgM antibodies is used as an indicator of acute infection or new infection<sup>21</sup>. The interpretation of acute or recent infection is if anti-HCMV IgM and IgG are positive (IgM+ IgG+), past or not acute infection is if IgM is negative and IgG is positive (IgM- IgG+), and uninfected or to early infected is if IgM and IgG are negative (IgM- IgG-)<sup>1</sup>. In this study, acute or recent infection (IgM+ IgG+) were 18/47 (38.3%) patients, past or not acute infection (IgM- IgG+) were 25/47 (53.1%) patients, and uninfected or early infection (IgM-IgG-) were 4/47 (8.5%) patients. Another study from 35 extrahepatic neonatal cholestasis patients showed that acute infection was 14/35 (40%), past infection was 9/35 (25.7%) and uninfected or early infection was 12/35 (34.3%)<sup>5</sup>. Neonatal cholestasis patients showed that 19/59 (32%) patients were on going infection, 34/59 (57.6%) patients were past infection, and uninfected or early infection was 6/59 patients<sup>22</sup>. Patients with acute infection appears to have greater liver damage. The infection of HCMV is associated with intrahepatic bile duct destruction and ductal paucity, indicating a possible role in the pathogenesis and progression of extrahepatic BA<sup>20</sup>.

The presentation of HCMV in liver of cholestasis infants was detected by PCR HCMV from liver biopsy tissues. PCR is a sensitive method for detecting HCMV based on amplification of nucleic acids that usually perform on the conserved regions as target gene<sup>21</sup>. In this study, the major immediate early gene was used as PCR targets. It showed that 32/47 (68.1%) patients had positive for DNA HCMV in their liver. Another study in cholestatic infants showed that 12/25 (48%) liver DNA was positive for HCMV<sup>22</sup> and 16/31 (52%)<sup>24</sup>. Another study reported the presentations of HCMV DNA in 50 liver specimens from transplant patients with HCMV infection. It was evaluated by immunohistochemistry (IHC) staining and in situ DNA hybridization using DNA-CMV probe. IHC staining uses two anti-CMV antibodies to detect early and late CMV antigens in the nucleus and cytoplasm and CMV antibody to detect nuclear antigen. The positive cytomegalovirus-infected cells using IHC staining and ISH were detected in the livers of patients who had viremia (as measured by viral culture) during life and at autopsy<sup>19</sup>. Using IHC and in situ DNA HCMV can detect the presence of HCMV DNA in liver cells, it can directly affect liver cells at real time examination. However, the role of HCMV infection in the liver diseases can be more complex, because HCMV-induced

immune mechanism may affect the liver, even if the virus is no longer demonstrable<sup>22</sup>. It shows that HCMV can be found in the liver in infected cells.

Most of the patients in this study on EHBA and non-EHBA were infected by HCMV. The results of this study showed 11/18 (61.1%) of the EBA patients and 21/29 (72.4%) of the non-EBA patients were infected by HCMV. This showed that HCMV contributed to the development of cholestasis in infants, including EHBA and non-EHBA. This was also in accordance with several previous studies. DNA HCMV was presented in 8/15 (53.3%) EHBA patients and 8/16 (50%) non-EHBA patients<sup>24</sup>, 9/18 (50%) EHBA patients and 3/7 (42.9 %) non-EHBA patients<sup>22</sup>, and 9/33 (27.3%) in the liver of EHBA patients<sup>5</sup>.

Some patients in acute or recent infection of HCMV group based on serological examination showed that it was also positive for HCMV DNA in their liver biopsy specimens. In addition, in the past or not acute infection of HCMV group, it was also still found HCMV DNA in their liver specimens. Another study was also found the same result that DNA HCMV was positive in the liver specimens, not only found in the acute infection patients, but also found in past infection patients<sup>5,22</sup>. DNA HCMV was detected in the liver specimens of acute and past patients, including the portal hepatitis<sup>5</sup>. HCMV detected by IHC and ISH deposits in the liver tissue in viremia patients<sup>19</sup>. The primary infection of HCMV leads to a lifelong latency that is characterized by maintenance of the viral genome without active infectious virus production. The latent human HCMV genome is distributed at low copy numbers that DNA HCMV is still detectable in the past infection patients<sup>25</sup>.

