Submissions

#16851 | Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants

Alphania Rahniayu , Gondo Mastutik, Anny Setijo Rahaju, S....

Submission Review Copyediting Production

Submission Files

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150458-1	2022gondo123, Original article Coressponding author Gondo Mastutik JIDC May 2022.docx	May 17, 2022	Article Text
151020-4	icasu, 16851-Figure-150998-1-18-20220524.tif (4)	May 26, 2022	Figure
151179-1	16851-Cover-151148-1-18-20220526.pdf	May 26, 2022	Other

Name	From	Last Reply	Replies	Closed
Comments for the Editor	2022gondo123 2022-05-17 02:05 BST	-	0	☐
#16851	icasu 2022-05-20 16:23 BST	2022gondo123 2022-05-26 03:25 BST	3	☐

Submissions

#16851 | Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants

Alphania Rahniayu , Gondo Mastutik, Anny Setijo Rahaju, S....

Submission Review Copyediting Production

Round 1

Round 1 Status

Submission accepted.

Notifications

[JIDC] Editor Decision	2022-07-25 13:58 BST
[JIDC] Editor Decision	2022-07-26 09:51 BST

Ibnu Ariyanto	**Review Submitted** Recommendation: Resubmit for Review	Open	Read Review
Eda Yazıcı Ozcelik		Open	Read Review
Jure	**Review Submitted** Recommendation: Resubmit for Review	Open	Read Review

Reviewer's Attachments

Search

153640-1	, 16851-Article Text-152544-1-4-20220517.docx	July 10, 2022
153642-1	, 16851-Figure-152545-1-4-20220526 (1) (1).docx	July 10, 2022

Revisions

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Name	From	Last Reply	Replies	Closed
152544-1 Fig. 1	jurearapovic 2022-07-10 10:08 BST	2022gondo123 2022-07-12 11:11 BST	2	☐

#16851 | Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants

Alphania Rahniayu , Gondo Mastutik, Anny Setijo Rahaju, S....

Copyediting Discussions

Add discussion

Name	From	Last Reply	Replies	Closed
JIDC #16851 - Copyediting	niyati 2022-09-14 11:26 BST	2022gondo123 2022-09-23 10:49 BST	2	☐

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Notifications

[JIDC] Editor Decision

2022-07-26 09:53 BST

Alphania Rahniayu , Gondo Mastutik, Anny Setijo Rahaju, S. Eriaty N. Ruslan, Priangga Adi Wiratama, Erna Sulistiyani, Bagus Setyoboedi:

The editing of your submission, "Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants: Infection of cytomegalovirus in cholestatic infant," is complete. We are now sending it to production.

Submission URL: https://jidc.org/index.php/journal/authorDashboard/submission/16851

Assoc. Prof. Dr. Tugba SARI, MD
Pamukkale University Faculty of Medicine Department of Infectious Diseases and Clinical Microbiology Denizli/Turkey.
Phone +90-505-852-54-30
drtugba82@gmail.com

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The Journal of Infection in Developing Countries

Notifications

[JIDC] Editor Decision

2022-07-26 09:51 BST

Alphania Rahniayu , Gondo Mastutik, Anny Setijo Rahaju, S. Eriaty N. Ruslan, Priangga Adi Wiratama, Erna Sulistiyani, Bagus Setyoboedi:

We have reached a decision regarding your submission to The Journal of Infection in Developing Countries, "Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants: Infection of cytomegalovirus in cholestatic infant".

Our decision is to:Accept Submission

Assoc. Prof. Dr. Tugba SARI, MD
Pamukkale University Faculty of Medicine Department of Infectious Diseases and Clinical Microbiology Denizli/Turkey.
Phone +90-505-852-54-30
drtugba82@gmail.com

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The Journal of Infection in Developing Countries

JIDC #16851 - Copyediting

Participants

Niyati (niyati)

Gondo Mastutik (2022gondo123)

Messages

Note	From
Dear Dr. Mastutik, An initial review of your recent submission to the Journal of Infection in Developing Countries has made it clear it is missing basic technical requirements. We strongly recommend authors whose native language is not English to have their manuscript checked by a language editing service, or by an English native speaker colleague. You are kindly requested to carefully check the following document: https://jidc.org/index.php/journal/about/submissions use the MS Word manuscript template provided. I am attaching a revised version of the manuscript with some comments for you. Please work on it using the track changes option of MS Office Word and not erasing comments, reply to them if needed. You can attach the new files to your next reply message, please do not start a new discussion, just reply to this one. Please do not hesitate to contact me for any additional information. Best Regards, Niyati niyati, 16851-Article Text-154866-1-6-20220914.docx	niyati 2022-09-14 11:26 BST
Dear Dr. Mastutik, I am writing to follow up on my previous message regarding the	niyati 2022-09-20

JIDC #16851 - Copyediting

Participants

Niyati (niyati)

Gondo Mastutik (2022gondo123)

Messages

Note	From
Dear Dr. Mastutik, An initial review of your recent submission to the Journal of Infection in Developing Countries has made it clear it is missing basic technical requirements. We strongly recommend authors whose native language is not English to have their manuscript checked by a language editing service, or by an English native speaker colleague. You are kindly requested to carefully check the following document: https://jidc.org/index.php/journal/about/submissions use the MS Word manuscript template provided. I am attaching a revised version of the manuscript with some comments for you. Please work on it using the track changes option of MS Office Word and not erasing comments, reply to them if needed. You can attach the new files to your next reply message, please do not start a new discussion, just reply to this one. Please do not hesitate to contact me for any additional information. Best Regards, Niyati niyati, 16851-Article Text-154866-1-6-20220914.docx	niyati 2022-09-14 11:26 BST
Dear Dr. Mastutik, I am writing to follow up on my previous message regarding the	niyati 2022-09-20

JIDC #16851 - Proof Confirmation

Participants

Niyati (niyati)

Gondo Mastutik (2022gondo123)

Messages

Note	From
Dear Dr. Mastutik, We have now copyedited your submission for English and journal style. Please review the copyediting as indicated below and send the corrected proof version back to me at your earliest convenience. - Please check the completeness and correctness of the text, tables, figures, names of authors, and author affiliations. - If you find a problem with the typesetting or formatting, indicate this in a comment box. Please do not make any revisions that affect the formatting of the document; these issues are corrected by our typesetter when the article is converted to PDF files for uploading to the Internet. - Please make any changes or comments using the "Track Changes" tool in the Word program. If you have any queries regarding the correction tool, please contact the JIDC. - Significant changes to the article as accepted for publication will not be considered at this stage unless permission is first obtained from the Editor. The final editing is performed by the Technical Editor based on your comments and proofreading marks. - Please review your manuscript carefully before returning it; we will not be able to make any further revisions in the future. To meet the production schedule for your issue, you must respond within 48 hours (even if you have no corrections) so	niyati 2022-10-31 14:03 GMT

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Participants

Niyati (niyati)

Gondo Mastutik (2022gondo123)

