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Birth prevalence and characteristics of congenital cytomegalovirus infection in an urban birth cohort, Jakarta, Indonesia

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ABSTRACT

Objectives: Little is known about the birth prevalence and characteristics of congenital cytomegalovirus (CMV) infection in developing countries. To determine the prevalence and characteristics of congenital CMV infection in Indonesia, we conducted a prospective study in an urban birth cohort of neonates at a national referral hospital in 2016–2017, Jakarta, Indonesia.
Methods: Consecutively born neonates were screened for the presence of CMV by using pan-herpesvirus nested-PCR and Sanger sequencing in saliva and/or urine specimens. Both the neonatal clinical findings as well as maternal characteristics were also evaluated.
Results: From a total of 411 newborns screened, congenital CMV infection was confirmed in 5.8% of the neonates. These CMV-positive newborns were more likely to have ventriculomegaly and thrombocytopenia compared to CMV-negative neonates. Notably, 67% CMV-positive neonates in our study had clinical findings that required medical intervention, from which only nine presented with symptoms suggestive of congenital CMV infection. Furthermore, congenital CMV infected babies were almost four times more likely to be born to mothers that had placenta previa and placental abruption.
Conclusions: Our work highlights the high prevalence of congenital CMV infection in neonates born in one of the biggest referral hospitals in metropolitan Jakarta, Indonesia.

Introduction

Human cytomegalovirus (CMV) is a prototype species member of *Betaherpesvirinae*, a subfamily of the *Herpesviridae* family which is highly prevalent in the human population, with seroprevalence ranging from 45% to 100% (Cannon et al., 2010; Goodrum, 2016). Although CMV usually causes asymptomatic infection, it can establish lifelong persistence and be reactivated occasionally to cause disease in the absence of adequate cellular immunity (Goodrum, 2016). CMV is also a leading cause of congenital (present-at-birth) infections worldwide with overall incidence ranging from 0.6% to 6% of newborns (Manicklal et al., 2013; Lanzieri et al., 2014). Approximately 10% of these newborns have apparent clinical signs of infection at birth (referred to as symptomatic congenital CMV infection) from which 50% will have permanent sequelae including sensorineural hearing loss, motor/cognitive deficit, and vision impairment (Dollard et al., 2007; Manicklal et al., 2013). However, 10–15% of congenital CMV infected babies without clinical signs at birth (referred to as asymptomatic congenital CMV infection) are also known to develop long-term neurological sequalae (Dollard et al., 2007; Manicklal et al., 2013).

Systematic review study has shown a higher prevalence of congenital CMV infection in developing countries (Lanzieri et al., 2014), partly because of the higher maternal CMV seroprevalence.

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However, reliable estimates of congenital CMV prevalence and outcome from Southeast Asian countries, including Indonesia, are limited (Usman et al., 2014; Setyoboedi et al., 2015). Without reliable data, the disease burden cannot be clearly determined to make a better screening and intervention strategy for the Indonesian newborn population. The objective of this study was therefore to determine the prevalence and identify the characteristics of congenital CMV infection in an urban birth cohort of neonates from a national referral hospital in Jakarta, Indonesia.

Materials and methods

Patients, clinical data, and samples collection

A cross-sectional screening study in newborns was conducted at the Integrated Service Unit of the Perinatology and Neonatal Intensive Care Unit (NICU), Department of Pediatrics, Dr. Cipto Mangunkusumo National Central Hospital, Jakarta, Indonesia from October 2016 to April 2017. There was a total of 1,066 births registered in the hospital during the study period. Approximately 175,000 newborns are born in Jakarta every year (Kementerian Kesehatan Republik Indonesia, 2017, 2018). This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (no. 925/UN2.F1/ETIK/2016). Written informed consent for participating in the study was obtained from all parents of the newborns.

Neonates consecutively born in the hospital were selected for enrollment to the study based on the following inclusion criteria: parental consent to participate and possibility to obtain saliva and urine samples during the first 21 days of birth. Parents were approached for consenting and enrollment during hospital admission. Neonates were excluded from the study if the parents refused to participate or if specimens could not be obtained. No randomization was done during enrollment. To determine the prevalence of congenital CMV infection, the sample size was estimated based on 6% prevalence of congenital CMV infection in China (Zhang et al., 2007). The sample size necessary for 95% confidence, 3% margin of error, and accounting for clustering with a design effect of 1 was estimated to be 245 newborns.

