Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein

By

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I, Melisha Moodley, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Paediatrics at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

AUTHOR'S CONTRIBUTIONS

The contributions of each author of the article are stipulated below:

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All researchers declare that they have no conflict of interest and that no other situation of real, potential or apparent conflict of interest is known to them. They undertake to inform the University of any change in these circumstances

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ABSTRACT

Background: Cytomegalovirus (CMV) can be transmitted from mother to child and can be congenital or postnatally acquired. Emerging evidence has shown that CMV and other congenital and neonatal infections, are under-appreciated causes of morbidity and mortality in African children. Research regarding the mortality rate and characteristics of CMV infected infants, specifically in a population with high HIV prevalence, is limited.

Objective: The primary objectives of the study were to establish the mortality rate and final outcomes of inpatients with a positive CMV test. Secondary objectives were to determine the demographical, clinical and laboratory characteristics of these infants, as well as maternal characteristics. Tertiary objectives were to describe the special investigations, morbidity, complications and management of these infants.

Methods: A retrospective, descriptive study was conducted by reviewing hospital records of infants younger than 12 months, who had a positive CMV test and were admitted to the academic hospitals in Bloemfontein, South Africa.

Results: Inpatient mortality for CMV infected infants was 13.3% (18 of 135 patients). 66.6% (12/18) of patients who died were HIV exposed and 33.3% (6/18) had CMV/HIV co-infection. The most common causes of death were sepsis (38.9%), pneumonia/pneumonitis (33.3%) and multi-organ failure (11.2%). 60.7% (82/135) of all CMV positive infants were HIV exposed and 20.7% (28/135) were HIV infected. 55.6% had a birth weight of less than 2,5 kg and were preterm, and 33.3% were small for gestational age. 5.9% were classified as congenital CMV and 94.1% as postnatally acquired. The most common clinical presentations were CMV pneumonia/pneumonitis (60%) and hepatomegaly (50.4%). Thrombocytopenia was a common finding (41.5%). 33.3% of infants had intra-uterine growth restriction and postnatal growth was suboptimal in 62.2%; 25.2% were underweight, and 37% of infants had failure to thrive. Microcephaly was present at birth in 25.2%, but poor brain growth led to postnatal microcephaly in 46.6%. Infants that were untreated for

CMV infection were more likely to have developmental delay (P-value <0.05). 50% (9/18) of the infants that demised were not treated for CMV.

Conclusion:

CMV infection in infancy is under-appreciated in South Africa. It contributes to morbidity and mortality, particularly in preterm and low birth weight infants, and HIV exposed or infected infants. Clinicians should have a high index of suspicion for CMV infection in infants who have postnatal growth failure and postnatal microcephaly.

KEYWORDS
Cytomegalovirus, Infant mortality, Infant morbidity, Low birth weight, HIV exposed, HIV infected, Demographical, Clinical, Laboratory Characteristics

ABBREVIATIONS

1. CMV – Cytomegalovirus
2. NHLS – National Health Laboratory Service
3. UFS – University of the Free State
4. PCR – Polymerase Chain Reaction
5. IgM – Immunoglobulin M
6. ELISA – Enzyme Linked Immunosorbent Assay
7. NEC – Necrotizing Enterocolitis
8. CDC – Centre for Disease Control and Prevention
9. HIV – Human Immunodeficiency Virus
10. AST – Aspartate Aminotransferase
11. ALT – Alanine Aminotransferase
12. MRI – Magnetic Resonance Imaging
13. ABR – Auditory Brainstem Evoked Response
14. WHO – World Health Organization
15. LBW – Low birth weight
16. VLBW – Very low birth weight
17. CNS – Central nervous system
18. SGA – Small for gestational age

DEFINITIONS

1. Universitas/Pelonomi Academic Hospital Complex: will include Universitas Academic Hospital and Pelonomi Tertiary Hospital
   Three year period: from January 2017 until December 2019
2. Positive CMV test:
   - Detectable Viral load
   - CMV IgM Positive
   - CMV PCR Positive
3. Congenital CMV infection: Positive CMV test within the first three weeks of life [1]
4. Postnatal CMV infection: The detection of CMV after three weeks of life to under one year of age [1]
5. Meditech system: Computer operated program used at Universitas/Pelonomi Academic Hospital Complex, which contains written records, investigations and blood results
6. Infants: Age group from birth to twelve months old
7. Time of CMV test: Period will include two weeks prior to the test and two weeks after performing the test
8. Thrombocytopenia: Platelet count less than 150 $\times 10^9$/L on the full blood count during the time of the CMV test [2]
10. Elevated liver transaminases: Any of the following during the time the CMV test was done [2]:
    - Alanine aminotransferase (ALT) > 45 U/L (Birth to under one year of age)
    - Aspartate aminotransferase (AST) >150 U/L (Birth to under one year of age)
    - Total bilirubin
      - 0-1 day old: Preterm and Term >137 µmol/L
      - 1-2 days old: Preterm >205 µmol/L, Term >197 µmol/L
      - 3-5 days old: Preterm >274 µmol/L, Term >205 µmol/L
      - Other infant: Preterm >34 µmol/L, Term > 21 µmol/L
    - Conjugated bilirubin
      - Neonatal > 10 µmol/L
      - Infants > 4 µmol/L

11. Insignificant CMV viral load: Viral load between 200 – 3 000 copies/mL [3]
12. Significant CMV viral load: Viral load > 3 000 copies/mL[3]
13. Vertical transmission: Passage of a disease-causing agent (pathogen) from mother to baby during the period immediately before and after birth. Transmission might occur across the placenta, in the breast milk, or through direct contact during or after birth.
14. HIV exposed: A baby whose mother is infected with HIV and can transmit the virus to her baby during pregnancy, labour, delivery or through breastfeeding
15. HIV infected: A baby who is infected with HIV and has a positive PCR for HIV
16. Gravida: Indicates the number of times a woman has been pregnant regardless of the pregnancy outcome
17. Parity: Indicates the number of pregnancies reaching viable gestational age
18. Mortality rate: A measure of the number of deaths
19. Premature babies: Babies born alive before 37 weeks of pregnancy are completed (WHO) [4]
20. Low birth weight: Defined by WHO as weight at birth less than 2500g [5]
21. Very low birth weight: Defined by WHO as weight at birth less than 1500g [5]
22. Virolactia: presence of viral particles in the breast milk [6]

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cytomegalovirus (CMV) is a double stranded deoxyribonucleic acid (DNA) virus that forms part of the Herpesviridae family. It can be transmitted in saliva, blood, secretions, urine and breast milk.[7] Transmission from mother to child can be congenital or postnatally acquired.[8,9] Ninety percent of neonates who are infected with CMV are asymptomatic. The most common long term sequela is non hereditary neuro-sensory hearing loss. There is lack of awareness of CMV infection. The general public are not aware of simple principles that can prevent CMV, such as good hygiene and regular hand washing.[8] One study evaluated the awareness and knowledge of congenital CMV in health care providers in the form of a questionnaire. This study concluded that most of the health care providers were unaware of the precise route of transmission of CMV. There is a large knowledge gap with regards to CMV in pregnancy and the burden of disease.[10]

Mortality

There is limited data on mortality of CMV infected infants in South Africa.

A South African study published in 2014 showed that neonates who had congenital CMV were more likely to die than the CMV negative neonates (mortality rate 42% vs. 18%, p = 0.01), especially if they were HIV exposed (47% vs. 15%, p = 0.02) or HIV infected (62% vs. 11%, p = 0.02).[11] Emerging evidence has shown that CMV and other congenital and neonatal infections, are under-appreciated causes of morbidity and mortality in African children.

Three studies discussed the impact of HIV and CMV co-infection in infancy. These studies concluded that CMV infection led to progression of HIV disease and increased mortality in children especially when both these viruses were acquired in infancy. These studies demonstrated that CMV is a significant pathogen independently associated with poor outcomes in individuals with HIV. These studies were published (1999, 2009 and 2014) in an era where antiretroviral therapy was not easily accessible

to the maternal and infant populations. This emphasises the need for further research to evaluate the impact of HIV treatment on CMV infection and mortality.[12-15]

A study conducted in 2009 in Kenya showed that the detection of CMV DNA in maternal serum just before delivery was associated with a fourfold increase in mortality in HIV infected infants.[16] A further longitudinal study was done on the same subset of Kenyan women and infants. This study demonstrated that HIV infected mothers with detectable plasma CMV DNA had decreased maternal survival at 24 months postpartum and their infants were less likely to survive the first two years of life.[16]

Research in this area of study is limited and more studies need to be published in order to gain a better understanding of the scope of the problem.[17] Studies published in Europe and America showed an overall mortality rate of 4 to 8% in congenital CMV. The mortality rate could be as high as 30% with severe fulminant disease. Between 5 to 10% of premature and very low birth weight (VLBW) infants with symptomatic CMV infection may die. [18,19]

Epidemiology of CMV

A South African study in Johannesburg reported a prevalence of 0.26/1000 live births over a five year period.[11] Another South African study done in the Eastern Cape showed a prevalence of 5.96% (95% CI 3.29 – 8.63). However, due to this wide confidence interval this prevalence is likely due to chance, which may be attributed to the small study population that was used.[9] There is currently limited published data on the prevalence or incidence of CMV in infants in the Bloemfontein population. A review of several international studies showed the incidence and prevalence was influenced by geographic and racial variation. One study demonstrated CMV infection to be higher in lower income countries such as South America, Africa and Asia and lowest in Western Europe and the United States of America. Globally there is a high CMV sero-prevalence in infants ranging between 1 to 5%.[8]

Congenital Versus Acquired CMV

Most of the literature defines congenital CMV as an intrapartum infection with a positive CMV test before three weeks of life, because perinatally acquired infection

can also manifest around this time. A positive CMV test after 3 weeks of life is generally regarded as acquired CMV infection.[20] There is still controversy surrounding the differentiation of congenital versus acquired CMV infection. Acquired infection occurs postnatally and includes perinatal, postnatal, or childhood periods. Perinatally and postnatally acquired infection can occur by exposure to infected cervical secretions and breast milk of a mother infected with CMV. Some sources in the literature define acquired CMV infection as including the perinatal period and the postnatal age up to one year of age. Over the age of one year is considered to be childhood CMV infection and the clinical presentation and management in these children differ compared to congenital and postnatally acquired CMV.[20]

Maternal Characteristics

The risk of vertical transmission to the fetus increases with primary maternal infection compared to recurrent infection. Fetal transmission appears to increase with advancing gestational age. Intrauterine transmission can occur in mothers without pre-existing immunity as a primary infection or as reactivation of a previous CMV infection. CMV can be spread either intrapartum or postpartum.[8,9] Factors shown to influence transmission of congenital CMV are maternal age and parity, where there is an increased risk in younger primigravid women.[8,9]

Maternal HIV and CMV

A few studies investigated the association of HIV and CMV infection. One South African study showed that the maternal CD4 count < 200 cells/µL during pregnancy was independently associated with congenital CMV with an adjusted odds ratio of 2.9 (95 % CI 1.2 – 7.3). This study had a small population with a wide confidence interval and it is likely these findings were due to chance.[21]