Messages

Note	From
Dear Dr. Mastutik, We have now copyedited your submission for English and journal style. Please review the copyediting as indicated below and send the corrected proof version back to me at your earliest convenience. - Please check the completeness and correctness of the text, tables, figures, names of authors, and author affiliations. - If you find a problem with the typesetting or formatting, indicate this in a comment box. Please do not make any revisions that affect the formatting of the document; these issues are corrected by our typesetter when the article is converted to PDF files for uploading to the Internet. - Please make any changes or comments using the "Track Changes" tool in the Word program. If you have any queries regarding the correction tool, please contact the JIDC. - Significant changes to the article as accepted for publication will not be considered at this stage unless permission is first obtained from the Editor. The final editing is performed by the Technical Editor based on your comments and proofreading marks. - Please review your manuscript carefully before returning it; we will not be able to make any further revisions in the future. To meet the production schedule for your issue, you must respond within 48 hours (even if you have no corrections) so	niyati 2022-10-31 14:03 GMT

152544-1 Fig. 1

BUKTI REVIEW

Participants

Salvatore Rubino (srubino)

Assoc. Prof. Dr. Tugba SARI, MD (tugbasari)

Gondo Mastutik (2022gondo123)

Messages

Note	From
Dear Author Please provide a Figure 1. Sincerely	jurearapovic 2022-07-10 10:08 BST
Dear Editor, Herewith I attach the Figure 1 file. Thank you. Best regard, Gondo Mastutik 2022gondo123, 16851-Figure-150998-1-18-20220524.tif	2022gondo123 2022-07-11 15:56 BST
Dear Editor, Herewith I attach several files about the main article, tables, and sentence changes before and before the revision, as recommended by the reviewer (Eda Yazıcı Ozcelik). Thank you. Best Regard, Gondo Mastutik 2022gondo123, 16851-153640-1-5-20220710 main article GM.docx	2022gondo123 2022-07-12 11:11 BST

Review: Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants

Jure

Completed: 2022-07-22 10:53 BST

Recommendation: Resubmit for Review

Reviewer Comments

For author and editor

I had read this manuscript with great interest. Although the results are very interesting, I am little confused by authors' study approaching. In some moments, the readers could think that cholestasis is the same as CMV infection. In MM sections authors should declare inclusion criteria parameters.

Prior making some conclusions about sensitivity or specificity of different tests, the authors should explain how many infants treated with specific anti-CMV therapy or how many of them had CMV diagnosis at discharge from the Hospital? How many of them were congenital, perinatal or postnatal infected?! Are these all cholesteric infants were diagnosed as CMV infection?

Second, I think that the Authors should make conclusion in accordance to new algorithms, and somehow in accordance with COVID-19 diagnostic development, suggesting molecular diagnostics even in developing countries. CMV PCR diagnostics should be easier or at least similar to do as COVID19 diagnostic. Please make conclusion and part of discussion in that sense.

In other sense, serology should be good screening approach prior molecular diagnostics.

Reviewer Files

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No Files

Review: Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants

Eda Yazıcı Ozcelik

Completed: 2022-07-10 12:16 BST

Recommendation: Revisions Required

Reviewer Comments

For author and editor

The study titled 'Polymerase Chain Reaction of Human Cytomegalovirus from Liver and Urine Compared with Serological Test in Cholestasis Infants' was a scientific study with a well-designed purpose and methodology. I have included some minor revise notes in the manuscript.
Authors should avoid giving general information in the discussion chapter. Therefore, the discussion should be reconsidered.
Since it is not compatible with the content of the figure file, I could not make any evaluation. Therefore, the relevant file needs to be reloaded and evaluated.

Reviewer Files

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153640-1 , 16851-Article Text-152544-1-4-20220517.docx	July 10, 2022
153642-1 , 16851-Figure-152545-1-4-20220526 (1) (1).docx	July 10, 2022

Menjawab comments reviewer

BEFORE AND AFTER REVISION JULY 2022

	Before	After revision including comment
Methodology *Sample collection* **Paragraph 2**	The samples were 35 infants with cholestasis who were treated at the Pediatric Inpatient Installation, Department of Child Health Sciences, Dr. Soetomo General Academic Hospital Surabaya in the period December 2017-December 2018. The operational definition of cholestasis in this study was infants with jaundice, where the conjugated bilirubin level is 20% of the total bilirubin level (if the total bilirubin is >5mg/dL) or the direct bilirubin level is >2 mg/dL (if the total bilirubin is <5mg/dL). Specimen taken from patients were liver biopsy, urine, and serological data. Inclusion criteria were infants with cholestasis and age 1-6 months. Exclusion criteria were patients who had received antiviral therapy, HIV patients, 1ilitary tuberculosis patients, malnourished patients, history of using immunosuppressive drugs such as corticosteroids and cytostatic, platelets < 80,000 mg/dl, prolonged hemostasis function, and ascites.	The samples were 35 infants with cholestasis who were treated at the Pediatric Inpatient Installation, Department of Child Health Sciences, Dr. Soetomo General Academic Hospital Surabaya in the period December 2017 to December 2018. The operational definition of cholestasis in this study was infants with jaundice, where the conjugated bilirubin level is 20% of the total bilirubin level (if the total bilirubin is greater than (>) 5 milligrams per deciliter (mg/dL) or the direct bilirubin level is >2 mg/dL (if the total bilirubin is less than (<) 5 mg/dL). Specimen taken from patients were liver biopsy, urine, and serological data. Inclusion criteria were infants with cholestasis and age 1-6 months. Exclusion criteria were patients who had received antiviral therapy, HIV patients, 1ilitary tuberculosis patients, malnourished patients, history of using immunosuppressive drugs such as corticosteroids and cytostatic, platelets <80.000 mg/dL, prolonged hemostasis function, and ascites.

Methodology *Serological data collection*	*Serological data collection* Serological examination, which includes anti-HCMV IgM and IgG levels were examined by the Enzyme Linked Fluorescent Assay (ELISA) method using a solid phase receptable from VIDAS. The interpretation of IgM was that IgM index unit <0.7 was negative, <0.7 - 0.9 was equivocal, > 0.9 was positive. The interpretation of IgG was that IgG index unit <4 was negative, > 4 - <6 was equivocal, > 6 was positive.	**Thank you. I have revised (greater than and less than) in the previous paragraph.** *Serological data collection* Serological examination, which includes anti-HCMV IgM and IgG levels were examined by the Enzyme Linked Fluorescent Assay (ELISA) method using a solid phase receptable from VIDAS. The interpretation of IgM was that IgM index unit <0.7 was negative, <0.7 to 0.9 was equivocal, >0.9 was positive. The interpretation of IgG was that IgG index unit <4 was negative, >4 to <6 was equivocal, >6 was positive.
Methodology *HCMV PCR from liver biopsy and urine specimens*	The β globin gene PCR was performed using PCO3+ and PCO4+ primers and the PCR Mastermix (Promega) which product size were the 325-base pair (bp). The compositions were 10 µl master mix (Promega), 1 µl PCO3+ 10 pmol, 1 µl PCO4+ 10 pmol, 5 µl ddH2O, 3 µl DNA template. The initial denaturation 5 minutes in 94°C for 1 cycle, then 30 seconds denaturation in 94°C, 30 seconds annealing in 55°C, 45 seconds elongation in 72°C, for all were in 40 cycles, and then 7 minutes for final elongation in 72° C.	The β globin gene PCR was performed using PCO3+ and PCO4+ primers and the PCR Mastermix (Promega) which product size were the 325 base pair (bp). The compositions were 10 µl master mix (Promega), 1 µl PCO3+ (in a concentration of 10 picomole), 1 µl PCO4+ (in a concentration of 10 picomole), 5 µl ddH2O, 3 µl DNA template. The initial denaturation 5 minutes in 94°C for 1 cycle, then 30 seconds denaturation in 94°C, 30 seconds annealing in 55°C, 45 seconds elongation in 72°C, for all were in 40 cycles, and then 7 minutes for final elongation in 72°C.