Saliva and urine samples were collected from enrolled newborns during hospital stay after delivery. Saliva samples were collected using sterile cotton swab and preserved in vials containing viral transport medium (Eagle's minimum essential medium, 500 U/ml penicillin, 500 μg/ml streptomycin, and 10% fetal bovine serum all from Gibco, Carlsbad, CA), while urine samples were collected with a pediatric urine collector bag for males and with sterile cotton wool balls for females. Sterile cotton wool balls placed inside diapers were used for females because of the challenge of collecting adequate urine samples when using urine collector bag due to leakage. There was no difference between sensitivity and specificity to detect CMV DNA between the two methods as reported by a recent study (Ross et al., 2015). Data from routine clinical evaluations of the newborns, including gestational age, gender, weight, height, head circumference, blood analysis, Apgar score, prematurity, congenital anomalies, and concomitant diseases, were collected from the medical record. Data of the mothers including demographic and clinical information as well as maternal history of pregnancy were from interviews conducted by research staff and from medical records. Samples were stored at 4 °C before transportation within 24 h to the Emerging Virus Research Unit (EVRU), Eijkman Institute for Molecular Biology, where they were stored at −80 °C before further testing.

Newborns with positive CMV results were followed up with more thorough evaluations, including growth and development through routine physical examination, head ultrasonography, audiological, and ophthalmological examinations. Parents were also requested to provide urine and saliva samples of the neonates after 3 and 6 months of birth. Growth was evaluated by measuring weight, height, and head circumference based on Fenton growth chart (for preterm babies) and WHO chart (for term babies), while development was assessed by using Bayley Infant Neurodevelopmental Screener (Aylward, 1995; Fenton, 2003; WHO, 2014). Head ultrasonography was performed to assess central nervous system (CNS) abnormalities and was considered normal if showing: normal size, shape and position of ventricle system; absence of intra and extra axial focal lesion; normal interhemispheric sulci and fissure; normal basal ganglia and cerebellum; as well as normal echogenicity of parenchyma. Audiological examination was done by using brainstem evoked response audiometry (BERA) assessment and was considered normal if hearing threshold was $\leq$30 dB. Ophthalmological examination was performed to search for common eye abnormalities found in congenital CMV infection and was considered normal if the following were absent: chorioretinitis, retinal scars, optic atrophy, central vision loss, and strabismus. Echocardiography was performed according to the published guidelines (Lai et al., 2006).

Detection of CMV infection

All saliva and urine specimens were tested for the presence of CMV DNA by using pan-herpesvirus nested-PCR as described previously (Chmielewicz et al., 2001; Mawuntu et al., 2018). Briefly, total nucleic acids were extracted by using QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) from 200 μl of specimens. Two microliters of the extracted nucleic acids were used as a template for subsequent two rounds PCR with mixture consisting of GoTaq Green Master Mix (Promega, Madison, USA) and primer pair to amplify CMV terminase-enconding gene. The following oligonucleotide primers were used: TS-TERM_707s (5′-TTGTGGACGAGRSIMAYTTYA-3′) and TS-TERM_707as (5′-ACAGCCACGCCNGTICCIGAIGC-3′) for round 1; and TS-TERM_708s (5′-GCAAGATCATNTTYRTITCITC-3′) and TS-TERM_708as (5′-TGTTGGTCGTRWAIGCIGGRT-3′) for round 2. PCR amplicons from patient samples with expected size (419 base pair) were further purified and sequenced with TS-TERM_708s and TS-TERM_708as primer pair by Sanger sequencing method in at least one type of specimen to confirm the identification of human CMV. The technical sensitivity of this pan-herpesvirus nested-PCR has previously been determined by our lab to be able to detect at least one copy of in-house CMV DNA control with known concentration derived from purified PCR product of human CMV cell culture isolate (Mawuntu et al., 2018). This assay sensitivity has not been compared to any commercial CMV detection kit.

Precautions were taken to reduce the risk of cross-contamination by working in three designated separate rooms: PCR reagent preparation room, nucleic acid extraction room, and amplification/gel electrophoresis room. Negative and positive controls were included in each assay run. Synthetic DNA plasmid constructs kindly provided by PREDICT USAID were used as positive control. Amplified products of this positive control contain a series of primer-binding sites rather than a real viral sequence to allow easy recognition if sample contamination occurs (Anthony et al., 2015).

Statistical analysis

Statistical analysis was performed using OpenEpi v3.01, SPSS v21, and GraphPad Prism v8. Quantitative data differences between groups were compared by unpaired Student *t* test for normally distributed data (based on D'Agostino-Pearson normality test) or by Mann-Whitney test for non-Gaussian distributed data.

Categorical data were compared using Mantel-Haenszel chi-square test when all expected numbers are at least 1 or otherwise by using Fisher's exact test. *P*-values less than 0.05 were considered statistically significant.

Results

From October 2016 to April 2017, 552 pregnant women admitted for delivery were approached for enrollment, from which 411 neonates with consent from parents and sufficient volume of samples were included in the study. Of the 411 enrolled newborns, 304 had both urine and saliva samples collected, while 99 and 8 newborns had saliva and urine samples only, respectively (Figure 1). Overall, 5.8% (24/411, 95% CI 3.9–8.7%) of neonates were confirmed to have congenital CMV infection by pan-herpesvirus PCR and Sanger sequencing of PCR product in at least one type of specimen. No other pathogens were identified, although the pan-herpesvirus PCR can detect a broad range of herpesviruses including herpes simplex virus, Epstein-Barr virus, and varicella-zoster virus (Anthony et al., 2015; Mawuntu et al., 2018).

