PREVALENCE OF CYTOMEGALOVIRUS (CMV) INFECTION AMONG INFANTS AND CORRELATION BETWEEN CMV PCR WITH CLINICAL OUTCOMES IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

DR NORJIHAN BINTI AB HAMID

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PATHOLOGY (MEDICAL MICROBIOLOGY)

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Symbols/ Abbreviations	Meaning
-	Negative or subtraction
+	Positive or addition
±	Plus-minus
≥	Greater than or equal to
≤	Less than or equal to
‡	Fisher`s Exact Test
x^2	Pearson Chi Square
ε	Mann- Whitney U test
°C	Degree Celsius
µl	Microliter
x	Times or multiplication
%	Percentage
/	Division or `or´
=	Equal to
~	Approximately
AIDS	Acquired Immunodeficiency Syndrome
cCMV	Congenital CMV infection
CF	Complement-fixing
CMV	Cytomegalovirus
CNS	Central nervous system
COI	Cut off index
DNA	Deoxyribonucleic acid

Symbols/ Abbreviations	Meaning
DPOAE	Distortion product otoacoustic emissions
ECLIA	Electro-cheluminescence Immunoassay
ELISA	Enzyme- Linked Immunosorbent Assay
et. al.	Et alia (and others)
FBC	Full blood picture
G- CSF	Granulocyte colony stimulating factor
HSV	Human simplex virus
HUSM	Hospital Universiti Sains Malaysia
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU/ml	International units to ml
IUGR	Intrauterine Growth Retardation
LFT	Liver function test
MRI	Magnetic resonance imaging
NK	Natural Killer
OHC	Outer hair cells
PCR	Polymerase chain reaction
$(Ru(bpy)^{2\prime 3+})$	Ruthenium complex
SGA	Small for Gestational Age
SNHL	Sensory neural hearing loss
TPA	Tripropylamine
UI	Unit Interval

ABSTRAK

Kekerapan jangkitan Cytomegalovirus di kalangan bayi dan korelasi antara CMV PCR dengan hasil klinikal di Hospital pengajaran tertiari di Malaysia

Pengenalan: Infeksi Cytomegalovirus (CMV) kongenital adalah penyebab utama kehilangan pendengaran kongenital dan kecacatan perkembangan neuron. Tujuan kajian ini adalah untuk menentukan epidemiologi, manifestasi klinikal dan kesan klinikal bagi bayi dengan jangkitan CMV di Hospital Universiti Sains Malaysia (HUSM) dan menentukan korelasi antara CMV PCR dengan kesan klinikal.

Tatacara: Sejumlah 648 sampel darah bayi yang meminta pemeriksaan TORCHES daripada Januari 2018 hingga Disember 2018 telah dihantar ke Makmal mikrobiologi di Hospital Universiti Sains Malaysia (HUSM). Sampel diperiksa untuk IgM dan IgG anti-CMV oleh kaedah immunoassigensi elektrokimia (ECLIA). Bayi dengan sampel serum pertama IgM dan IgG antibodi titre yang menunjukkan kemungkinan jangkitan Cytomegalovirus diminta untuk menghantar sampel serum kedua pada selang dua hingga empat minggu bersama-sama dengan sampel plasma untuk longgokan virus CMV DNA dan sampel ibu untuk analisis serologi. Kuantiti plasma CMV DNA ditentukan menggunakan PCR masa nyata. Korelasi CMV PCR dengan hasil klinikal dianalisis dengan menggunakan korelasi titik-biserial.

Keputusan: Kelaziman gejala jangkitan CMV di kalangan bayi di HUSM ialah 6.48% (42/648). Daripada 648 kes, hanya 196 kes yang mempunyai sampel sera berpasangan dan 97 kes diuji untuk CMV PCR. Hanya 39 kes positif untuk CMV PCR. Berdasarkan keputusan serologi dan PCR, kes-kes tersebut diketogorikan ke dalam tiga kumpulan: jangkitan CMV akut (n = 42), imuniti pasif (n = 113) dan tidak dapat disimpulkan (n = 41). Tanda-tanda klinikal untuk jangkitan CMV akut adalah bayi kecil dibandingkan usia kehamilan (64%), jaundis (52.3%), sepsis (21.4%), penilaian pendengaran yang tidak normal (9.5%), hepatitis (9.5%), masalah okular (4.7% pra matang (4.7%), ruam (2%), saiz otak yang kecil (2.3%), kalsifikasi cerebral (2.3%) dan sel platelet yang rendah (2.3%). Terdapat korelasi yang signifikan sederhana di antara paras kuantiti DNA CMV dengan penilaian pendengaran yang tidak normal dan korelasi signifikan lemah di antara paras kuantiti CMV DNA dengan penemuan penglihatan yang tidak normal.