Commented [A1]: greater-than sign (>) less-than sign (<) should be revised.

Commented [A2R1]: For example: 4<IgG index<6

Commented [A3]: pmol amounts should be shown in parentheses

Commented [A4]: pmol amounts should be shown in parentheses

Results Paragraph 1	There were 35 infants with cholestasis involved in this study, consisting of 20 males and 15 females aged between 1-6 months (mean ± SD = 2.771 ± 1.087). The levels of direct/conjugated bilirubin (D Bil) were 7,955± 4,674 (mean + SD) and total bilirubin (T Bil) was 10,369 ± 5,896 (mean ± SD) **(Table 1**).	**I have changed, (comma) to . (point)** There were 35 infants with cholestasis involved in this study, consisting of 20 males and 15 females aged between 1-6 months (mean ± SD = 2.771 ± 1.087). The levels of direct/conjugated bilirubin (D Bil) were 7.955± 4.674 (mean + SD) and total bilirubin (T Bil) was 10.369 ± 5.896 (mean ± SD) **(Table 1**).
Results Paragraph 2	All samples in this study, showed positive results for PCR of the β globin gene, hence continued with detection of HCMV. The result of HCMV PCR from liver tissues and urine specimen were positive in 26/35 (74.3%) and in 30/35 (85.7%) infants, respectively. The product of HCMV PCR was showed in **Figure 1**.	I have attached. Thank you.
Results Paragraph 3	Serological data showed that the IgM positive were found in 16/35 (45.7%) infants and IgG positive were found in 31/35 (88.6%) infants. Acute infection (IgM+ IgG+), past infection (IgM- IgG+), and uninfected (IgM-IgG) were found in 16/35 (45.7%), 15/35 (42.9%), and 4/35 (11.4%) infants, respectively (**Table 2**).	Serological data showed that the IgM positive were found in 16/35 (45.7%) infants and IgG positive were found in 31/35 (88.6%) infants. Acute infection (IgM+ and IgG+), past infection (IgM- and IgG+), and uninfected (IgM- and IgG-) were found in 16/35 (45.7%), 15/35 (42.9%), and 4/35 (11.4%) infants, respectively (**Table 2**).

Commented [A5]: Evaluation could not be made because there is no related figure in the attached files.

Commented [A6]: (IgM- and IgG -)

Results Paragraph 4	There was concordance between anti-HCMV IgG with HCMV PCR from liver biopsy (p < 0,05; p= 0,017) and from urine specimens (p < 0,05; p= 0,030) with kappa coefficient were 0,360 for HCMV PCR from liver biopsy and 0,364 for HCMV PCR from urine specimens (fair: 0,21 – 0,4) **(Table 4).**	**I have changed position this paragraph to be paragraph 5.** **Paragraph 4:** There was no concordance between anti-HCMV IgM with HCMV PCR from liver biopsy (p >0.05; p= 0.929), but there was concordance between anti-HCMV IgM with HCMV PCR from urine specimens (p<0.05, p= 0.027) which kappa coefficient was 0.246 (fair: 0.21 – 0.4) **(Table 3).** **Paragraph 5:** There was concordance between anti-HCMV IgG with HCMV PCR from liver biopsy (p<0.05; p=0.017) and from urine specimens (p< 0.05; p=0.030) with kappa coefficient were 0.360 for HCMV PCR from liver biopsy and 0.364 for HCMV PCR from urine specimens (fair: 0.21 – 0.4) **(Table 4).**
Results Paragraph 6	McNemar (exact sig 2 sided) test showed that there was significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgM (p < 0,05, Liver: 0,031, Urine: <0,001), but there was no significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgG	McNemar (exact sig 2 sided) test showed that there was significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgM (p<0.05, liver: 0.031, urine: <0.001), but there was no significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgG (p>0.05, liver: 0.125, urine: 1.000) (**Table 5**).

Commented [A7]: Table numbers should be given in the order described in the text.

	(p>0,05, Liver: 0,125, Urine: 1,000) (**Table 5**).	
Discussion Paragraph 3	An accurate diagnosis of congenital HCMV infection is very important to determine the appropriate management of this disease. The standard method for determining congenital HCMV infection is the tissue culture from a urine and or saliva specimen from infant at age 2 to 3 weeks of life [6,8]. This method using clinical sample such as saliva or urine which inoculated into human fibroblast cells, incubated and observed the presentation of cytopathic effect that characterized in HCMV infection for 2 until 21 days [11]. In addition, this method is less effective and time consuming, because it requires around 3 weeks to obtain the true negative result [11]. Another method for detecting HCMV infection is PCR. This method is able to detect the HCMV DNA virus in approximately 24 o 48 hours, even though the viral load is low [4,9,10]. Materials used for PCR examination include urine, blood, saliva, liquor, amniotic fluid, and tissue [9,10].	It was already mentioned in the introduction, therefore I prefer to remove it from the discussion section.

Commented [A8]: General information should be avoided in the discussion chapter. This paragraph can be included in the introduction if necessary.

References Number 9	Soetens O, Fellous CV, Foulun I (2008) Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. Journal of Clinical Microbiology 46: 943-946. https://doi.org/10.1128/JCM.01391-07	**Thank you. I removed it.** **I revised all reference citation styles like JIDC style.** Soetens O, Fellous CV, Foulun I (2008) Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. J Clin Microbiol 46: 943-946.
References	1. Oliveira NL, Kanawaty FR, Costa SC, Hessel G (2002) Infection by cytomegalovirus in patients with neonatal cholestasis. Arq Gastroenterol. Apr-Jun; 39:132-136. 2. Chen J, Hu L, Wu M, Zhong T, Zhou YH, Hu Y (2012) Kinetics of IgG antibody to cytomegalovirus (CMV) after birth and seroprevalence of anti-CMV IgG in Chinese children. Virol J. Dec 10; 9:304. 3. Kenneson A, Cannon MJ (2007) Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. Jul-Aug; 17:253-76. 4. Revello MG, Gerna G (2002) Diagnosis and management of human cytomegalovirus	1. Oliveira NL, Kanawaty FR, Costa SC, Hessel G (2002) Infection by cytomegalovirus in patients with neonatal cholestasis. Arq Gastroenterol 39:132-136. 2. Chen J, Hu L, Wu M, Zhong T, Zhou YH, Hu Y (2012) Kinetics of IgG antibody to cytomegalovirus (CMV) after birth and seroprevalence of anti-CMV IgG in Chinese children. Virol J 10; 9:304. 3. Kenneson A, Cannon MJ (2007) Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 17:253-276. 4. Revello MG, Gerna G (2002) Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 15:680-715. 5. Gantt S, Bitnun A, Renaud C, Kakkar

Commented [A9]: All references should be written in the same format.

infection in the mother, fetus, and newborn infant. Clin Microbiol Rev. Oct;15:680-715.

