

The agreement of cytomegalovirus (CMV) serology examination and CMV polymerase chain reaction of liver tissue in infants with cholestasis

*Bagus Setyo boedi¹, Reny Widayanti¹, Sjamsul Arief¹, Dwi yanti Puspitasari¹, Rendi Aji Prihaningtyas¹

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Abstract

Introduction: The incidence of cholestasis in infancy is associated with congenital or viral infection. Commonest cause is *Cytomegalovirus* (CMV) infection. In Indonesia, diagnosis of cholestasis due to CMV infection is still not optimal because the support of virological examination is still limited, so serological examination is an option.

Objective: Analysing the agreement of CMV IgG or IgM serological examination and CMV polymerase chain reaction (PCR) examination of liver tissue in infants with cholestasis

Method: This is an analytic observational research using cross sectional design. Samples were infants with cholestasis, 1-12 months of age, in the Department of Child Health, Dr. Soetomo General Hospital, who met the inclusion criteria. Samples were taken from December 2017- May 2018. Liver biopsy was done in Department of Child Health, Dr. Soetomo Hospital. CMV PCR examination of liver tissue was carried out in the Tropical Disease Laboratory at Airlangga University. Data were analysed by calculating the kappa coefficient CMV IgG or IgM serology examination and CMV PCR examination of liver tissue.

Results: There were 30 infants with cholestasis, consisting of 16 (53%) boys and 14 (47%) girls. Positive CMV IgG was found in 28 subjects and CMV IgM was found in 15 subjects. CMV PCR was positive in 20 (67%) subjects. The most common age range with positive CMV IgG and IgM as well as CMV PCR was 1-3 months. Agreement of CMV

IgG compared to CMV PCR of liver tissue showed weak result with kappa value of 0.25. The agreement of CMV IgM compared to CMV PCR of liver tissue was not found with kappa value 0.00.

Conclusions: In infants with cholestasis, there is no agreement for CMV serological examination with CMV PCR of liver tissue. CMV serologic examination cannot replace CMV PCR of liver tissue to determine the presence of CMV virus in infant with cholestasis.

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(Key words: cholestasis, CMV serology, CMV PCR liver tissue, agreement, infant).

Introduction

Cholestasis is a decrease or obstruction of bile flow at each level to the extrahepatic biliary tract and duodenum¹. Cholestasis is found in 1 in 2500 births². Incidence of cholestasis in infancy is associated with congenital or viral infection. Commonest cause is *Cytomegalovirus* (CMV) infection. Research conducted in Brazil on 76 infants with cholestasis showed 29.4% positive for CMV serology in intrahepatic cholestasis and 28.5% positive for CMV serology in extrahepatic cholestasis³. CMV infection is diagnosed in neonates by isolating the virus from urine, detecting IgM in blood, detecting CMV antigen in blood and identifying CMV-DNA by polymerase chain reaction (PCR)⁴. CMV-DNA can be taken from blood or tissue⁵.

Research conducted in Brazil showed a positive frequency of CMV-DNA of 34.3% of 35 samples of liver tissue examined⁶. Research in Poland in nine children with cholestasis showed 9 CMV IgG positive, 4 CMV IgM positive, and 9 CMV DNA positive in the blood but on immunohistochemistry and molecular examination in liver biopsy tissue, CMV was not found⁷. Research in Brazil showed that in comparison with PCR, serology had a low sensitivity (33.3%) and a low positive predictive value (28.6%)⁸. In Indonesia, diagnosis of cholestasis due to CMV infection is still not optimal because the support of virological examination is still limited, so serological examination is an option.

¹Airlangga University, Indonesia

*Correspondence: bagus.setyo boedi@fk.unair.ac.id

 <https://orcid.org/0000-0002-3923-6913>

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Objectives

Analysing the agreement of CMV IgG or IgM serological examination and CMV PCR examination of liver tissue in infants with cholestasis

Method

This is an analytic observational research using cross sectional design. Subjects were 1-12 months old infants with cholestasis in the Department of Child Health, Dr. Soetomo General Hospital, who met the inclusion criteria. Samples were taken during a 6 month period from December 2017-May 2018.

Ethical issues: Ethical approval was obtained from the Ethics Committee of Dr. Soetomo General Hospital (ERC No. 729/Panke. KKE/XII/2017). Parental written informed consent was obtained prior to data collection.

