FACTORS ASSOCIATED WITH CYTOMEGALOVIRUS (CMV) INFECTION IN NEONATES

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DECLARATION

I, Hitesh Amrat Diar, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University. Any information used in this research report has been obtained by me, Hitesh Amrat Diar, while employed by the Chris Hani Baragwanath Academic Hospital and the University of the Witwatersrand.

Signed: _____

On this: _____ day of: _____, 2013

In memory of My Dearest Brother and Friend

Pritesh Amrat Diar

1981 – 2000

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Publications:

None

Conference presentation:

- H Diar, R Thomas, S Velaphi. Factors Associated With Cytomegalovirus (CMV) Infection in Neonates. The 27th Conference on Priorities in Perinatal Care in Southern Africa; 2008 Mar 14; Indaba Hotel, Johannesburg, South Africa.

ABSTRACT

Background: Congenital Cytomegalovirus (CMV) infection is common in neonates. Factors associated with congenital CMV infection in a human immunodeficiency virus (HIV) prevalent setting, are not known.

Objective: To determine characteristics and outcome of congenital CMV-infected neonates.

Methods: The Hospital records of neonates tested for CMV in first 21 days of life from January 2004 to December 2008 were retrospectively reviewed for the following variables; maternal and neonatal characteristics, clinical presentation, laboratory findings and in-patient mortality. Newborns that were CMV-positive and CMV-negative were compared for the above variables.

Results: From the 177 patients suspected to have congenital CMV, 28 were confirmed to be congenital CMV-infected. The hospital records were retrieved for 24/28 (86%) CMV-positive and 62/149 (42%) CMV-negative patients (86 study participants). In CMV-positive group, 22 patients (92%) were low birth weight, 15 (63%) were preterm and 7 (29%) were small for gestational age. There were no significant differences noted for birth-weight, gestational age and growth between CMV-positive and CMV-negative patients. Hepatosplenomegaly was more common in CMV-positive than CMV-negative patients (n =9/24 (38%) vs n =10/62 (16%); p =0.03). The platelet count was lower in CMV-positive than in negative patients (median =71 $x10^9$/L vs median =49 $x10^9$/L; p =0.003). Congenital CMV-infected patients were more likely to be HIV-exposed (n =19/24 (79%) vs n =27/62 (44%); p =0.003) and HIV-infected (n =13/19 (68%) vs n =6/19 (32%); p =0.02) than CMV-negative patients. The in-hospital mortality was significantly higher in symptomatic congenital CMV-infected (n =10/24 (42%) vs n =11/62 (18%); p =0.01) and HIV co-infected (n =8/13 (62%) vs n =1/9 (11%); p =0.02) neonates.

Conclusions: The presence of hepatosplenomegaly and/or persistent thrombocytopaenia in HIV-exposed patients is suggestive of congenital CMV with HIV co-infection. Neonates' co-infected with HIV and CMV are less likely to survive to hospital discharge.

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CHAPTER 1

1.0 INTRODUCTION

Congenital CMV infection has been reported to occur in as high as 23% of neonates born to mothers infected with human immunodeficiency virus (HIV) [1, 2]. Congenital CMV infection has also been shown to be more common in HIV-infected than in HIV-uninfected neonates[1]. This has also been reported to lead to a more rapid progression of HIV infection in these newborns[1]. The association between CMV and HIV infection has been supported by reduction of congenital CMV in areas where prevention of mother to child transmission (PMTCT) of HIV has been implemented. Following the introduction of HAART in developed countries in 1997 to curb the MTCT of HIV infection, there has been a decline in the prevalence of congenital CMV infection[2].

In the pre-highly active antiretroviral therapy (pre-HAART) era, the most important maternal factors impacting on congenital CMV infection were young age, single marital status, lower social class and lower parity[2, 3, 4]. However, in the HAART era, the main factor impacting on congenital CMV infection was the time of mother to child transmission of HIV, that is, either in-utero or intrapartum[1, 5, 6].

About 30% of mothers attending antenatal clinics in South Africa (SA) are HIV positive[3, 7]. A similar percentage has been noted amongst pregnant women attending ante-natal care and/ or delivering at Chris Hani Baragwanath Academic Hospital (CHBAH). It is common clinical practice in the Neonatal Unit of this hospital that neonates born with thrombocytopaenia, and/ or hepato-/splenomegaly and/ or conjugated hyperbilirubinaemia, are investigated for CMV infection.

As a clinician working in the Neonatal Unit, it was my impression that there were a significant number of patients that were diagnosed with congenital CMV infection during the era of HIV infection (pre-HAART era). Therefore, I sought to determine the incidence, characteristics and the outcome of patients with congenital CMV infection admitted to the Neonatal Unit at Chris Hani Baragwanath Academic Hospital.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Epidemiology of congenital cytomegalovirus infection

Infection with CMV in the neonate is usually acquired through vertical transmission from the mother, which can occur congenitally or perinatally. Congenital CMV infection, which implies transplacental transmission, results in more than 70% of these symptomatic newborns developing long-term neurological hearing and/ or visual impairments[8]. The global birth prevalence of congenital CMV infection, representing the global burden of congenital CMV, is 0.64%[9]. South Africa contributes 0.13% to this global disease prevalence[9]. The global birth incidence of congenital CMV infection, representing the risk of acquiring congenital CMV infection, has been reported to be 0.15-2%[10]. Developed countries contribute 0.15-0.5% to the global incidence of congenital CMV, while developing countries contribute 0.5-1.8%[10].

Amongst women of child-bearing age in developed countries, the prevalence of CMV has been reported to be 40% and 80% in high and low socio-economic population groups, respectively[10]. In developing countries the prevalence in this same group of women has been reported to be between 90 and 100%[10]. A mother with primary CMV infection is more likely to transmit infection to her unborn child at a risk of 40%, compared to the mother with reactivation or recurrent infection with a risk of 3%[10]. In contrast to this transmission risk of CMV infection from mother to child, the incidence of congenital CMV infection has been shown to be directly proportional to the prevalence of previous maternal CMV infection. The existence of numerous different strains of CMV virus in populations with high prevalence of CMV antibodies has been postulated to explain this phenomenon[10, 11].

Perinatal CMV infection typically presents during the period of three weeks to one month after birth[12]. Perinatal CMV infection is commonly transmitted vertically to the

newborn by exposure to CMV-infected maternal genital tract secretions during vaginal delivery. Perinatal acquired CMV infection is excluded by the finding of CMV-negative swabs at birth from the mucosal surfaces in newborn and the vagina or cervix in the mother[12]. The shedding of CMV in the genital tract secretions of pregnant women represents either reactivation of latent virus or reinfection with different CMV strain. The CMV infection is unlikely to be of the primary type as these women are usually found to be seropositive during pregnancy. Viral shedding occurs in 13-40% of these seropositive pregnant women[13]. Perinatal CMV infection acquired by exposure to these CMV-infected genital tract secretions occurs in 5-10% of live-births[14, 15].

Postnatal CMV infection typically presents during the period of one month to twelve months after birth[12]. Most postnatal CMV infections have been shown to be transmitted vertically through CMV-infected breastmilk[13]. Viral shedding in breastmilk occurs in newborns in 13-40% of these seropositive pregnant women[15]. The presence of CMV virolactia, that is the presence of CMV-DNA in breastmilk, can be present in up-to 88% of CMV-seropositive women[16]. These women usually do not excrete virus in other body fluids such as saliva or urine and, are usually asymptomatic. The viral reactivation in seropositive women who are breastfeeding occurs at the level of the mammary glands[13]. Term infants that acquire CMV through breastmilk are usually asymptomatic. In the study reported by Yasuda et al. most breast milk became CMV-DNA positive by 2 weeks after delivery, peaked at 4-6 weeks and this was followed by a nadir in virolactia[16]. In the study looking at very-low-birth-weight (VLBW) preterm infants, Hamprecht et al. reported that the MTCT rate of CMV through breastmilk was 37%. This resulted in symptomatic postnatal infection in 48% of the infants[13]. The Japanese cohort of preterm infants in the Yasuda et al. study revealed a transmission rate of only 10% with none of the infants being symptomatic[16]. The latter study used a freeze-thawing technique (-20°C) which has been shown to reduce the viral titre and

the infectivity of the CMV-infected breastmilk[16]. Less commonly, postnatal CMV-infection in the newborn is acquired horizontally by the transfusion of CMV-infected blood or blood products[17]. The study by Benson et al. reported 12.5% and 25% of neonates became CMV-infected through blood transfusion during simple transfusion and exchange transfusion, respectively[18]. This was during the era when the policy of using blood from CMV-negative donors had not been implemented. After the policy of using blood from CMV-negative donors had been implemented, no neonate became infected with CMV infection through blood transfusion[18]. Kim et al. sought to determine to whether filtering and irradiation blood products could help prevent CMV infection in a population of Korean VLBW infants. In their study they compared VLBW infants transfused with filtered-irradiated blood to VLBW infants transfused with nonfiltered, nonirradiated blood. They reported that the irradiation and filtering of the blood products did not decrease the transfusion-related CMV infection rate[19]. Overall, there are few studies addressing the value of irradiating and/ or using seronegative units to prevent transfusion transmission of CMV infection (TT-CMV) infection in neonates. The failure to prevent TT-CMV using seronegative units is due to the donors being in the window period of infection. Leuko-depleted blood units may transmit CMV if the leuko-reduction filters fail to remove a sufficient fraction of CMV-infected white blood cells (WBC's) either because of mechanical problems or high viral loads[20].