Another South African study showed that a lower maternal CD4 count correlates with an increase in CMV viral load in infants.[22] A further South African study, conducted in the Eastern Cape, showed equal prevalence in HIV exposed and HIV unexposed infants. However, it is worth noting that these findings were statistically insignificant ($p = 0.869$), most likely due to the small sample size.[11]

Post-natal acquisition of CMV has little clinical significance and is not associated with long term disability and illness in term infants. However, this route of transmission still holds significance for low birth weight and premature infants.[8]

A study published in 2018 showed that infants who were HIV infected postnatally were four times more likely to acquire CMV infection compared to those who were HIV exposed but not infected. This article also showed that infants who were born with HIV infection had a six times greater chance of acquiring CMV infection compared to infants who were HIV exposed but not infected at birth with HIV.[23]

Breast Feeding Related CMV

One study described the rate of CMV virolactia and prevalence of breast milk-transmitted CMV infection after freezing and pasteurization of breast milk. This study concluded that the intervention, namely freezing and pasteurization of breast milk, reduced the risk of CMV infection transmission, but did not eliminate it.[24]

The American Academy of Paediatrics recommend routine breastfeeding from CMV sero-positive mothers even for preterm babies, as benefits outweigh risks. The Austrian Society of Paediatrics recommends pasteurization of breast milk from CMV sero-positive mothers until corrected gestational age of 34 weeks. [25,26,27]

One prospective cohort study in Atlanta, United States of America, evaluated both breast milk and blood transfusions as sources of postnatal CMV infection in VLBW infants. This study demonstrated an association between maternal plasma CMV DNA levels and breast milk transmitted CMV infections. However, this study was not able to establish a cut off level for the DNA levels in the milk that could cause the infection. It was concluded that any detectable level of DNA in the milk may cause infection.[25]

Blood Product Transfusion Related CMV

A pilot study in South Korea investigated transfusion related CMV infection among very low birth weight infants in an endemic area. This study concluded that filtration or irradiation of CMV seropositive blood products (including red blood cells and platelets) did not significantly reduce transfusion related CMV infection in infants. This study was

a small study and had several limitations including lack of randomization and lack of groups receiving irradiated only and filtered only blood products.[28]

Another older study published in 1981, concluded that the total volume of more than 50 millilitres (from multiple transfusions) of red blood cells from sero-positive donors was an important risk factor for the development of transfusion related CMV disease in low birth weight infants. This study did not use irradiated or leucodepleted blood.[29]

Newer studies demonstrated that the residual risks were estimated between 1-3% when CMV seronegative or leucodepleted transfusions were performed.[6,30]

The Atlanta cohort showed that the use of blood products that are leucodepleted or CMV seronegative (not in the window period of the infection), can prevent transfusion related CMV. However this study had several limitations. The relative risk of transfusion related CMV between the seronegative and leucodepleted blood products was not compared. This was due to the CMV untested donors in the leucodepleted blood product group. Therefore the relative safety of this study could not be evaluated accurately.[25]

Clinical Characteristics for CMV Infection in Infants

The clinical findings in symptomatic infants are similar to those of other congenital infections.[31] With regards to clinical presentation for congenital CMV infection, studies have shown that petechiae are present in 54 to 76%, jaundice at birth in 38 to 67%, and hepatosplenomegaly in approximately 39 to 60% of patients. Small size for gestational age may be present in 39 to 50%, and microcephaly in 36 to 53% of cases.[31]

Infants who acquire CMV perinatally may have signs and symptoms of disease such as lymphadenopathy, hepatitis and pneumonitis. These infants may have considerable morbidity especially if born prematurely. Further research needs to be done to assess the long term neurological outcomes of these infants.

Transfusion acquired CMV infection presented similarly to CMV mononucleosis. The incubation period is generally 20 to 60 days. The clinical features include fever and severe malaise.[20]

CMV enterocolitis is not well documented in the literature. One study reviewed a case report of CMV associated colitis mimicking necrotizing enterocolitis (NEC), which was confirmed by the presence of CMV inclusion bodies on histology. CMV enterocolitis usually presents with ulceration leading to stricture formation, whereas NEC leads to gangrene and perforation of bowel.[32]

Investigations

Laboratory findings in infants with congenital CMV included elevated liver enzymes with elevated direct and indirect serum bilirubin in 50 to 83% of cases and thrombocytopenia in 48 to 77% of cases.[31]

One study described the spectrum of cranial ultrasound and magnetic resonance imaging (MRI) abnormalities of the brains of infants with congenital CMV. This was a small study that reviewed eleven infants with confirmed CMV on viral isolate or PCR. Cranial ultrasound examinations were performed on all infants and MRI brain scans on six infants. The spectrum of abnormalities on cranial ultrasound included: periventricular calcification, lenticulostriate vasculopathy associated with mild to moderate ventricular dilation and periventricular cysts. The MRI provided additional information including polymicrogyria, hippocampal dysplasia and cerebellar hypoplasia. This study also screened for hearing loss by automated auditory brainstem response assessment (AABR) using the ALGO test. If the infant failed this test a full auditory brainstem evoked response (ABR) was performed by an audiologist to assess neurosensory hearing loss.[33] Chorioretinitis is the most common ocular manifestation of CMV.[31]

Confirmatory Testing for CMV

The Centre of Disease Control and Prevention (CDC) recommends performing CMV PCR on saliva as a screening test, whilst urine may be tested for diagnostic confirmation of CMV in children less than one year old. The reason for the confirmatory test on urine is that most CMV seropositive mothers shed CMV in their breast milk. This can cause a false-positive CMV result on saliva collected shortly after the baby has breastfed.[34] The accuracy of the CMV PCR has been described extensively in the literature. One such study compared CMV PCR and CMV IgM detection using ELISA

diagnosis of CMV infection in high risk neonates. This study showed that the PCR in relation to viral culture (which is the gold standard) had one hundred percent sensitivity and specificity with excellent agreement between both tests, with a kappa coefficient being between 0 to 1 and significant p-values.[1]

Management:

Congenital CMV - Whom to treat:

There is controversy in the literature surrounding the treatment of congenital CMV. Congenital CMV is usually categorized as symptomatic versus asymptomatic at birth. The literature has differing definitions and opinions of what constitutes symptomatic CMV thus making decisions regarding treatment difficult.[18,35] The European Expert Consensus Guideline and The Red Book report of the Committee on Infectious Diseases from the American Academy of Pediatrics recommend treating babies with evidence of central nervous system (CNS) disease, evidence of life threatening disease or severe single organ disease or multiple organ disease.[18]

Treatment of congenital CMV infection is based on the presence of symptoms and on the immune status of the infant. The current guideline for congenital CMV recommends treating infants with virologically confirmed CMV that have at least one end organ symptom (examples include pneumonitis, hepatitis, thrombocytopenia).[35]

Evidence for treating babies with multiple, but not severe manifestations of the disease (jaundice, hepatosplenomegaly without significantly raised liver enzymes and SGA) is limited. It is recommended that these cases be individualized and discussed with experts like a Paediatric Infectious Disease Specialist.[18]

Early Postnatal Infection

The current guideline recommends treatment with ganciclovir or valganciclovir in severe symptomatic infections in preterm or very low birth weight infants.[35]

A study in 2013 evaluated the effectiveness and safety of ganciclovir for treatment of severe CMV pneumonia in children that were immunocompetent. This was a small study population of 43 patients with the mean age of infants between 45 to 85 days.

The mean duration of ganciclovir treatment was 12 days. The outcome was to show a normal x-ray at the end of treatment or clinical improvement at discharge. Eighty percent of infants showed a normal chest x-ray at end of the treatment and 97 were discharged. The study found that there was no severe toxicity associated with ganciclovir treatment.[36] The limitation was that this study design was not a randomized controlled trial.

Children and Adolescents

In immune-competent patients the guideline recommends supportive care with hydration and fever control. Treatment with ganciclovir is recommended in immune-compromised patients.[35]

Congenital CMV – Antiviral treatment

Treatment with ganciclovir and valganciclovir has shown to improve neuro-developmental outcomes of infected infants.[35]

A randomized trial conducted in 2003 in neonates more than 32 weeks gestation, compared the effect of six weeks of ganciclovir versus no treatment in symptomatic congenital CMV disease involving the CNS. CNS disease included microcephaly, intracranial calcification, abnormal cerebrospinal fluid (CSF) for age, hearing deficit and chorioretinitis. This study showed improvement from baseline in neurodevelopment and hearing in the treated group. This trial unfortunately had a significant loss to follow up.[37]

A more recent trial published in 2005 compared 6 weeks to 6 months of valganciclovir therapy in infants with symptomatic congenital CMV infection with and without CNS involvement. Children who received six months of antiviral therapy were more likely to have improved hearing or maintain normal hearing (odds ratio 2.61, 95% CI 1.05 - 6.43) and higher language and receptive communication scores at 24 months.[38]

The current recommendation is to treat with ganciclovir or valganciclovir for 6 months. Valganciclovir is the drug of choice, but intravenous ganciclovir can be used in babies unable to tolerate the oral drug, who have life threatening disease or if gastrointestinal absorption is uncertain.[18]

After an extensive review through the literature, it is worth noting that there is a dearth of information regarding the demographic representation of CMV infection in infants in Bloemfontein. It is common practice in the Universitas Academic and Pelonomi Tertiary Hospital Complex to test infants who have recurrent sepsis failing to respond to antibiotics for CMV infection. The CMV viral load is then requested. It is also common practice to investigate infants with hepatosplenomegaly and conjugated hyperbilirubinemia for congenital infections, and CMV is usually included in the screen.

The National Health Laboratory Service (NHLS) started testing for CMV viral load instead of using the PCR test in 2016, as this is more effective. This aids in deciding, timeously, who to treat based on significant viral loads. The literature shows a lack of standardization to establish a viral load cut-off associated with active disease.[3] There is differing evidence on who should be treated based on viral load and clinical disease. The recommendation is to assess each case individually and decide on starting treatment based on risk versus benefit.

The Future: CMV Vaccine

Many experimental CMV vaccines have been evaluated in clinical trials, but are not licensed. There is still hope for a vaccine for prevention of CMV disease in new-borns and immunocompromised patients.[31]

The phase one of the randomized, double-blind, placebo-controlled trial was conducted in 1999. The vaccine was based on the envelope glycoprotein, gB, combined with a novel adjuvant, MF59. The study population (adults 18 to 50 years old) received either placebo or gB at specific intervals from one to four doses. It was noted that the fourth dose produced an increased level of antibody. This study reported no serious adverse effects and has mild side effects which were similar in both groups.[39]

During phase two of the trial, conducted in 2009, three doses of the CMV vaccine or placebo were given at 0, 1, and 6 months to CMV seronegative women within 1 year after they had given birth. The study population were tested quarterly during a 42-month period, using an assay for IgG antibodies against CMV proteins other than glycoprotein B. The primary end point was the time until the detection of CMV infection.