5. Gantt S, Bitnun A, Renaud C, Kakkar F, Vaudry W (2017) Diagnosis and management of infants with congenital cytomegalovirus infection. Paediatr Child Health. May; 22:72-74.
6. Marsico C, Kimberlin DW (2017) Congenital Cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. Ital J Pediatr. Apr 17; 43:38.
7. Gunkel J, van der Knoop BJ, Nijman J, de Vries LS, Manten GTR, Nikkels PGJ, Murk JL, de Vries JIP, Wolfs TFW (2017) Congenital Cytomegalovirus Infection in the Absence of Maternal Cytomegalovirus-IgM Antibodies. Fetal Diagn Ther. Jun17; 42:144-149.
8. Bilavsky E, Watad S, Levy I, Linder N, Pardo J, Ben-Zvi H, Attias J, Amir J (2017) Positive IgM in Congenital CMV Infection. Clin Pediatr (Phila). Apr; 56:371-375.
9. Soetens O, Fellous CV, Foulun I

F, Vaudry W (2017) Diagnosis and management of infants with congenital cytomegalovirus infection. Paediatr Child Health 22:72-74.

6. Marsico C, Kimberlin DW (2017) Congenital Cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. Ital J Pediatr 43:38.
7. Gunkel J, van der Knoop BJ, Nijman J, de Vries LS, Manten GTR, Nikkels PGJ, Murk JL, de Vries JIP, Wolfs TFW (2017) Congenital Cytomegalovirus Infection in the Absence of Maternal Cytomegalovirus-IgM Antibodies. Fetal Diagn Ther 42:144-149.
8. Bilavsky E, Watad S, Levy I, Linder N, Pardo J, Ben-Zvi H, Attias J, Amir J (2017) Positive IgM in Congenital CMV Infection. Clin Pediatr (Phila) 56:371-375.
9. Soetens O, Fellous CV, Foulun I (2008) Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. J Clin Microbiol 46: 943-946.
10. Goegebuer T, Van Meensel B, Beuselinck K, Cossey V, Van Ranst M, Hanssens M, Lagrou K (2008) Clinical

(2008) Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. Journal of Clinical Microbiology 46: 943-946. https://doi.org/10.1128/JCM.01391-07

10. Goegebuer T, Van Meensel B, Beuselinck K, Cossey V, Van Ranst M, Hanssens M, Lagrou K (2008) Clinical predictive value of real-time PCR quantification of human cytomegalovirus DNA in amniotic fluid samples. J Clin Microbiol. Mar; 47:660-665.
11. Ross SA, Novak Z, Pati S, Boppana SB (2011) Diagnosis of cytomegalovirus infections. Infectious Disorders Drug Targets 11:466–74.
12. Crough T, Khanna R (2009) Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev. Jan; 22: 76-98.
13. Mastutik G, Kurniasari N, Rahniayu A, Rahaju AS, Ruslan SEN, Ilmiah K, Setyoboedi B, Sulistyani E (2022) Detection of cytomegalovirus in urine predictive value of real-time PCR quantification of human cytomegalovirus DNA in amniotic fluid samples. J Clin Microbiol 47:660-665.
11. Ross SA, Novak Z, Pati S, Boppana SB (2011) Diagnosis of cytomegalovirus infections. Infect Disord Drug Targets 11:466-474.
12. Crough T, Khanna R (2009) Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev 22:76-98.
13. Mastutik G, Kurniasari N, Rahniayu A, Rahaju AS, Ruslan SEN, Ilmiah K, Setyoboedi B, Sulistyani E (2022) Detection of cytomegalovirus in urine specimen of cholestasis infants by polymerase chain reaction. Res J Phar Tech 15:1419-3.
14. Situmorang L, Setyoboedi B, Arief S, Mastutik G (2019) Infection of Cytomegalovirus (CMV) in cholestasis infant with biliary atresia. Indones. J Clin Pathol Med Lab 26:175-181.
15. Davis AR, Rosenthal P, Escobar GJ, Newman TB (2011) Interpreting conjugated bilirubin levels in newborns. J Pediatr 158:562-565.
16. Suchy FJ (2004) Neonatal cholestasis. Pediatr Rev 25:388-396.
17. Rashed YK, Saber MA, Tawfik M,

Commented [A10]: All references should be written in the same format.

specimen of cholestasis infants by polymerase chain reaction. Res J Phar Tech. May; 15:1419-3.

14. Situmorang L, Setyoboedi B, Arief S, Mastutik G (2019) Infection of Cytomegalovirus (CMV) in cholestasis infant with biliary atresia. Indones. J. Clin. Pathol. Med. Lab. Mar; 26: 175-181.
15. Davis AR, Rosenthal P, Escobar GJ, Newman TB (2011) Interpreting conjugated bilirubin levels in newborns. J Pediatr. Apr; 158:562-565.
16. Suchy FJ (2004) Neonatal cholestasis. Pediatr Rev. Nov; 25:388-96.
17. Rashed YK, Saber MA, Tawfik M, Mourad WS (2013) Histopathological features and accuracy for diagnosing biliary atresia by prelaparotomy liver biopsy in Egypt. Egyptian Pediatric Association Gazette. 61:42-45.
18. Pereira TN, Walsh MJ, Lewindon PJ, Ramm GA (2010) Pediatric cholestatic liver disease: Diagnosis, assessment of disease progression and mechanisms of fibrogenesis.

Mourad WS (2013) Histopathological features and accuracy for diagnosing biliary atresia by prelaparotomy liver biopsy in Egypt. Egyptian Pediatric Association Gazette 61:42-45.

18. Pereira TN, Walsh MJ, Lewindon PJ, Ramm GA (2010) Pediatric cholestatic liver disease: Diagnosis, assessment of disease progression and mechanisms of fibrogenesis. World J Gastrointest Pathophysiol 1:69-84.
19. Götze T, Blessing H, Grillhösl C, Gerner P, Hoerning A (2015) Neonatal Cholestasis - Differential Diagnoses, Current Diagnostic Procedures, and Treatment. Front Pediatr 3:43.
20. Fischler B, Lamireau T (2014) Cholestasis in the newborn and infant. Clin Res Hepatol Gastroenterol 38:263-267.
21. Liu P, Guo L, Huang L, Zhao D, Zhen R, Hu X, Yuan X (2015) Analysis of factors affecting the prognosis of neonatal cholestasis. Int J Clin Exp Med 8:8005-8009.
22. Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A (1998) The viral association of neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 27:57-64.
23. De Tommaso AM, Andrade PD, Costa

World J Gastrointest Pathophysiol. Jun 15; 1:69-84.

19. Götze T, Blessing H, Grillhösl C, Gerner P, Hoerning A (2015) Neonatal Cholestasis - Differential Diagnoses, Current Diagnostic Procedures, and Treatment. Front Pediatr. Jul; 3:43.
20. Fischler B, Lamireau T (2014) Cholestasis in the newborn and infant. Clin Res Hepatol Gastroenterol. Jun; 38:263-267.
21. Liu P, Guo L, Huang L, Zhao D, Zhen R, Hu X, Yuan X (2015) Analysis of factors affecting the prognosis of neonatal cholestasis. Int J Clin Exp Med. May 15; 8:8005-9.
22. Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A (1998) The viral association of neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr. Jul; 27:57-64.
23. De Tommaso AM, Andrade PD, Costa SC, Escanhoela CA, Hessel G (2005) High frequency of human cytomegalovirus DNA in the liver of infants with extrahepatic neonatal cholestasis.

SC, Escanhoela CA, Hessel G (2005) High frequency of human cytomegalovirus DNA in the liver of infants with extrahepatic neonatal cholestasis. BMC Infect Dis 5:108.