2.2 Virology of CMV

Cytomegalovirus (CMV) is a ubiquitous double-stranded DNA virus first isolated in the mid-20th century from the epithelium of the salivary glands in susceptible infants. The enlarged infected cells with intra-cytoplasmic and intra-nuclear inclusions were originally coined the term 'cytomegalia' leading to the clinical cytomegalic inclusion disease[21]. These inclusions render the classical 'owl's eye' appearance to the infected cells. Human

cytomegalovirus, also known as human herpes virus 5, has humans as the only reservoir. The viral incubation period is approximately 40 days following exposure and being a member of the Herpesviridae family of viruses, CMV has the ability to cause primary infection followed by a life-long latent state with intermittent reactivation[22]. In congenitally infected neonates, viral shedding may persist for months to years, following primary infection[22].

Structurally, the linear double-stranded DNA viral genome is surrounded by an icosahedral capsid, amorphous material (tegument) and lipid envelope. The capsid is composed of three proteins, namely; p155, p34 and p37. The tegument is composed of four phosphoproteins, namely; pp65, pp71, pp28 and pp150. The lipid envelope is composed of three glycoproteins, namely; gB, gH and gL. The genome contains over 200 genes all with the propensity to isomerize into multiple human strains responsible for the clinical reinfections[22, 23]. The virus lacks the enzyme thymidine kinase, which renders it resistant to antiviral agents that utilize this particular enzyme for their action[24].

2.3 Clinical characteristics associated with congenital CMV infection

The typical clinical picture of cytomegalic inclusion disease (CID) is characterized by involvement of the reticuloendothelial and central nervous system (CNS) which may be associated with ocular and auditory damage[8]. Asymptomatic congenital CMV infection occurs in 90% of all CMV-infected neonates at birth, while symptomatic congenital CMV infection occurs in 10% of cases. Fifty percent of all symptomatic neonates present with the classical cytomegalic inclusion disease while the remaining neonates demonstrate mild or atypical signs. The typical clinical manifestations include; low-birth-weight (LBW) either due to intrauterine growth retardation (50%) or prematurity (34%), a purpuric rash (13%), petechiae (76%), jaundice (67%) and hepatosplenomegaly (60%) [8]. The neurological manifestations include; microcephaly (53%), chorioretinitis, seizures (7%) and hypotonia

(27%) and later sensorineural hearing loss. It has been reported that about 30% of symptomatic congenitally infected neonates will develop sensorineural hearing loss[24].

Congenital CMV causes intracranial pathology which include; intracranial calcification, neuronal migrational abnormalities, white matter disease, periventricular cysts, cerebral atrophy, ventriculomegaly, ventricular adhesions and lenticulostriate vasculopathy[25]. Intra-uterine CMV infection before 18-24 weeks of pregnancy can cause migrational disturbances such as lissencephaly and pachygyria in the fetal brain, leading to cerebral malformations. Later in pregnancy (after 26 weeks) when myelination is occurring, white matter lesions and periventricular calcification can occur[25]. Van der Knaap et al. used magnetic resonance imaging (MRI) criteria as a proxy to diagnose congenital CMV infection by correlating MRI findings suggestive of white matter disease with CMV-PCR testing using CMV-DNA on neonatal blood using Guthrie card[26]. The presence of white matter disease in the anterior part of the temporal lobe increased the likelihood of congenital CMV infection being the cause[26].

Term infants with perinatal CMV infection are typically asymptomatic because of maternally transmitted CMV-IgG antibody. However, 15-25% of infected preterm infants may present clinically either with pneumonia or sepsis-like illness[22]. Severe congenital CMV infections leading to hepatic dysfunction, bleeding, disseminated intravascular coagulopathy or secondary bacterial infections, have a mortality rate of 30%[22].

2.4 Laboratory abnormalities associated with congenital CMV infection

The commonly reported laboratory abnormalities in congenital CMV-infected neonates reflect the involvement of the hepatobiliary and reticulendothelial systems. These include; anaemia, thrombocytopaenia, hepatic transaminitis, conjugated hyperbilirubinaemia and an elevated cerebrospinal fluid (CSF) protein[4, 8, 27].

Bopanna et al. in their cohort of congenital CMV-infected infants, defined thrombocytopaenia as: a platelet count <100, 000 cells/ mm^3, an elevated aspartate transaminase (AST) level as: >80 U/ L, conjugated hyperbilirubinaemia as: Direct bilirubin >4 mg/ dL and an elevated CSF protein level as: >120 mg/ dL. They reported thrombocytopaenia, elevated AST, elevated DB and an elevated CSF protein to occur in 77%, 83%, 81% and 46% of patients, respectively[8]. Mussi-Pinhata et al. in their Brazilian cohort of congenital CMV-infected infants defined; thrombocytopaenia as: a platelet count <100, 000 cells/ mm^3, elevated ALT level as: >108 IU/ L, elevated AST as: >130 IU/ L, direct bilirubin level as: >2 mg/ dL and elevated CSF protein level as: >120 mg/ dL and they reported these to occur in 10%, 20%, 5% and 43% of patients, respectively[4]. Ranjit et al. in their Canadian cohort of CMV-infected infants defined thrombocytopaenia as: a platelet count <100, 000 cells/ mm^3, elevated ALT level as: >100 IU/ L, elevated AST as: >100 IU/ L and direct bilirubin level >3 mg/ dL and these occurred in 50%, 48%, 50% and 47% of cases, respectively[27].

Based on the above mentioned studies, thrombocytopaenia (defined as <100 000 cells/ mm^3) occurs in 10-77% of infants with congenital CMV. An elevated ALT or AST concentration (defined as >100 IU) occurs in 20-50% of infants with congenital CMV. The presence of a conjugated hyperbilirubinaemia (defined as >2 mg/ dL) occurs in 5-47% of congenital CMV infected infants. An elevated CSF protein concentration (defined as >120mg/ dL) is present in 43-46% of infants with congenital CMV infection[4, 8, 27].

2.5 Diagnosis of congenital CMV infection

The diagnosis of congenital CMV infection is typically based on serological and virological methods. Demonstration of CMV-IgG antibodies after infection, cannot distinguish between active and past infection. Antibodies of the IgM class are produced

immediately after primary infection and their presence in the newborn before 2-3 weeks of age suggests congenital infection. The detection of CMV-IgM antibodies is neither specific nor sensitive as demonstrated by being either present in only 70% of the neonates with congenital CMV at birth or when present, persist for several months[24, 28].

The gold standard for detection of fetal infection is culturing CMV from urine which has a specificity of 100%[29]. However, because CMV is a slow growing virus, it takes up to six weeks to grow and demonstrate a typical cytopathic effect (CPE) using standard tissue cultures. The other more rapid method of culturing CMV is using the "shell-vial assay", which involves low speed centrifugation for 40-60 minutes at 37°C, leading to amplification of virus in cell cultures using diploid human embryonic fibroblasts. This procedure facilitates the detection of viral antigens produced early in the replication of CMV prior to the development of the characteristic cytopathic effect. The infected cells are detected within 16-48 hours by immunofluorescence using monoclonal antibodies directed to an immediate-early antigen (72kDa) of CMV[30].