Kesimpulan: Kelaziman jangkitan CMV di kalangan bayi di hospital kami adalah sama dengan kajian- kajian terdahulu. Walaubagaimanapun, kelaziman CMV kongenital adalah lebih rendah mungkin disebabkan oleh tidak dapat didiagnosis. Penilaian pendengaran yang tidak normal, sepsis, CMV hepatitis dan ruam adalah signifikan kaitan dengan jagkitan CMV akut bersimptom di kalangan bayi. Terdapat korelasi yang signifikan antara beban viral CMV dengan penilaian pendengaran yang tidak normal dan penemuan optik yang tidak normal.

Kata kunci: Cytomegalovirus, bayi, PCR, serologi

ABSTRACT

Prevalence of Cytomegalovirus (CMV) infection among infants and correlation between CMV PCR with clinical outcomes in a tertiary teaching Hospital in Malaysia

Introduction: Congenital Cytomegalovirus (CMV) infection is the leading infectious cause of congenital hearing loss and neurodevelopmental disability. The aims of this study were to describe the prevalence and clinical manifestations of infants with CMV infection in Hospital Universiti Sains Malaysia (HUSM) and to determine its correlation between CMV PCR with clinical outcomes.

Materials and Methods*:* A total of 648 blood samples of infants requested for TORCHES screening from January 2018 to December 2018 were sent to Microbiology laboratory in Hospital Universiti Sains Malaysia (HUSM). The samples were tested for anti-CMV IgM and IgG by electrochemiluminiscence immunoassay (ECLIA) method. Infants with first serum sample of IgM and IgG antibody titre suggestive of Cytomegalovirus infection were requested for second serum sample at two to four weeks interval together with plasma samples for Cytomegalovirus DNA viral load and maternal sample for serological analysis. The plasma CMV DNA quantification was performed using real-time PCR assays. Correlation of CMV viral load with clinical outcomes were analysed using the point- biserial correlation

Results*:* The prevalence of symptomatic Cytomegalovirus infection among infant in HUSM was 6.48 % (42/648). Out of 648 cases, 196 cases had paired sera samples and 97 cases were tested for CMV PCR. Only 39 cases were positive for CMV PCR. Based on serological and PCR results, the cases were categorised into three groups: acute CMV infection (n = 42), passive immunity (n = 113) and inconclusive (n =41). Clinical

presentations for acute Cytomegalovirus infection were small for gestational age (64%), jaundice (52.3%), presumed sepsis (21.4%), abnormal hearing assessment (9.5%), hepatitis (9.5%), ocular problem (4.7%), prematurity (4.7%), rash (2%), microcephaly (2.3%), cerebral calcification (2.3%) and thrombocytopenia (2.3%). There was positive moderate significant correlation between CMV viral load and abnormal hearing assessment and weak positive significant correlation between CMV viral load and abnormal ocular finding.

Conclusion*:* The prevalence of CMV infection among infants in our center was comparable with previous studies. However, the prevalence of cCMV among infants was lower that might be due underdiagnosed. Abnormal hearing assessment, presumed sepsis, CMV hepatitis and rash were significantly associated with acute symptomatic CMV infection among infants. There were significant correlation between CMV viral load with abnormal hearing assessment and abnormal ocular findings.

Keywords*:* Cytomegalovirus, infants, PCR, serology

CHAPTER 1: INTRODUCTION

LITERATURE REVIEW

1.1 Background of the study

Cytomegalovirus (CMV) is a double stranded DNA virus also known as human herpesvirus 5. It is a natural human pathogen belonging to the β-herpesviridae subfamily. It is one of the eight herpesviruses that frequently infect human being. Immunocompromised patients mainly advanced AIDS patient, transplant recipients, and congenitally infected newborn is well recognized for its tendency to cause disease even though infection in immunocompetent person are usually asymptomatic.

CMV is discovered universally but the seroprevalence differ widely between various geographical areas. A systematic review and meta-analysis estimated a global CMV seroprevalence of 83% for the general population.[1] The Eastern Mediterranean region had the highest mean seroprevalence which was 90% (95%UI: 85-94) whereas the European region had the lowest mean seroprevalence which was 66% (95%UI: 56-74).[1]

The South-East Asian region showed the seroprevalence of 86% for CMV in general population.[1] Besides, CMV seroprevalence also differ between countries. It had propensity to be higher in developing countries (80–90% in South Africa, 70–80% in Ghana, 49% in Brazil, 49% in Turkey and 49% in India) and lower in developed countries (50–60% in the United States, 40–70% in Western Europe, 60–70% in Australia, 60–70% in Canada).[2]