From 304 neonates with both urine and saliva samples available, 1.3% (4/304) had detectable CMV DNA in both samples while 5.3% (16/304) had detectable CMV DNA in either urine or saliva only. From 107 neonates with only urine or saliva sample available, CMV DNA was detected in saliva of three neonates and in urine of one neonate. The median time of urine and saliva sample collection of the CMV-positive was 1 day (range 0–20 days) and 0 days (0–14 days) after birth, respectively. Seven out of nine CMV-positive saliva samples (with negative result in urine or without available urine samples) were collected at day 0 after birth. The other two CMV-positive saliva samples were collected at day 1 and 2 after birth.

Comparison between CMV-positive (N = 24) and CMV-negative neonates (N = 387) is shown in Table 1. Notably, significantly higher frequency of ventriculomegaly (38% vs 4%, $P < 0.000$) and thrombocytopenia (33% vs 9%, $P = 0.001$) were found in CMV-positive compared to CMV-negative neonates. There were also a higher proportion of female patients (71% vs 51%, $P = 0.063$) as well as heart (80% vs 38%, $P = 0.066$), hearing (11% vs 0%, $P = 0.063$), and musculoskeletal (4% vs 0%, $P = 0.058$) defects in CMV-positive cases, although not statistically significant. The frequency of admission to NICU or special care, prematurity, fatal outcome, and presence of other comorbidities was not significantly different between the two groups. Furthermore, no other significant differences were found in median birth weight, birth length, head circumference, Apgar score, and other laboratory results.

Clinical characteristics of the 24 neonates with congenital CMV infection are described in Table 2. Of these 24 CMV-positive cases, 16 (67%) were symptomatic at birth and required admission to NICU or special care unit because of the presence of birth defects, jaundice, sepsis, and respiratory distress. Notably, all of these 16 symptomatic CMV-positive cases had more than one comorbidity, from which three died within the first month of life (case no. 6, 15, 18) and two died at the age of 3 months (case no. 4 and 5). No significant abnormalities were found from ten CMV-positive neonates with available follow-up evaluations. Moreover, from six CMV-positive cases with available follow-up specimens (median 5; range 2–7 months), only one case (17%) still had detectable CMV in his urine 4 months after birth (Table 2; case no. 13). Parents of the CMV-positive patients were contacted to inform the CMV screening result and the option for antiviral treatment was discussed with eligible patients. However, none of the patients received antiviral treatment.

Clinical characteristics of all mothers of the neonates enrolled in this study are shown in Table 3. The numbers of mothers and