The efficacy of this vaccine was found to be 50 % (95 % confidence interval) in healthy postpartum females based on infection rates per 100-person years. This study showed that glycoprotein B vaccine has potential to decrease incident cases of maternal and congenital cmv in neonates .Both of these studies concluded that gB remains a cornerstone component of all CMV vaccines and should be used to develop a future gB vaccine .[40]

AIMS AND OBJECTIVES OF STUDY

The aim of the study was to investigate and describe CMV infection in infants admitted to Universitas Academic and Pelonomi Tertiary Hospital. The primary objective was to determine the mortality rate and final outcomes of infants admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex, who tested positive for cytomegalovirus over a three year period, namely 2017 to 2019.

Secondary objectives were to determine the demographical, clinical and laboratory characteristics of these infants. The maternal characteristics were also described.

Tertiary objectives were to describe the morbidity, complications and management of these infants. Special investigations that were captured included MRI, cranial ultrasound, hearing tests, vision screening and developmental assessments. The management of infants with CMV infection were described under this objective. This included the pretreatment viral load, the number of patients that were treated and duration of treatment. The initial viral load could be categorized as significant versus insignificant.

RESEARCH QUESTIONS

- What is the mortality rate and the final outcomes of infants who tested positive for CMV in the Universitas Academic and Pelonomi Tertiary Hospital Complex over a three year period?
- What is the mortality rate and the final outcomes for infants who are HIV exposed and who tested positive for CMV?

- What is the mortality rate and the final outcomes for infants who are HIV infected and who tested positive for CMV?
- What are the demographic, clinical and laboratory characteristics of infants who had positive CMV tests in the Universitas Academic and Pelonomi Tertiary Hospital Complex over the three year period?
- How many infants with a positive CMV test developed complications that resulted in morbidity? How many of these infants were treated? What was the duration of treatment?
- How many infants with positive CMV tests had a significant viral load?
- How many infants received antiviral medication?
- What was the duration of antiviral medication treatment in these infants?
- How many of the infants who died, received antiviral medication for CMV?
- How many patients who were not treated had developmental delay?

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CHAPTER 2

Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein

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ABSTRACT

Background: Congenital and postnatally acquired CMV may significantly contribute to morbidity and mortality in infancy. Outcomes and characteristics of CMV infected infants in a HIV prevalent population are not well known.

Methods: A retrospective, descriptive study was conducted by reviewing hospital records of infants younger than 12 months, who had a positive CMV test and were admitted to the academic hospitals in Bloemfontein, South Africa.

Results: Inpatient mortality for CMV infected infants was 13.3% (18 of 135 patients). 66.6% (12/18) of patients who died were HIV exposed and 33.3% (6/18) had CMV/HIV co-infection. The most common causes of death were sepsis (38.9%), pneumonia/pneumonitis (33.3%) and multi-organ failure (11.2%). 60.7% (82/135) of all CMV positive infants were HIV exposed; 20.7% (28/135) were HIV infected. 55.6% had a birth weight of less than 2,5 kg and were preterm. 33.3% were small for gestational age at birth, with suboptimal postnatal growth in 62.2%. Microcephaly was present at birth in 25.2%, but poor brain growth lead to postnatal microcephaly in 46.6% of patients. The most common clinical presentations were CMV pneumonia/pneumonitis (60%) and hepatomegaly (50.4%). Thrombocytopenia was a common finding (41.5%). 50% (9/18) of the infants who demised were not treated with antiviral medication.

Conclusion: CMV infection in infancy is under-appreciated in South Africa. It contributes to morbidity and mortality, particularly in preterm and low birth weight infants, and HIV exposed or infected infants. Clinicians should have a high index of suspicion for CMV infection in infants who have postnatal growth failure and postnatal microcephaly.

INTRODUCTION

Cytomegalovirus (CMV) is a double stranded DNA virus that forms part of the Herpesviridae family.[1] CMV can be transmitted in saliva, blood, secretions, urine and breast milk. Infection in neonates and infants can be congenital or be postnatally acquired, but the impact on morbidity and mortality in African children is under-appreciated due to lack of research.[2][3][4]

Congenital CMV, generally defined as a positive test before three weeks of life, has an overall mortality rate of 4 to 8% in developed countries. Premature and very low birth weight (VLBW) infants with symptomatic infection are at higher risk of dying (8 to 10%). In severe fulminant disease, mortality could be as high as 30%.[5][6][7] Postnatally acquired CMV has less clinical significance. Studies done in South Africa proved that neonates with congenital CMV are more likely to die, and are particularly at risk if HIV exposed or HIV infected.[8] Detection of CMV DNA in maternal serum before delivery was associated with a fourfold increase in mortality in HIV infected infants, in a study performed in Kenya.[9] Antiretroviral medications were not readily accessible when these studies were performed. CMV transmission transplacentally is more likely in younger, primigravid women and later in gestation.[2][3] Infants with congenital or postnatally acquired HIV infection are more likely to acquire CMV, than HIV exposed-uninfected counterparts.[8] CMV can be transmitted through breast milk.[10] Detection of CMV DNA in breast milk is associated with postnatal acquisition of CMV in VLBW infants.[11] CMV seronegative or leucodepleted blood products minimize the risk for transfusion-related CMV infection, with residual risk in one study suggested to be 1 to 3%. [11][12][13]

Congenital CMV presents most commonly with petechiae due to thrombocytopenia, conjugated jaundice, hepatomegaly, splenomegaly, intra-uterine growth restriction and microcephaly. Lymphadenopathy, hepatitis with elevated liver enzymes, and pneumonitis can occur in perinatal infection. CMV associated colitis may occur in the neonatal period.[14] Transfusion related CMV viraemia may present after 20 to 60 days with fever and malaise.[11][14] Morbidity may also include chorioretinitis and non-hereditary sensorineural hearing loss.[15] Neuroimaging may reveal significant brain abnormalities which may predict adverse neurodevelopmental outcome.[15]

Current recommendations support antiviral treatment of infants with evidence of central nervous system (CNS) involvement, life threatening infection, severe single organ disease or multiple organ involvement. Treatment with ganciclovir or valganciclovir for six months is suggested.[6] [16][17]

MATERIALS AND METHODS

This was a descriptive study using a retrospective study design. The study population included all infants less than one year of age, who had a positive CMV test and were admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex in Bloemfontein, South Africa, from the 1st of January 2017 until the 31st of December 2019. Study subjects with positive CMV tests were identified by accessing the National Health Laboratory Service (NHLS) database, after which hospital records were obtained for data collection.

Information from these sources was entered on a data collection sheet. A coded number was assigned to each study participant. A pilot study was performed using three patients one from each year of the study period. After which recommendations were made and modifications were implemented.

Final outcomes of infants diagnosed with CMV were captured to determine the inpatient mortality and causes of death. Demographic particulars included maternal and infant characteristics, including birth parameters and postnatal growth. Data was captured regarding risk factors for acquiring CMV infection (specifically HIV exposure or co-infection), clinical and biochemical features of CMV infection in infancy and special investigations to identify CMV-related complications. Information was also gathered regarding CMV testing, and the management with antiviral medication.

The study was approved by the Health Sciences Research Ethics Committee of the University of the Free State, the Free State Department of Health and the NHLS.

Due to the retrospective design, parental consent was not required. Confidentiality was maintained by assigning a unique study number to each audited file. After entering data on a Microsoft Office Excel 2018 spreadsheet, statistical analysis was done by the Department of Biostatistics at the University of the Free State.

Descriptive statistics namely means, standard deviations, medians and percentiles were calculated for continuous data. Frequencies and percentages were calculated for categorical data. Chi-Square test used for comparison of categorical variables.

RESULTS

During the 3 year study period (2017-2019), 156 infants younger than 1 year of age had a positive CMV test according to the NHLS data base. 15 patients were duplicated due to changes in names or hospital numbers. Of the remaining 141 possible study participants, 6 were excluded: 4 patients were not inpatients, 1 was transferred to a private hospital and 1 was post liver transplant and receiving valganciclovir prophylaxis. Therefore, 135 patient files were included for final data collection.

61.5% of the infants were admitted to Pelonomi Tertiary Hospital, 33.3% to Universitas Academic Hospital and 5.2 % were transferred between the two for differing levels of care. The median age of infants testing positive for CMV was 75 days (10.7 weeks). 94.1% tested positive after 3 weeks of age and were presumed to have acquired CMV postnatally, while 5.9% were classified as congenital CMV.

Mortality and causes of death

The inpatient mortality rate for CMV infected infants admitted to the Academic Complex was 13.3% (18 of 135 patients). The rest of the infants were either discharged or transferred to other institutions. The most common causes of death were sepsis (38.9%), pneumonia/pneumonitis (33.3%) and multi-organ failure (11%). Less common were liver failure (5.6%), CMV colitis (5.6%) and severe gastro-oesophageal reflux (5.6%) (Figure 1). 12/18 (66.7%) of the infants who demised were HIV exposed and 6/18 (33.3%) had CMV/HIV co-infection.

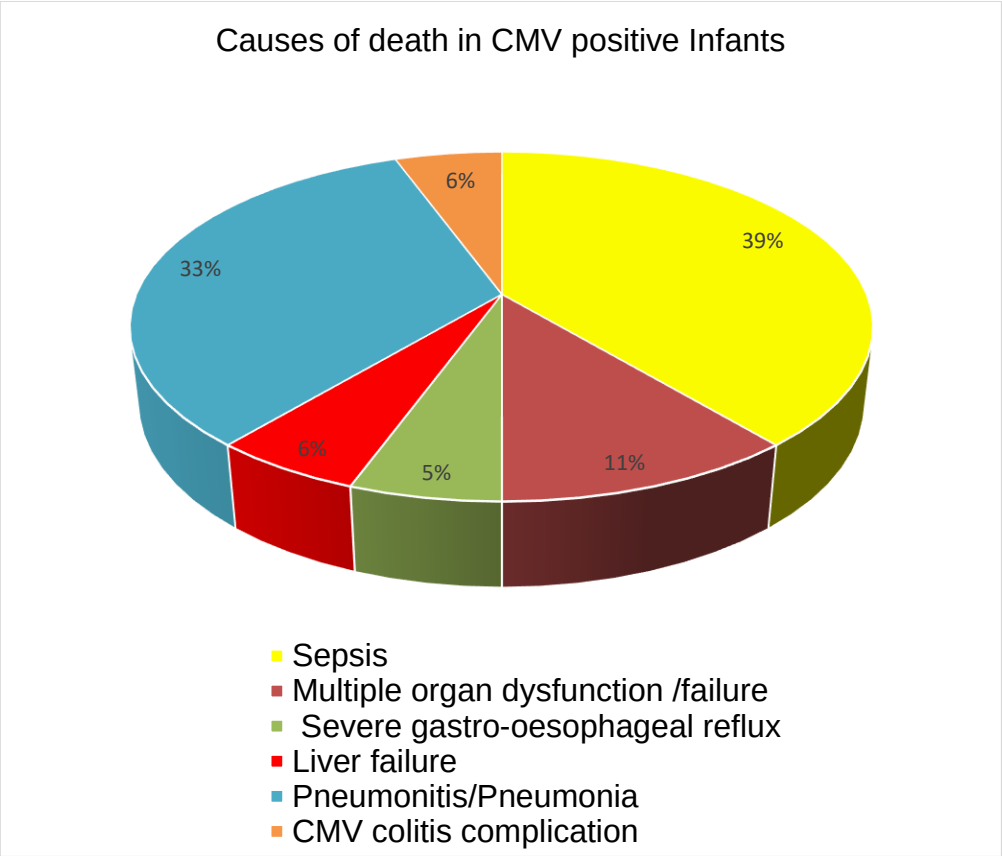

Figure 1. Causes of death in CMV positive infants

Maternal characteristics of infants with CMV infection

Mothers of CMV infected infants were of child bearing age (20-34 years) in 65.9%. 11.1% were teenage pregnancies, and 7.4% of mothers were of advanced maternal age. Only 20% were primigravid, and it was the second pregnancy in 40% of mothers. Most had a vaginal delivery in a medical facility (57%), with caesarean sections performed in 33.3% (Table 1).