CMV infection is distinctly endemic in Malaysia. A cross-sectional serological view from 1961 to 1979 of different age groups from 0 to 55 years of the average Malaysian population showed the seroprevalence was 71.6%, and a preliminary blinded study showed that the seroprevalence of CMV in the Malaysian state of Selangor and Wilayah Persekutuan was 92%.[3, 4]

Global mean seroprevalence for women of childbearing age was 86%.[1] The prevalence of CMV among childbearing women in Malaysia was also high, evidenced by extremely high incidence of CMV (99.5%) Complement fixing (CF) antibodies in the neonates of all females in three races (Malay, Indian, Chinese)[3] with 84% of all pregnant woman had CMV specific IgG antibody positive.[5]

The most frequent viral infection in infant is infection with CMV. CMV infection can happen in divergent stages of life such as during intrauterine growth (prenatal or vertical infection), which may lead to congenital infection, during delivery or in the first breastfeed (perinatal infection) or at a next stage (postnatal or horizontal infection).

Congenital CMV (cCMV) infection can be an outcome of primary infection in pregnancy, reactivation from latency, and reinfection with a new strain.[5, 6] These three types of maternal infection contribute to the high prevalence of infection in a population. It is different from what was believed before, which was congenitally CMV infection acquired from women who developed primary infection during pregnancy [7].

The majority of babies may be infected as an outcome of maternal CMV reactivation or reinfection, even in countries with relatively low seroprevalence. [6] Furthermore, more infants with congenital infection were born from mothers who had reactivation and reinfection with a new strain compare to mothers who had a primary infection in a community with high seroprevalence.[8]

Universally, 0.7 per 1000 live births was approximated to be affected by cCMV.[9] The rates of congenital infection in developing countries were about 1-5 % of live births, whereas the rate for developed countries were 0.6-0.7 %.[10] cCMV infection became the essential cause of congenital infections among the congenital Toxoplasmosis, Rubella, CMV and Herpes Simplex (TORCHES) diseases in Malaysia. The highest prevalence of cCMV infection was detected in 193 (11.4%) infants compare to the prevalence of congenital syphilis (4%), congenital rubella infection (3.7%), congenital toxoplasma (1.0%) and congenital herpes simplex virus infection (0%).[11] Out of the 193 cases, 10.4 % had CNS defects.[11] Secondary infection is the leading factor for intrauterine CMV infection in Malaysia compare to primary infections or reactivation.[11]

The outcome of cCMV infection can be terrible. Infection in early pregnancy will lead to clinical manifestation such as intrauterine growth retardation, microcephaly, petechiae, thrombocytopenia, jaundice, chorioretinitis or hepatosplenomegaly, with a mortality rate of 20–30% in ten to fifteen percent of newborn.[12] About 10−15% of infants with cCMV born with symptoms but another 85−90% of newborn infants with cCMV were asymptomatic.[12] Long term sequelae such as sensorineural hearing loss, developmental delay, cerebral palsy, and the ophthalmic problem will develop in more than 90% of symptomatic infants.[13] Meanwhile around 10-15% asymptomatic congenital infection will later presented with long term sequelae.[12]

Perinatal and postnatal CMV infection also can cause severe infection in premature infants. Infection can be acquired through during or after birth via the birth canal, breast milk feeding, or rarely via blood transfusion.[14] CMV infection via postnatal transmission is also frequent in early life even though vertical transmission is the main route of virus distribution within the society. Prevalence of CMV-associated diseases among postnatal infected preterm infants ranging from 0-87%.[15]

1.1.1 Cytomegalovirus

Cytomegalovirus is a member of the Herpesviridae family. It has a double stranded DNA core of 200 kilobase pairs which surrounded by an icosahedral capsid. This capsid contains double-stranded and linear and consists of 230 kbp which is a viral genome that contain over 190 coding region.

Transposition of two genomic regions by four isomers build up the viral particles, i.e., the unique long (UL) and unique short (US) parts. There are sequences encoding of the virion proteins, proteins of the capsid, tegument, and envelope in these areas. Six of the high molecular weight glycoproteins (gcI, gcII, and gcIII. The gB and gH glycoprotein) out of twelve main glycoproteins play the principal role in the virus-host connection. Its functions were essential for penetration of the virus into the target cell, syncytia development, and communication from cell to cell.

Phosphoproteins in the viral tegument are essential for the control of HCMV genes and adjustment of the host cell metabolism. Pp150 and pp65 phosphoproteins play a significant immunogenically role, whereas pp71 plays a significant role in the early moment of the replicative revolution for virus replication and initiate gene expression. Apart from a role in immunogenicity such as avoidance of the virus from the immune system, the pp65 also has control for the latency process.

The incubation period of this infection is four to twelve weeks. Most infections are asymptomatic in immunocompetent subjects, but the symptomatic infected person may develop continuous fever, myalgia, and enlargement of a cervical lymph node. It causes significant infection and clinical manifestation among infected fetuses and immunodeficiency patients.