In this study, 12 patients presented positive for anti-HCMV serology in their serum (6 patients with IgM+ IgG+ and 6 patients with IgM-IgG +), but negative for HCMV DNA in the liver biopsy. PCR analysis of the  $\beta$ -globin gene in these patients showed a strong positive so that it showed that the specimen was sufficiently qualified for the study. In this case, patients with IgM+ IgG-, might be because the viral load is low, therefore, HCMV DNA was not detected in the liver tissue. The new born IgG is obtained maternally from the mother, whereas IgM is still in the learning process to synthesize slowly<sup>1</sup>, so serological examination of IgM and IgG anti-HCMV in the first week of the new born still cannot reflect the condition HCMV infection in infants. Another factor to be considered in patients with positive IgM serology and negative PCR is the cross-reaction examination with other herpesviruses<sup>5</sup>.

Some patients with positive for PCR HCMV DNA were negative for anti-HCMV antibodies. In this study, one

patient that showed positive for DNA HCMV in the liver biopsy, but not presented IgM and or IgG in the serum. The data showed that the patient had a diagnosis of non-extrahepatic biliary atresia and was 2 months old. This result was also the same as others, like in Korea showing that a few young patients under 3 months old had negative for the anti-HCMV IgM but positive for PCR HCMV DNA<sup>3</sup>. In Brazil, it showed that there were 3 patients with positive PCR DNA HCMV but negative for serology of HCMV<sup>5</sup>. Newborn children have a low ability to produce IgM or IgG. Young infants have immature immune systems, which may fail to produce antibodies during acute infection, so that anti-HCMV IgM has presented negative results<sup>3</sup> or the patient in the early stages of HCMV infection, therefore anti-HCMV IgM had not been produced<sup>23</sup>.

There were 3 patients who serologic and HCMV PCR results were equally negative but showed symptoms of cholestasis. The results of the data search showed that 2 patients had a diagnosis of non-extrahepatic biliary atresia and 1 patient with a diagnosis of extrahepatic biliary atresia. This may be due to any of these causes, including  $\alpha$ 1-antitrypsin deficiency (AATD), viral infections and genetic cholestatic disorders, such as Alagille's syndrome and various types of progressive familial intrahepatic cholestasis (PFIC)<sup>2</sup> that progress to extrahepatic biliary atresia or non-extrahepatic biliary atresia.

This study showed that there was no association between serological anti-HCMV and PCR HCMV liver with histopathological diagnosis and between serological anti-HCMV and PCR HCMV liver, while in our previous study showed association between serological anti-HCMV and PCR HCMV from urine specimens<sup>26</sup>. Other studies shown that no correlation between HCMV positivity by PCR and the histological findings<sup>5</sup>. The accuracy of serology for detecting HCMV antibodies is low<sup>5</sup>. Other studies examining the correlation between severity of cholestasis, the degree of liver damage, and the presence of the virus in the liver tissue, showed that there was no correlation between the degree of cholestasis and CMV infection. Correlation between serology and virus detection in the liver is poor<sup>17</sup>. This indicates that serological examination and PCR HCMV liver cannot be used to determine the type of cholestasis, neither EBA nor non-EBA.

#### CONCLUSION:

Most of cholestasis patients in this study were seropositive anti-HCMV IgG, with acute or past infections of HCMV. HCMV DNA is detected in more than 50% of liver biopsy tissue. Some patients in recent or past infection showed positive for HCMV DNA in the liver tissues, but conversely some patients who were

negative for HCMV DNA in the liver tissues showed positive serology anti-HCMV in the sera. PCR HCMV DNA in the liver tissues indicates the presentation of HCMV, inactivity or latent infection status. It suggests that PCR can be used as routine tools to detect the presentation of HCMV DNA. Type of cholestasis infants, both EBA and non-EBA, cannot be determined based on the serological and PCR examination, but based on histopathological feature.

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#### CONFLICT OF INTEREST:

We declare no conflict of interest.

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