The diagnosis of congenital CMV infection is usually based on viral isolation from urine samples collected within the first three weeks of life[22, 24]. In congenitally infected children, viral shedding may persist for years after a primary infection[22]. In these cases the presence of viral proteins in peripheral blood leukocytes (PBL) specifically, pp65 (antigenaemia), indicates active infection[30]. The pp65 antigenaemia test is based on the immunocytochemical detection of a 65kDa lower matrix phosphoprotein in the nuclei of peripheral blood leukocytes, using monoclonal antibodies[30]. The other diagnostic test for CMV is CMV-PCR which can be done using blood, urine and saliva. The process of CMV-DNA detection was first reported in 1994 by Shibata et al. using neonatal dried blood on filter paper[31]. The sensitivity and specificity of CMV-PCR testing on urine for the diagnosis of congenital CMV is 97% and 100% respectively[28]. The CMV-PCR test on serum for the

diagnosis of congenital CMV has been shown to have a sensitivity and specificity of 100%[28]. Boppanna et al. reported on a prospective multicenter screening trial in neonates, which compared real-time CMV-PCR on the liquid-saliva and on dry-saliva to rapid culture of saliva done at birth. The results showed that the real-time PCR assay of both liquid-saliva and dried-saliva samples have excellent sensitivity (>97%) and specificity (99.9%) as compared with the standard saliva rapid culture. The study also demonstrated the shortcoming of the dried-blood-spot PCR assays which identified at most 40% of CMV-infected newborns[32].

2.6 Predictors of poor outcome in neonates with congenital CMV infection

The predictors of adverse neurological outcome in children with symptomatic congenital CMV infection include microcephaly, chorioretinitis and cranial abnormalities detected on computerized tomography (CT) within the first month of life.

Noyola et al. performed a longitudinal cohort study involving neonates with symptomatic congenital CMV infection[33]. The aim of the study was to determine the clinical and radiological findings that would predict an unfavourable neurodevelopmental outcome[33]. The abnormalities found on cranial CT done during the neonatal period included; white matter lucencies, ventriculomegaly, intracranial calcifications, destructive encephalopathy, brain atrophy, and neuronal migration disorders. The presence of these abnormalities in cranial CT, were the most sensitive predictor of mental retardation and motor disability[33]. The presence of microcephaly at birth was the most specific predictor of poor cognitive outcome in newborns with symptomatic congenital CMV infection[34, 35]. Intrauterine growth retardation and petechiae are more likely associated with the development of sensorineural hearing loss[36]. Muruyama et al. performed a retrospective study to assess which fetal manifestations were associated with death or neurodevelopmental impairment. The study revealed that the presence of either abdominal findings such as ascites and hepatosplenomegaly or, abnormal

cerebral findings such as ventriculomegaly, intracranial calcification and microcephaly was more likely associated with a poor outcome in congenital CMV-infected infants[37]. Female infants had a poorer outcome than male infants[37].

The mortality associated with symptomatic congenital infection is less than 5% in the newborn period, and above 10% in the first year of life. The most severely affected infants have a mortality rate of 30%. In most instances death is due to hepatic dysfunction, bleeding, disseminated intravascular coagulation or secondary bacterial infections[34, 35].

CHAPTER 3

3.0 STUDY HYPOTHESIS

Neonates with congenital CMV infection have specific characteristics that distinguish them from those neonates without congenital CMV infection.

3.1 AIMS AND OBJECTIVES

3.1.1 Aims

To determine the characteristics and survival to hospital discharge in neonates with congenital CMV infection admitted to the Neonatal Unit at Chris Hani Baragwanath Academic Hospital, Johannesburg, between January 2004 and December 2008.

3.1.2 Objectives

- To determine the number of patients with congenital CMV infection amongst the infants admitted to the Neonatal Unit at CHBAH.
- To determine the demographic features/ characteristics, clinical characteristics and laboratory findings in neonates with congenital CMV infection.
- To determine the in-hospital mortality rate in neonates with congenital CMV infection
- To compare the demographic features/ characteristics, clinical characteristics laboratory findings and, survival to hospital discharge between neonates with congenital CMV and those who were CMV-uninfected.

3.2 MATERIALS AND METHODS

3.2.1 Study design

This is a descriptive study using a retrospective study design. Hospital clinical records of patients who had specimens sent to the laboratory requesting for urine 'shell-vial' culture and/ or pp65 antigen test, were reviewed.

3.2.2 Study sample

The study sample included all patients that were admitted to Chris Hani Baragwanath Academic Hospital Neonatal Unit and had been investigated for suspected congenital CMV infection (had urine shell-vial culture and/ or pp65 results) during the period January 2004 to December 2008. The reason for using this study period was two-fold. Firstly, the urine shell-vial culture and pp65 antigenaemia tests were both available from January 2004. Secondly, I felt that a five year period will provide one with enough numbers to achieve the study objectives.

3.2.3 Study procedures

The names, hospital numbers of patients, who had specimens processed and tested by the National Institute of Communicable Diseases (NICD) for pp65 antigen and/ or shell-vial culture, were requested from NICD. Hospital records of these patients were retrieved and reviewed for the information listed below under data collection. In order to determine the factors associated with congenital CMV infection, neonates with positive CMV results (shell-vial urine culture and/ or pp65) were compared with those who tested CMV-negative.

3.2.4 Data collection

The information that was collected included; maternal characteristics, neonatal demographics, neonatal clinical and laboratory data and neonatal outcome at/ or prior to hospital discharge.

The maternal characteristics that were collected included; maternal age, maternal parity, mode of delivery, maternal rapid plasma reagin (RPR) and maternal HIV status. The neonatal demographics included; chronological age (in days) at testing, gender, gestational age (in weeks) at birth, birth weight (in grams), length (in centimeters) at birth and head circumference (centimeters) at birth. The clinical and laboratory findings that were recorded in the neonates' hospital charts at the time of CMV testing were collected for analysis.

3.2.5 Definitions

- Congenital CMV infection was defined as a positive urine 'shell-vial' culture and/ or positive pp65 antigen test, within the first three weeks of life.
- The incidence of congenital CMV infection was expressed as all CMV-infected neonates within the first three weeks of life at testing, as both a subset of the total number of admissions and, as a subset of the total number of live-births, during the 5 year period.
- The clinical signs were extracted from what was recorded in the charts and were therefore not predefined.
- Hepato-/splenomegaly implied the presence of either hepatosplenomegaly or hepatomegaly or splenomegaly.
- The outcomes in neonates with congenital CMV infection were described as either survival or death prior to hospital discharge.
- Leukopenia was defined as total white cell count $<5.0 \times 10^9/ L$[38].

- Persistent thrombocytopaenia was defined as a platelet count <150 $x10^9$/ L reported on two consecutive blood tests[38].
- An elevated alanine aminotransferase (ALT) was defined as ALT >25 U/ L[38].
- An elevated aspartate aminotransferase (AST) was defined as AST >140 U/ L[38].
- An elevated gamma-glutamyltransferase (γGT) was defined as γGT >132 U/ L[38].

3.2.6 Data capturing and analysis

- Data was captured onto *Microsoft® Office Excel®* 2007 from predesigned data collection forms (see APPENDIX A) and statistical analysis was done using *Statistica®* 9.1.210.0 and *Stata/SE®* 12.0 for Windows (64-bit x86-64).
- In the case of a normal distribution, the means and standard deviations were used. In the case of a non-normal distribution, medians and interquartile ranges (IQR) were used.
- The Student t-test, independent by groups, with its non-parametric counterpart, the Mann-Whitney U test, was used to compare continuous numerical variables. A p-value <0.05 represented a statistically significant difference.
- The Pearson Chi-square test was used to compare two categorical variables. For categorical variables with expected frequencies ≤5 (from the cross tabulations), the two-tailed Fischer exact test was used. A p-value <0.05 represented a statistically significant difference.

3.2.7 ETHICS

3.2.7.1 Patient confidentiality

A coded number was allocated to each study patient to maintain confidentiality. The patients' identifiable factors were recorded and kept separately from the primary data for analysis.

3.2.7.2 Study approval

The approval to perform this study was granted by the University of the Witwatersrand Human Research Ethics Committee (ethics clearance number: M080102; see APPENDIX B) and the hospital protocol review committee.