Only 10% of affected infant in prenatal infection may show symptom in different body systems may be due to infection of different cell types, such as epithelial cells, endothelial cells, white blood cells, fibroblasts and specific cells (e.g., neurons, retina, smooth muscle, gastrointestinal system, and hepatocytes) by CMV. Examples of clinical manifestations involving CNS infection were cerebral and periventricular calcifications, microcephaly, hypotonia, difficulty with sucking, spasticity, hemiparesis, and seizures. The infection of the hematopoietic system leads to the clinical manifestation of jaundice, hepatomegaly, and/or splenomegaly. Other clinical manifestations are chorioretinitis, sensorineural hearing loss, pulmonary complications, low birth weight, and prematurity.

The virus was taken up by the host cell through the attachment of complex glycoproteins on the viral envelope to the cell membrane receptors for the process of infection by using of specific membrane receptors such as soluble heparin, annexins, epidermal growth factor receptor (EGFR) and integrins which attach the virus to the host cell. (FIG.1)

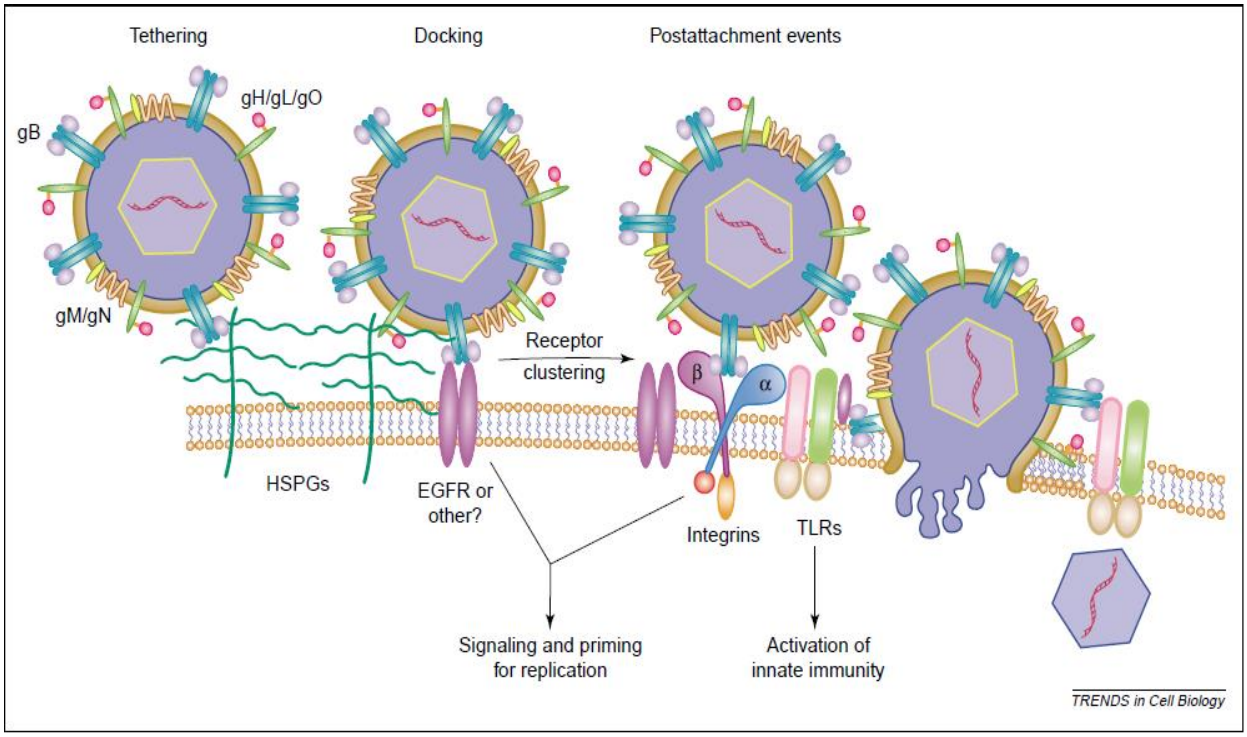

FIG 1: Mechanism of CMV infection in host cell (Adopted from Compton T. Receptors and immune sensors: the complex entry path of human cytomegalovirus. Trends in cell biology. 2004; 14(1):5-8)

After attachment to the host's cell, the derangements of viral and cell membranes will trigger cell signalling pathways that cause further changes in transcription, causing lytic infection and latency. The Capsid and tegument proteins were introduced into the cytoplasm of the cell together with the release of viral DNA for infection to start.