60.7% of mothers were HIV positive during pregnancy. 90.2% did receive antiretroviral treatment (ART), with 4.9% who were not yet initiated and 4.9% who defaulted treatment. HIV viral loads were available in all but three mothers, with lower than

detectable levels or < 1 000 copies/mL in 53.7% and 42.7% with > 1 000 copies/mL. Virological failure in women exceeding 12 weeks of ART was documented in 18.6% of mothers.

TABLE 1. Maternal characteristics

Maternal age in years	n (percentage)
< 20	15 (11.1)
20-29	64 (47.4)
30-34	25 (18.5)
35-39	8 (5.9)
≥ 40	2 (1.5)
Unknown	21 (15.6)
Gravidity	
Primigravid	27 (20)
G2	54 (40)
G3	28 (20.7)
G4	8 (5.9)
> G4	5 (3.7)
Unknown	13 (9.7)
Delivery method	
Vaginal	77 (57)
Born before arrival	5 (3.7)
Caesarean section	45 (33.3)
Unknown	8 (6)
HIV status during pregnancy	
Positive	82 (60.7)
Negative	53 (39.3)
HIV viral load in positive patients	
Lower than detectable/ ≤ 1 000 copies/mL	44 (53.7)
> 1 000 copies//mL	35 (42.7)
Unknown	3 (3.6)
CD4 count in HIV positive mothers (cells/mm^3)	
< 200	24 (29.3)
> 200	32 (39)
Unknown	26 (31.7)
Received HIV treatment during pregnancy	
Yes	74 (90.2)
No	8 (9.8)
Duration of HIV treatment	
Never initiated treatment	4 (4.9)
≤ 12 weeks of treatment (high risk of vertical transmission)	17 (20.4)
> 12 weeks of treatment (low risk of vertical transmission)	42 (51.2)
> 12 weeks of treatment (virological failure/poor adherence)	15 (18.6)
Defaulted treatment	4 (4.9)

Infant characteristics

There was a slight male predominance in CMV infected infants (54.8% vs 45.2%), with 54.8% born prematurely, and 55.5% weighing less than 2.5 kg at birth. 33.3% had intra-uterine growth restriction and postnatal growth was suboptimal in 62.2%, with 25.2% being underweight and 37% having failure to thrive. Microcephaly was present at birth in 25.2%, but poor brain growth lead to postnatal microcephaly in 46.6% of patients. 28 infants (20.9%) tested HIV positive (Table 2).

Exclusive breastfeeding was documented in 92.6% of cases and mixed feeding from birth in 6.7%. Blood products were administered to half of the patients during admission, with 66.2% of patients receiving packed red blood cells (PC), and 26.5% PC and platelets. 43 patients (63.2%) required 2 to 5 blood product transfusions.

Clinical characteristics

Infants most commonly presented with signs of pneumonia/pneumonitis (60%). Hepatomegaly was also common in half of the patients, but only 20.7% had associated increase in liver functions and 23.7% had elevated conjugated bilirubin levels. Thrombocytopenia was present in 56 of 135 patients (41.5%); 22 of the 56 cases (39.3%) were classified as severe (< 50 $x10^9$/L).

Table 2. Infant characteristics	n (percentage)
Gender	
Male	74 (54.8)
Female	61 (45.2)
Gestational age	
Preterm	74 (54.8)
Term	60 (44.4)
Unknown	1 (0.8)
HIV status	
Positive	28 (20.7)
Negative	107 (79.3)
Weight category	
< 1 kg	13 (9.6)
1-1.499 kg	25 (18.5)
1.5-2.499 kg	37 (27.4)
≥ 2.5 kg	60 (44.5)
Type of feeding	
Exclusive breastfeeding	125 (92.6)
Exclusive formula feeding	1 (0.7)
Mixed feeding	9 (6.7)
Duration of breastfeeding	
≤1 month	7 (5.2)
> 1 month	114 (84.4)
Unknown	14 (10.4)
Fetal growth	
Small for gestational age (symmetrical)	15 (11.1)
Small for gestational age (asymmetrical)	30 (22.2)
Appropriate for gestational age	81 (60)
Large for gestational age	4 (2.9)
Unknown	5 (3.8)
Head circumference at birth	
Microcephaly	34 (25.2)
Normal	90 (66.7)
Macrocephaly	3 (2.2)
Unknown	8 (5.9)
Length at birth	
< 10^{th} centile	23 (17)
10^{th}-90^{th} centile	103 (76.3)
> 90^{th} centile	0 (0)
Unknown	9 (6.7)
Postnatal growth	
Underweight	34 (25.2)
Normal	45 (33.3)
Overweight	1 (0.7)
Failure to thrive	50 (37)
Not applicable	2 (1.5)
Unknown	3 (2.3)

Head circumference growth	
Microcephaly	63 (46.6)
Normal	61 (45.2)
Macrocephaly	2 (1.5)
Not applicable	2 (1.5)
Unknown	7 (5.2)
Transfusion of blood products administered	
Yes	68 (50.3)
No	67 (49.7)
Type of blood products administered	
Packed red blood cells	45 (66.2)
Platelets and packed cells	18 (26.5)
Unknown	1 (1.5)
Number of transfusions	
1	16 (23.5)
2-5	43 (63.2)
5-10	5 (7.4)
>10	0 (0)
Unknown	4 (5.9)
Presence of clinical characteristics	
Petechiae	12 (8.8)
Jaundice	36 (26.6)
Hepatomegaly	68 (50.4)
Splenomegaly	30 (22.2)
Colitis	16 (11.9)
Pneumonitis	81 (60)
Lethargy	15 (11)
Hypotonia	11 (8.1)
Poor neonatal sucking reflex	17 (13)
Convulsions	6 (4.4)
Fever	20 (14.8)
Malaise	11 (8.1)
Lymphadenopathy	14 (10.4)
Laboratory characteristics	
Thrombocytopenia	56 (41.5)
Severity of thrombocytopenia ($x10^9$/L)	
Mild (100-150)	11 (19.6)
Moderate (50-100)	23 (41.1)
Severe (< 50)	22 (39.3)
Hepatitis	28 (20.7)
Elevated liver enzymes	28 (20.7)
Elevated direct bilirubin	32 (23.7)
Elevated indirect bilirubin	32 (23.7)

Special investigations

Cranial ultrasound examinations were performed in 54.8% of which 36.5% were normal. Haemorrhage, periventricular calcifications, and cystic lesions were identified using this modality. Only 6.6% and 2% of patients received MRI and CT brain imaging, respectively. Furthermore, only 17.7% of patient had documented hearing screen results. Hearing loss was confirmed in 41.7% of screened infants. Otoacoustic emission (OAE) tests were most commonly performed (62.5%). Regarding ophthalmology screening, 7 of 63 (11.1%) screened infants had loss of vision. Developmental assessment was documented in 43.7% of cases, and 74.6% of infants evaluated had delayed milestones. (Table 3)

Table 3. Special investigations	n (percentage)
Cranial ultrasound performed	74 (54.8)
Cranial ultrasound findings	
Normal	27 (36.5)
Calcifications: periventricular	12 (16.2)
Periventricular cysts	5 (6.6)
Multi-cystic lesions	2 (2.7)
Interventricular haemorrhage	19 (25.7)
Periventricular calcifications and cysts	1 (1.4)
Hydrocephalus and periventricular cysts	2 (2.7)
Periventricular cysts leucomalacia	1 (1.4)
Hydrocephalus	2 (2.7)
Periventricular flaring	1 (1.4)
Ventriculitis	2 (2.7)
MRI brain performed	5 (6.6)
MRI findings	
Normal scan	3 (60)
Periventricular cysts	1 (20)
Poor myelination	1 (20)
CT brain performed	3 (2)
CT scan results	
Normal	2 (66.6)
Ventriculomegaly	1 (33.3)
Hearing screen	24 (17.7)
Type of hearing test	
OAE	15 (62.5)
BERA	8 (33.3)
Unknown	1 (4.2)
Hearing loss	10 (41.7)
Ophthalmology screen done	63 (46.6)
Vision loss	7 (11.1)
Developmental assessment	59 (43.7)
Delayed milestones in assessment	44 (74.6)

Management of CMV

A significant viral load (> 3 000 copies/mL) was documented in 61.6% of cases, with the median viral load calculated as 7 394 copies/mL. In 23 (17%) patients a diagnosis was based on a positive IgM test for CMV.

71 (52.6%) infants were treated with antiviral medication: 54.5% received both ganciclovir and valganciclovir. Most treated patients received only 4 weeks or less of treatment (49.3%).The Chi-Square test was used to assess the relationship between the infants who were not treated for CMV and developmental delay. Infants not treated for CMV infection are more likely to have developmental delay. The relation between these variables was statistically significant with a P value < 0.05 ($p = 0.0067$).

Table 4. Management of CMV	n (percentage)
Viral load at diagnosis (copies/mL)	
200-3 000	43 (38.4)
> 3 000	69 (61.6)
Other test (IgM CMV)	23 (17)
Infants treated for CMV	71 (52.6)
Antiviral medication in treated patients	
Ganciclovir only	28 (39.9)
Valganciclovir only	4 (5.6)
Ganciclovir and valganciclovir	39 (54.5)
Duration of treatment	
< 4 weeks	35 (49.3)
4-6 weeks	16 (22.5)
6 weeks to 3 months	14 (19.8)
3 months to 6 months	6 (8.4)
Viral load done at follow up	29 (21.5)
Viral load value at follow up (copies/mL)	
200-3 000	24 (82.8)
≥ 3 000	5 (17.2)

DISCUSSION

There is limited data published on infant mortality due to CMV infection, as most studies report morbidity and mortality in congenital CMV. Notably, there is also limited data regarding the outcomes of CMV infected infants who are preterm, HIV exposed or HIV infected.