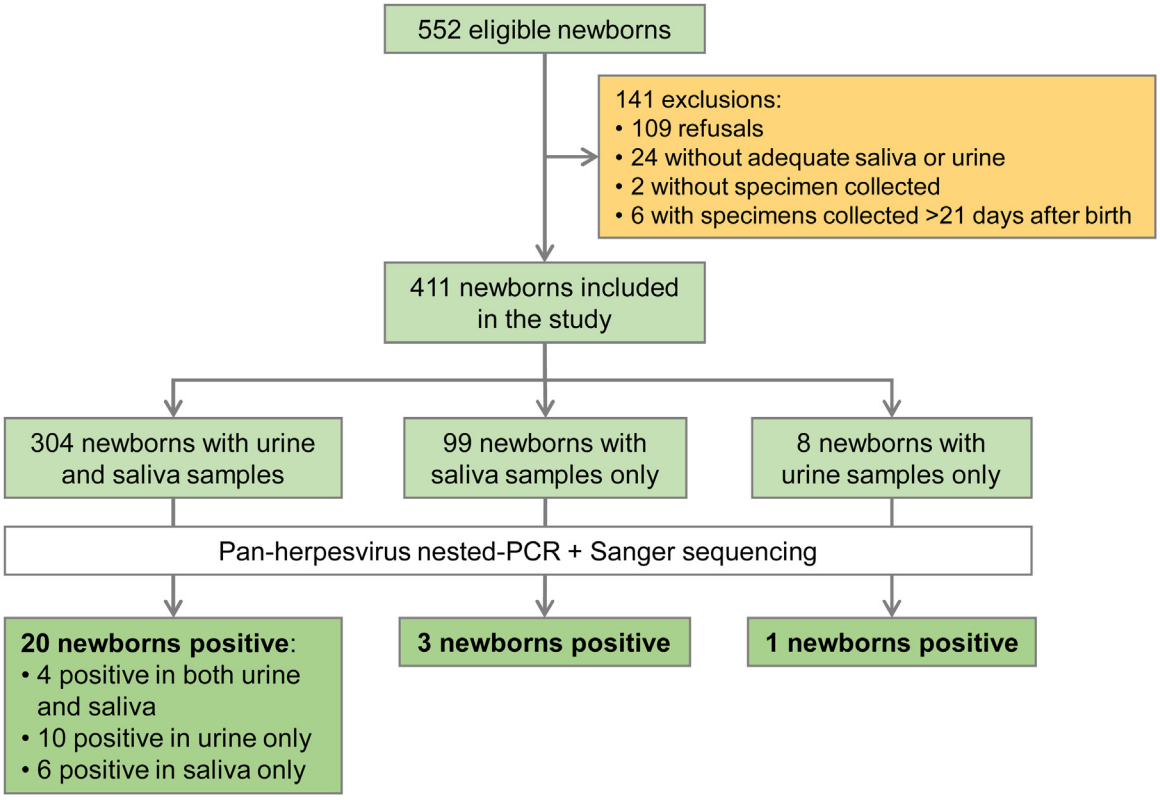

Figure 1. Flow diagram of total cases enrolled, collected samples, and outcome of the testing.

Table 1
Comparison of clinical characteristics, congenital anomalies, and laboratory findings between CMV-positive and CMV-negative neonates.

Parameter	CMV-positive, N = 24	CMV-negative, N = 387	*P*-value
Clinical characteristics			
Neonates gender, female[a]	17 (71)	198/386 (51)	0.063
Mode of delivery			
Vaginal	6 (25)	123 (32)	0.488
Caesarean	18 (75)	264 (68)	
Gestational age at birth in weeks	36 (29–41)	37 (25–41)	0.790
Normal (36–42 weeks)	14 (58)	237 (61)	0.777
Premature (<36 weeks)	10 (42)	150 (39)	
Birth weight in gram	2,540 (1,625–4,235)	2,446 (550–6640)	0.328
Birth length in cm	44 (31–53)	46 (26–56)	0.356
Head circumference in cm	32 (23–37)	32 (16–59)	0.124
Apgar score at 1 minute	8 (1–9)	8 (0–9)	0.455
Apgar score at 5 minutes	9 (2–10)	9 (1–10)	0.342
Require resuscitation	9 (38)	113 (29)	0.388
Require admission to NICU or special care	16 (67)	213 (55)	0.266
Sepsis	11 (46)	163 (42)	0.767
Respiratory distress	11 (46)	158 (41)	0.675
Jaundice	5 (21)	76 (20)	0.931
Other comorbidities at birth	8 (33)	81 (21)	0.182
Congenital anomalies			
Neonates with at least 1 birth defect	5 (21)	46 (12)	0.198
Microcephaly	0 (0)	14 (4)	1.000
Macrocephaly	0 (0)	5 (1)	1.000
CNS defect	3/8 (38)	7/86 (8)	**0.010**
Ventriculomegaly	3/8 (38)	3/86 (4)	<**0.000**
Hydrocephalus	0/8 (0)	1/86 (1)	1.000
Multiple periventricular cyst	0/8 (0)	1/86 (1)	1.000
Hypoxic ischemic encephalopathy	0/8 (0)	1/86 (1)	1.000
Agenesis of the corpus callosum	0/8 (0)	1/86 (1)	1.000
Heart defect	4/5 (80)	21/56 (38)	0.066
Gastrointestinal defect	0 (0)	5 (1)	1.000
Genitourinary defect	1 (4)	3 (0.8)	0.212
Eye defect	0/3 (0)	2/66 (3)	1.000
Hearing defect	1/9 (11)	0/133 (0)	0.063
Musculoskeletal	1 (4)	0 (0)	0.058
Maxillofacial	0 (0)	1 (0.3)	1.000
Laboratory findings			
Hemoglobin in mg/dl	15 (10–21), N = 16	16 (8–23), N = 202	0.913
Leukocyte in $10^3/\mu l$	11 (5–38), N = 15	13 (3–83), N = 203	0.325
Thrombocyte in $10^3/\mu l$	217 (11–493), N = 15	247 (8–450), N = 202	0.386
Thrombocytopenia	5/15 (33)	18/202 (9)	**0.001**
CRP in mg/l	0.3 (0.1–5), N = 15	0.2 (0–46), N = 198	0.237
Immature to total neutrophil ratio	0.15 (0.02–0.4), N = 15	0.1 (0.01–0.5), N = 194	0.222
Procalcitonin in ng/ml	0.6 (0.1–4), N = 8	1 (0–55), N = 73	0.245
Total bilirubin in mg/dl	10 (0–18), N = 9	10 (1–19), N = 132	0.909
Blood glucose mg/dl	80 (6–100), N = 15	86 (11–175), N = 199	0.295

Data are presented as median (range), n (%), or n/N (%) where data are missing. *P*-value below 0.05 is highlighted in bold.
[a] Sex from one case could not be determined from CMV-negative group.

neonates were different because there were 29 mothers who had a twin pregnancy. However, specimens were collected from only 55 neonates. Among the twins, there were two twins in whom congenital CMV infection was confirmed in both; one where only one of the twins was infected; and another where one of the twins was infected while the status of the other one could not be determined because of unavailable samples. In total, 22 mothers of 24 CMV-positive neonates and 363 mothers of 387 CMV-negative neonates were evaluated.