24. Goel A, Chaudhari S, Sutar J, Bhonde G, Bhatnagar S, Patel V, Bhor V, Shah I (2018) Detection of cytomegalovirus in liver tissue by polymerase chain reaction in infants with neonatal cholestasis. Pediatr Infect Dis J 37: 632-636.
25. Sira MM, Sira AH, Elhenawy IA, Khalil FO (2016) Prevalence of serological markers of TORCH infections in biliary atresia and other neonatal cholestatic disorders. Open J Pediatr Child Heal 2:13-17.
26. Ross SA, Ahmed A, Palmer AL, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Fowler KB, Boppana SB, National Institute on Deafness and Other Communication Disorders CHIMES Study (2014) Detection of congenital cytomegalovirus infection by real-time polymerase chain reaction analysis of saliva or urine specimens. J Infect Dis 210:1415-1418.
27. Boppana SB, Ross SA, Shimamura M, Palmer AL, Ahmed A, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Britt WJ,

BMC Infect Dis. Dec1; 5:108.

24. Goel A, Chaudhari S, Sutar J, Bhonde G, Bhatnagar S, Patel V, Bhor V, Shah I (2018) Detection of cytomegalovirus in liver tissue by polymerase chain reaction in infants with neonatal cholestasis. Pediatr Infect Dis J 37: 632-636.
25. Sira MM, Sira AH, Elhenawy IA, Khalil FO (2016) Prevalence of serological markers of TORCH infections in biliary atresia and other neonatal cholestatic disorders. Open J Pediatr Child Heal. 2:13–17.
26. Ross SA, Ahmed A, Palmer AL, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Fowler KB, Boppana SB, National Institute on Deafness and Other Communication Disorders CHIMES Study (2014) Detection of congenital cytomegalovirus infection by real-time polymerase chain reaction analysis of saliva or urine specimens. J Infect Dis. Nov 1; 210:1415-1418.
27. Boppana SB, Ross SA, Shimamura M, Palmer AL, Ahmed A, Michaels MG, Sánchez PJ, Bernstein DI, Tolan Fowler KB (2011) Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med 364:2111-2118
28. Bhatia P, Narang A, Minz RW (2010) Neonatal cytomegalovirus infection: diagnostic modalities available for early disease detection. Indian J Pediatr 77:77-79.
29. Setyoboedi B, Widayanti R, Arief S, Puspitasari D, Prihaningtyas RA (2021) The agreement of cytomegalovirus (CMV) serology examination and CMV polymerase chain reaction of liver tissue in infants with cholestasis. Sri Lanka J Child Heal 50:43-48.

RW Jr, Novak Z, Chowdhury N, Britt WJ, Fowler KB (2011) Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med. Jun 2; 364:2111-2118

28. Bhatia P, Narang A, Minz RW (2010) Neonatal cytomegalovirus infection: diagnostic modalities available for early disease detection. Indian J Pediatr. Jan; 77:77-79.
29. Setyoboedi B, Widayanti R, Arief S, Puspitasari D, Prihaningtyas RA (2021) The agreement of cytomegalovirus (CMV) serology examination and CMV polymerase chain reaction of liver tissue in infants with cholestasis. Sri Lanka J. Child Heal. 50:43-48

To the layout editor: 1 Figure; 5 Tables

Original Article

Polymerase chain reaction of human cytomegalovirus from liver and urine compared with serological test in cholestasis infants

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Abstract

Introduction: The most common infection in cholestatic infants is caused by human cytomegalovirus (HCMV). The aims were to detect the presentation of HCMV in cholestatic infants and to evaluate the concordance, sensitivity, and specificity between serology and polymerase chain reaction (PCR) of HCMV from liver biopsy and urine specimens.
Methodology: A descriptive observational study with a cross-sectional approach was conducted on 35 cholestatic infants with ethical approval. Specimens were liver biopsy, urine, and anti-HCMV serology. Liver and urine specimens were performed to nested PCR, followed by statistical analysis.
Results: PCR from the liver biopsy and urine specimen were positive in 74.3% and 85.7%, respectively. There was no concordance between IgM with the liver PCR, but there was a concordance between IgM with the urine PCR and between IgG with the liver and urine PCR. The sensitivity and specificity of IgM with the liver PCR were 46 % and 56%, respectively, with a diagnostic accuracy of 49%. While IgG sensitivity was 96% with a diagnostic accuracy of 80%. IgG sensitivity and IgM specificity compared with the urine PCR were 93% and 100%, respectively, with a diagnostic accuracy of more than 60%.
Conclusion: It demonstrates a high prevalence of HCMV DNA in urine and liver biopsy from cholestatic infants. HCMV PCR assay is more sensitive and specific than the anti-HCMV IgM, but IgG has high sensitivity and accuracy diagnostic. Therefore, serological examination is an option for diagnosing HCMV infection in cholestatic infants in developing countries with no PCR facilities.

Key words: infant mortality; infectious disease; developing country; human cytomegalovirus

Running Title: Infection of cytomegalovirus in cholestatic infant

Introduction

The infant mortality rate in developing countries is still high. One of the causes is cholestasis. The incidence of cholestasis in infants is associated with congenital abnormalities or viral infections. The most common cause of infection was Human cytomegalovirus (HCMV) infection [1]. Data showed that the seroprevalence of HCMV in women of childbearing age is approximately 40-80% in a developed country and 90-100% in developing countries [2]. Therefore, it causes the congenital transmission of the virus from mothers who are primary HCMV infected to the fetus [3,4]. This congenital infection of HCMV occurs in approximately 0.5-0.7% of live births [3,5,6]. Most infected newborns are asymptomatic [3,5], but approximately 11% of live birth with congenital HCMV infection were symptomatic [3] such as jaundice (62%), petechiae (58%), and hepatosplenomegaly (50%) [1] and up to 20% develop sensorineural hearing loss or other permanent neurologic sequelae [5] and lead to permanent disabilities [3]. Therefore, proper early diagnosis is very important in order to provide appropriate therapy and reduce the occurrence of permanent disability.

Currently in Indonesia, the most frequently used method for diagnosing HCMV infection is a serological examination of anti-HCMV immunoglobulin M (IgM) and immunoglobulin (IgG) from blood samples. The presentation of IgM antibody to HCMV is formed approximately 1-2 weeks after infections, the titer peaks in 1-3 months, then begins to decrease, and remains detectable up to 4 months [4,5,7]. In addition, anti-HCMV IgG can be detected 2-3 weeks after the appearance of symptoms [4,5] and maternally anti-HCMV IgG from the mother can be detected up to 8 months [2]. However, the sensitivity of IgM detection is still low where IgM was found to be negative in more than 50% of symptomatic children, while in asymptomatic children it was 78% [8]. Therefore, serological examination of anti-CMV IgM and IgG in newborns still cannot fully indicate the presence of HCMV infection in infants.

Polymerase chain reaction (PCR) examination is a virological detection method that is useful in diagnosing viral diseases because of its ability to detect very small amounts of viral DNA. HCMV DNA from infants can be isolated from body tissues such as liver biopsy tissues and body fluids such as tears, salivary, and urine [9,10]. The most common gene target area is the immediate early (IE) gene. On 2-4 hours after infection, the IE gene begins to activate the replication process, and intact virions spread in all body fluids within 48-72 hours after infection [11,12]. Therefore, the presentation of HCMV DNA can be detected from body fluids on the second or third day after infection. The objective of this study was to detect the presentation of HCMV DNA in the liver tissues and urine specimens from infants with cholestasis by PCR and to evaluate the concordance of the IgM and IgG anti-HCMV with PCR of HCMV from liver tissues and urine, as well as the sensitivity and specificity of serologycal test compared to PCR.