This study suggests that postnatally acquired CMV infection contributed to significant mortality and morbidity, with an inpatient mortality in our academic hospitals of 13.3%. This was high compared to other countries. Studies performed in first world countries showed a low overall mortality of congenital CMV ranging from 4 to 8%. Some studies suggest that in severe fulminant disease the mortality rate was as high as 30%. With regard to premature and VLBW infants with symptomatic CMV infection, the mortality was between 5-10%. [6,18] Of the 18 patients who died, 12 (66.7%) of the infants were HIV exposed and 6 (33.3%) had CMV/HIV co-infection. The burden of HIV contributes to CMV infection associated mortality.

A South African study published in 2014 showed that neonates who had congenital CMV were more likely to die than the CMV negative neonates (mortality rate 42% vs. 18%, p = 0.01), especially if they were HIV exposed (47% vs. 15%, p = 0.02) or HIV infected (62% vs. 11%, p = 0.02).[8]

Three studies discussed the impact of HIV and CMV co-infection in infancy, concluding that CMV infection led to progression of HIV disease and increased mortality in children, especially when both these viruses were acquired in infancy. It demonstrated that CMV is a significant pathogen independently associated with poor outcomes in individuals with HIV. These studies were published (1999, 2009 and 2014) in an era

where antiretroviral therapy was not easily accessible to the maternal and infant populations.[19][20][21][22]

A significant number of mothers (60.7%) had HIV before or during pregnancy, with most (90.2%) receiving ART, suggesting good accessibility to treatment and successful programs for prevention of mother-to-child-transmission (PMTCT). However, high HIV viral loads were still noted during pregnancy (42.7%), and documented virological failure after 12 weeks of treatment was still significant (18.6%). Possible reasons for high viral loads in pregnancy have been described in an observational South African study that hypothesized that ART may be compromised due the physiological changes in pregnancy. The increase in blood volume and body mass index (BMI) in pregnant women, as well as changes in drug metabolism, may affect efficacy. Social determinants negatively impacting on adherence to drug regimens, must not be underestimated.[23]

Although a study conducted in the Eastern Cape, South Africa, could not find higher prevalence of CMV infection in HIV exposed compared to non-exposed infants, literature describes possible associations between maternal HIV and congenital CMV, particularly if the CD4 count is < 200 cells/µL. A lower maternal CD4 count has also been shown to correlate with a higher CMV viral load in the infant.[8][24][25]

In this study, 29.3% of women still had a CD4 count below 200 cells/µL in pregnancy, while 20.7% of infants had HIV/CMV co-infection. Adherence to exclusive breastfeeding was surprisingly high, with 84.4% continuing beyond 1 month. Literature suggests that any detectable CMV DNA in maternal breast milk has a risk for postnatal infection to the infant.[26] Despite this, the American Academy of Paediatrics still supports breastfeeding, even in preterm infants, as benefits outweigh the risks.

Alternatively, pasteurization of expressed breast milk might minimize risks in preterm infants.[10]

History of prematurity and low birth weight was common in this group, with intra-uterine growth restriction present in 33.3%, compared to 39-50% described in literature. Poor postnatal growth was noted in 62.2% of infants. Documented microcephaly (25.2%) and poor brain growth (46.6%), correlated with other studies which suggest an occurrence of 36-53%. Jaundice was recorded in 26.6%, which is slightly lower than the 38-67% recorded in other studies. Hepatomegaly was present in 50.4% and splenomegaly in 22.2%, in keeping with other studies describing hepatosplenomegaly in 39-60% of cases.[17]

Exposure to blood products was common during admission. Neonates received leucodepleted packed cells as standard of care, but there is insufficient information about the type of packed cells administered to older infants in this cohort.[12] Fever, lymphadenopathy and malaise, which could indicate CMV seroconversion after blood product transfusion, were uncommon in clinical presentation and documented in 14.8%, 10.4% and 8.1%, respectively. Due to the retrospective nature of the study, the causal relationship of blood product transfusion could not be correlated with these non-specific signs.[13]

Colitis was noted in 11.9%, but this was not proven histologically as not all patients required surgical intervention. CMV enterocolitis is not well documented in literature, and a higher index of suspicion with efforts to confirm the diagnosis is warranted.[27]

Thrombocytopenia is commonly described in patients with CMV (48-77%). In this study, 41.5% of infants had thrombocytopenia of which 39.3% were classified as severe (platelet count < 50 $x10^9$/L). CMV hepatitis with elevation in liver enzymes and

hyperbilirubinemia was less common than described in literature (21-24% vs 50-83%).[14]

Central nervous system involvement significantly impacts morbidity and mortality.[28] Only 8 of the 135 cases (6.6%) had a MRI or CT brain scan result, possibly reflecting constraints inherent to a resource-limited setting. Cranial ultrasound was performed in 74 (55%) of the study group; 47 (63.5%) had abnormal findings with periventricular calcifications in 12 (16.2%) cases. Neurosensory hearing deficits were identified in 10 patients, but a hearing screen was only documented in 24 patients (18%).

CMV whole blood viral load or a serum IgM are routinely performed to indicate CMV infection in infants, with confirmatory molecular testing recommended for infants with positive IgM results. The majority of patients (61.6%) had significant CMV viral loads exceeding 3 000 copies/mL, and CMV IgM yielded positive results in 30 patients. The advantage of the quantitative viral load test is that it has higher sensitivity and specificity than serology and enables monitoring of response to treatment. Quantitative PCR (viral load) has been found to be more useful in immunocompromised patients, for example in HIV, with a high CMV viral load correlating with clinical symptoms and the presence of disease. The IgM has a low sensitivity with most of the studies having a wide range between 25-84%.[29] Although not all infants shed the virus in urine, the urine shell vial assay was 94.5% sensitive and 100% specific. When using blood PCR in making the diagnosis, it has sensitivity and specificity of 89.2% and 95.8%, respectively, when compared to tests performed on saliva. Blood PCR may be falsely negative in the absence of viremia.

The management of congenital CMV and acquired CMV in infancy is controversial. An individualized approach with discussion with a paediatric infectious disease specialist is recommended. Of the hospitalised patients in the academic complex, 71 (52.6%) were treated for CMV, with the majority of patients receiving ganciclovir and valganciclovir. It would be expected that infants that were critically ill and requiring admission, would have all been treated with CMV antiviral medication. However, only half of the patients who demised received antiviral treatment in the study period. Most of the patients were treated for less than 4 weeks, despite current recommendations supporting 6 months of treatment for patients with CNS involvement, life threatening infection, severe single organ disease or multiple organ involvement.

Limitations

This was a retrospective descriptive study design, collecting data on a selected population of hospitalised infants testing CMV positive, which did not allow for comparisons to be made between CMV infected individuals and CMV negative patients. One could also not compare the mortality risk in HIV exposed or HIV co-infected patients.

The study did not include information on whether HIV infected infants were receiving ART. HIV viral load results were also not captured.

During the study period (2017-2018) the OAE machine was out of order, which can explain the lack of screening results.

Data from outpatient records were not available, and therefore screening tests with BERA may be under reported in this study.

CONCLUSION

CMV infection in infancy is under-appreciated in South Africa and a higher index of suspicion is warranted. It contributes to morbidity and mortality in infancy, particularly in preterm and low birth weight infants. CMV/HIV co-infection remains significant and may be associated with adverse outcomes. Early diagnosis of congenital and acquired CMV infection, appropriate special investigations, and appropriate management with antiviral medication may improve the prognosis. Further research would be necessary to determine if CMV infection in infancy increases mortality and morbidity.

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ABBREVIATIONS AND DEFINITIONS

ABR	Auditory brainstem evoked response
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BERA	Brainstem Evoked response audiometry
CDC	Centre for Disease Control and Prevention
CMV	Cytomegalovirus
CNS	Central nervous system
ELISA	Enzyme Linked Immune-sorbent Assay
HIV	Human immunodeficiency virus
IgM	Immunoglobulin M
LBW	Low birth weight
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
NHLS	National Health Laboratory Service
OAE	Otoacoustic emission
PC	Pack red blood cells
PCR	Polymerase chain reaction
SGA	Small for gestation age
UFS	University of the Free State
VLBW	Very low birth weight
WHO	World Health Organization

Congenital CMV infection	Positive CMV test within the first three weeks of life
Elevated liver transaminases	Any of the following during the time the CMV test was done: • ALT > 45 u/l • AST > 150 u/l • Total bilirubin

Age	Preterm	Term
0-1 day old	> 137 µmol/l	> 137 µmol/l
1-2 days old	> 205 µmol/l	> 197 µmol/l
3-5 days old	> 274 µmol/l	> 205 µmol/l
Other infant	> 34 µmol/l	> 21 µmol/l
• Conjugated bilirubin	Neonatal	Infants

	> 10 µmol/l > 4 µmol/l
Gravida	Indicates the number of times a woman has been pregnant regardless of the pregnancy outcome
HIV exposed	A baby whose mother is infected with HIV and can transmit the virus to her baby during pregnancy, labour, delivery or through breastfeeding
HIV infected	A baby who is infected with HIV and has a positive PCR for HIV
High-risk infants for HIV	infants identified at birth whos mothers had no recent VL available in the last 12 weeks of pregnancy or maternal VL ≥ 1000 copies/mL
Infants	Age group from birth to twelve months old
Insignificant CMV viral load	Viral load between 200 - 3000 copies/ml
Low birth weight	Weight at birth less than 2500g
Low-risk infants for HIV	Infants born to mothers with a suppressed HIV VL within the last 12 weeks of delivery, or at delivery
Meditech system	Computer operated program used at Universitas/Pelonomi Academic Hospital Complex, which contains written records, investigations and blood results
Mortality rate	A measure of the number of deaths
Parity	Indicates the number of pregnancies reaching viable gestational age
Positive CMV test	• Detectable viral load • CMV IgM Positive • CMV PCR Positive
Postnatal CMV infection	The detection of CMV after three weeks of life to under one year of age
Premature babies	Babies born alive before 37 weeks of pregnancy are completed
Significant CMV viral load	Viral load > 3000 copies/ml

Three year study period	From 1st January 2017 until 31st December 2019
Thrombocytopenia	Platelet count less than 150 $\times 10^9$/l on the blood result during the time of the CMV test
Time of CMV test	Period will include two weeks prior to the test and two weeks after the test
Universitas/Pelonomi Academic Hospital Complex	Includes Pelonomi Tertiary Hospital and Universitas Academic Hospital
Vertical transmission	Passage of a disease-causing agent (pathogen) from mother to baby during the period immediately before and after birth. Transmission might occur across the placenta, in the breast milk, or through direct contact during or after birth.
Very low birth weight	Weight at birth less than 1500g
Virolactia	Presence of viral particles in the breast milk

Appendix A:

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

28-May-2020

Dear **Dr Melisha Moodley**

Ethics Clearance: **Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein**
Principal Investigator: **Dr Melisha Moodley**
Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2020/0097/3006**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00011992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

Appendix B:

health
Department of
Health
FREE STATE PROVINCE

18 May 2020

Dr M Moodley
Dept. of Paediatric and Child Health
UFS

Dear Dr M Moodley

Subject: Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of **Pelonomi and Universitas Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeelats@fshealth.gov.za / makenamr@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- **Please discuss your study with Institution Manager on commencement for logistical arrangements see 2nd page for contact details.**
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- **As part of feedback you will be required to present your study findings/results at the Free State Provincial health research day**

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 25/05/2020

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

Appendix C:

NATIONAL HEALTH LABORATORY SERVICE

Practice No. 5200296

Office of the Business Manager
UNIVERSITAS ACADEMIC LABORATORIES
PO BOX 339(G3)
C/O: CHEMICAL PATHOLOGY
1st FLOOR
BLOCK C
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF FREE STATE
BLOEMFONTEIN
9301

REQUEST FOR APPROVAL OF LABORATORY RESOURCES FOR ACADEMIC PURPOSES

Date: 17 February 2020

Requestor: Dr. Melisha Moodley

Project Name: "CHARACTERISTICS AND OUTCOMES OF INFANTS WITH CYTOMEGALOVIRUS INFECTION IN BLOEMFONTEIN."