The proportion of placenta previa and placental abruption cases which were diagnosed by clinical presentation and ultrasonography were found to be significantly higher in mothers of the CMV-positive compared to the CMV-negative group (18% vs 5%, $P = 0.013$) (Table 3). In our study, placenta previa was diagnosed if the placenta was inserted completely or partially in the lower uterine segment during pregnancy, while placental abruption was defined by bleeding caused by premature separation of a normally sited placenta from its attachment to the uterus.

Frequency of twin pregnancy was higher in mothers of the CMV-positive compared to CMV-negative group (18% vs 7%), although the difference was not quite statistically significant ($P = 0.052$). No other significant differences were observed including mothers' age, socioeconomic, educational status, obstetrical history, complications and medical treatment history during pregnancy, and other complications during delivery between mothers of the CMV-positive and CMV-negative group.

Discussion

There is very limited information in Indonesia on congenital CMV infection, which has been recognized as the most frequent infectious cause of neonatal birth defects in both developing and developed countries (Lanzieri et al., 2014). We believe that our study is the first to provide a report on congenital CMV infection from a prospective screening study in Indonesia. By using a sensitive molecular assay to detect CMV DNA in newborns' saliva and urine samples, we found that one in every seventeen newborns in our study population was congenitally infected with CMV.

Table 2
Clinical characteristics of neonates with confirmed congenital CMV infections.

No.	CMV-positive sample		Twin	Gestational age in week	Birth weight in gr	Birth length in cm	Head circum-ference in cm	Admission to NICU or special care	Birth defect	Comorbidities during hospitalization	Follow-up findings (age)[b]						
	Urine	Saliva									Growth development	Eye	BERA	Head USG	Echo-cardiography	Other	CMV DNA detection
1	+	–	No	37	2,715	49	32	Yes	Musculoskeletal (CTEV)	Respiratory distress, hyperthyroidism	NA	NA	NA	NA	NA	NA	NA
2	+	–	No	39	3,645	50	35	No	No	NA	NA	NA	NA	NA	NA	NA	Negative in urine (7 m); saliva NA
3	+	–	No	29	1,625	39	31	Yes	No	Sepsis, respiratory distress, and hemangioma	Normal (11 m)	Normal (5 and 14 m)	Normal (6 and 14 m)	Normal (7 and 14 m)	NA	NA	NA
4[a]	+	+	Yes	38	4,235	43	32	Yes	Heart (CAVSD, TF, PDA, and mild pericardial effusion) and ventriculomegaly	NA	NA	NA	NA	NA	NA	Deceased (3 m)	NA
5[a]	+	+	Yes	38	4,235	43	32	Yes	Heart (CAVSD, TF, PDA, and mild pericardial effusion) and ventriculomegaly	NA	NA	NA	NA	NA	NA	Deceased (3 m)	NA
6	+	+	No	36	3,000	53	32	Yes	No	Sepsis, respiratory distress, anoxic brain damage, pneumonia, anemia, and thrombocytopenia	NA	NA	NA	NA	NA	Deceased (1 m)	NA
7	–	+	No	38	3,020	50	32	No	No	NA	NA	NA	NA	NA	NA	NA	Negative in urine (5 m); saliva NA
8	+	–	No	39	3,393	50	33	Yes	No	Sepsis	NA	NA	NA	NA	NA	NA	NA
9	+	–	No	41	3,935	50	37	No	No	NA	Normal (6 m)	NA	Normal (6 m)	Normal (4 m)	NA	NA	Negative in urine (6 m; saliva NA
10	+	+	Yes	36	1,654	41	32	Yes	No	Prematurity	Normal (2 m)	Normal (2 m)	Normal (3 m)	Normal (2 m)	Normal (2 m)	NA	NA
11	+	+	Yes	36	2,010	43	23	Yes	No	Sepsis	Normal (2 m)	Normal (2 m)	Normal (3 m)	Normal (2 m)	Normal (2 m)	NA	NA
12	NA	+	No	37	3,160	47	32	No	No	NA	NA	NA	NA	NA	NA	NA	NA
13	+	–	No	33	2,327	45	31	Yes	Heart (PDA and PFO), ventriculomegaly, and genitourinary	Sepsis, respiratory distress, cholestasis, cardiomegaly, anemia, and thrombocytopenia	Normal (6 m)	Normal (6 and 9 m)	Normal (6 and 9 m)	Normal (7 and 9 m)	Normal (7 m)	NA	Positive in saliva (4 m); urine NA
14	+	–	No	33	2,143	43	32	Yes	No	Sepsis, respiratory distress, jaundice, and thrombocytopenia	Normal (6 m)	NA	NA	Normal (14 d)	NA	NA	Negative in urine and saliva (6 m)
15	+	–	No	32	1,807	41	29	Yes	No	Sepsis, respiratory distress, and jaundice	NA	NA	NA	NA	NA	Deceased (7 d)	NA
16	–	+	No	41	3,465	31	32	No	No	NA	NA	NA	NA	NA	NA	NA	NA
17	NA	+	No	38	2,800	41	32	Yes	No	Sepsis, respiratory distress,	Normal (10 m)	NA	NA	Normal (10 m)	NA	NA	NA
18	+	–	No	33	1,960	44	30	Yes	No	Sepsis shock and respiratory distress	NA	NA	NA	NA	NA	Deceased (3 d)	NA
19	NA	+	No	34	2,130	45	30	No	No	NA	NA	NA	NA	NA	NA	NA	NA
20	–	+	No	35	2,365	43	30	No	No	NA	NA	NA	NA	NA	NA	NA	NA