Methodology

Sample collection

This study was a descriptive observational study with a cross-sectional approach. This study has received approval from the ethical commission with ethical clearance number 729/Panke.KKE/XII/2017. All parents or guardians of the subjects in this study have received an informed consent explanation and were willing to participate in this study.

The samples were 35 infants with cholestasis who were treated at the Pediatric Inpatient Installation, Department of Child Health Sciences, Dr. Soetomo General Academic Hospital Surabaya in the period December 2017 to December 2018. The operational definition of cholestasis in this study was infants with jaundice, where the conjugated bilirubin level is 20% of the total bilirubin level (if the total bilirubin is greater than (>) 5 milligrams per deciliter (mg/dL) or the direct bilirubin level is > 2 mg/dL (if the total bilirubin is less than (<) 5 mg/dL). Specimen taken from patients were liver biopsy, urine, and serological data. Inclusion criteria were infants with cholestasis and aged 1 to 6 months. Exclusion criteria were patients who had received antiviral therapy, HIV patients, miliary tuberculosis patients, malnourished patients, history of using immunosuppressive drugs such as corticosteroids and cytostatic, platelets < 80.000 mg/dL, prolonged hemostasis function, and ascites.

Serological data collection

Serological examination, which includes anti-HCMV IgM and IgG levels was examined by the Enzyme Linked Fluorescent Assay (ELISA) method using a solid phase receptacle from VIDAS. The interpretation of IgM was that IgM index unit < 0.7 was negative, < 0.7 to 0.9 was equivocal, > 0.9 was positive. The interpretation of IgG was that IgG index unit < 4 was negative, > 4 to < 6 was equivocal, and > 6 was positive.

HCMV PCR from liver biopsy and urine specimens

The liver biopsy and urine specimens were collected in a sterile collection tube and then taken to the Institute of Tropical Diseases, Airlangga University for identification of HCMV infection by nested PCR. Extraction was carried out using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany~~iagen~~) according to the protocol then followed by PCR using primer as reported previously [13,14].

The β globin gene PCR was performed using PCO3+ and PCO4+ primers and the PCR Mastermix (PROMEGA, Madison, USA~~romega~~) which product size were the 325 base pair (bp). The compositions

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were 10 µL master mix (Promega), 1 µL PCO3+ (in a concentration of 10 picomole), 1 µL PCO4+ (in a concentration of 10 picomole), 5 µL ddH_2O, 3 µl DNA template. The initial denaturation 5 minutes at 94 °C for 1 cycle, then 30 seconds of denaturation at 94 °C, 30 seconds of annealing at 55 °C, 45 seconds of elongation at 72 °C, for all were in 40 cycles, and then 7 minutes for final elongation at 72 °C.

The PCR of HCMV DNA was performed using the MIE4 and MIE5 primers which resulted in size 435 bp for the first round and the IE1 and IE2 primer for the second round which resulted in size 161 bp. The compositions were 10 µL master mix (Promega), 1 µL the forward primer, 1 µL the reverse primer, 4 µL ddH_2O, and 4 µL the DNA template. The PCR conditions were 5 minutes of initial denaturation at 94 °C, 30 seconds of denaturation at 94 °C, 30 seconds of annealing at 67 °C, and 45 seconds of elongation at 72 °C. All were carried out for 40 cycles, then 7 minutes for final elongation at 72 °C.

Statistical Analysis

The presentation of HCMV DNA in liver biopsy and urine specimens was shown in percentage. The concordance of anti-HCMV IgM and IgG with HCMV PCR from liver and urine specimens was analyzed by the Fisher's Exact Test 2-sided and McNemar. The sensitivity and specificity were shown in percentage.

Results

There were 35 infants with cholestasis involved in this study, consisting of 20 males and 15 females aged between 1 to 6 months (mean $\pm$ SD = 2.771 ± 1.087). The levels of direct/conjugated bilirubin (D Bil) were 7.955 ± 4.674 (mean + SD) and the total bilirubin (T Bil) was 10.369 ± 5.896 (mean $\pm$ SD) (Table 1).

All samples in this study showed positive results for PCR of the β globin gene, hence continued with detection of HCMV. The result of HCMV PCR from liver tissues and urine specimens were positive in 26/35 (74.3%) and in 30/35 (85.7%) infants, respectively. The product of HCMV PCR is shown in Figure 1.

Serological data showed that IgM positive were found in 16/35 (45.7%) infants and IgG positive were found in 31/35 (88.6%) infants. Acute infection (IgM+ and IgG+), past infection (IgM- and IgG+), and uninfected (IgM- and IgG-) were found in 16/35 (45.7%), 15/35 (42.9%), and 4/35 (11.4%) infants, respectively (Table 2).

There was no concordance between anti-HCMV IgM with HCMV PCR from liver biopsy ($p > 0.05$; $p = 0.929$), but there was concordance between anti-HCMV IgM with HCMV PCR from urine specimens ($p < 0.05$, $p = 0.027$) which kappa coefficient was 0.246 (fair: 0.21 – 0.4) (Table 3).

There was concordance between anti-HCMV IgG with HCMV PCR from liver biopsy ($p < 0.05$; $p = 0.017$) and from urine specimens ($p < 0.05$; $p = 0.030$) with kappa coefficient were 0.360 for HCMV PCR from liver biopsy and 0.364 for HCMV PCR from urine specimens (fair: 0.21 – 0.4) (Table 4).

McNemar (exact sig 2-sided) test showed that there was a significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgM ($p < 0.05$, liver: 0.031, urine: < 0.001), but there was no significant difference between HCMV PCR from liver biopsy and urine specimens with anti-HCMV IgG ($p > 0.05$, liver: 0.125, urine: 1.000) (Table 5).

The sensitivity and specificity of IgM anti-HCMV compared with HCMV PCR of liver biopsy specimens were 46.15% and 55.55%, respectively, with a diagnostic accuracy of 48.57%. While the sensitivity of anti-HCMV IgG is 96.15% with a diagnostic accuracy of 80%. In addition, the sensitivity of IgG and specificity of IgM compared to HCMV PCR of urine specimen showed 93.33% and 100%, respectively, with a diagnostic accuracy of more than 60% (Table 5).

Discussion

Cholestasis is a decrease or obstruction of bile flow at any stage to the extrahepatic biliary tract and duodenum with the main symptoms of cholestasis are jaundice, acholic stools, and dark urine [15-17]. This condition is the most common cause of morbidity and mortality in infants and children. The accumulation of bile acids has an impact on hepatotoxicity. Therefore, it becomes the underlying cause of liver disorders [18]. The identity of prolonged neonatal jaundice more than 2 weeks of early life is an essential procedure for an early diagnosis of cholestasis diseases [10]. The inability to detect and monitor the progression of liver damage will hinder the appropriate management of cholestatic disease.

The most common causes of cholestasis are biliary atresia, α-1 antitrypsin deficiency, and infection, including HCMV infection [20]. HCMV can be transmitted horizontal or maternal from mother to fetus or infant [21]. In this study, the time of infection could not be determined whether during prenatal, natal or postnatal periods, due to the age of infants involved in this study was variable from 1 to 6 months, even though more than 50% of cholestasis occurred in 1 to 2 months infants. This requires further confirmation.