Then, phosphoproteins pp65 and pp71, which are situated in the viral tegument, will travel to the nucleus of the cell and commence the infection. The pp71 protein causes both lytic infection and latent infection in the host by the expression of an immediate-early (IE) cluster of genes, whereas pp65 protein help in avoiding the host's immune system by inhibiting natural killer (NK) cells on the infected cells besides its essential role during early stage of the infection by breakdown of the alpha chain of HLA-DR and

the phosphorylation of viral proteins. All of these lead to the persistence of the virus in the cell for a long duration and escape from the host's immune system.

1.1.2 Clinical manifestations of CMV infection in infants

Around 10−15% of infants with cCMV developed symptoms at delivery. The clinical manifestation include thrombocytopenia, petechiae, hepatomegaly, splenomegaly, hepatitis, intrauterine growth restriction (IUGR), central nervous system involvement such as intracranial calcifications, microcephaly, sensorineural hearing loss, and chorioretinitis.[16]

Asymptomatic cCMV infection is defined as a newborn with cCMV infection that has little or no signs and symptoms at birth, and it comprises of 85−90% of newborns with cCMV infection and 15% from these infants can develop significant outcome especially sensorineural hearing loss.[12]

The perinatal infection is due to horizontal infection of newborn and infant. Most cases are asymptomatic. Symptomatic forms of illness involved localized infection such as pneumonia, hepatosplenomegaly syndrome, purple haemolytic anemia syndrome or disabling conditions such as microcephaly, deafness and chorioretinitis. Postpartum infection is due to horizontal infection. Most of the cases are asymptomatic. When they presented with symptoms, prolonged febrile syndrome or mononucleosis-like syndrome are the clinical manifestations.

1.1.3 Kinetic of antibody respond to CMV

Cytomegalovirus has the capacity to produce two types of infections namely primary and recurrent due to characteristic of replication and cytopathogenic they possess. (Refer FIG.2) In the symptomatic patients, CMV-specific IgM detection was in close proximity to onset of symptoms and peak at three to four weeks after the onset. Three to four months later, the IgM antibodies usually will diminish to low or undetectable levels. In primary CMV infection, CMV IgG was detected in an early phase of infection, and identification of CMV IgG is applicable as immune status screening due to it may be detected for the lifelong but seropositive person is not immune from reactivation or reinfections [17]

The diagnosis of cCMV infection is not recommended using serological tests. It may reflect maternal antibody status since IgG antibodies from the mother will cross the placenta during pregnancy and do not represent an infection in the infant whereas detection of CMV IgM in the newborn would indicate cCMV infection because maternal IgM antibodies do not cross the placenta but are only present in 25-40% of newborns with congenital infection.

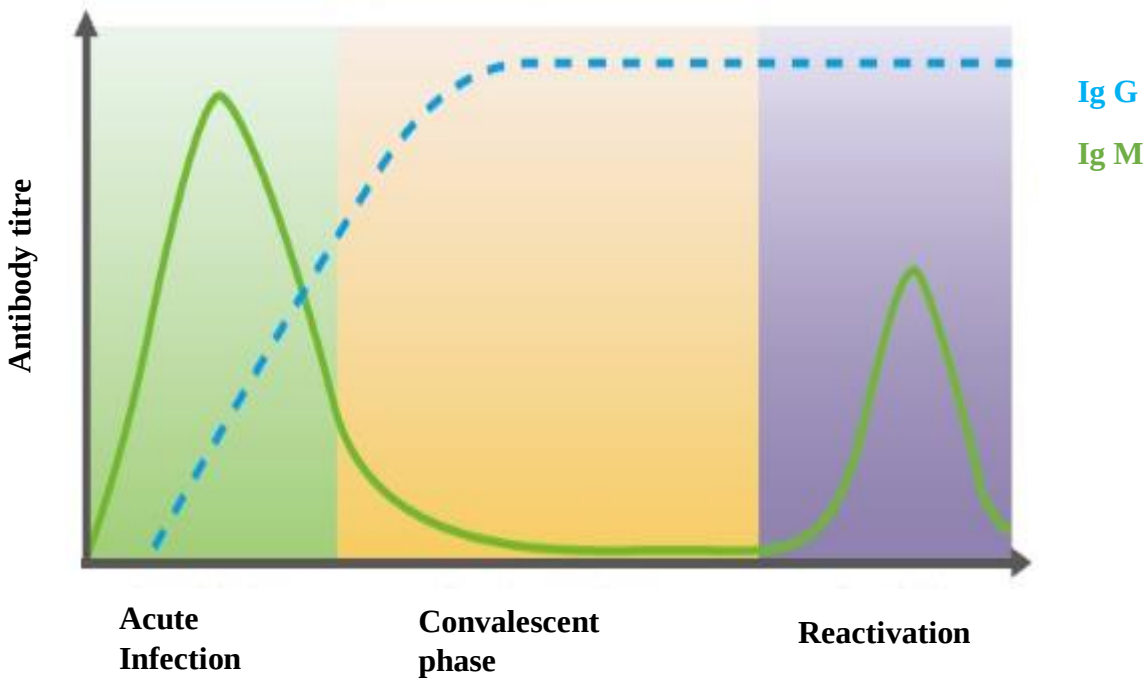

FIG. 2: Antibody titre during a CMV infection (Adopted from http://sigmadiagnosticsinc.com/product/cmv-igg/)