Table 2 (*Continued*)

No.	CMV-positive sample		Twin	Gestational age in week	Birth weight in gr	Birth length in cm	Head circum-ference in cm	Admission to NICU or special care	Birth defect	Comorbidities during hospitalization	Follow-up findings (age)[b]						
	Urine	Saliva									Growth development	Eye	BERA	Head USG	Echo-cardiography	Other	CMV DNA detection
21	+	–	Yes	33	2,032	43	31	Yes	Heart (PDA) and hearing (abnormal OAE results)	Sepsis, respiratory distress, pneumonia, cholestasis, abnormal liver function, embolism and thrombosis of arteries of lower extremities, thrombocytopenia, anemia, and hepatosplenomegaly	NA	NA	NA	NA	NA	NA	NA
22	–	+	No	33	1,992	44	32	Yes	No	Sepsis, respiratory distress, anemia, and thrombocytopenia	Normal (3 w)	NA	NA	NA	NA	NA	NA
23	–	+	No	37	2,955	46	30	No	No	NA	Normal (5 m)	NA	NA	NA	NA	NA	Negative in urine (2 m); saliva NA
24	–	+	Yes	33	1,627	43	32	Yes	No	Respiratory distress and jaundice	Normal (6 m)	Normal (5 m)	Normal (5 m)	Normal (5 m)	NA	NA	NA

NICU, Neonatal Intensive Care Unit; BERA, brainstem evoked response audiometry; NA, not available; PDA, patent ductus arteriosus; CAVSD, complete atrioventricular septal defect; PFO, patent foramen ovale; OAE, otoacoustic emission; CTEV, congenital talipes equinovarus; TF, Tetralogy of Fallot; US, ultrasound.

[a] Neonates number 4 and 5 are thoracopagus conjoined twins. Birth weight from these twins could not be assessed individually.

[b] Age in month (m), week (w), or day (d).

The 5.8% prevalence of congenital CMV infection found in our study from an urban birth cohort in Indonesia is higher compared to other newborn screening studies in Finland (0.2%) (Puhakka et al., 2018), Japan (0.3%) (Yamaguchi et al., 2017), Iran (0.5%) (Karimian et al., 2016), Panama (0.6%) (Estripeaut et al., 2007), China (0.7%) (Wang et al., 2017), Brazil (1.1%) (Mussi-Pinhata et al., 2009), India (2.1%) (Dar et al., 2008), and Nigeria (3.8%) (Olusanya et al., 2015), but similar to those observed in a peri-urban birth cohort in Gambia (5.4%) (van der Sande et al., 2007) and in a high-risk population in China (6.1%) (Zhang et al., 2007). Although maternal CMV immune status was not assessed in our study, an earlier report on healthy female blood donors has documented a 97.7% CMV seroprevalence in metropolitan Jakarta (Noviar et al., 2017). It is thus very likely that nearly all of the mothers in our study were CMV seropositive, which might explain the high prevalence of congenital CMV infection. As one review study has shown, congenital CMV birth prevalence paradoxically increased with maternal CMV seroprevalence (Kenneson and Cannon, 2007).

The use of saliva samples for detection of congenital CMV infection has been shown to be very sensitive (>97%) and specific (99%) in a prospective multicenter study (Boppana et al., 2011). Although CMV shedding in mothers' breastmilk could lead to false-positive results when using saliva for detection of congenital infection, the false-positive rates has been shown to be very low, which ranged from 0.03% to 0.14% (Ross et al., 2018). In our study, the median time of saliva sample collection of the CMV-positive was 0 days (0–14 days) after birth. From nine CMV-positive saliva samples (with negative result in paired urine or without available urine samples), seven samples were collected at day 0 after birth while the other two were collected at days 1 and 2 after birth, suggesting a minimal contribution of CMV DNA contamination from breastmilk in our study.