Total sampling was performed in this study. Inclusion criteria were infants with cholestasis 1-12 months of age. The exclusion criteria were septicaemia, HIV infection, miliary tuberculosis, severe malnutrition, thrombocytopenia, prolonged clotting time, ascites and a history of using immunosuppressants including steroids and cytostatic agents. Liver biopsy was carried out in the Department of Child Health, Dr. Soetomo General Hospital. Serology examination was carried out in the clinical pathology laboratory in the Dr. Soetomo General Hospital. CMV PCR examination of liver tissue was carried out in the Tropical Disease Laboratory at Airlangga University. Descriptive statistics and calculating the kappa coefficient CMV IgG or IgM serology examination and CMV PCR examination of liver tissue were done.

Results

During the 6 month period, 33 subjects were sampled, 30 subjects met the inclusion criteria and 3 subjects were excluded, 2 with severe infection (sepsis) and 1 with ascites. The basic characteristics of the sample are shown in Table 1. The average age in this study was 3.6 months.

Based on the results of CMV serology, out of 30 study subjects, CMV IgG was positive in 28 patients, and CMV IgM was positive in 15 patients. Age distribution based on CMV serology results shows that the most common age range with CMV IgG and CMV IgM positive is at 1-3 months of age (Figure 1).

Table 1: Basic characteristics of the sample (n=30)

Variable	Result
<i>Sex - n (%)</i>	
Male	16 (53)
Female	14 (47)
<i>Age in months - n (%)</i>	
1-3	20 (66.7)
4-6	06 (20.0)
7-9	02 (06.7)
10-12	02 (06.7)
<i>AST in IU - Mean (SD)</i>	221.7 (153.3)
<i>ALT in IU - Mean (SD)</i>	161.4 (86.5)
<i>Direct bilirubin in mg/dl Mean (SD)</i>	8.16 (4.51)
<i>Total bilirubin in mg/dl Mean (SD)</i>	11.11 (5.77)

AST: Aspartate transaminase, ALT: Alanine transaminase

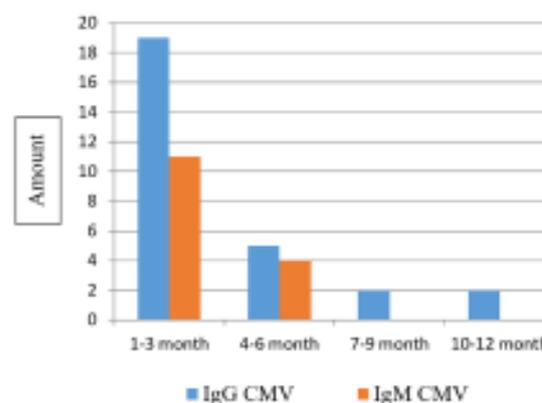


Figure 1: Age distribution based on CMV serology

Based on CMV PCR results of liver tissue, CMV PCR was positive in 20 (67%) and CMV PCR was negative in 10 (33%). The most positive CMV PCR was found in the 1-3 month age range in 15 (75%) subjects (Figure 2).

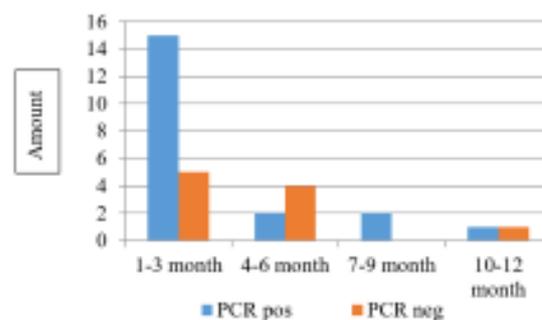


Figure 2: Age distribution based on CMV PCR in liver tissue

From CMV serologic examination compared with CMV PCR liver tissue was obtained, from 28 subjects with positive CMV IgG, CMV PCR positive liver tissue as much as 20 (71.4%). Based on CMV IgM results compared to CMV PCR liver tissue, from 15 subjects with positive CMV IgM, positive CMV PCR of liver tissue as many as 10 (66.7%) subjects (Table 2).

Table 2: Results between CMV serology and CMV PCR liver tissue

Variable		PCR CMV		Total
		Negative	Positive	
IgG CMV	Negative	02	0	02
	Positive	08	20	28
IgM CMV	Negative	05	10	15
	Positive	05	10	15

The agreement of CMV IgG compared to CMV PCR of liver tissue obtained a weak agreement with kappa value of 0.25. The agreement of CMV IgM

compared to CMV PCR of liver tissue was not in agreement with a kappa value of 0.00 (Table 3).