This study used specimens from liver biopsy and urine. It showed high prevalence of HCMV DNA in cholestatic infants. HCMV DNA detected in more than a half of patient that was 74.3% of liver tissue and 85.7% of urine. Another study in liver biopsy tissues in cholestasis infants showed that 48% [22], 34.3% [23], and 52% [24] patients were positive for HCMV DNA. In a Brazilian study on patients with extrahepatic cholestasis, of 33 liver biopsy samples examined by HCMV PCR, 27.3% were positive for HCMV DNA [23]. Research in Egypt involving 94 patients with biliary atresia and 91 patients with neonatal

cholestasis due to other causes (non-biliary atresia), the frequency of HCMV DNA by PCR examination of liver biopsy in patients with biliary atresia was 5.3%, non-biliary atresia 23% [25]. In addition, PCR of urine samples was considered the optimal sample for the detection of HCMV infection in newborns. The PCR results showed that there were 79 of 80 (98.8%) positive urine samples [26].

The use of PCR as a diagnostic method in developing countries is not routinely carried out due to limited equipment and funds. Therefore, serological examination is still used as an alternative method for diagnosing HCMV infection. Serological tests are very useful to determine infection condition, acute infection, or recent infection by examining the IgM or in past infections by examining the presence of HCMV IgG [11,15]. This study showed that there were 45.7% of cholestatic infants in acute infection and 42.9% in past infection. In addition, the data showed that anti-HCMV IgM was in 45.7% and IgG was 88.6% of cholestatic infants. Other studies showed that IgM positive for HCMV in neonatal cholestasis in Sweden was 32.2% and IgG positive was 90% [22] and in Brazil, 28.9% was positive for IgM, both in intra and extra hepatic cholestasis [1]. Neonatal cholestasis in Egypt, IgM HCMV was positive in 12.4% [25]. In addition, other studies in India showed that anti-HCMV IgM was positive in 42% of patients and anti-HCMV IgG was positive in 84% of patients in neonatal cholestasis [24]. The IgM in primary infection of neonatal, showed the IgM reaches the peak at the first of 1 to 3 months, and later the titer begins to decrease [7], but persistent anti-HCMV IgM in the low level usually can be detected in more than 3 months or up to a year [7]. On the other hand, the maternal IgG of HCMV in infants will disappear at 8 months [2].

This study showed that there was concordance between anti-HCMV IgM with HCMV PCR from urine specimens with fair strength of agreement (0.246). It showed all infants with IgM positive were positive PCR from urine specimens. Furthermore, there was no concordance between anti-HCMV IgM with HCMV PCR from liver biopsy, that 12/16 (46.2%) infants with positive anti-HCMV IgM were positive for HCMV PCR. There were 4 infants who showed IgM positive and HCMV PCR negative. This may be because IgM can persist for 6 to 9 months after primary infection [11]. Therefore, IgM serology results are still positive while viral DNA is negative. In addition, there were 5 out of 19 infants with IgM negative, but PCR from liver and urine specimens were positive. This is in accordance with other studies which suggested that the serological examination of HCMV turned out to be a less accurate marker of HCMV infection in liver tissue [24]. The accuracy of serology for detecting HCMV antibodies was low [23]. The positivity of anti-HCMV IgM or HCMV DNA does not indicate the cause of cholestasis, but it implies that the virus may have influenced the severity of the original pathology [25].

This study showed the concordance of anti-HCMV IgG with HCMV PCR from liver and urine specimens with fair strength of agreement. Among 31 cholestatic infants with positive anti-HCMV IgG,

there were 25 (96.2%) infants were positive for HCMV PCR from liver tissue specimens and 28 (93.3%) infants were positive for HCMV PCR from urine specimens. The liver and urine specimens of some infants showed HCMV PCR negative and IgG was positive. The presentation of IgG anti-CMV indicates a past infection, where anti-CMV IgG antibodies were produced for 2 weeks post-infection and persisted for years [11]. Data showed the infants were 3 to 5 months age. This suggested that the virus may have infected in the past. In addition, there were 3 of liver and 2 urine specimens with IgM, IgG, and HCMV DNA were negative. This might indicate that the infants were not infected with HCMV, while the cholestasis was caused by others etiologies [24].

In this study, HCMV PCR from urine specimen had sensitivity higher than specimen from liver, that was 92,31% with the accuracy diagnostic was 77%. HCMV PCR is a highly sensitive method for detecting HCMV in variable clinical samples [11]. In addition, urine specimens are easy to collect, non-invasive, and large amounts of viral shedding are found in body fluids including urine [11]. It is different from a liver biopsy. It is difficult, invasive, painful, require the proper skills and radiological equipment. Therefore, urine sample was more feasible to use as specimen for PCR in diagnosing HCMV infection of cholestatic infants.

In this study, sensitivity and specificity of IgM anti-HCMV compared with HCMV PCR of liver biopsy specimens were 46.15% and 55.55%, respectively, with a diagnostic accuracy of 48.57%. In addition, anti-HCMV IgG still had a high sensitivity of 96.15% in the liver and 93.33 % in urine specimens. This is in accordance with previous studies which stated that the HCMV PCR test was more sensitive and specific than the anti-HCMV serological test [24]. Sensitivity and specificity of anti-HCMV IgM compared with HCMV PCR from liver samples were 69% and 61%, respectively [24]. The sensitivity and specificity of PCR is higher than that of antigenemia, the sensitivity can reach 100%, the specificity is 72-90%, the positive predictive value is 69-90%, and the negative predictive value is 100% [27,28]. In addition, these results indicates that serological examination, when compared with HCMV PCR from urine specimens, shows high diagnostic accuracy that more than 60%. Therefore, in remote areas or area that do not have PCR equipment, the serological examination can still be an option for detecting HCMV infection in cholestatic infants. However, the anti-HCMV serological examination cannot replace PCR [29], so in health centers that have an access to perform PCR, PCR remains a necessity in diagnosing cholestatic infants because it has higher sensitivity and specificity.

Conclusion

This study demonstrated a high prevalence of HCMV DNA in the urine and liver biopsy specimens of cholestatic infants. HCMV PCR in urine had higher sensitivity than in the liver with a diagnostic accuracy of about 77%. Considering this and the patient is an infant, urine is the more widely available specimen for use in the diagnosis of CMV infection in cholestatic infants.

This study found no concordance between IgM with the PCR liver, but there was concordance between IgM with the PCR urine, and between IgG with the PCR liver and urine. In addition, HCMV PCR test was more sensitive and specific than the anti-HCMV serological test which IgM compared with the PCR liver has sensitivity and specificity of about 50%, and compared with the urine PCR has a sensitivity of 53% and specificity of 100%, with the diagnostic accuracy of 60%. Furthermore, IgG compared with the PCR urine has a high sensitivity of 95% with a high accuracy diagnostic of more than 80%, but has a low specificity. Considering the vast territory of Indonesia which consists of thousands of islands, there are still many health centers that lack equipment to perform PCR. Therefore, serological examination is an option for diagnosing HCMV infection in infants with cholestasis. This can also be applied in other developing countries that have not yet reached PCR testing services.

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Conflict of interest

Authors declare there is no conflict of interest.

Ethical permission

The ethical was obtained from the Dr. Soetomo General Academic Hospital, Surabaya, number 729/Panke.KKE/XII/ 2017.