CHAPTER 4

4.0 RESULTS

4.1 Descriptive statistics of neonates with congenital CMV infection

During the 5 year study period, from January 2004 to December 2008, a total of one hundred and seventy seven (N =177) neonates were tested for a suspected CMV infection within the first twenty one days of life. From the one hundred and seventy seven (N =177) neonates that were suspected to have CMV infection within the first twenty one days of life, twenty eight were confirmed CMV cases (16%). The hospital records were retrieved for 86% (24/28) and 42% (62/149) of the patients who were confirmed to be congenital CMV-positive and congenital CMV-negative, respectively (Figure 1).

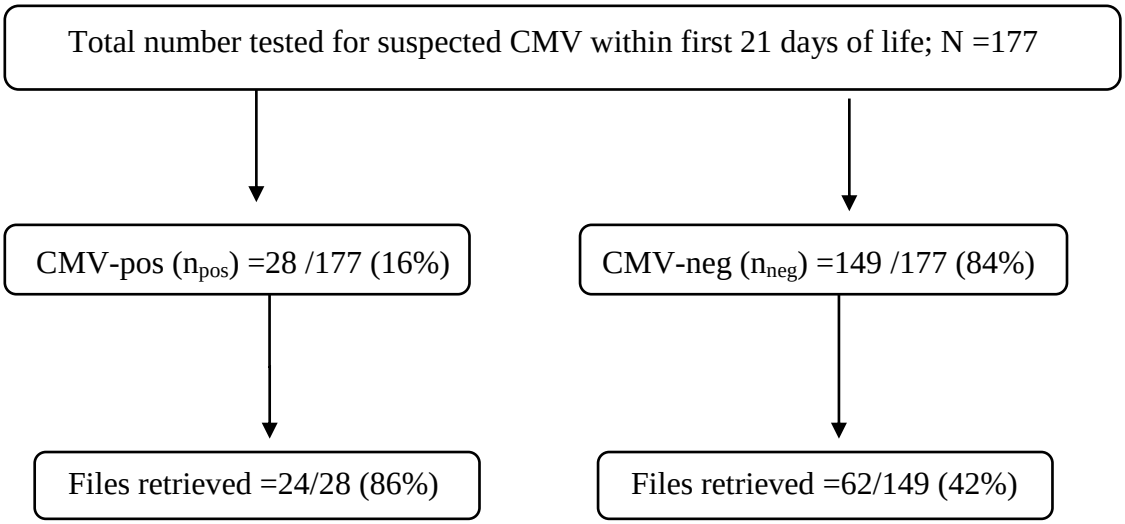

Figure 4.1 Number of patients tested for CMV infection and number of hospital records retrieved.

4.1.1 Indications for testing for CMV in neonates with congenital CMV

Table 4.1 shows the indications for testing patients for CMV in infants who were diagnosed with congenital CMV. The commonest reason for suspecting the presence of congenital CMV infection was the presence of persistent thrombocytopaenia (62%). This was followed by the clinical presence of hepato-/splenomegaly (38%).

Table 4.1 Indications for testing in neonates with congenital CMV

Indications; n (%)	CMV-negative n =62	CMV-positive n =24
1) Persistent thrombocytopaenia	40 (65)	15 (62)
2) Hepato-/splenomegaly	10 (16)	9 (38)
3) Persistent jaundice	5 (8)	0 (0)
4) Chronic lung disease	2 (3)	0 (0)
5) Hydrops fetalis	2 (3)	0 (0)
6) Leukopenia	1 (2)	0 (0)
7) Unknown	2 (3)	0 (0)

4.1.2 Incidence of congenital CMV infection amongst neonates admitted at CHBAH

During the 5 year study period, the numbers of neonates admitted to the Unit were 19215 and the numbers of live-births were 106159. Based on this data, the incidence of congenital CMV infection in the Neonatal Unit was calculated at 1.50 per 1000 admissions or 0.26 per 1000 live-births or 0.026%. Table 4.2 and Table 4.3 show the incidence of congenital CMV infection expressed as either per 1000 admissions or per 1000 live-births, respectively.

Table 4.2 Incidence of congenital CMV infection (per 1000 admissions)

Year	Congenital CMV-pos	Total suspected	Admissions	Incidence / 1000 admissions	Incidence Percent (%)
2004	11	47	3843	2.86	0.286
2005	3	23	3543	0.85	0.085
2006	6	22	3671	1.63	0.163
2007	5	57	3974	1.26	0.126
2008	3	28	4184	0.72	0.072
Total	28	177	19215	1.50	0.150

Table 4.3 Incidence of congenital CMV infection (per 1000 live-births)

Year	Congenital CMV-pos	Total suspected	Live-births	Incidence / 1000 Live-births	Incidence Percent (%)
2004	11	47	18565	0.59	0.059
2005	3	23	19767	0.15	0.015
2006	6	22	22060	0.27	0.027
2007	5	57	22918	0.22	0.022
2008	3	28	22849	0.13	0.013
Total	28	177	106159	0.26	0.026

4.1.3 Maternal characteristics of neonates with congenital CMV

Table 4.4 shows the maternal characteristics for those neonates with congenital CMV infection. The categories are representative of teenage (13-19 years), child-bearing age (20 – 34 years) and advanced maternal age (≥35 years) pregnancies. The majority of pregnancies were mothers of child-bearing age (71%) with less than three pregnancies (50%). Just over a third of patients were born through caesarian-section. One patient was born to a mother who had a positive RPR. The majority of neonates with congenital CMV infection (19/24; 79%) were born to HIV-positive mothers.

Table 4.4 Maternal characteristics of neonates with congenital CMV

Characteristics (n =24)		n (%)
Age (years)		
	13 – 19	2 (8)
	20 – 34	17 (70)
	≥35	5 (20)
Parity (no.)		
	0	10 (42)
	1 – 2	12 (50)
	≥3	2 (8)
Mode of delivery		
	vaginal	15 (62)
	caesarean	9 (38)
RPR		
	positive	1 (4)
	negative	23 (96)
HIV		
	positive	19 (79)
	negative	5 (21)

4.1.4 Characteristics of neonates diagnosed with congenital CMV

Table 4.5 shows the characteristics of neonates with congenital CMV infection. Among the neonates with congenital CMV infection 92% were low birth weight with 83% born preterm and 29% born SGA.

Microcephaly was present in 17% of congenital CMV-neonates. Screening cranial ultrasounds for intracranial calcifications were performed on 19 (79%) of the congenital CMV-infected neonates. All the patients with microcephaly had normal cranial ultrasounds. Intra-cranial calcification was detected in 1 (4%) of the congenital CMV-infected neonates in the absence of microcephaly.

Table 4.5 Characteristics of neonates diagnosed with congenital CMV

Characteristics (n =24)		n (%)
Age at CMV testing (days)		
	<7	11 (46)
	7 – 14	10 (41)
	15 – 21	3 (13)
Gender		
	male	14 (58)
	female	10 (42)
Gestational age (weeks)		
	<30	2 (8)
	30 – 34	7 (29)
	35 – 37	12 (50)
	>37	3 (13)
Birth-weight (g)		
	<1500	9 (38)
	1500 – 2500	13 (54)
	>2500	2 (8)
Birth-head circumference		
	microcephaly	4 (17)
	macrocephaly	0 (0)
	normocephaly	12 (50)
	unknown	8 (33)
Growth		
	small-gestational-age (SGA)	7 (29)
	appropriate-for-gestational-age (AGA)	15 (63)
	large-for-gestational-age (LGA)	0 (0)
	unknown	2 (8)

4.1.5 Haematological and biochemical indices in neonates with congenital CMV

Table 4.6 shows the laboratory parameters of those neonates with congenital CMV infection, at the time of CMV testing. The majority of patients had thrombocytopaenia (n =23/24; 96%). They also presented with direct hyperbilirubinaemia (n =10/24; 42%), and/or abnormal alanine aminotransferase concentration (n =8/24; 33%) and/or abnormal gamma-glutamyl transferase concentration (n =12/24; 50%).