1.1.4 Diagnosis of Acute CMV infection in infants

Similar with other herpes viruses, acute CMV infection is associated with abundant levels of lytic viral replication and after that dissemination throughout the body, together by the beginning of viral latency in long lived cell types that is accountable for prolonged preservation of infection.[18]

The serological method was used to diagnose CMV infection. Other than that, repeated IgG avidity testing may assist in detecting the timing of infection in specific circumstances. The identification of IgG from the mother that was obtained passively by cross the placenta creates a diagnostic dilemma for the infant's CMV serology testing. Therefore, diagnosis of acute CMV infection in infants demand either a turn from negative to positive serologic testing (seroconversion) or by the identification of CMV DNA in blood, urine or saliva.[20]

The meaning of `active` infection is reflected by lytic viral replication and by confirmation of identification of CMV DNA or antigen or isolation of CMV in culture. Reactivation of latent virus or reinfection with an exogenous strain of CMV usually caused active CMV infection in the chronically infected individual. However, CMV viremia and end-organ disease are usual in immunocompromised patients and congenitally infected infants. [21]

cCMV infection can be diagnosed by the detection of CMV DNA via PCR in body fluids or tissue samples (saliva, urine, blood, CSF, and placenta) that were acquired before Day 21 of life in newborn.[22] Diagnosis for perinatal or postnatal CMV infections are more demanding as it requires verification of a negative sample taken before three weeks of life and a positive sample obtained after three weeks of life.[23]

1.1.5 Relevant investigations to determine end-organ disease

Additional investigations to point out end-organ diseases are crucial for further management which include full blood count (FBC) to look for thrombocytopenia (<100,000/mm³ or neutropenia (<1.0 x 10^9/L) and liver function test (LFT) to look for elevated transaminases level that often came with the evidence of conjugated hyperbilirubinaemia.[23]

Imaging such as MRI is essential to look for confirmation of changes in white or grey matter, presence of cysts as well as migration defects such as polymicrogyria.[23] Cerebral calcification or ventriculomegaly that may present in CMV infection can be detected using a cranial ultrasound.[23]

1.1.6 The association between CMV Viral load and clinical outcomes in infants

Some children with cCMV infection will develop long term permanent neurological outcomes such as the auditory deficit, ophthalmic impairment, and neurological disabilities. Around 40- 60% cCMV infants will develop sequelae such as hearing loss, mental retardation and development delay.[10] Whereas ten to fifteen percents of asymptomatic cCMV infants will also develop these permanent outcomes.[10]

Viral load refers to net viral replication rate which may be affected by few factors such as timing of the in utero infection as well as quality and quantity of the specific immune response. Infants with symptomatic cCMV have higher viral load than asymptomatic infants, and late sequelae of cCMV infection such as sensory neural hearing loss were suggested attributable by the presence of higher viral load during infancy. [24, 25]

One study in Japan found that prolonged observation of CMV DNA in blood during the Valganciclovir and Ganciclovir therapy showed that the possibility for sensory neural hearing loss because of prolonged CMV viremia but CMV viral load at diagnosis could not evaluate the auditory outcome.[26]

1.1.7 Treatment for CMV infection in infants

Treatment available for CMV infection in the infant is intravenous Ganciclovir and oral prodrug, Valganciclovir. Its inhibit CMV replication by altering the viral DNA synthesis. Kimberlin et al. have shown that treatment for infants with symptomatic cCMV

infection who has central nervous system involvement using intravenous Ganciclovir for six weeks will impede auditory deterioration at 12 months.[27] The infants who obtained treatment begin to surpass developmental delay at both six and twelve months in contrast to infants who did not get the anti-viral treatment. [27, 28]

The latest clinical trial also showed Valganciclovir treatment for six months to treat symptomatic congenital CMV disease, as compared with six weeks duration, happen to improve hearing and neurodevelopment outcomes modestly in the longer term, at two years age although did not improve hearing in the short term.[16]

It is crucial to inform the potential toxicities associated with Valganciclovir therapy to the family members. The examples of short term side effects are neutropenia, liver toxicity, and thrombocytopenia, whereas long term side effect associated with therapy is undefined, but there is a possible effect on fertility and risk of carcinogenicity. These side effects may need therapy, for example, with granulocyte-colony stimulating factor (G-CSF) for neutropenia. It is vital to monitor full blood count, liver and renal function frequently while on treatment.