Dear Dr. Moodley,

Your request for use of laboratory facilities / data is hereby granted under following conditions:

1) That University Ethical Committee approval and approval from the Universitas Hospital management is obtained
2) All laboratory data remain confidential to the patient and doctor (anonymity is maintained)
3) This Office must be notified before any publication of any results / findings are made.
4) NHLS is recognised in all publications
5) That a successful K-Project application be made and relevant NHLS project cost centre be created to utilise testing at NHLS as per your protocol.

May your project be successful.

Regards,

NATIONAL HEALTH LABORATORY SERVICE
20 FEB 2020
BUSINESS MANAGER
UNIVERSITAS ACADEMIC

Mr. Pakiso Letanta
Acting Business Manager

Appendix D:

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

[redacted]

The Chair: Health Sciences Research Ethics Committee
Dr SM Le Grange
For Attention: Mrs M Marais
Block D, Room 104,
Francois Retief Building
Po Box 339 (G40)
Nelson Mandela Drive
Faculty of Health Sciences
University of the Free State
Bloemfontein
9300

21 Jan 2020

Dear Dr SM Le Grange

Dr M Moodley (Student number: **_2017558814_**)

Project Title:

Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein

I, Dr. Mosia, hereby grant Melisha Moodley permission to conduct the above mentioned research project. The research will be completed in accordance with myself as Head of Department of Paediatrics and Child Health and Dr Van der Byl as supervisor of this study.

Yours faithfully

Dr Mosia

22/01/2020

Date

Department of Paediatrics and Child Health / Departement Pediatrie en Kindergesondheid
205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
P.O. Box/Posbus 339 (G69), Bloemfontein 9300, South Africa/Suid-Afrika, www.ufs.ac.za, F: +27(0)51 444 3230
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Appendix E

Characteristics and outcomes of infants with cytomegalovirus infection in Bloemfontein

Researcher: Dr. Melisha Moodley
Supervisor: Dr. Van Der Byl

2020

RESEARCH PROTOCOL

Full Title: The final outcomes and characteristics of infants with cytomegalovirus infection in the Universitas/Pelonomi Academic Hospital Complex

DOCUMENT VERSION:

Version	Date	Author	Reason	Sections
49	12/01/2020	Melisha Moodley		All

STUDY INVESTIGATOR(S)

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1. ABBREVIATIONS

1. CMV – Cytomegalovirus
2. NHLS – National Health Laboratory Service
3. UFS – University of the Free State
4. PCR – Polymerase Chain Reaction
5. VL – Viral Load
6. IgM – Immunoglobulin M
7. ELISA – Enzyme Linked Immune-sorbent Assay
8. NEC – Necrotizing Enterocolitis
9. CDC – Centre for Disease Control and Prevention
10. HIV – Human Immunodeficiency Virus
11. AST – Aspartate Aminotransferase
12. ALT – Alanine Aminotransferase
13. MRI – Magnetic Resonance Imaging
14. ABR – Auditory Brainstem Evoked Response
15. WHO – World Health Organization
16. LBW – Low birth weight
17. VLBW – Very low birth weight
18. CNS – Central nervous system
19. SGA – Small for gestation age

2. DEFINITIONS

1. Universitas/Pelonomi Academic Hospital Complex: will include Pelonomi Tertiary Hospital and Universitas Academic Hospital
2. Three year period: from January 2017 until December 2019
3. Positive CMV test:
   a. Detectable Viral load
   b. CMV IgM Positive
   c. CMV PCR Positive
4. Congenital CMV infection: Positive CMV test within the first three weeks of life[1]
5. Postnatal CMV infection: The detection of CMV after three weeks of life to under one year of age[1]
6. Meditech system: Computer operated program used at Universitas/Pelonomi Academic Hospital Complex, which contains written records, investigations and blood results
7. Infants: Age group from birth to twelve months old
8. Time of CMV test: Period will include two weeks prior to the test and two weeks after the test
9. Thrombocytopenia: Platelet count less than 150 $\times 10^9$/l on the blood result during the time of the CMV test[2]
10. Elevated liver transaminases: Any of the following during the time the CMV test was done[2]:
    a. Alanine aminotransferase (ALT) > 45 u/l (Birth to under one year of age)

b. Aspartate aminotransferase (AST) >150 u/l (Birth to under one year of age)
c. Total bilirubin
    - 0-1 day old: Preterm and Term >137 µmol/l
    - 1-2 days old: Preterm >205 µmol/l, Term >197 µmol/l
    - 3-5 days old: Preterm>274 µmol/l, Term >205 µmol/l
    - Other infant: Preterm >34 µmol/l, Term > 21 µmol/l
d. Conjugated bilirubin
    - Neonatal > 10 µmol/l
    - Infants > 4 µmol/l

11. Insignificant CMV viral load: Viral load between 200 - 3000 copies/ml[3]
12. Significant CMV viral load: Viral load > 3000 copies/ml[3]
13. Vertical transmission: Passage of a disease-causing agent (pathogen) from mother to baby during the period immediately before and after birth. Transmission might occur across the placenta, in the breast milk, or through direct contact during or after birth.
14. HIV exposed: A baby whose mother is infected with HIV and can transmit the virus to her baby during pregnancy, labour, delivery or through breastfeeding
15. HIV infected: A baby who is infected with HIV and has a positive PCR for HIV
16. Gravida: Indicates the number of times a woman has been pregnant regardless of the pregnancy outcome
17. Parity: Indicates the number of pregnancies reaching viable gestational age
18. Mortality rate: A measure of the number of deaths
19. Premature babies: Babies born alive before 37 weeks of pregnancy are completed (WHO)[4]
20. Low birth weight: is defined by WHO as weight at birth less than 2500g[5]
21. Very low birth weight: is defined by WHO as weight at birth less than 1500g[5]
22. Virolactia: presence of viral particles in the breast milk[6]

3. INTRODUCTION AND LITERATURE REVIEW

Cytomegalovirus (CMV) is a double stranded DNA virus that forms part of the herpesviridae family. It can be transmitted in saliva, blood, secretions, urine and breast milk.[7] Transmission from mother to child can be congenital or postnatally acquired.[8],[9] Ninety percent of neonates who are infected with CMV are asymptomatic. The most common long term squealae is non hereditary neuro-sensory hearing loss. There is lack of awareness of CMV infection. The general public are not aware of simple principles that can prevent CMV, such as good hygiene and regular hand washing.[8] One study evaluated the awareness and knowledge of congenital CMV in health care providers in the form of a questionnaire. This study concluded that most of the health care providers were unaware of the precise route of transmission of CMV. There is a large gap of knowledge with regards to CMV in pregnancy and the burden of disease.[10]

Mortality

A South African study showed that neonates who had congenital CMV were more likely to die than the CMV negative neonates (mortality rate 42% vs. 18%, p = 0.01) especially if they were HIV exposed (47% vs. 15%, p = 0.02) or HIV infected (62% vs. 11%, p = 0.02).[11] No other data on mortality of CMV infected infants has been published in South Africa. Emerging evidence has shown that CMV and other congenital and neonatal infections, are under-appreciated causes of morbidity and mortality in African children. Research in this area of study is limited and more studies need to be published in order to gain a better understanding.[12] Studies published in Europe and America showed an overall mortality rate of 4 to 8% in congenital CMV. Mortality rate could be as high as 30% with severe fulminant disease. Between 5 to 10% of premature and VLBW infants with symptomatic CMV infection may die.[13][14]

Epidemiology of CMV

A South African study in Johannesburg reported a prevalence of 0.26/1000 live births over a five year period.[11] Another South African study done in the Eastern Cape Province showed a prevalence of 5.96% (95% CI 3.29 – 8.63). However, due to this wide confidence interval this prevalence is likely due to chance, which may be attributed to the small study population that was used.[9] There is currently no published data on the prevalence or incidence of CMV in infants in the Bloemfontein population. A review of several international studies showed the incidence and prevalence was influenced by geographic and racial variation. One study demonstrated CMV infection to be higher in lower income countries such as South America, Africa and Asia and lowest in Western Europe and the United States of America. Globally there is a high CMV sero-prevalence ranging between 1 to 5%.[8]

Congenital versus acquired CMV

Most of the literature defines congenital CMV as an intrapartum infection with a positive CMV test before three weeks of life, because perinatal acquired infection can also manifest around this time. A positive CMV test after 3 weeks of life is generally regarded as acquired CMV infection.[15] There is still controversy surrounding the differentiation of congenital versus acquired CMV infection. Acquired infection occurs postnatally and includes perinatal, postnatal, or childhood periods. Perinatally and postnatally acquired infection can occur by exposure to infected cervical secretions and breast milk of a mother infected with CMV. Some

sources in the literature define acquired CMV infection as including the perinatal period and the postnatal age up to one year of age. Over the age of one year is considered to be childhood CMV infection and the clinical presentation and management in these children differ compared to congenital and postnatally acquired CMV.[15]

Maternal characteristics

The risk of vertical transmission to the fetus increases with primary maternal infection compared to recurrent infection. Fetal transmission appears to increase with advancing gestational age.