Table 4.6 Haematological and biochemical indices in neonates with congenital CMV

Laboratory parameters (n =24)		n (%)
White cell count ($x10^9$/ L)		
	<5.0	2 (8)
	5.0 – 25.0	22 (92)
	>25.0	0 (0)
Hb (g/ dL)		
	<14.0	10 (42)
	14.0 – 18.0	11 (46)
	>18.0	3 (13)
Platelets ($x10^9$/ L)		
	<50	12 (50)
	50 – 100	10 (42)
	101 – 150	1 (4)
	>150	1 (4)
C-reactive protein (mg/ L)		
	<10.00	12 (50)
	10.00 – 20.00	2 (8)
	>20.00	3 (13)
	unknown	7 (29)
Direct bilirubinaemia >20% Total serum bilirubin (µmol/ L)		
	yes	10 (42)
	no	7 (29)
	unknown	7 (29)
ALT (U/ L)		
	normal	10 (42)
	abnormal	8 (33)
	unknown	6 (25)
γ-GT (U/ L)		
	normal	6 (25)
	abnormal	12 (50)
	unknown	6 (25)

4.1.6 Neonatal HIV status and mortality in congenital CMV subgroup

Among the 24 congenital CMV-infected patients, 19 (79%) were born to HIV-positive mothers and 13 (68%) of these HIV-exposed infants were HIV-infected (positive HIV-PCR at age 6 weeks). The crude mortality rate was 42%.

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4.2 Comparative analysis for neonates with positive CMV tests to those who were tested for CMV and were negative in the first three weeks of life

4.2.1 Comparison of maternal characteristics between CMV-negative and CMV-positive neonates

There were no differences in maternal age, number of pregnancies, mode of delivery and maternal RPR results between the CMV-negative and CMV-positive neonates. The neonates who were CMV-positive were more likely to be born to mothers who were HIV-positive (HIV-exposed) (n =19/24 (79%) vs n =27/62 (44%); p =0.003). Table 4.7 shows the comparison of maternal characteristics between CMV-negative and CMV-positive neonates.

Table 4.7 Comparison of maternal characteristics between CMV-negative and CMV-positive neonates

	CMV-negative n_{neg} =62	CMV-positive n_{pos} =24	P-value
Age (years)			0.26*
median	26.0	30.0	
IQR	12.0	11.0	
unknown; n	1	1	
Parity (n)			0.60*
median	1.0	1.0	
IQR	1.0	1.6	
unknown; n	1	0	
Mode of delivery			0.57**
Vaginal; n (%)	34 (55)	15 (62)	
Caesarean; n (%)	27 (44)	9 (38)	
unknown; n (%)	1 (1)	0 (0)	
RPR			1.00***
positive; n (%)	5 (8)	1 (4)	
negative; n (%)	57 (92)	23 (96)	
HIV			0.003**
positive; n (%)	27 (44)	19 (79)	
negative; n (%)	35 (56)	5 (21)	

Asterisks denote tests used to make comparisons: * - Mann-Whitney U test**;**

**** -** Pearson Chi-square**; *** -** Fisher exact 2-tailed

4.2.2 Comparison of clinical indications for testing for CMV and characteristics of CMV-negative and CMV-positive neonates

Table 4.8 shows the comparison of clinical indications for congenital CMV testing and characteristics between CMV-negative and CMV-positive neonates. The clinical presence of hepato-/splenomegaly was more likely the reason to suspect congenital CMV in those neonates with subsequent confirmed congenital CMV infection ($p = 0.03$). There were no significant differences in the other indications listed in table 4.10 between the CMV-negative and the CMV-positive neonates ($p > 0.05$).

Table 4.8 Comparison of clinical indications for congenital CMV testing and characteristics between CMV-negative and CMV-positive neonates

	CMV-negative n_{neg} =62	CMV-positive n_{pos} =24	P-value
Indications for CMV testing; n (%)			
Hepato-/splenomegaly	10 (16)	9 (38)	0.03*****
Thrombocytopaenia	40 (65)	15 (62)	0.71*****
Persistent jaundice	5 (8)	0 (0)	0.69******
Chronic lung disease	2 (3)	0 (0)	0.37******
Hydrops fetalis	2 (3)	0 (0)	0.37******
Leukopenia	1 (1)	0 (0)	0.37******
Unknown	2 (3)	0 (0)	0.37******
Age at CMV testing (days)			0.22*******
median	9.0	9.5	
IQR	7.2	8.0	
Gender			0.91*
male; n (%)	37 (60)	14 (58)	
female; n (%)	25 (40)	10 (42)	
Gestational age (weeks)			0.74*******
median	34.0	34.0	
IQR	8.0	3.0	
Birth-weight (g)			0.70*******
median	1572.5	1700.0	
IQR	981.3	1007.5	
Head circumference (cm)			0.84*******
mean	30.26	29.89	
standard deviation	±3.23	±3.01	
unknown; n	12	6	

Asterisks denote type of test used to make comparisons: * - Pearson Chi-square;

**** -** Fisher exact 2-tailed; ***** -** Mann-Whitney U test

4.2.3 Comparison of neonatal laboratory parameters other than HIV-related tests

Table 4.9 shows the comparisons of the laboratory parameters between CMV-negative and CMV-positive neonates. The neonates in the congenital CMV-positive group were more likely to have lower platelet counts (median =71 $x10^9$/L vs median =49 $x10^9$/L; p =0.003) when compared to the neonates in the congenital CMV-negative group. There were no significant differences for the other neonatal laboratory parameters.

Table 4.9 Comparison of haematological and biochemical indices between CMV-negative and CMV-positive neonates

	CMV-negative n_{neg} =62	CMV-positive n_{pos} =24	P-value
White cell count (x10^9/ L)			0.32*****
median	8.3	7.17	
IQR	6.4	3.80	
unknown; n	3	0	
Hb (g/ dL)			0.65*****
mean	14.55	14.13	
standard deviation	±3.28	±2.68	
unknown; n	3	0	
Platelets (x10^9/ L)			0.003*****
median	71.0	48.0	
IQR	58.0	35.2	
unknown; n	3	1	
C-reactive protein (mg/ L)			0.81*****
median	6.5	5.00	
IQR	9.4	9.97	
unknown; n	9	7	
Direct serum bilirubin (µmol/ L)			0.39†
median	24.5	14.0	
IQR	63.7	117.0	
unknown; n	23	7	
ALT (U/ L)			0.32†
median	21.5	30.0	
IQR	80.0	54.3	
unknown; n	23	6	
γGT (U/ L)			0.18†
median	143.0	167.5	
IQR	173.3	152.8	
unknown; n	22	6	

Asterisks denote type of test used to make comparisons: * - Mann-Whitney U test**;**

† **-** Student-t test, independent, by groups

4.2.4 Comparison of HIV status based on HIV-PCR performed at age six weeks between CMV-negative and CMV-positive neonates

Table 4.10 shows the comparison of the CMV-negative and CMV-positive neonates according to their HIV status. The neonates in the congenital CMV-positive group were more likely to be HIV-infected (n =13/19 (68%) vs n =6/19 (32%); p =0.02) when compared to the neonates in the congenital CMV-negative group.

Table 4.10 Comparison of HIV status based on HIV-PCR performed at age six weeks between CMV-negative and CMV-positive neonates

n =46	CMV-negative, HIV-exposed n_{neg} =27	CMV-positive, HIV-exposed n_{pos} =19	P-value
HIV-PCR			0.019*****
positive; n (%)	9 (33)	13 (68)	
negative; n (%)	18 (67)	6 (32)	

Asterisks denote type of test used: * - Pearson Chi-square

4.2.5 Outcome at hospital discharge

- In the 62 CMV-negative infants, 3 HIV-unexposed neonates did not have a recorded outcome. Due to a prolonged hospital stay, more than one hospital bed-letter was utilised. Hence, the hospital bed-letters where the outcomes would have been recorded were not found.

4.2.5.1 Comparison of outcomes between CMV-negative and CMV-positive neonates

The neonates with congenital CMV were more likely to die before discharge (p=0.01) (Table 4.11)

Table 4.11 Comparison of outcomes between CMV-negative and CMV-positive neonates

	CMV-negative n_{neg} =62	CMV-positive n_{pos} =24	P-value
Outcome; n (%)			0.01*****
Death before discharge	11 (18)	10 (42)	
Survival up-to discharge	48 (77)	14 (58)	
unknown	3 (5)	0 (0)	

Asteriks denote type of test used: * - Pearson Chi-square

4.2.5.2 Comparison of mortality between congenital CMV-negative and congenital CMV-positive neonates according to their HIV status

The neonates with both congenital CMV (congenital CMV-positive) and HIV infection (HIV-PCR positive) were more likely to die before hospital discharge (n =8/13 (62%) vs n =1/9 (11%); p =0.02) compared to those who were CMV-positive and HIV-negative. Table 4.12 shows the comparison in mortality to hospital discharge between the congenital CMV-uninfected, and congenital CMV-infected neonates according to their HIV status.