Table 3: Results of kappa suitability between CMV serology and CMV PCR liver tissue based on age

Age	IgG CMV with PCR CMV	IgM CMV with PCR CMV
	Kappa	Kappa
1-3 months	0.273	0.053
1-4 months	0.362	0.015
1-6 months	0.272	0.031
1-12 months	0.250	0.000

In terms of age, at 1-3 months and 1-4 months of age, CMV IgG compared to CMV PCR liver tissue had a weak agreement with kappa values of 0.273 and 0.362. At the age of 1-6 months also CMV IgG compared to CMV PCR liver tissue had a weak agreement with kappa value 0.272 (Table 3).

Based on the sensitivity test and specificity test IgG CMV against CMV PCR, sensitivity was 100%, specificity was 20%, positive predictions were 71.4%, negative predictions were 100% and accuracy was 73.3%. Whereas for IgM CMV against CMV PCR, sensitivity was 50%, specificity was 50%, positive predictions were 66.7%, negative predictions were 33.4% and accuracy was 50%.

Discussion

From this study, the age range in as many as 20 (66.7%) subjects, was 1-3 months. The mean age of the study population was 3.6 months. In research conducted in Brazil, the median age of subjects at the first visit was 82.5 days (\pm 3 months)⁶. In a study conducted in India, in 101 infants with neonatal cholestasis, the mean age of the subjects was 2.8 \pm 1.7 months⁹. In another study, the average age at time of liver biopsy was 2.5 \pm 1 month¹⁰. In this study there were 53% males. Studies by Liberek (2002), Brandao (2009) and Goel (2018) also showed greater male prevalence^{8,10,11}. In this study, the mean AST and ALT values were 221.7 mg/dl and 161.4 mg/dl respectively. A study by Liberek (2002) had AST 156 mg/dl / ALT 169 mg/dl, a study by Talachian (2014) had AST 280 mg/dl / ALT 238 mg/dl and a study by Sira (2016) had AST 134 mg/dl / ALT 230 mg/dl^{11,12,13}.

From the 30 study subjects, CMV IgG was positive in 28 (93%) and CMV IgM was positive in 15

(50%). Research in Brazil on 76 subjects with neonatal cholestasis was divided into 2 groups, intrahepatic cholestasis (34) and extrahepatic cholestasis (42); in the intrahepatic cholestasis group, CMV IgM was positive in 29.4%, while in the extrahepatic cholestasis group, CMV IgM was positive in 28.5%³. In the study conducted in Poland, all 9 subjects with cholestasis were CMV IgG positive and 4 were CMV IgM positive⁷. In an African study of 78 infants with prolonged jaundice, 72 (92.3%) were CMV IgG positive and 31 (39.7%) were CMV IgM positive; the 92.3% positive CMV IgG is likely due to maternal IgG transfer via the placenta or IgG production because the baby is infected, even though 39.7% positive for IgM in the baby, presumably because there was a possibility of infection in the uterus¹⁴. CMV IgG is useful to determine whether the patient has been infected with CMV in the past, whilst IgM antibodies indicate an acute condition or new infection⁵.

In the current study, the highest number of positive CMV IgG and IgM subjects were found in the 1-3 month age range. Research in China showed that primary infection with CMV in children occurred in the age range 1-3.5 months and that there was very high anti-CMV IgG seroprevalence in young infants (<6 months) due to persistent maternal antibodies¹⁵. Generally, moderate to high levels of IgM antibodies can be detected at 1-3 months after the onset of infection, after which IgM levels begin to fall but remain positive for more than 9 months after primary infection¹⁶.

In our study of 30 subjects, 20 (67%) were liver tissue CMV PCR positive. These results indicate high CMV frequency in infants with cholestasis. In a study in Taiwan, 26 liver tissue subjects with

biliary atresia were positive for HCMV DNA in liver tissue with PCR of 2 (7.6%)¹⁷. In research from Brazil, from 33 liver tissue in subjects with extrahepatic cholestasis, 9 (27.3%) subjects with PCR were positive for HCMV DNA⁶. Research in India, from 31 subjects with neonatal cholestasis, found positive CMV PCR liver tissue with PCR in 16 (52%)¹⁰. Variations in CMV PCR results in liver tissue may be explained because of the few patients involved in study as well as the geographic variation of subjects¹⁸.