Antiviral should begin in the first month of life and the latest guideline suggest the extension of treatment for six months of oral Valganciclovir.[27] Presently, the benefit of treating CMV infection with anti-viral therapy after the first month of life is uncertain due to insufficient studies. A phase II randomized controlled trial (NCT01649869) in infants and children with cCMV infection by using Valganciclovir and the relationship with hearing loss age is currently in progress for the age of one month up to four years of age.[29]

1.2 Rationale of the study

CMV infection is the significant non-genetic cause of childhood hearing loss and a major cause of neurodevelopmental delay since the eradication of rubella in the developed countries.[30] It causes more cases of congenital disease compare to the integration of 29 currently screened conditions in most American states. [31] In European countries, it is one of the common disorder included in newborn screening.[32] CMV transmission rate from maternal to the fetus are remarkably higher than *Rubella* and *Toxoplasma gondii* infection.

Lack of awareness on cCMV among healthcare workers and the public can be due to three main factors. CMV infection is not recognized at birth due to the nature of the illness; most of maternal and newborn infections were asymptomatic. Secondly, the sequelae of CMV infection are delayed. Thirdly, most people assumed that cCMV born from women with non- primary infection have normal outcome.[10]

cCMV possess a serious burden on developing countries due to high birth rates and high seroprevalence. The occurrence of congenital infection is precisely correlated with the seroprevalence of CMV antibodies in the community.[33]

Hence, the purpose of this study is to determine the epidemiology, clinical manifestations and clinical outcomes for infants with CMV infection in Hospital Universiti Sains Malaysia (HUSM). Besides that, this study will also explore the correlation between CMV viral loads with the clinical outcomes of the infection.

1.3 Study flowchart

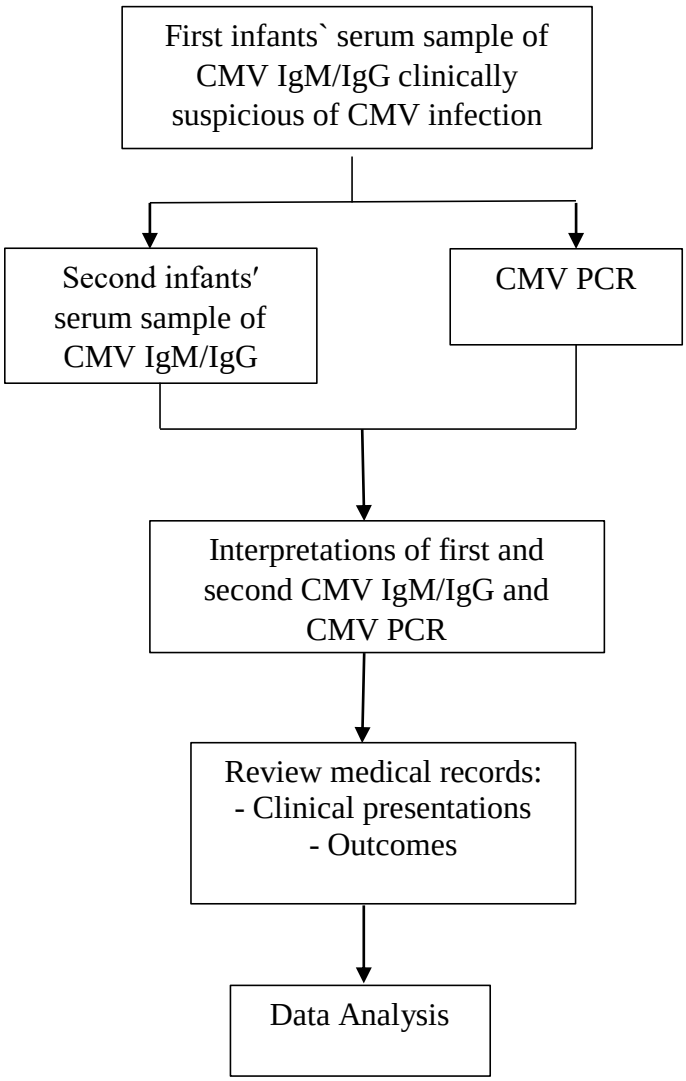

FIG. 3: Study flowchart

1.4 Study Objectives

General objective

To study the prevalence of Cytomegalovirus (CMV) infection among infants and the correlation between Cytomegalovirus (CMV) PCR with clinical outcomes in Hospital Universiti Sains Malaysia.