Table 4.12 Comparison of mortality between congenital CMV-negative and congenital CMV-positive neonates according to their HIV status

Death before discharge (n =46)	Congenital CMV-negative, HIV exposed n=27	Congenital CMV-positive, HIV exposed n=19	P-value
Death before discharge			0.021*****
HIV-PCR pos	1 (11)	8 (62)	
HIV-PCR neg	3 (17)	1 (17)	

Asterisks denote type of test used: * - Fisher exact 2-tailed

CHAPTER 5

5.1 DISCUSSION

In neonates with congenital CMV signs and symptoms are present in only 10% of cases[22], with up-to 90% of these symptomatic newborns developing neurosensory and/ or neuromotor impairment[39]. In this single centre study, the hospital files of neonates with suspected congenital CMV infection being based on specific clinical, haematological and biochemical parameters were retrospectively reviewed. The maternal and neonatal characteristics, neonatal clinical findings, laboratory findings and mortality associated with congenital CMV infection are reported. Comparisons were made to those neonates that were deemed congenital CMV-negative.

During the 5 year study period, the incidence of symptomatic congenital CMV was 0.026%. These were neonates suspected to have CMV infection within the first three weeks of life, based on clinical and/ or haematological and/ or biochemical indices. The presence of thrombocytopaenia was the most common reason for suspecting congenital CMV infection. The findings of significance included; the tendency for the symptomatic congenital CMV-infected neonates to be both HIV-exposed and/ or HIV-infected and hepato-/splenomegaly being the dominant finding on clinical examination. Neonates who were CMV-infected had much lower platelet counts compared to the CMV-uninfected. Mortality before hospital discharge was higher in the congenital CMV-infected and HIV co-infected neonate.

The incidence rate in this study was 0.026%, which is much lower than that reported by Schoub et al. in a study that tested 2250 asymptomatic neonates[40]. Schoub et al. reported an incidence of 0.13% (95% CI: 0.5 – 0.39) [41]. The difference in incidence in this study and the study by Schoub et al. is three-fold. Firstly, Schoub et al. used serology to diagnose CMV infection whereas in this study we used a shell-vial culture and/ or pp65. CMV serology tends to give false positive and false negative results. Secondly, Schoub et al. tested all babies born

to mothers with reactivated CMV infection during their pregnancy, while in this study only the symptomatic newborns were tested irrespective of time of acquisition of maternal CMV infection. Therefore this study could have underestimated the incidence of congenital CMV. Our study reported on symptomatic congenital CMV-infected newborns' as reflected in Table 4.3 above, where the incidence of congenital CMV was 0.026%. This is similar to that of the incidence rate of 0.07% (95% CI: 0.03 – 0.56) as reported by Kennesen et al. from twenty-seven study groups[9]

The SGA rate of 29% in this study for neonates with congenital CMV is identical to the Australian cohort reported by Munroe et al.[41] However, studies by Bopanna et al.[8] and Ranjit et al.[27] report much higher SGA rates in their congenital CMV-infected newborn cohorts, at 50% and 43%, respectively. These might be related to acquiring infection either within the first or subsequent trimesters of pregnancy.

In this retrospective review, the most common indications for suspecting CMV in neonates within the first three weeks of life were; either the clinical presence of hepato-/splenomegaly or evidence of persistent thrombocytopaenia. The comparative analysis confirmed a significant association between the presence of severe thrombocytopaenia and/ or hepatosplenomegaly, and congenital CMV infection. In the study by Bopanna et al. thrombocytopaenia and hepatosplenomegaly was present in 76% and 60% of their congenital CMV-infected cohort, respectively[8]. The Australian cohort of Munroe et al. reported thrombocytopaenia and hepato-/splenomegaly to be present in up-to 80% of congenital CMV infected neonates[41]. In the American cohort of Ranjit et al. thrombocytopaenia and hepato-/splenomegaly was reported as 40-45% and 90%, respectively[27]. The presence of thrombocytopaenia and hepto-/splenomegaly is a common finding in other reports[8, 27, 41].

The presence of microcephaly in the patients with congenital CMV infection in this study was present in 17% of the cases which is similar to the 22% reported in Australian

cohort by Munro et al[27]. In their study, utilising real-time-PCR, Al-Hareth et al. found no link between the presence of low-birth-weight and microcephaly and, congenital CMV infection in their cohort of neonates that were compared to equivalent number of controls. However, their numbers of CMV-infected neonates were small (n =3) compared to our study (n =24) [42]. The Bopanna et al. study reported a higher rate of microcephaly at 53%, which probably implied earlier (first trimester) intra-uterine acquisition of the virus[8]. The presence of microcephaly implies foetal acquisition of infection within the first trimester of pregnancy.

The presence of a conjugated hyperbilirubinaemia with either a transaminitis and/ or an elevated gamma-glutamyl transferase concentration tended to dominate the liver function abnormalities (40-50%) of congenital CMV-infected neonates in this study. Bopanna et al. reported higher rates of transaminitis and direct hyperbilirubinaemia in excess of 80%, in their cohort[8]. Incidentally, this study found the presence of direct-hyperbilirubinaemia in only 42% of congenital CMV-infected subjects. More importantly, the presence of direct-hyperbilirubinaemia as an indication for CMV testing in this study was in less than 10% of neonates with suspected congenital CMV infection.

These clinical and laboratory findings likely represents the spectrum of disease manifestations based on different study populations due to disease severity and/ or geographic location and/ or available resources for testing and therapy.

During the study period the prevalence of HIV infection in mothers attending antenatal clinic in the Gauteng province of South Africa was 30.4%[43]. Importantly, this record review was done during the era when the standard-of-care for PMTCT of HIV, encompassed an intrapartum single-dose nevirapine (NVP) to the HIV-infected mother followed by a single oral dose of NVP syrup to the HIV-exposed neonate at birth. Subsequent testing of the HIV-exposed newborns for the presence of HIV infection was performed at the chronological age of six weeks. In the study by Gray et al. neonates were randomized within 24 hours of

delivery to receive either a single oral dose of NVP or Zidovudine (ZDV). The authors reported a transmission rate of 11.9% in the NVP arm of the study at age 6 weeks[44]. This is lower than the transmission rate in our study of 54% in the congenital CMV-infected group of neonates. Where the Gray et al. study tested neonates for the presence of HIV infection with the HIV-PCR test before the age of 6 weeks, this study performed the HIV-PCR test only at 6 weeks of age. This may have missed some of the patients that were HIV-infected prior to the age of six weeks[44].

The presence of congenital CMV infection has been shown to increase the likelihood of MTCT of HIV infection in the Kamduang et al. study[6]. Doyle et al. reported on a cohort of HIV-exposed neonates that demonstrated a higher incidence of congenital CMV infection in neonates with HIV infection (50% versus 3.5%) [2]. Guibert et al. reported on a multicentre cohort of HIV-exposed neonates who showed a higher prevalence of in-utero HIV transmission in congenital CMV infected neonates compared to those neonates without congenital CMV infection (67% v/s 42%; $p < 0.001$) [1]. However, all three studies did not include a control population to assess the transmission of in-utero CMV infection in neonates born to HIV-uninfected mothers. Also, there is no comment on the association of congenital CMV infection in HIV-exposed neonates who are subsequently deemed HIV-negative at six weeks of age[1, 2, 6].

Slyker et al. reported on a cohort of fifty one Kenyan HIV-exposed infants that were ultimately either HIV-exposed, uninfected or HIV-exposed, infected with a control group that was HIV-unexposed and therefore HIV-uninfected (n =13). The authors reported congenital CMV in 29% and 2.7% of HIV-exposed, infected and HIV-exposed, uninfected neonates, respectively. All neonates were congenital CMV-negative in the control group[45]. Their results alluded to the fact that, the risk of acquiring congenital CMV in HIV-exposed, uninfected neonates were higher than in HIV-unexposed neonates. Duryea et al. echoed these findings

when they examined a cohort of HIV-exposed neonates. They reported 7% of neonates with congenital CMV as being HIV-exposed but HIV-uninfected with no neonates with congenital CMV in the HIV-infected group. However, the neonates with HIV infection (1%) comprised a small number in relation to the HIV-uninfected (99%) neonates[46].