Our study also revealed that CMV-positive neonates were more likely to have ventriculomegaly and thrombocytopenia compared to CMV-negative neonates at birth as previously reported in a number of studies (Enders et al., 2001, 2017; Lipitz et al., 2002; Malinger et al., 2003). Interestingly, we also found a higher proportion of heart defects in CMV-positive neonates although the difference was not quite statistically significant. Apart from a few studies in neonates and case reports that have demonstrated fetal cardiac involvement (Campbell et al., 1995; Barnett et al., 2010), congenital heart defect with CMV infection was rarely reported. Moreover, it has also been shown that CMV infection of the heart is common in immunocompetent patients with fatal myocarditis (Kytö et al., 2005), suggesting the potential of CMV to infect heart tissue and causing abnormalities.

In our study, we did not find any significant association between congenital CMV infection and maternal age, primiparity, or socioeconomic status, which have been shown as maternal risk factors for congenital CMV infection in developing countries (Kenneson and Cannon, 2007; van der Sande et al., 2007; Dar et al., 2008; Mussi-Pinhata et al., 2009; Wang et al., 2017). However, we found that mothers of CMV-positive neonates were almost four times more likely to have placenta previa and placental abruption compared to mothers of CMV-negative neonates. A single study investigating the exposure of herpesvirus in antepartum hemorrhage (caused by both placenta previa and placental abruption) did not find any association between the two (Gibson et al., 2008). Whether placenta previa and placental abruption facilitate greater CMV transmission to the fetus or are simply a direct consequence of CMV infection is not known. Nevertheless, it has been suggested that viral infection of extravillous trophoblast cells, specialized fetally derived cells that invade the maternal uterus allowing direct contact with the maternal blood, might negatively impact the process of placental invasion (Koi et al., 2001). It has also been shown that CMV infection inhibits migration and invasion of

Table 3
Comparison of clinical and demographic characteristics as well as complications during pregnancy and delivery between mothers of CMV-positive and CMV-negative neonates.

Parameter	Mother of CMV-positive cases, N = 22	Mother of CMV-negative cases, N = 363	*P*-value
Clinical and demographic characteristics			
Age, median (range)	29 (20 – 39)	29 (15 – 47)	0.745
<25-year-old	4 (18)	88 (24)	0.518
Educational status			
Primary-secondary school	17 (77)	280 (77)	0.988
Tertiary school	5 (23)	83 (23)	
Number of people in household	3 (2 – 6)	3 (2 – 11)	0.866
≥3 people	18 (82)	263 (73)	0.337
Monthly family income in million IDR, median (range)	4 (2 – 12)	3 (0.2 – 20)	0.318
Lower socioeconomic status[a]	10 (45)	186 (50)	0.599
Obstetrical history			
Gravidity, median (range)	2 (1 – 8)	2 (1 – 8)	0.475
Parity, median (range)	1 (0 – 5)	1 (0 – 6)	0.235
Abortions, median (range)	0 (0 – 2)	0 (0 – 6)	0.489
Primiparity	6 (27)	143 (39)	0.258
Twin pregnancy	4 (18)	25 (7)	0.052
Complication and medical treatment during pregnancy			
All complications	17 (77)	319 (88)	0.148
Hypertension	4 (18)	59 (16)	0.813
Diabetes	2 (9)	17 (5)	0.355
Pre-eclampsia/eclampsia	4 (18)	53 (15)	0.646
HIV infection	1 (5)	5 (1)	0.245
Urinary tract infection	0 (0)	22 (6)	0.627
Fever without known cause	0 (0)	24 (7)	0.382
Upper respiratory illness	0 (0)	5 (1)	1.000
Gastroenteritis	0 (0)	7 (2)	1.000
Dengue fever	0 (0)	1 (0.3)	1.000
Varicella	0 (0)	2 (0.6)	1.000
Abnormal vaginal discharge	14 (64)	263 (73)	0.372
Other complications	3 (14)	84 (23)	0.301
Antibiotic treatment	1 (5)	23 (6)	0.736
Complication during delivery			
All complications	17 (77)	325 (90)	0.077
Hypertension	3 (14)	51 (14)	0.957
Diabetes	1 (5)	15 (4)	0.925
Pre-eclampsia/eclampsia	3 (14)	66 (18)	0.590
Fever	0 (0)	6 (2)	1.000
Premature rupture of membrane	7 (32)	189 (52)	0.065
Hemorrhage (post-delivery)	1 (5)	6 (2)	0.325
Placenta previa and placental abruption	4 (18)	19 (5)	**0.013**

Data are n (%) unless otherwise stated. *P*-value below 0.05 is highlighted in bold. NA, not available. IDR, Indonesian rupiah.
[a] Based on a standard minimum regional income during the study period. Monthly income below IDR 3,350,000 was categorized as lower socioeconomic status.

human extravillous cytotrophoblasts, suggesting that the virus can impair placentation and contribute to some of the placental defects seen in CMV-positive pregnancies (Warner et al., 2012).