Intrauterine transmission can occur in mothers without preexisting immunity as a primary infection or as reactivation of a previous CMV infection. CMV can be spread either intrapartum or postpartum.[8,9] Factors shown to influence transmission of congenital CMV are maternal age and parity, where there is an increased risk in younger primigravid women. [8,9]

Maternal HIV and CMV

Many studies investigated the association of HIV and CMV infection. One South African study showed that the maternal CD_4 count <200 cells/µL during pregnancy was independently associated with congenital CMV with an adjusted odds ratio of 2.9 (95 % CI 1.2 – 7.3). This study had a small population with a wide confidence interval and it is likely these findings were due to chance.[16] Another South African study showed that a lower maternal CD_4 count correlates with an increase in CMV viral load in infants.[17] A further South Africa study, conducted in the Eastern Cape Province, showed equal prevalence in HIV exposed and HIV unexposed infants. However, it is worth noting that these findings were statistically insignificant (p = 0.869), most likely due to the small sample size.[11]

Post-natal acquisition of CMV has little clinical significance and is not associated with long term disability and illness in term infants. However, this route of transmission still holds significance for low birth weight and premature infants.[8]

One study described the rate of CMV virolactia and prevalence of breast milk transmitted CMV infection after freezing and pasteurization of breast milk. This study concluded that the intervention, namely freezing and pasteurization of breast milk, reduced the risk of CMV infection transmission, but did not eliminate it.[18]

Blood product transfusion related CMV

A pilot study in South Korea investigated transfusion related CMV infection among very low birth weight infants in an endemic area. This study concluded that filtration or irradiation of CMV seropositive blood products (including red blood cells and platelets) did not significantly reduce transfusion related CMV infection in infants. This study was a small study and had several limitations including lack of randomization and lack of groups receiving irradiated only and filtered only blood products.[19]

Another older study in 1981, concluded that the total volume of more than 50 milliliters (from multiple transfusions) of red blood cells from sero-positive donors was an important risk factor

for the development of transfusion related CMV disease in LBW infants. This study did not use irradiated or leucodepleted blood.[20]

Clinical characteristics for congenital and acquired CMV infection in infants

The clinical findings in symptomatic infants are similar to those of other congenital infections.[21] With regards to clinical presentation for congenital CMV infection, studies have shown that petechiae is present in 54 to 76%, jaundice at birth in 38 to 67%, and hepatosplenomegaly in approximately 39 to 60% of patients. Small size for gestational age may be present in 39 to 50%, and microcephaly in 36 to 53% of cases.[21]

Infants who acquire CMV perinatally may have signs and symptoms of disease such as lymphadenopathy, hepatitis and pneumonitis. These infants may have considerable morbidity especially if born prematurely. Further research needs to be done to assess the long term neurological outcomes of these infants.[15]

Transfusion acquired CMV infection presented similarly to CMV mononucleosis. The incubation period is generally 20 to 60 days. The clinical features include fever and severe malaise.[15]

CMV enterocolitis is not well documented in the literature. One study reviewed a case report of CMV associated colitis mimicking necrotizing enterocolitis (NEC), which was confirmed by the presence of CMV inclusion bodies on histology. CMV enterocolitis usually presents with ulceration leading to stricture formation, whereas NEC leads to gangrene and perforation of bowel.[22]

Investigations

Laboratory findings in infants with congenital CMV included elevated liver enzymes with elevated direct and indirect serum bilirubin in 50 to 83% of cases and thrombocytopenia in 48 to 77% of cases.[21]

One study described the spectrum of cranial ultrasound and magnetic resonance imaging (MRI) abnormalities of the brains of infants with congenital CMV. This was a small study that reviewed eleven infants with confirmed CMV on viral isolate or PCR. Cranial ultrasound examinations were performed on all infants and MRI brain scans on six infants. The spectrum of abnormalities on cranial ultrasound included: periventricular calcification, lenticulostriate vasculopathy associated with mild to moderate ventricular dilation and periventricular cysts. The MRI provided additional information including polymicrogyria, hippocampal dysplasia and cerebellar hypoplasia. This study also screened for hearing loss, by automated auditory brainstem response assessment (AABR) using the ALGO test. If the infant failed this test a full auditory brainstem evoked response (ABR) was performed by an audiologist to assess neurosensory hearing loss.[23] Chorioretinitis is the most common ocular manifestation of CMV.[21]

Confirmatory testing for CMV

The Centre of Disease Control and Prevention (CDC) recommends performing CMV PCR on saliva as a screening test, whilst urine may be tested for diagnostic confirmation of CMV in

children less than one year old. The reason for the confirmatory test on urine is that most CMV seropositive mothers shed CMV in their breast milk. This can cause a false-positive CMV result on saliva collected shortly after the baby has breastfed.[24] The accuracy of the CMV PCR has been described extensively in the literature. One such study compared CMV PCR and CMV IgM detection using ELISA diagnosis of CMV infection in high risk neonates. This study showed that the PCR in relation to viral culture (which is the gold standard) had one hundred percent sensitivity and specificity with excellent agreement between both tests, with a kappa coefficient being between 0 to 1 and significant p-values.[1]

Management

Congenital CMV - Whom to treat:

There is controversy in the literature surrounding the treatment of congenital CMV. Congenital CMV is usually categorized as symptomatic versus asymptomatic at birth. The literature has differing definitions and opinions of what constitutes symptomatic CMV thus making treatment difficult.[13],[25] The European Expert consensus Guideline and The Red Book report of infectious disease from the American Academy of Paediatrics recommend treating babies with evidence of central nervous system (CNS) disease, evidence of life threatening disease or severe single organ disease or multiple organ disease.[13]

Treatment of congenital CMV infection is based on the presence of symptoms and on the immune status of the infant. The current guideline for congenital CMV recommends treating infants with virologically confirmed CMV that have at least one end organ symptom (examples include pneumonitis, hepatitis, thrombocytopenia).[25]

Evidence for treating babies with multiple, but not severe manifestations of the disease (jaundice, hepatosplenomegaly without significantly raised liver enzymes and SGA) is limited. It is recommended that these cases be individualized and discussed with experts like a Paediatric Infectious Disease Specialist.[13]

Early postnatal infection

The current guideline recommends treatment with ganciclovir or valganciclovir in severe symptomatic infections in preterm or VLBW infants.[25]

Children and adolescents

In immune-competent patients the guideline recommends supportive care with hydration and fever control. Treatment with ganciclovir is recommended in immune-compromised patients.[25]

Congenital CMV – Antiviral treatment

Treatment with ganciclovir and valganciclovir has shown improvement in neuro-developmental outcomes.[25]

A randomized trial conducted in 2003 in neonates more than 32 weeks gestation compared the effect of six weeks of ganciclovir versus no treatment in symptomatic congenital CMV disease involving the CNS. CNS disease included microcephaly, intracranial calcification,

abnormal cerebrospinal fluid (CSF) for age, hearing deficit and chorioretinitis. This study showed improvement from baseline in neurodevelopment and hearing in the treated group. This trial unfortunately had a significant loss to follow up.[26]

A more recent trial published in 2005 compared 6 weeks to 6 months of valganciclovir therapy in infants with symptomatic congenital CMV infection with and without CNS involvement. Children who received six months of antiviral therapy were more likely to have improved hearing or maintain normal hearing (odds ratio 2.61, 95% CI 1.05 - 6.43) and higher language and receptive communication scores at 24 months.[27]

The current recommendation is to treat with ganciclovir or valganciclovir for 6 months. Valganciclovir is the drug of choice but intravenous ganciclovir can be used in babies unable to tolerate the oral drug, who have life threatening disease or if gastrointestinal absorption is uncertain.[13]

After an extensive review through the literature, it is worth noting that there is a dearth of information regarding the demographic representation of CMV infection in infants in Bloemfontein. It is common practice in the Universitas Academic and Pelonomi Tertiary Hospital Complex to test infants who have recurrent sepsis failing to respond to antibiotics for CMV infection. The CMV viral load is then requested. It is also common practice to investigate infants with hepatosplenomegaly and conjugated hyperbilirubinemia for congenital infections, and CMV is usually included in the screen.

The National Health Laboratory Service (NHLS) started testing for CMV viral load instead of using the PCR test in 2016, as this is more effective. This aids in deciding, timeously, who to treat based on significant viral loads. The literature shows a lack of standardization to establish a viral load cutoff associated with active disease.[3] There is differing evidence on who should be treated based on viral load and clinical disease. The recommendation is to assess each case individually and decide on starting treatment based on risk versus benefit. The researcher will consider a CMV viral load as significant if it exceeds 3000 copies/ml.[3]

This study will assist health care workers by identifying the demographic, clinical and biochemical markers of CMV infection in the local setting and prompt a response to screen for CMV. This may lead to earlier intervention with initiation of early treatment if indicated, in order to prevent CMV related complications.

4. AIMS AND OBJECTIVES OF STUDY

The aim of the study is to investigate and describe CMV infection in infants admitted to Universitas Academic and Pelonomi Tertiary Hospital.

The primary objective is to determine the mortality rate and final outcomes of infants admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex, who tested positive for cytomegalovirus over a three year period, namely 2017 to 2019.

Secondary objectives are to determine the demographical, clinical and laboratory characteristics of these infants. The maternal characteristics will also be described.

Tertiary objectives will describe the morbidity, complications and management of these infants. Special investigations including MRI, cranial ultrasound, hearing tests, vision screening and developmental assessments will be captured. The management of the infant with CMV infection will also be described under this objective. This will include the pretreatment viral load, the number of patients that were treated and duration of treatment. The initial viral load will be categorized as significant versus insignificant.

5. RESEARCH QUESTIONS

- What is the mortality rate and the final outcomes of infants who tested positive for CMV in the Universitas Academic and Pelonomi Tertiary Hospital Complex over a three year period?
- What is the mortality rate and the final outcomes for infants who are HIV exposed and who tested positive for CMV?
- What is the mortality rate and the final outcomes for infants who are HIV infected and who tested positive for CMV?
- What are the demographic, clinical and laboratory characteristics of infants who had positive CMV tests in the Universitas Academic and Pelonomi Tertiary Hospital Complex over the three year period?
- How many infants with a positive CMV test developed complications that resulted in morbidity? How many of these infants were treated? What was the duration of treatment?
- How many infants with positive CMV tests had a significant viral load?
- How many infants received antiviral medication?
- What was the duration of antiviral medication treatment in these infants?
- How many of the infants who died, received antiviral medication for CMV?

6. STUDY DESIGN

This will be a retrospective, descriptive cross sectional study design.

7. STUDY LOCATION AND POPULATION

All infants younger than one year who had a positive CMV test according to the NHLS data base and who were admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex in Bloemfontein over the three year period, from 2017 to 2019, will be included. A sample size of approximately 25 patients is expected.

8. ELIGIBILITY CRITERIA

Inclusion criteria:

All infants aged between 0 and 12 months of age who had a positive CMV test (as per definition) from the NHLS data base, and who were admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex over the three year study period (2017 -2019).

Exclusion criteria:

1. Any samples done on patients while not admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex.

2. Positive CMV tests not performed by the National Health Laboratory Service.
3. Children older than one year old at the time of the CMV test.
4. Patients with no documentation, including no discharge summary on the Meditech system, or no clinical file found. These cases will however be noted.

9. STUDY PROCEDURES AND MEASUREMENT

The primary measurement is to determine the mortality rate and final outcomes of infants admitted to the Universitas Academic and Pelonomi Tertiary Hospital Complex, who tested positive for cytomegalovirus over the three year period, namely 2017 to 2019. Measurement will include the percentage of the infants that demised who were HIV exposed and HIV infected.

The maternal characteristics will include maternal age, parity, mode of delivery and HIV status and history of previous CMV infection or current infection.

The demographic characteristics for the infant population will include sex of the infant, HIV status, chronological age at the time of testing for CMV and the infant's anthropometry (weight, length and head circumference) at birth. This will include gestational age and whether the infant's anthropometry parameters were appropriate for gestational age or not. It will be described if these infants infected with CMV are born preterm and/or have intrauterine growth restriction. The choice of feeding in infancy will be noted. It will be recorded whether these infants received a blood product transfusion prior to a CMV test and how many times the infant was transfused.