The Australian study by Munro et al. reported a mortality rate of 1.6% in their cohort which translated to one patient who died from CMV-related complications[41]. Bopanna et al. reported a mortality rate of <5% in the symptomatic neonates[8]. Ranjit et al. reported a mortality rate of 7% in their cohort, with significant risk of adverse outcomes if there was abnormal BAER (OR 8.7), head ultrasound (OR 8.5) or brain CT scan (OR 21.0) at presentation[27]. The presence of female gender, abnormal abdominal or cerebral findings was reported by Maruyama et al. as predictors of adverse outcome[37]. Bristow et al. assessed congenital CMV-associated mortality rates in the United States for the period 1990-2009. The authors reported that 41% of neonates with congenital CMV died within the first month of life with specific racial/ ethnic disparities[47]. From the United Kingdom and Ireland, Townsend et al. reported a mortality rate of 10.5% in congenital CMV-infected neonates[48].

The current study showed the outcome of mortality before hospital discharge, to be significantly higher in the congenital CMV-infected subgroup of neonates (42% versus 16%; p =0.01). This difference in mortality rate between our study and other studies is that 54% of our patients were HIV-infected and 38% were VLBW. These HIV-infected infants could have been infected in-utero. Infants infected in-utero with HIV had been reported to have a high mortality rate[49].

Based on this study findings, neonates with hepato-spleno/megaly and/ or persistent thrombocytopaenia from birth and are born to HIV positive mothers should be tested for CMV and HIV (PCR). The testing for HIV (PCR) in this scenario should not wait for 6 weeks which is the current recommendation for all HIV-exposed infants.

5.1.1 Strengths and Limitations

The number of newborns' with symptomatic congenital CMV infection from a single centre is one of the main strengths of this study. Also, comparisons were made with neonates that actually tested negative for the presence of congenital CMV infection. The comparison was not made with normal patients where congenital CMV infection was not suspected.

The main limitation of this study is that it is retrospective. The inability to retrieve all the patient records for the congenital CMV-negative neonates led to the major discrepancy in files procured between CMV positive and negative neonates. The indications for performing CMV testing in these patents were not always specific and detailed in the patient records. The HIV-exposed neonates were only tested for the presence of HIV infection at six weeks of life. This may have resulted in an underestimate of HIV transmission and positivity status as HIV may have been acquired much earlier than six weeks of life. The clinical information in the patient hospital files was not transferred and stored to an interactive database where this information could be easily retrieved. The filing was done manually where the files are arranged according to the patients' date of birth in individual file-boxes. The patient files were also being utilised by other study groups to retrieve information. The handling of these patient files by more than one individual leads to files being lost, damaged or misplaced. Also, the storage of files is done at a facility not within the Neonatal complex. Hence, some of the CMV-negative files could either not be retrieved or incompletely retrieved.

CHAPTER 6

6.1 CONCLUSION

What we already know and what this study reinforces is that the incidence of symptomatic congenital CMV infection is higher in HIV-infected neonates. Thrombocytopaenia and/ or hepato-/splenomegaly are common presentation scenarios at birth in neonates with congenital CMV infection.

What this study adds, is that the blood investigations in HIV-exposed neonates with hepato-spleno/megaly and/ or thrombocytopaenia should include an HIV-PCR soon after birth, in addition to testing for CMV. These neonates with congenital CMV and HIV co-infection are less likely to survive to hospital discharge.

- The high mortality rate in patients with HIV and CMV co-infection was noted before the early use of HAART in neonates, therefore it will be important to repeat the similar study to assess mortality in this group after early use of HAART.

6.1.1 Recommendation

- In resource-limited settings, HIV-exposed neonates presenting with hepatosplenomegaly and/ or persistent thrombocytopaenia at birth, warrant early investigation for both CMV and HIV infection as these tend to occur concurrently.

CHAPTER 7

7.0 REFERENCES

1. Guibert G, Warszawski J, Le Chenadec J, Blanche S, Benmebarek Y, Mandelbrot L et al. Decreased Risk of Congenital Cytomegalovirus Infection in Children Born to HIV-1–Infected Mothers in the Era of Highly Active Antiretroviral Therapy. *Clin Infect Dis* 2009;48:1516 – 25.
2. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. *Pediatr Infect Dis J* 1996;15:1102 – 6.
3. Adhikari M, Kauchali S, Moodley S. Clinical Profile and Morbidity Pattern of Infants Born to HIV-Infected Mothers in urban South Africa. *Indian Pediatr* 2006;43:804 – 8.
4. Preece PM, Tookey P, Ades A, Peckham CS. Congenital cytomegalovirus infection: predisposing maternal factors. *J. Epidemiol Community Health* 1986;40:205 – 9.
5. Mussi-Pinhata MM, Yamamoto AY, Brito RMM, Isaac MDL, Oliveira PFDC, Suresh Boppana S et al. Birth Prevalence and Natural History of Congenital Cytomegalovirus Infection in a Highly Seroimmune Population. *Clin Infect Dis* 2009;49:522 – 8.
6. Khamduang W, Jourdain G, Sirirungsi W, Layangool P, Kanjanavanit S, Krittigamas P et al. The Interrelated Transmission of HIV-1 and Cytomegalovirus During Gestation and Delivery in the Offspring of HIV-Infected Mothers. *J Acquir Immune Defic Syndr Hum Retrovirol* 2011;58:188 – 92.
7. Rice BD, Bätzing-Feigenbaum J, Hosegood V, Tanser F, Hill C, Barnighausen T et al. Population and antenatal-based HIV prevalence estimates in a high contracepting female population in rural South Africa. *BMC Public Health* 2007;18(7):160.

8. Boppana S, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *Pediatr Infect Dis J* 1992;11:93 – 9.
9. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol* 2007;17:253 – 57.
10. Gaytant MA, Steegers EAP, Semmekrot BA, Merkus HMMW, Galama JMD. Congenital Cytomegalovirus Infection: Review of the Epidemiology and Outcome. *Obstet Gynecol Surv* 2002;57(4):245 – 56.
11. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *N Engl J Med* 2001;344:1366 – 71.
12. Trincado DE. Rawlinson RD. Congenital and perinatal infections with cytomegalovirus. *J Paediatr Child Health* 2001;37:187 – 92.
13. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. *Lancet* 2001;357:513 – 18.
14. Shen CY, Chang SF, Yen MS, Ng HT, Huang ES, Wu CW. Cytomegalovirus Excretion in Pregnant and Nonpregnant Women. *J Clin Microbiol* 1993;31(6):1635 – 36.
15. Montgomery R, Youngblood L, Medearis DN Jr. Recovery of cytomegalovirus from the cervix in pregnancy. *Pediatrics* 1972;49(4):524 – 31.
16. Yasuda A, Kimura H, Hayakawa M, Ohshiro M, Kato Y, Matsuura O, Suzuki C, Morishima T. Evaluation of Cytomegalovirus Infections Transmitted via Breast Milk in Preterm Infants With a Real-Time Polymerase Chain Reaction Assay. *Pediatrics* 2003;111:1333 – 36.

17. Reynolds DW, Stagno S, Hosty TS, Tiller M, Alford CA Jr. Maternal cytomegalovirus excretion and perinatal infection. *N Engl J Med* 1973;289(1):1 – 5.
18. Benson JWT, Bodden SJ, Tobin JOH. Cytomegalovirus and blood transfusion in neonates. *Arch Dis Child* 1979;54:538 – 41.
19. Kim ARE, Lee YK, Kim KA, Chu YK, Baik BY, Kim ES et al. Transfusion-related Cytomegalovirus Infection Among Very Low Birth Weight Infants in an Endemic Area. *J Korean Med Sci* 2006;21:5 – 10.
20. Josephson CD, Castillejo MI, Caliendo AM, Waller EK, Zimring J, Easley KA et al. Prevention of Transfusion-Transmitted Cytomegalovirus in Low–Birth Weight Infants (≤1500 g) Using Cytomegalovirus-Seronegative and Leukoreduced Transfusions. *Transfus Med Rev* 2011;25(2):125 – 32.
21. Dudgeon JA. Cytomegalovirus Infection. *Arch Dis Child* 1971;46:581 – 83.
22. Malm G, Engman ML. Congenital cytomegalovirus infections. *Semin Fetal Neonat Med* 2007;12:154 – 59.
23. Stagno S. Cytomegalovirus. In: Remington JS, Klein JO, eds. *Infectious Diseases of the Fetus and Newborn Infant*, 4th Ed. Philadelphia: WB Saunders, 1995, pp 312 – 53.
24. Syggelou A, Lacovidou N, Kloudas S, Christoni Z, Papaevangelou V. Congenital cytomegalovirus infection. *Ann N Y Acad Sci* 2010;1205:144 – 47.
25. Fink KR, Thapa MM, Ishak GE, Pruthi S. Neuroimaging of Pediatric Central Nervous System Cytomegalovirus Infection. *RadioGraphics* 2010;30:1779 – 96.
26. Van der Knaap MS, Vermeulen G, Barkhof F, Hart AAM, Loeber JG, Weel JFL. Pattern of white matter abnormalities at MR imaging: use of polymerase chain reaction testing of Guthrie cards to link pattern with congenital cytomegalovirus infection. *Radiology* 2004;230(2):529 – 36.