Author contribution

All of the authors contributed to reading and approved the final manuscript.

Alphania Rahniayu: main idea, writing, and editing manuscript, histopathological diagnoses.

Gondo Mastutik: main idea, laboratory examinations, writing, and editing manuscript, reviewing.

Anny Setijo Rahaju: reviewing and histopathological diagnoses.

S.iti Eriaty Nur. Ruslan: laboratory examinations.

Priangga Adi Wiratama: statistical analysis.

Erna Sulistiyani: reviewing manuscript.

Bagus Setyoboedi: collecting patients.

References

1. Oliveira NL, Kanawaty FR, Costa SC, Hessel G (2002) Infection by cytomegalovirus in patients with neonatal cholestasis. Arq Gastroenterol 39: 132-136.
2. Chen J, Hu L, Wu M, Zhong T, Zhou YH, Hu Y (2012) Kinetics of IgG antibody to cytomegalovirus (CMV) after birth and seroprevalence of anti-CMV IgG in Chinese children. Virol J 9: 304.
3. Kenneson A, Cannon MJ (2007) Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 17: 253-276.
4. Revello MG, Gerna G (2002) Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 15: 680-715.
5. Gantt S, Bitnun A, Renaud C, Kakkar F, Vaudry W (2017) Diagnosis and management of infants with congenital cytomegalovirus infection. Paediatr Child Health 22: 72-74.
6. Marsico C, Kimberlin DW (2017) Congenital cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. Ital J Pediatr 43: 38.
7. Gunkel J, van der Knoop BJ, Nijman J, de Vries LS, Manten GTR, Nikkels PGJ, Murk JL, de Vries JIP, Wolfs TFW (2017) Congenital cytomegalovirus infection in the absence of maternal cytomegalovirus-IgM antibodies. Fetal Diagn Ther 42: 144-149.
8. Bilavsky E, Watad S, Levy I, Linder N, Pardo J, Ben-Zvi H, Attias J, Amir J (2017) Positive IgM in congenital CMV infection. Clin Pediatr (Phila) 56: 371-375.
9. Soetens O, Fellous CV, Foulun I (2008) Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. J Clin Microbiol 46: 943-946.
10. Goegebuer T, Van Meensel B, Beuselinck K, Cossey V, Van Ranst M, Hanssens M, Lagrou K (2008) Clinical predictive value of real-time PCR quantification of human cytomegalovirus DNA in amniotic fluid samples. J Clin Microbiol 47: 660-665.
11. Ross SA, Novak Z, Pati S, Boppana SB (2011) Diagnosis of cytomegalovirus infections. Infect Disord Drug Targets 11: 466-474.
12. Crough T, Khanna R (2009) Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev 22: 76-98.
13. Mastutik G, Kurniasari N, Rahniayu A, Rahaju AS, Ruslan SEN, Ilmiah K, Setyoboedi B, Sulistyani E (2022) Detection of cytomegalovirus in urine specimen of cholestasis infants by polymerase chain reaction. Res J Phar Tech 15: 2151-2157.
14. Situmorang L, Setyoboedi B, Arief S, Mastutik G (2019) Infection of cytomegalovirus (CMV) in

cholestasis infant with biliary atresia. Indones J Clin Pathol Med Lab 26: 175-181.

15. Davis AR, Rosenthal P, Escobar GJ, Newman TB (2011) Interpreting conjugated bilirubin levels in newborns. J Pediatr 158: 562-565.
16. Suchy FJ (2004) Neonatal cholestasis. Pediatr Rev 25: 388-396.
17. Rashed YK, Saber MA, Tawfik M, Mourad WS (2013) Histopathological features and accuracy for diagnosing biliary atresia by prelaparotomy liver biopsy in Egypt. Egypt Pediatr Assoc Gaz 61: 42-45.
18. Pereira TN, Walsh MJ, Lewindon PJ, Ramm GA (2010) Pediatric cholestatic liver disease: Diagnosis, assessment of disease progression and mechanisms of fibrogenesis. World J Gastrointest Pathophysiol 1: 69-84.
19. Götze T, Blessing H, Grillhösl C, Gerner P, Hoerning A (2015) Neonatal Cholestasis - Differential Diagnoses, Current Diagnostic Procedures, and Treatment. Front Pediatr 3: 43.
20. Fischler B, Lamireau T (2014) Cholestasis in the newborn and infant. Clin Res Hepatol Gastroenterol 38: 263-267.
21. Liu P, Guo L, Huang L, Zhao D, Zhen R, Hu X, Yuan X (2015) Analysis of factors affecting the prognosis of neonatal cholestasis. Int J Clin Exp Med 8: 8005-8009.
22. Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A (1998) The viral association of neonatal cholestasis in Sweden: A possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 27: 57-64.
23. De Tommaso AM, Andrade PD, Costa SC, Escanhoela CA, Hessel G (2005) High frequency of human cytomegalovirus DNA in the liver of infants with extrahepatic neonatal cholestasis. BMC Infect Dis 5:108.
24. Goel A, Chaudhari S, Sutar J, Bhonde G, Bhatnagar S, Patel V, Bhor V, Shah I (2018) Detection of cytomegalovirus in liver tissue by polymerase chain reaction in infants with neonatal cholestasis. Pediatr Infect Dis J 37: 632-636.
25. Sira MM, Sira AH, Elhenawy IA, Khalil FO (2016) Prevalence of serological markers of TORCH infections in biliary atresia and other neonatal cholestatic disorders. Open J Pediatr Child Heal 2: 13-17.
26. Ross SA, Ahmed A, Palmer AL, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Fowler KB, Boppana SB, National Institute on Deafness and Other Communication Disorders CHIMES Study (2014) Detection of congenital cytomegalovirus infection by real-time polymerase chain reaction analysis of saliva or urine specimens. J Infect Dis 210: 1415-1418.
27. Boppana SB, Ross SA, Shimamura M, Palmer AL, Ahmed A, Michaels MG, Sánchez PJ, Bernstein DI,

Tolan RW Jr, Novak Z, Chowdhury N, Britt WJ, Fowler KB (2011) Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med 364: 2111-2118

28. Bhatia P, Narang A, Minz RW (2010) Neonatal cytomegalovirus infection: diagnostic modalities available for early disease detection. Indian J Pediatr 77: 77-79.
29. Setyoboedi B, Widayanti R, Arief S, Puspitasari D, Prihaningtyas RA (2021) The agreement of cytomegalovirus (CMV) serology examination and CMV polymerase chain reaction of liver tissue in infants with cholestasis. Sri Lanka J Child Heal 50: 43-48.

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Figures

Figure 1. The β-globin gene PCR result size 110 base pair (bp) in lane 2, 3, 4, 5 (A) and the HCMV PCR result size 435 bp for first round in lane 2, 3, 4 and 161 bp for second round in lane 5, 6 (B). The line 1 is PCR marker.

Tables

Table 1. Patient Characteristics.

Table 2. HCMV PCR and anti-HCMV serological from infants with cholestasis.

Table 3. The concordance of anti-HCMV IgM with HCMV PCR from liver biopsy and urine specimens.

Table 4. The concordance of anti-HCMV IgG with HCMV PCR from liver biopsy and urine specimens.

Table 5. Sensitivity and Specificity of anti-HCMV serology compared with HCMV PCR from liver biopsy and urine specimens.