The CMV-positive neonates identified in our study had multiple symptoms and comorbidities that were non-specific for symptomatic congenital CMV infection (Rawlinson et al., 2017), including sepsis and respiratory distress. Although many published studies have used several different criteria to define symptomatic CMV infection, a consensus definition has been proposed by the International Congenital Cytomegalovirus Recommendations Group which includes multiple manifestations of thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis (raised transaminases or bilirubin), or CNS involvement, abnormal cerebrospinal fluid indices for age, chorioretinitis, and sensorineural hearing loss (Rawlinson et al., 2017). Notably, 16 out of 24 (67%) CMV-positive neonates in our study had symptoms and/or comorbidities that required medical intervention, from which only nine presented characteristic symptoms of congenital CMV infection, including CNS involvement (ventriculomegaly), thrombocytopenia, hepatosplenomegaly, jaundice (raised bilirubin), and abnormal otoacoustic emission test. The presence of asymptomatic CMV-positive neonates as well as those with uncharacteristic manifestations in our study highlights the importance for better screening strategies for congenital CMV infection. As systematic review studies have shown, 1 out of 10 asymptomatic children with congenital CMV infection will later experience hearing loss (Goderis et al., 2014; Bartlett et al., 2017). Although all CMV-positive neonates with follow-up examinations in our study had normal growth and development, head ultrasound, eye, and BERA evaluations, as well as echocardiography, we believe that these were probably related to the short follow-up period (all were less than 1.5 year). A recent large longitudinal study has found that 7.8% asymptomatic congenital CMV infected children developed delayed-onset hearing loss which was diagnosed around 20 months after birth (Goderis et al., 2016), suggesting that an extended follow-up period around 2 years is necessary to determine some late-onset birth defects.

There were some limitations in our study. First, since Dr. Cipto Mangunkusumo National Central Hospital is a referral hospital for management of high-risk pregnancies or those with complications, the 5.8% prevalence of congenital CMV reported in this study could not be extrapolated to other cities or regions in the country. Second, the detection of CMV DNA in saliva without a confirmatory result in urine and/or subsequent samples might contribute to low-rate false-positivity and hence overestimation of overall CMV prevalence. Third, we did not test maternal anti-CMV IgG to determine CMV serostatus although it is very likely that >90% of

the mothers in this study were CMV seropositive as reported in an earlier study (Noviar et al., 2017). Fourth, we could not conclusively determine the long-term impact of congenital CMV infection since we were unable to do follow-up examinations in most of the CMV-positive neonates. Lastly, we did not perform other tests to exclude other common congenital infections including rubella virus, *Toxoplasma gondii*, syphilis, lymphocytic choriomeningitis virus, and parvovirus B19. Nevertheless, with all the limitations, our study revealed the high prevalence of congenital CMV infection and to our knowledge is the first to describe the clinical characteristics from an Indonesian population.

Conclusions

CMV infection is a major cause of congenital infection, yet is understudied in developing countries. Our study revealed that one in every seventeen (5.8%) newborns in our study population was congenitally infected with CMV, which is in the higher range compared to other developing countries. We further discovered that congenital CMV infection was associated with ventriculomegaly and thrombocytopenia. Furthermore, congenital CMV infected babies were found to be more likely to be born to mothers with placenta previa and placental abruption. Our study is the first to highlight the high prevalence of congenital CMV infection in Jakarta, the biggest city with largest population in Indonesia, but needs to be expanded to other regions or cities in Indonesia to optimize public health measures as well as better screening and intervention strategies.

Author contribution

Conceptualization and study design: NDP; Data curation and management: NDP, AW, RD, IYS, AKD, MMR, NJ, YYA; Data analysis: NDP, AW, RD; Funding acquisition: NDP, DS; Laboratory experiments: AW, IYS, AKD; Patient' samples and clinical data collection: NDP, MMR, NJ, YYA, SH, ATPI, AP, MRK, HIS; Supervision: NDP, SH, ATPI, AP, MRK, HIS, SRH, KSAM, DS; Writing (original draft preparation): NDP, AW, RD; Writing (review & editing): NDP, RD, SRH, KSAM, DS.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (no. 925/UN2.F1/ETIK/2016). Written informed consent for participating in the study was obtained from all parents of the newborns.

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Conflict of interest statement

The authors have declared that no competing interests exist.

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