27. Ranjit I, Kelly K, Kelly EN, Ford-Jones EL. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus (SCCMV) infection. *Eur J Pediatr* 2006;165:773 – 78.
28. Nelson CT, Istas AS, Wilkerson MK, Demmler GJ. PCR detection of Cytomegalovirus DNA in Serum as a Diagnostic Test for Congenital Cytomegalovirus Infection. *J Clin Microbiol* 1995;33(12):3317 – 18.
29. de Vries JJC, van der Eijk AA, Wolthers KC, Rusman LG, Pas SD et al. Real-time PCR versus viral culture on urine as a gold standard for the diagnosis of congenital cytomegalovirus infection. *J Clin Virol* 2012;53:167 – 70.
30. Bhatia P, Narang A, Minz RW. Neonatal Cytomegalovirus Infection: Diagnostic Modalities Available for Early Disease Detection. *Indian Pediatri* 2010;77:77 – 9.
31. Shibata M, Takano H, Hironaka T, Hirai K. Detection of human cytomegalovirus DNA in dried newborn blood filter paper. *J Virol Methods* 1994;46(2):279 – 85.
32. Boppana SB, Ross SA, Shimamura M, AL Palmer, Ahmed A, Michaels, Sánchez PJ, Bernstein DI, Tolan RW, Novak S, Chowdhury N, Britt WJ, Karen B. Fowler KB. Saliva Polymerase-Chain-Reaction Assay for Cytomegalovirus Screening in Newborns. *N Engl J Med* 2011;364:2111 – 8.
33. Noyola DE, Demmler GJ, Nelson CT, Griesser C, Williamson WD, Atkins JT et al. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *J Pediatr* March 2001;138:325 – 31.
34. Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF. Symptomatic Congenital Cytomegalovirus Infection in Infants Born to Mothers With Preexisting Immunity to Cytomegalovirus. *Pediatrics* 1999;104:55 – 60.

35. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. *Scand J Infect Dis* 1999;31:443 – 57.
36. Rivera LB, Boppana SB, Fowler KB, Britt WJ, Stagno S et al. Predictors of hearing loss in children with symptomatic congenital cytomegalovirus infection. *Pediatrics* 2002;110:762 – 67.
37. Maruyama Y, Sameshima H, Kamitomo M, Ibara S, Kaneko M, Ikenoue T et al. Fetal manifestations and poor outcomes of congenital cytomegalovirus infections: Possible candidates for intrauterine antiviral treatments. *J Obstet Gynaecol Res* 2007;33(5):619 – 23.
38. Martin RJ, Fanaroff AA, Walsh MC. Neonatal-Perinatal Medicine. Diseases of the fetus and infant. Vol two. 8th Ed. Mosby Elsivier Philadelphia 2006. Appendix B. Tables of Normal Values.p1804 and p1810.
39. Lombardi GF, Stronati M. Congenital cytomegalovirus infection: treatment, sequelae and follow-up. *J Matern Fetal Med* October 2010;23(S3):45 – 8.
40. Schoub BD, Johnson S, McAnerney JM, Blackburn NK, Guidozzi F, Ballot D et al. Is antenatal screening for rubella and cytomegalovirus justified? *S Afr Med J* 1993;83:108 – 10.
41. Munro SC, Trincado D, Hall B, Rawlinson WD. Symptomatic infant characteristics of congenital cytomegalovirus disease in Australia. *J Paediatr Child Health* 2005;41:449 – 52.
42. Al-Hareth, Monem F, Meguid NA. Is low birth weight a risk indicator for congenital cytomegalovirus infection? *J Infect Dev Ctries* 2010;4(1):44 – 47.
43. Goga A, Dinh TH, Jackson D. South African Prevention of Mother-to-child-transmission Evaluation study group (SAPMTCTE study group). "Evaluation of the Effectiveness of

the National Prevention of Mother-to-Child Transmission (PMTCT) Programme at Six Weeks Postpartum in South Africa 2010." South African Medical Research Council, National Department of Health of South Africa and PEPFAR/US Centers for Disease Control and Prevention. 2012. <http://www.doh.gov.za/docs/reports/2012/pmtcteffectiveness.pdf> [Accessed 01 Oct 2012].

44. Gray GE, Urban M, Chersich MF, Bolton C, van Niekerk R et al. for the PEP Study Group. A randomized trial of two postexposure prophylaxis regimens to reduce mother-to-child HIV-1 transmission in infants of untreated mothers. *AIDS* 2005;19:1289 – 97.
45. Slyker JA, Lohman-Payne BL, John-Stewart GC, Maleche-Obimbo E, Emery S, Richardson BA et al. Acute cytomegalovirus infection in Kenyan HIV-infected infants. *AIDS* 2009;23:2173 – 81.
46. Duryea EL, Sanchez PJ, Sheffield JS, Jackson GL, Wendel GD, McElwee BS et al. Maternal Human Immunodeficiency Virus Infection and Congenital Transmission of Cytomegalovirus. *Pediatr Infect Dis J* 2010;29:915 – 18.
47. Bristow BN, O'Keefe KA, Shafir SC, Frank J. Sorvillo FJ. Congenital Cytomegalovirus Mortality in the United States, 1990–2006. *PLoS Negl Trop Dis* 2011;5(4):e1140.
48. Townsend CL, Peckham CS, Tookey PA. Surveillance of congenital cytomegalovirus in the UK and Ireland. *Arch Dis Child Fetal Neonatal Ed* 2011;96:F398 – 403.
49. Chandwani S, Aditya K, Bebenrot D, Kim M, John D, Fidelia A et al. Cytomegalovirus infection in human immunodeficiency virus type-1 infected children. *Pediatr Infect Dis J* 1996;15:310 – 14.

APPENDIX A

Data Collection Sheet

Factors associated with Cytomegalovirus (CMV) Infection in Neonates

Study Number: CMV ___ ___ ___ ___

CMV Positive: shell-vial culture/ pp65

Indications for CMV test: _____

- **Maternal Details**

Age: _____ Parity: _____ Mode of delivery: Vaginal/ C-section

RPR Results: Neg. / Pos. / Unknown HIV results: Neg. / Pos. / Unknown

- **Infant Details**

Date of birth: _____ Birth weight: _____grams, Percentile: _____

Head Circumference: _____ cm, Percentile: _____

Length Percentile: _____ cm, Percentile: _____

Gestational age: _____ Ballard / Obstetrics; HIV PCR: pos / neg; Gender: Male / Female

- **Clinical**

Ventilated: Yes/ No, if Yes, state diagnosis or indication for ventilation:

_____

Signs of congenital infection: Jaundice / Petechiae / Purpura / Hepatosplenomegaly

CNS signs: Microcephaly/ Hydrocephalus/ Seizures / Hypotonia

Other Clinical signs: _____

_____

- **Investigations**

Cranial sonar findings: _____

CT scan findings if done: _____

FBC and CRP at time of culturing CMV

WCC: ___ Hb: ___Platelets: ____CRP: ____

HIV PCR done: Yes / No, if Yes, Pos / Negative

- **Management**

Blood product/ s: Yes / No, if Yes, date & type of transfusion:

_____

Breastfeeding: Yes/ No

- **Outcome**: Survival/ Death (Hospital discharge)

APPENDIX B

Ethics Clearance

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

6 July 2009

Dr Hitesh A Diar
Department of Paediatrics
Division of Neonatology
CH Baragwanath Hospital
University

Dear Dr Diar

RE: Protocol M080102:'Factors Associated with Cytomegalovirus Infection in Neonates' Amendment to research project

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved the extension of the abovementioned protocol from January 2003 to January 2009 as requested in your letter dated 29 May 2009.

Thank you for keeping us updated and informed.

Yours sincerely,

Anisa Keshav
Secretary
Human Research Ethics committee (Medical)