Specific objectives

1. To describe the prevalence of symptomatic CMV infection among infants in HUSM.

2. To describe the clinical manifestation and clinical outcomes of CMV infection among infants in HUSM.

3. To determine the correlation between CMV viral load and clinical outcomes of CMV infection among infants in HUSM.

1.5 Research questions

1. What is the prevalence of symptomatic CMV infection among infants in HUSM?

2. What are the clinical manifestation and clinical outcomes of CMV infection among infants in HUSM?

3. Is there any correlation between CMV viral load and clinical outcomes of CMV infection among infants in HUSM?

1.6 Research hypothesis

There is a correlation between CMV viral load and clinical outcomes of CMV infection among infants in HUSM.

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CHAPTER 2

STUDY PROTOCOL

2.1 Title

Prevalence of Cytomegalovirus (CMV) infection among infants and correlation between CMV PCR with clinical outcomes in Hospital Universiti Sains Malaysia

2.2 Objective

2.2.1 General objective

To study the prevalence of Cytomegalovirus (CMV) infection among infants and the correlation between Cytomegalovirus (CMV) PCR with clinical outcomes in Hospital Universiti Sains Malaysia.

2.2.2 Specific objectives

1. To describe the prevalence of symptomatic CMV infection among infants in HUSM.

2. To describe the clinical manifestation and clinical outcomes of CMV infection among infants in HUSM.

3. To determine the correlation between CMV viral load and clinical outcomes of CMV infection among infants in HUSM.

2.3 Methodology

2.3.1 Study design

This is a hospital- based prospective cohort study involving paired sera serological samples and plasma for CMV PCR (viral load monitoring) that were sent for detection of CMV infection conducted for a duration of one year starting from 1st January 2018 until 31st December 2018

2.3.2 Reference population

Serum samples sent for IgM and IgG CMV antibody titre detection and plasma for CMV PCR (viral load) among infant's patients in Hospital Universiti Sains Malaysia (HUSM).

2.3.3 Target population

Serum samples sent for IgM and IgG CMV antibody titre detection and plasma for CMV PCR (viral load) among infant's patients sent to Microbiology Laboratory in Hospital Universiti Sains Malaysia (HUSM).

2.3.4 Source population

Serum samples sent for IgM and IgG CMV antibody titre detection and plasma for CMV PCR (viral load) among infant's patients in HUSM from 1st January 2018 until 31st December 2018.

2.3.5 Sampling frame

The sampling frame were infants with first serum sample of IgM and IgG antibody titre suggestive of CMV infection will be requested for second serum sample at two to four weeks interval together with plasma samples for CMV viral load.

2.3.6 Inclusion criteria

1. All first serum samples for IgM and IgG antibody titre suggestive of CMV infection among infants were requested for second serum sample at two to four weeks interval with CMV PCR.
2. Mother`s serum sample was requested to confirm the evidence of passive immunity from the mother.

3. All first serum samples for IgM and IgG antibody titre suggestive of CMV infection without second serum samples but with plasma sample for CMV viral load detection were included in this study.

2.3.7 Exclusion criteria

1. Single serum sample from infants whom had passed away.
2. Infants that are already initiated on treatment before the first serum sample.
3. A serial serum sample was received more than four weeks duration.
4. Missing patient's folders.

2.3.8 Sample size estimation

Objective 1

In view of the small reference population for this study and no previous study done for seroprevelance in paired sera, sample size calculation for this objective was based on representative sample size (n_0) and adjusted sample size (n) using finite population correction.[1]

$$\text{Representative sample size } (n_0) = \frac{Z^2 p}{e^2}$$

$$\text{Adjusted sample size } (n) = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Whereby:

p: Prevalence assumed to be 50 % with maximum variability

e: precision of 0.05 (5%)

Z: Distribution when α is 0.05 = 1.96,

N: Population size

Drop out: 10% of sample size

Sample size for the target population were calculated

Newborn and infants

$n_0 = 92.19$ [2]

N = adjusted to HUSM population, admission of newborn and infant is 4000

n = 90, adding 10% dropout = 99 (~ 100)

P= 0.06; 6% CMV infection in NICU (Verboon-Maciolek,M.A.et al, 2005)y

Total sample size: 100

Objective 2

Sample size for clinical demographics, clinical outcomes and the clinical manifestations of CMV infection among infants in HUSM were not calculated because these only involve descriptive statistic analysis using the checklist. (Refer Appendix 1)

Objective 3

The correlation between CMV viral load and clinical outcomes CMV infection among infants in HUSM sample size was measured using the correlation sample size. [3]

The standard normal deviate for $\alpha = Z_\alpha =$

The standard normal deviate for $\beta = Z_\beta =$

$C = 0.5 * \ln[(1+r)/(1-r)] =$

Total sample size = $N = [(Z_\alpha+Z_\beta)/C]^2 + 3$

α (two-tailed) = 0.05

$\beta = 0.2$

r = 0.4 (the value of r : is from expert opinion)

N= 47

Total sample size : 47

(Cummings, S.R. and S.B. Hulley, 1988)