The clinical characteristics will include petechiae, jaundice and hepatosplenomegaly (which will be sub classified as hepatomegaly and splenomegaly). The clinical notes will be reviewed to assess whether these infants had microcephaly and other neurological deficits, such as lethargy, hypotonia, a poor sucking reflex and seizures. The Meditech discharge summaries will be reviewed and noted if the patients had features of lymphadenopathy, fever, malaise, colitis and pneumonitis.

The laboratory characteristics will include thrombocytopenia which will be defined as a platelet count less than 150 $\times 10^9$/l on the blood result during the time of the CMV test (defined as within a two week period from the time of test). Biochemical characteristics will include: elevated liver transaminases defined as an increased alanine aminotransferase (ALT), an increased aspartate aminotransferase (AST), or elevated direct and indirect serum bilirubin as per definition. Elevated liver transaminases defined as any of the following during the time the CMV test was done: ALT more than 45 u/l from birth to under one year of age; AST more than 150 u/l from birth to under one year of age. Elevated bilirubin levels will be defined as follows: Total bilirubin from 0–1 day old in term and preterm more than 137 µmol/l. Age from 1-2 days old in preterm babies more than 205 µmol and in term babies more than 197 µmol/l. Age 3-5 days old in preterm more than 274 µmol/ and in term more than 205 µmol/l. Other infants that were preterm more than 34 µmol/l and term more than 21 µmol/l. Conjugated bilirubin in neonates more than 10 µmol/l and in infants more than 4 µmol/l.

The study will describe the morbidity and complications of infants with a positive CMV test. The percentage of infants who had complications and whether they were treated or not will be captured. The duration of treatment will be noted. The measure will include the percentage of infants who had a positive CMV test that demised and were not treated.

10. CONSENT

Consent will be obtained from NHLS, Virology Department to access the NHLS data base and the Free State Department of Health for hospital records.

11. METHODS OF DATA COLLECTION

A data capture sheet created in Microsoft Word will be used to collect information from the NHLS data base, files, Meditech system, PACS radiology reports and the audiology outpatient book at the Speech and Occupational Therapy Department. This information will then be transferred to a Microsoft Excel spread sheet. Data collection will start as soon as ethical approval has been granted by the Health Sciences Research Ethics Committee of the University of the Free State, as well as approval by the Free State Department of Health and the NHLS.

12. DATA ANALYSIS

Descriptive statistics namely means and standard deviations or medians and percentiles will be calculated for continuous data. Frequencies and percentages will be calculated for categorical data. The analysis will be done by the Department of Biostatistics at the University of the Free State.

The patient records will be collected on the data sheet using the patients' file and the Meditech system. This data will be entered into the Excel spreadsheet and the data analyzed thereafter. The Excel spreadsheet that is drawn up will be kept by the principal researcher until completion and publication of the study.

13. ETHICAL CONSIDERATIONS

As this is a retrospective, descriptive study, no consent from parents of infants will be required. Confidentiality will however be maintained throughout the study by making use of a unique study number for each file audited.

The protocol will be submitted for ethical consideration and approval by the Health Sciences Research Ethics Committee of the University of the Free State, after which approval will be sought from the Free State Department of Health and the NHLS Virology Department.

14. IMPLEMENTATION

The demographics associated with CMV infection in infants in the local setting are not well defined and clinical features of the infection may be easily overlooked. This study will allow us to be vigilant and add clinical significance to early testing for CMV, with appropriate special investigations to evaluate for related morbidities. Early treatment which will prevent complications and improve outcomes will be prioritized. There is a lack of evidence to describe the mortality associated with CMV infected infants in South Africa and this study aims to explore this crucial aspect.

The study will be written in the form of a publishable paper with the aim to publish the article in a reputable medical journal. Ideally we would like this study to be part of the foundation of South African research, documenting the burden of disease caused by congenital and acquired CMV in infants living in a developing country. This may ultimately influence national policy-making to consider screening for CMV infection in expectant mothers in the same manner that HIV is screened during prenatal and antenatal visits in the public sector.

15. LIMITATION OF THE STUDY

The limitation of the study is related to the retrospective nature of the study design. Data collected may be incomplete or incorrectly recorded in the patient's file or discharge summary. The Meditech system may also have incomplete records and this can be overcome by including patient files and records to collect information.

A small sample size is expected, which may make interpretation of results difficult. It will not be possible to calculate the prevalence of CMV.

16. BUDGET

Expenses will include:

1. Transport to Universitas and Pelonomi Hospitals for data collection = R100
2. Printing of the data collection sheets and pens to fill in data information = R300
3. Transport for regular contact session with supervisor and statistician = R100

The total estimated amount is R500. This will be covered by the researcher.

17. PILOT STUDY

Once ethical approval has been established, a pilot study will be conducted with three patient records, one from each year included in the study. This data will be entered on an Excel spread sheet and analyzed thereafter. This pilot study will provide the framework for the rest of the study. Changes will be made to the data capture sheet if any flaws are identified, and after correction, the patient files will be recaptured and will form part of the final results.

18. TIME FRAME

1. Preliminary literature review:	2018
2. Writing of protocol:	January to October 2019
3. Presentation to Paediatrics Department:	October 2019
4. Submission of protocol to Study leader:	November 2019
5. Submission to Departmental Evaluation Committee:	November/December 2019
6. Final submission to Department of Biostatistics:	January 2020
7. Submission to Ethics Committee:	February 2020
8. Submission to Department of Health:	March 2020
9. Approval by NHLS:	March 2020
10. Pilot study and analysis:	April 2020
11. Data collection:	April to May 2020
12. Data analysis:	June 2020
13. Writing up of results:	July to September 2020

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1 Appendix F

16.1 DATA CAPTURE SHEET

STUDY NUMBER ALLOCATED ☐ Initials ☐ Year: 2017 / 2018 / 2019

Basic Data

Place of admission: Universitas Hospital / Pelonomi Hospital

Date of birth: _____ Date of admission: _____ Date of discharge/death: _____

Date of CMV test:_____ Age at time of test: _____ Outcome: **Alive / Demised**

Cause of death if demised: _____

If less than 3 weeks of age: Congenital CMV? Yes / No

If more than 3 weeks of age: Congenital CMV / Acquired CMV / Unsure

☐ Headings

☐ Risk factors for CMV infection

Maternal characteristics during pregnancy					
Maternal age	<20 years	20-29 years	30-34 years	35-39 years	40years +
Parity	Unknown	Primigravida	G2	G3	G4+
Delivery	Unknown	Vaginal	BBA	Assisted	C/Section
Mother with cmv infection	Unknown	Yes	Primary or Recurrent	No	
HIV	Unknown	Positive	Negative		
HIV+ viral load	Unknown	LDL/ <1000	>1000copies/ml	Value:	
HIV+ CD4	Unknown	CD4 <200	CD4>200		
HIV+ treatment	Unknown	Untreated	Treated	Duration:	

Demographics of infants testing positive for CMV						
Gender		Unknown	Male	Female		
Gestational age		Unknown	Preterm	Term		
HIV status		Unknown	Positive	Negative		
Low birth weight		Unknown	Yes	No		
Birth weight		Unknown	<1 kg	1 – 1.499 kg	1.5 – 2.499 kg	>2.5 kg
Feeding choice		Unknown	Exclusive BF	Exclusive formula	Mixed feeding	Exclusive BF duration:
BF yes	No					
Fetal growth		Unknown	SGA: symmetrical	SGA: asymmetrical	AGA	LGA
SGA/IUGR YES	NO					
Head circumference		Unknown	Microcephaly	Normal	Macrocephaly	
Length (birth)		Unknown	<10th centile/yes	10-90th centile	>90th centile	
Postnatal growth		Unknown	Underweight	Normal	Overweight	Failure to thrive
HC growth		Unknown	Microcephaly	Normal	Macrocephaly	
Packed Cells?		Unknown	No	Yes	If yes: nr of t/f	
Platelets?		Unknown	No	Yes	If yes: nr of t/f	

Clinical characteristics of CMV infection in infants					
Petechiae	Unknown	No	Yes		
Jaundice	Unknown	No	Yes		
Hepatomegaly	Unknown	No	Yes		
Splenomegaly	Unknown	No	Yes		

Colitis	Unknown	No	Yes		
CMV pneumonitis	Unknown	No	Yes		
Others					
Lethargy	Unknown	No	Yes	Specify	Unspecified
Hypotonia	Unknown	No	Yes	Specify	Unspecified
Poor sucking reflex as neonate	Unknown	No	Yes	Specify	Unspecified
Convulsions	Unknown	No	Yes	Specify	Unspecified
Fever	Unknown	No	Yes	Specify	Unspecified
Malaise	Unknown	No	Yes	Specify	Unspecified
Lymphadenopathy	Unknown	No	Yes	Specify	Unspecified

Laboratory characteristics on special investigations of infants with CMV					
Thrombocytopenia	Unknown	No	Yes		
			100-150	50-100	<50
Hepatitis	Unknown	No	Yes	Unsure	
AST/ALT raised	Unknown	No	Yes		
Direct bilirubin high	Unknown	No	Yes		
Indirect bilirubin high	Unknown	No	Yes	Diagnosis?	

Special investigations for morbidity related to CMV infection in infancy					
Cranial u/s performed	Unknown	No	Yes		

Cranial u/s result if done	Unknown	Normal	Calcifications periventricular	Periventricular cysts	Other:	
MRI performed	Unknown	No	Yes			
MRI result if done	Unknown	Normal	Periventricular cysts	Poor myelination	Migrational abnormalities	Other
CT performed	Unknown	No	Yes	Findings		
Hearing screen done	Unknown	No	Yes	If yes: OAE	If yes: BERA	
Hearing loss	Unknown	No	Yes			
Ophthalmology screen	Unknown	No	Yes	Chorioretinitis		
Vision loss	Unknown	No	Yes	Other:		
Developmental assessment	Unknown	No	Yes			
Delayed milestones	Unknown	No	Yes			

Management of infant with CMV infection					
Viral load at diagnosis	Unknown	Insignificant (200-3000)	Significant (yes) (>3000)	Value:	Log:
Treatment for CMV	Unknown	No	Yes		
Antiviral medication	Unknown	Ganciclovir	Valganciclovir	Both	
Duration of treatment: approximately	4 weeks	6 weeks	3 months	6 months	Other:
Viral load follow up	Unknown	Insignificant (200-3000)	Significant (>3000)	Value:	Log:

The Pediatric Infectious Disease Journal (PIDJ)- INSTRUCTION FOR AUTHORS

Online Submission and Review System

SCOPE

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Abstract	250 words	250 word limit
Written Article excluding abstract and tables ,figures and references	2860 words	3000 word limit

I Dr Melisha Moodley declare that the word count for research report in the format of a published article for UFS and report for the Pediatric Infectious Disease Journal (PIDJ) is true and correct.