CMV PCR positive liver tissue was mostly found in the 1-3-month age range. Research in India on 31 infants with neonatal cholestasis, where the average age at the time of liver biopsy was 2.5 ± 1.0 months, positive CMV PCR was 16 (52%)¹⁰. CMV serological examination compared with CMV PCR of liver tissue, from 28 subjects with positive CMV IgG, 20 positive PCR (71.4%) subjects. CMV IgG compared to CMV PCR obtained a weak agreement with a kappa value of 0.25. Research conducted in India 26 CMV IgG positive subjects, 15 (58%) positive CMV PCR liver tissue with a kappa 0.2 value¹⁰. In the Brazilian study, 9 CMV IgG positive subjects, 3 subjects were positive for HCMV DNA. CMV IgG represents the presence of transplacental maternal IgG⁶.

CMV IgM results compared to CMV PCR of liver tissue, from 15 subjects with positive CMV IgM, 66.7% positive PCR subjects. In this study, CMV IgM compared with CMV PCR did not obtain agreement with kappa value 0.00. Research in Poland, in 9 children with cholestasis, obtained a weak correlation between positive serological results and virus detection in liver tissue⁷. Other studies have shown a positive association between CMV IgM and CMV PCR in liver tissue even though the relationship was very weak. It is suspected that CMV IgM is a poor indicator of the presence or absence of CMV in liver tissue¹⁰. In infants with CMV serology positive but liver tissue PCR negative, possibly at the time of the study, subjects passed the acute phase of hepatitis due to CMV where the CMV virus was eliminated from liver tissue by the host immune system or CMV infection may be outside liver tissue or hepatitis caused by other aetiologies. Infants with positive HCMV DNA with PCR on liver tissue but negative CMV serology may not produce enough antibodies for some unknown reason¹⁷.

This study showed that at the age of 1-3 months, 1-4 months, 1-6 months and 1-12 months, CMV IgG compared to CMV PCR liver tissue, obtained a weak agreement. There was no agreement obtained in CMV IgM in that age range. Other studies have

shown that serological examination has weak accuracy to detect active CMV virus infections⁶. There was weak correlation between positive serology and detection of CMV virus in liver tissue⁷. CMV blood serology or PCR is not an accurate marker for CMV infection in liver tissue¹⁰. CMV IgM and CMV IgG serology examination is not recommended for diagnosing CMV infection in infants. CMV IgG positivity reflects IgG antibodies obtained from the mother through the placenta and not diagnosis of CMV infection. Maternal IgG antibodies can last up to 18 months in infants. IgG antibodies that remain in infants cannot distinguish congenital, natal or postnatal infections¹⁹.

In this study, CMV IgM sensitivity to CMV PCR liver tissue was 50%, specificity 50%, accuracy 50%, positive predictive value 66.7% and negative predictive value 33.4%. Other studies also showed CMV IgM sensitivity to CMV PCR liver tissue was 69% and specificity was 61%¹⁰. Research in Brazil showed CMV PCR of liver tissue compared to serology with sensitivity of 54%, specificity of 61% and accuracy of 59%⁶. Compared to PCR, serology has a high accuracy of 82.4%, sensitivity of 33.3%, specificity of 88.9%, positive predictive value of 28.6%, and negative predictive value of 90.01%⁸. Examination of CMV IgM antibodies for primary infection has less specificity, because IgM can be positive in active CMV infection⁵. In patients with positive CMV IgM but negative DNA PCR, results are likely to be due to false positive results from IgM. False positives can be secondary to the presence of rheumatoid factors (infant IgM against maternal IgG) or cross-reaction with herpesviruses. In patients with IgM negative but positive PCR DNA, results are likely to be due to false negative. False negative results are likely secondary due to competition between high maternal IgG antibody levels and relatively low infant IgM levels⁶.

One limitation of this study is that the biopsy sample was taken with a special needle biopsy on the liver tissue so that the sample obtained was not as good as if the sample was taken during surgery. Ideally, samples should be taken from the bile duct tissue during Kasai surgery. Another drawback of this study is not to examine the types of intrahepatic or extrahepatic cholestasis. The results of this study found no agreement of CMV serological examination and CMV PCR liver tissue in infant cholestasis.

Conclusions

In infants with cholestasis, there was no agreement for CMV IgM examination with CMV PCR of liver tissue. However, for CMV IgG with CMV PCR of liver tissue, there was low agreement. Serologic

examination of CMV IgG or IgM is not sensitive and specific, and cannot replace CMV PCR of liver tissue to detect the presence of CMV virus in infant cholestasis.

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